Final Report Part I: Short Report

Preclinical systematic review and meta-analysis of the effects of age and comorbidities on ischaemic stroke outcome and treatment efficacy

Acronym: StrokeCoMorb

Funding programme: Federal Ministry of Education and Research (BMBF) CONFIRMATORY PRECLINICAL STUDIES - QUALITY IN HEALTH RESEARCH - Module 2: Systematic reviews and meta-analyses

Project number: 01KC190

Project summary – EN

Scientific and technical background

Ischaemic stroke is a leading cause of death and disability worldwide and limited treatment options are available. Over 1000 drugs have been tested using models of stroke involving cells or animals and many are reported to be effective. However, these drugs have failed to show benefit when tested in human stroke patients. Systematic review and meta-analysis are techniques to analyse large numbers of studies and have helped to identify possible reasons for this 'translational failure'.

One aspect of translation where research is lacking is the generalisability of experimental results: can we accurately predict outcome in humans based on animal models? A number of factors, including how well we model relevant patient characteristics, may affect generalisability. Stroke affects women and men, who are usually elderly, and often suffer from other health conditions such as high blood pressure (hypertension), obesity, and diabetes, known as comorbidities. In contrast, the most commonly used models of stroke are young healthy male rodents. In this project, we investigated the possible consequences of this discordance by systematically reviewing the impact of modelling advanced age and comorbidities on i) stroke severity in cell-based (in vitro) and animal (in vivo) models and ii) the effectiveness of experimental treatments. We also contributed to the development of state-of-the-art technologies to automate aspects of the review process and improve the speed and accuracy with which reviews can be completed in the future.

Project progress

We divided the project into five sub-projects to more efficiently review the data and to implement any necessary improvements as we progressed:

1. Effect of age, in vivo
2. Effect of diabetes, obesity, and metabolic syndrome (DOMS), in vivo
3. Effect of hypertension, in vivo
4. Effect of age and comorbidities, in vitro
5. Automated approaches

We have published six protocols associated with this project, explicitly pre-specifying the design and analysis plan for each sub-project, in order to minimise bias, help reduce duplication of efforts, and enhance the prospects for collaboration. For all of the in vivo sub-projects (numbers 1-3), we have completed data collection and are in the final stages of data analysis and writing manuscripts for publication. In collaboration with our University of Edinburgh co-applicant, we determined that current

strategies to electronically search for relevant studies to include in a systematic review were much less effective for in vitro studies than for in vivo studies. This led us to focus on developing a classifier to automatically identify in vitro studies for systematic review (sub-project 4). Also in collaboration with our co-applicants, we worked on a framework involving an automated pipeline to identify and visualise in vivo studies in an online database, known as a Systematic Online Living Evidence Summary (SOLES; sub-project 5).

Main results and collaborations

We systematically searched and screened the literature for in vivo studies describing ischaemic stroke in animal models of advanced age or comorbidities. We included 139 studies (sub-project 1), 188 studies (sub-project 2) and 248 studies (sub-project 3). We have presented preliminary meta-analyses of the synthesised results of these studies at two international conferences. The results suggest that advanced age and comorbidities have effects on the severity of the injury induced by stroke, when compared to young healthy animals. They also suggest that the strength of response to treatments tested in aged or comorbid animals differs compared to the response in young healthy animals. We have started to identify some of the factors that contribute to these differences, including study design elements such as the species of animal used, and study quality elements such as whether measures were taken to reduce the risk of bias in the results.

These results will provide important information for preclinical stroke researchers to identify the aspects of their studies that might affect the generalisability of their results and the experimental variables that should be considered in future studies. We developed a collaboration with the Stroke Preclinical Assessment Network (SPAN), a network of preclinical stroke researchers who test the efficacy of treatments for stroke in animals using a rigorous approach similar to how treatments are tested in human clinical trials. Our results will help to inform experimental design in next round of treatments tested by the SPAN, to make sure they are as clinically relevant as possible.

These results also helped inform a successful grant application to investigate sex differences in preclinical stroke studies and thus continue our determination of the factors affecting the generalisability of findings from preclinical to clinical settings. This project will include a SOLES of the synthesised evidence related to sex differences and also the evidence on advanced age and comorbidities from the current project. Further, we have developed a collaboration with an in vivo stroke research laboratory and will co-supervise a PhD candidate who will systematically review the effects of sex on stroke before undertaking in vivo experiments informed by the results of the systematic review.

Our collaborative effort to develop a classifier to more easily identify in vitro studies in the context of systematic reviews has recently been published and is now ready to be applied (sub-project 4). This work also helped inform a successful grant application, where we will develop a framework for the systematic review of in vitro studies, including tools to systematically identify in vitro studies (e.g., through our classifier) and to assess study quality.

In addition, we collaborated on a classifier to identify in vivo animal studies that has been incorporated into a pipeline for Stroke-SOLES (https://camarades.shinyapps.io/STROKE-SOLES/), where in vivo ischaemic stroke studies are automatically identified and presented in a dashboard format (sub-project 5). Stroke-SOLES is freely available for users to search and download articles. It provides a resource for

the research community that is expected to substantially reduce the amount of time spent identifying stroke articles relevant to different research questions, both in the context of systematic reviews and more broadly.

Project summary – DE

Wissenschaftlicher und technischer Hintergrund

Der ischämische Schlaganfall stellt weltweit eine der Haupttodesursachen und eine der Hauptursache für bleibende Behinderungen dar, und bietet nur begrenzte Behandlungsmöglichkeiten. Mehr als 1000 Medikamente wurden an Schlaganfallmodellen von Zellen oder Tieren getestet, und viele von ihnen haben sich als wirksam erwiesen. Bei der Erprobung an menschlichen Schlaganfallpatient:innen haben diese Medikamente jedoch keinen Nutzen gezeigt. Systematische Reviews und Meta-Analysen sind Techniken zur Analyse einer großen Anzahl von Studien und haben dazu beigetragen, mögliche Gründe für dieses "Translationsversagen" zu ermitteln.

Ein Aspekt der Translation, an dem es der Forschung mangelt, ist die Generalisierbarkeit von Versuchsergebnissen: Können wir auf der Grundlage von Tiermodellen die Ergebnisse beim Menschen genau vorhersagen? Die Generalisierbarkeit kann durch eine Reihe von Faktoren beeinflusst werden, unter anderem dadurch, wie gut wir relevante Patientenmerkmale modellieren. Ein Schlaganfall betrifft sowohl Frauen wie Männer, die in der Regel höheren Alters sind und häufig an anderen Krankheiten wie Bluthochdruck (Hypertonie), Übergewicht und Diabetes - den so genannten Komorbiditäten - leiden. Im Gegensatz dazu sind die am häufigsten verwendeten Modelle für Schlaganfälle junge, gesunde, männliche Nagetiere. In diesem Projekt untersuchten wir die Folgen dieses Unterschieds, indem wir systematisch die Auswirkungen der Modellierung von fortgeschrittenem Alter und Komorbiditäten auf i) die Schwere des Schlaganfalls in zellbasierten (in vitro) und tierischen (in vivo) Modellen und ii) die Wirksamkeit experimenteller Behandlungen analysierten. Wir haben auch zur Entwicklung neuer Technologien beigetragen, um Aspekte des involvierten Überprüfungsprozesses zu automatisieren und die Geschwindigkeit und Genauigkeit zu verbessern, mit der die Überprüfungen in Zukunft durchgeführt werden können.

Projektfortschritt

Wir haben das Projekt in fünf Teilprojekte unterteilt, um die Daten effizienter zu prüfen und notwendige Verbesserungen im Laufe des Projekts umsetzen zu können:

1. Auswirkung des Alters, in vivo
2. Auswirkung von Diabetes, Übergewicht und metabolischem Syndrom (DOMS), in vivo
3. Auswirkung von Bluthochdruck, in vivo
4. Auswirkung von Alter und Komorbiditäten, in vitro
5. Automatisierte Ansätze

Wir haben sechs Protokolle im Zusammenhang mit diesem Projekt veröffentlicht, in denen das Design und der Analyseplan für jedes Teilprojekt definiert sind, um Verzerrungen zu minimieren, doppelte Arbeit zu vermeiden und die Aussichten auf Kollaborationen mit externen Partnern zu verbessern. Für alle In-vivo-Teilprojekte (Nummern 1-3) haben wir die Datenerfassung abgeschlossen und befinden uns

in der Endphase der Datenanalyse und der Erstellung von Manuskripten für die Veröffentlichung. In Zusammenarbeit mit unseren Mitantragsteller:innen von der Universität Edinburgh haben wir festgestellt, dass die derzeitigen Strategien für die elektronische Suche nach relevanten Studien, die in ein systematisches Review aufgenommen werden sollen, für In-vitro-Studien weit weniger effektiv sind als für In-vivo-Studien. Daher konzentrierten wir uns auf die Entwicklung eines Klassifikators zur automatischen Identifizierung von In-vitro-Studien für das systematische Review (Teilprojekt 4). Ebenfalls in Zusammenarbeit mit unseren Mitantragsteller:innen arbeiteten wir an einem System mit einer automatisierten Pipeline zur Identifizierung und Visualisierung von In-vivo-Studien in einer Online-Datenbank, einer sogenannten Systematic Online Living Evidence Summary (SOLES; Teilprojekt 5).

Wichtigste Ergebnisse und Kollaborationen

Wir haben die Literatur systematisch nach In-vivo-Studien durchsucht, die einen ischämischen Schlaganfall in Tiermodellen mit fortgeschrittenem Alter oder Begleiterkrankungen beschreiben. Wir schlossen 139 Studien (Teilprojekt 1), 188 Studien (Teilprojekt 2) und 248 Studien (Teilprojekt 3) ein. Auf zwei internationalen Konferenzen haben wir vorläufige Ergebnisse der Meta-Analysen dieser Studien vorgestellt. Die Ergebnisse deuten darauf hin, dass fortgeschrittenes Alter und Komorbiditäten Auswirkungen auf die Schwere der durch einen Schlaganfall hervorgerufenen Schädigung haben, wenn man sie mit jungen, gesunden Tieren vergleicht. Sie deuten auch darauf hin, dass die Stärke der Reaktion auf Behandlungen, die bei alten oder komorbiden Tieren getestet wurden, sich von der Reaktion bei jungen gesunden Tieren unterscheidet. Wir haben damit begonnen, einige der Faktoren zu ermitteln, die zu diesen Unterschieden beitragen und darunter Elemente des Studiendesigns wie der verwendeten Tierart und Elemente der Studienqualität wie der Frage, ob Maßnahmen ergriffen wurden, um das Risiko einer Verzerrung der Ergebnisse zu verringern, gefunden.

Diese Ergebnisse liefern wichtige Informationen für präklinische Schlaganfallforscher:innen, um Aspekte ihrer Studien zu identifizieren, die eine Generalisierbarkeit ihrer Ergebnisse beeinträchtigen könnten, sowie Faktoren, die in zukünftigen Studien berücksichtigt werden sollten. Wir haben eine Zusammenarbeit mit dem Stroke Preclinical Assessment Network (SPAN) aufgebaut, einem Netzwerk von präklinischen Schlaganfallforscher:innen, die die Wirksamkeit von Schlaganfallbehandlungen im Tierversuch mit einem methodisch-strikten Ansatz ähnlich wie in klinischen Studien am Menschen testen. Unsere Ergebnisse werden in die Versuchsplanung für die nächste Runde der vom SPAN getesteten Behandlungen einfließen, um sicherzustellen, dass sie so klinisch relevant wie möglich sind.

Diese Ergebnisse haben auch dazu beigetragen, einen erfolgreichen Zuschussantrag zu stellen, um Geschlechtsunterschiede in präklinischen Schlaganfallstudien zu untersuchen und so die Faktoren weiter zu bestimmen, die die Verallgemeinerbarkeit der Ergebnisse von präklinischen auf klinische Bedingungen beeinflussen. Dieses Projekt wird eine SOLES der synthetisierten Evidenz zu Geschlechtsunterschieden und auch die Evidenz zu fortgeschrittenem Alter und Komorbiditäten aus dem aktuellen Projekt beinhalten. Wir haben eine Zusammenarbeit mit einem In-vivo-Schlaganfall-Forschungslabor aufgebaut und werden eine Doktorarbeit mitbetreuen, die die Auswirkungen des Geschlechts auf den Schlaganfall systematisch untersuchen wird, bevor die In-vivo-Experimente durchgeführt werden, die auf den Ergebnissen des Reviews basieren.

Unsere gemeinsame Arbeit an der Entwicklung eines Klassifikators zur leichteren Identifizierung von In-vitro-Studien im Rahmen systematischer Reviews wurde kürzlich veröffentlicht und kann nun angewendet werden (Teilprojekt 4). Diese Arbeit hat auch zu einem erfolgreichen Förderantrag

beigetragen, bei dem wir eine Handreichung für systematische Reviews von In-vitro-Studien entwickeln werden, welche Instrumente zur systematischen Identifizierung von In-vitro-Studien (z. B. durch unseren Klassifikator) und zur Bewertung der Studienqualität umfasst.

Darüber hinaus haben wir an einem Klassifikator zur Identifizierung von In-vivo-Tierstudien mitgearbeitet, der in eine Pipeline für Stroke-SOLES (https://camarades.shinyapps.io/STROKE-SOLES/) integriert wurde, in der In-vivo-Studien zum ischämischen Schlaganfall automatisch identifiziert und in einem Dashboard-Format dargestellt werden (Teilprojekt 5). Stroke-SOLES steht den Nutzer:innen frei zur Verfügung, um Artikel zu suchen und herunterzuladen, und stellt eine Ressource für die Forschungsgemeinschaft dar, von der erwartet wird, dass sie den Zeitaufwand für die Identifizierung von Schlaganfallartikeln, die für verschiedene Forschungsfragen relevant sind, sowohl im Zusammenhang mit systematischen Reviews als auch im weiteren Sinne erheblich verringern wird.

Final Report Part II: Detailed Presentation

Preclinical systematic review and meta-analysis of the effects of age and comorbidities on ischaemic stroke outcome and treatment efficacy

Acronym: StrokeCoMorb

Funding programme: Federal Ministry of Education and Research (BMBF) CONFIRMATORY PRECLINICAL STUDIES - QUALITY IN HEALTH RESEARCH - Module 2: Systematic reviews and meta-analyses

Project number: 01KC190

Scientific and technical background

Ischaemic stroke is a leading cause of death and disability worldwide and limited treatment options are available. Attempts to develop new treatments using in vivo animal models have been unsuccessful; systematic review and meta-analysis have provided important information on factors that may be contributing to this translational failure. The impact of low internal validity has been well established: studies at high risk of bias often report larger treatment effects than studies where measures to reduce bias, such as randomisation and blinding, have been carried out. In combination with small sample sizes and publication and reporting biases, this can result in a body of evidence predicting overly or falsely positive outcomes for experimental therapies.

Less well established is the role of external validity in translational research. External validity is the extent to which experimental inferences can be generalised, e.g. to other laboratories, from in vitro to in vivo models and, ultimately, to humans. Our ability to generalise results beyond the laboratory may be affected by several factors, including the construct and predictive validity of our models. In stroke research, an important aspect of construct validity is how well we model relevant patient characteristics. Stroke affects women and men, who are usually elderly, and often suffer from comorbidities such as hypertension and diabetes. The most commonly used models of stroke, in contrast, are young, healthy, male rodents. There is evidence that the physiological effects of aging and comorbidities can affect stroke progression and responses to treatment. However, they are rarely modelled in stroke with factors including time, cost, and level of experimental difficulty cited as barriers.

In addition to animal models, stroke researchers use animal and human-derived in vitro brain cell or slice models exposed to ischaemia-like conditions to investigate stroke pathophysiology and treatments. The results from these experiments often inform the decision to take forward interventions to test in animals, and may have the capacity to replace animal experiments under some circumstances. However, there has been little discussion on what constitutes sufficient validity of in vitro evidence to warrant the progression to animal studies, or what factors should be modelled in vitro to provide the most robust, generalisable evidence possible.

In this project, we aimed to assess in the in vivo and in vitro literature the impact of modelling age and comorbidities on experimental stroke outcomes and treatment efficacies.

We hypothesised that modelling age and comorbidities would increase stroke severity, and that treatment efficacy in these models would be limited.

Our specific **objectives** were:

1. In vivo – investigate in models of middle cerebral artery occlusion (MCAo; the most common cause of stroke in humans) the impact of modelling advanced age and comorbidities on:

a) Stroke outcome (histological and functional)

b) Treatment efficacy with and without co-treatments for comorbidities

2. In vitro – investigate if age and comorbidities are modelled in animal or human-derived cell-based platforms incorporating ischaemia-like conditions or ischaemia-induced processes, and their impact on:

a) Cell survival and death

b) Treatment efficacy with and without co-treatments for comorbidities

3. Investigate concordance between in vitro and in vivo modelling systems incorporating age and comorbidities

Systematic reviews are time and resource intensive and often out of date by the time they are published. An important feature of this project was to apply new automation tools to improve the accuracy and speed of the review, and to contribute to the development and validation of these tools. We aimed to apply methods to prioritise screening to streamline the review process and to assess study design criteria related to risk of bias (RoB). We also aimed to collect data to help train automated tools for systematic review, especially in vitro reviews, for which few validated tools existed. Research and development into automating systematic reviews continues to represent an area of great interest for researchers. It has the potential to reduce substantially the time and resources needed to complete preclinical systematic reviews in the future.

Project execution:

Initially, the project was planned to investigate all studies related to ageing and comorbidities in vivo together. To more effectively review these data in experimental stroke and to implement any necessary improvements as we progressed, we split the project into several sub-projects in the beginning:

6. Effect of age, in vivo
7. Effect of diabetes, obesity, and metabolic syndrome (DOMS), in vivo
8. Effect of hypertension, in vivo
9. Effect of age and comorbidities, in vitro
10. Automated approaches

The project plan, revised from our initial submission, is detailed in Figure 1. A cost neutral extension of the project of eight months was requested as a consequence of the Corona pandemic, starting from 2020. Even though all tasks could be executed also from the home office, the need for simultaneous work load and child care limited the effectiveness of our involved personnel. The conditions imposed by the Corona pandemic remained challenging for our staff also throughout the extension period.

Due to a change in student assistant personnel and the need to tackle pandemic-related delays, we trained one co-worker, five student assistants, and one master's student throughout the project. Training these health and biomedical research students has the benefit of teaching skills related to systematic review and meta-analysis, but also provides a deep understanding of how to assess the validity of research and, importantly, how to design their future research in a rigorous manner.

We registered and / or published a full protocol for each sub-project.

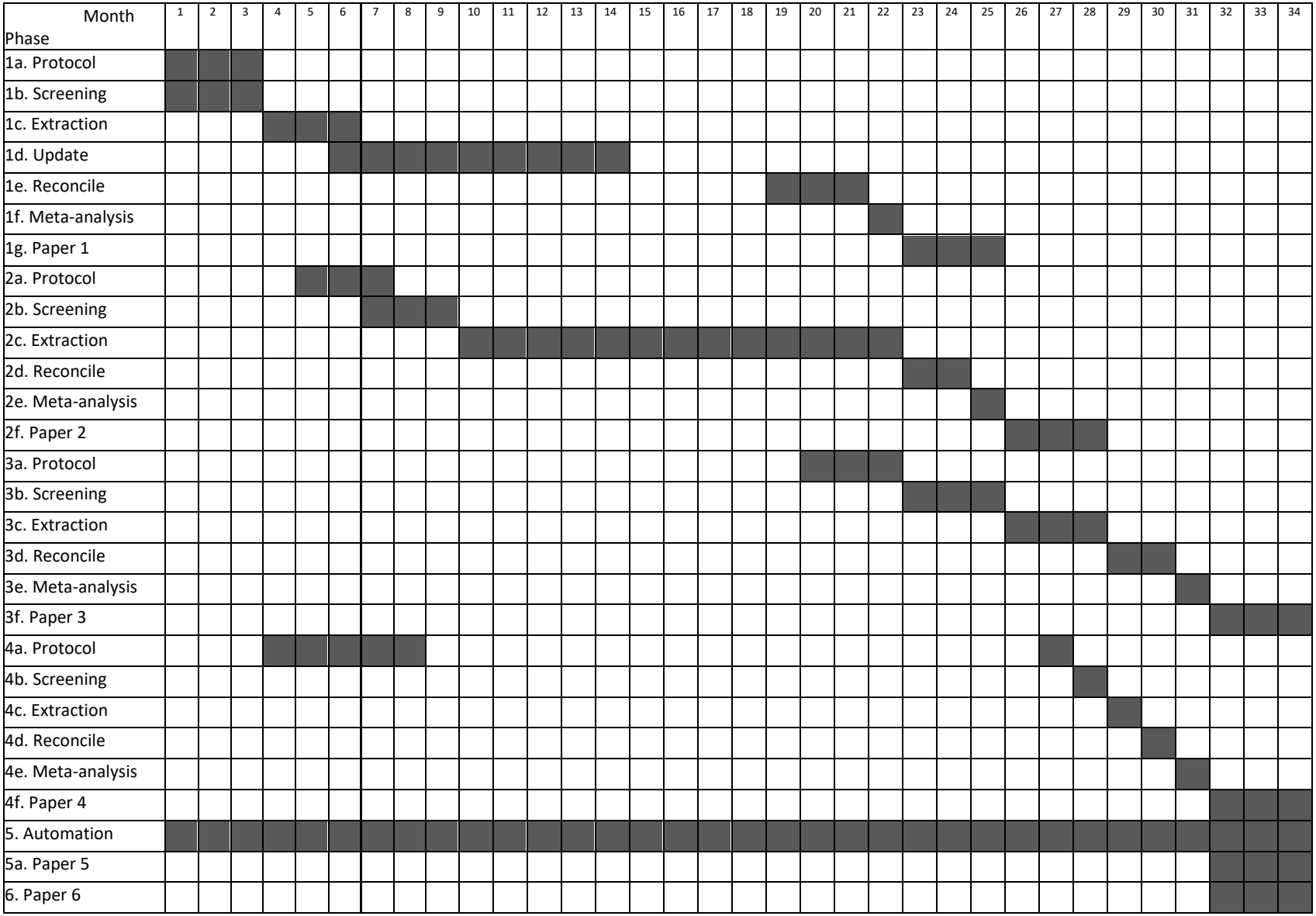

Figure 1: GANTT Chart of StrokeCoMorb project including request for extension

Project outcomes:

1. **Effect of age, in vivo**

For our first sub-project, the protocol "Systematic review and meta-analysis of the effects of ageing on stroke outcome and treatment efficacy in animal models of ischaemic stroke" was published on the SyRF website (McCann et al 2020).

We conducted our original search in January 2019, which returned 1398 unique records, and a search update in April 2020, which returned 1102 records. These 2500 rescords were screened for inclusion by a minimum of two independent reviewers and a total of 139 studies have been included after full-text screening. We evaluated changes in the outcomes infarct size, neurobehaviour, and mortality in response to stroke in young vs. aged animals and in treated vs. untreated aged animals. We also evaluated the difference in treatment efficacy in young vs. aged animals, where these data were reported.

Data extraction and assessment of RoB have been completed by two independent reviewers for each included study, disagreements have been reconciled by a third reviewer, and data analysis is currently underway. Preliminary results have been presented at two conferences and indicate that advanced age affects both stroke outcomes and treatment efficacy. Manuscript preparation has begun and a final search update will be conducted prior to submission.

Protocol:

McCann, S.K., Heine, K., Cruz, F., Sena, E.S., Bannach-Brown, A., & Dirnagl, U. Systematic review and meta-analysis of the effects of ageing on stroke outcome and treatment efficacy in animal models of ischaemic stroke. (2020, January 31) SyRF protocol registry; https://syrf.org.uk/protocols

Conference abstracts:

McCann, S., Cruz, F., Rackoll, T., Hair, K., Dirnagl, U., & Sena, E. (2022, June). Effect of age on ischaemic stroke outcomes: A systematic review and meta-analysis of animal studies. In JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM (Vol. 42, No. 1_ SUPPL, pp. 231-231).

McCann, S.K., Heine, K., & Dirnagl, U. Preclinical systematic review and meta-analysis of the effects of age and comorbidities on ischaemic stroke outcome and treatment efficacy. REWARD | EQUATOR Conference, Berlin, Germany 2020

2. **Effect of diabetes, obesity, and metabolic syndrome, in vivo**

Diabetes and obesity were combined in this second project, due to the overlap in their occurrence in human populations and animal models. A pilot search also revealed a third overlapping co-morbidity relevant to human stroke patients: metabolic syndrome, which is also modelled in animals. We therefore extended this project to include metabolic syndrome and adjusted the protocol and search strategy accordingly.

In this sub-project, we searched the databases in July 2020 and retrieved 6406 unique records. Title and abstract screening conducted by at least two independent reviewers limited the number of results to 313, for which full texts were retrieved. After full text screening, data from 188 independent studies were extracted by two reviewers and discrepancies in the data resolved. In the meta-analysis, we aimed to assess the effect of each metabolic disease on stroke outcome compared to healthy animals and the treatment effect in comorbid and in comorbid vs. healthy animals.

A student of the extra-occupational master program “Laboratory Animal Science” at RWTH Aachen University, Joachim Wahl, completed their MSc dissertation on this sub-project under our supervision (McCann primary supervisor). Dr Wahl looked specifically at studies modelling obesity and was graded 1.1 for his thesis.

Preliminary findings were also presented at the ‘Brain & Brain PET’ conference 2022 in Glasgow, UK, and suggest that diabetes and obesity both have an effect on stroke outcome. The search did not retrieve enough comparisons to justify a meta-analysis for the comparison of models of metabolic syndrome against healthy animals. Equally, the number of studies including neurobehavioral outcomes were not sufficient in obesity models for quantitative comparisons.

An update of the search is currently underway to include also the most recent publications. This will potentially increase the robustness of the findings of a publication that will be written after completion of the update.

Protocol:

Rackoll, T., Sena, E., Macleod, M. R., Lawrence, C., Bannach-Brown, A., Dirnagl, U., Cruz, F., Vojvodic, S., & McCann, S. (2020, July 14). Systematic review and meta-analysis of the effects of diabetes mellitus, obesity and metabolic syndrome on stroke outcome and treatment efficacy in animal models of ischaemic stroke. https://doi.org/10.17605/OSF.IO/9BWGS

Registration:

Rackoll, T., McCann, S., Bannach-Brown, A., Sena, E., Cruz, F., Dirnagl, U., & Lawrence, C. Systematic review and meta-analysis of the effects of diabetes mellitus, obesity and metabolic syndrome on stroke outcome and treatment efficacy in animal models of ischaemic stroke. PROSPERO 2020 CRD42020191339: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020191339

MSc Thesis:

Wahl, J. 2021. *Systematic Review of the Effects of Obesity on Stroke Outcome in Animal Models of Ischaemic Stroke*. Master thesis, RWTH Aachen University.

Conference abstract:

Rackoll, T., Wahl, J., Cruz, F., Vojvodic, S., Iqbal, S., Hobby, D., Lawrence, C., Sena, E., Dirnagl, U. and McCann, S. (2022, June). The effect of metabolic diseases on stroke outcome in preclinical stroke: Systematic Review and meta-analysis. In JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM (Vol. 42, No. 1_ SUPPL, p. 202).

3. Effect of arterial hypertension, in vivo

This third section looked at the effect of arterial hypertension on stroke outcome and treatment efficacy. The protocol was published on osf.io and registered on PROSPERO for animals. An initial search revealed that the number of studies surpassed the volume of results from the previous two sub-projects, which justified the division in several sub-projects. The final search was conducted in October 2021 and revealed 8287 unique records. After title and abstract screening by two independent reviewers, 739 studies remained for full text screening. After screening by two reviewers and reconciliation by a third reviewer, 248 independent studies remained.

Data analysis is currently underway. Given the large number of retrieved studies, more possibilities for quantitative effect measures using meta-analysis are to be expected and will be summarised in a subsequent publication.

Protocol:

Rackoll, T., Bannach-Brown, A., Lawrence, C., Vojvodic, S., Iqbal, S., Hobby, D., Sena, E.S., Dirnagl, U., & McCann, S. (2021, October 8). Systematic review and meta-analysis of the effects of arterial hypertension on stroke outcome and treatment efficacy in animal models of ischaemic stroke. https://osf.io/e4rxw

Registration:

Rackoll, T., Bannach-Brown, A., Lawrence, C., Vojvodic, S., Iqbal, S., Hobby, D., Sena, E.S., Dirnagl, U., & McCann, S. Systematic review and meta-analysis of the effects of arterial hypertension on stroke outcome and treatment efficacy in animal models of ischaemic stroke. PROSPERO 2021 CRD42021283853: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021283853

4. Effect of age and comorbidities, in vitro

The systematic review of in vitro studies has become increasingly common in recent years, however little guidance exists on the most appropriate way to identify and synthesise in vitro data. In collaboration with our Edinburgh University-based co-applicant, we determined that the relevance of search results is much lower for in vitro than in vivo studies. This means that using search and screening methods traditionally used for in vivo reviews – methods based on title and abstract [tiab] – may fail to identify a large proportion of in vitro studies. Correctly and completely identifying relevant studies is one of the most critical aspects of a systematic review. Failing to include relevant studies leads to incomplete reviews and conclusions that may be misleading or biased. Often, in vitro studies are included as a component of an animal or human study and not reported in the [tiab], necessitating screening the full-text of a paper to identify such experiments. This dramatically increases the length of time taken to carry out a review, especially in the preclinical space where there are often many search results to screen.

Therefore, we developed a classifier to automatically detect in vitro studies based on [tiab] (protocol: Wilson et al 2022; publication: Wilson et al 2023). We manually screened a random sample of 2000 publication records, in duplicate, to identify studies involving in vitro experiments and supplemented this dataset with a further 453 known in vitro studies. We used this combined dataset to train a machine learning algorithm to identify in vitro experiments. The algorithm currently performs with sensitivity of 0.954, specificity of 0.850 and precision of 0.700. We compared the performance of the algorithm with manual human screening and regular expression (RegEx)-based methods, which search for patterns of characters in text.

We propose that this tool may be used as a first selection phase in in vitro systematic reviews to limit the extent of full-text screening necessary. As this tool is very new, we have not yet been able to apply it to our in vitro systematic review on the effect of age and comorbidities. We have published the protocol for this sub-project (McCann et al 2023) and will proceed as detailed, with the aim to investigate if current in vitro models capture age and comorbidities, the methods used, and the concordance between in vivo and in vitro results.

Publication:

Wilson, E., Cruz, F. A., Maclean, D., Ghanawi, J., McCann, S.K., Brennan, P.M., Liao, J., Sena, E., & Macleod, M; Screening for in vitro systematic reviews: a comparison of screening methods and training of a machine learning classifier. Clin Sci (Lond) 31 January 2023; 137 (2): 181–193. doi: https://doi.org/10.1042/CS20220594

Protocols:

Wilson, E., Cruz, F. A., Liao, J., McCann, S., Macleod, M. R., & Sena, E. (2022, February 5). Development and validation of methods for identification and quality assessment of in vitro research. https://doi.org/10.17605/OSF.IO/AHFR3

McCann, S., Hair, K., Rackoll, T., Sena, E., Vojvodic, S., & Wilson, E. (2023). Protocol for a preclinical systematic review and meta-analysis of the effects of advanced age and comorbidities on in vitro ischaemic stroke model outcomes. https://osf.io/h3tdr

5. Automated approaches

In this part of the project, we aimed to test the use of recently available automation tools in the context of systematic review. We initially aimed to use a RegEx-based tool to assess the reporting of randomisation, blinding, and sample size calculation and to record the phrases used to report these data in the included papers, to help improve the performance of the RegEx libraries behind the tool. Since submitting our application, a different automated tool to assess reporting of measures to reduce RoB, using natural language processing, was published by our collaborators (Wang et al 2022). This tool outperforms RegEx for assessing most RoB items.

However, while the development of more advanced automation tools is an exciting move towards streamlining systematic reviews, their performance levels mean that their application is currently recommended for research improvement activities where several hundred publications are to be evaluated. They are not yet at the level where they are appropriate for the evaluation of individual publications (Wang et al 2022; https://doi.org/10.1002/jrsm.1533).

During this project, it also became apparent through the extensive deliberations required to reach consensus on assessing RoB in many experiments, that the unstandardised, poor reporting quality for most studies made automated assessments problematic. For example, an automated tool will provide a threshold for the likelihood that randomisation is reported in a study, however to assess RoB, we need to know the method of randomisation and whether this method is appropriate.

Due to these issues, the automated assessment of RoB items in our systematic reviews was not pursued. When automatically assessing larger cohorts of studies, RegEx-based identification of some items will likely remain optimal in some cases (e.g. assessing reporting of a sample size calculation) due to the scarcity of training data (especially “positive” cases) for machine learning algorithms. In these instances, the phrases we recorded during our reviews to describe these events will add useful information to tool development. We also recorded reporting of additional items including animal housing facility type, method of stroke induction, and temperature control during stroke induction, that will all contribute to automatic annotations of stroke studies in the future.

During this project we also developed flowchart-based decision trees for every item of the SYRCLE RoB Tool. These flowcharts proved instrumental in training reviewers and improving the agreement ratio between independent reviewers. We now have plans to implement a Shiny app based on these flowcharts to streamline RoB assessment and visualisation, which can be integrated into the CAMARADES Systematic Review Facility (SyRF) online platform for general use.

Since submitting our initial application, there has also progress in automatically identifying, screening and visualising preclinical studies in the context of systematic reviews. Led by our collaborators at the University of Edinburgh, Systematic Online Living Evidence Summaries (SOLES), are a new way to accelerate evidence synthesis (Hair et al 2022; https://doi.org/10.31222/osf.io/nbe5q), superseding our planned approach of using keyword frequency to prioritise screening.

During this project, we collaborated on the development of a classifier to identify in vivo studies. The algorithm has achieved a sensitivity of 95%, a specificity of 98%, and an accuracy of 97%. This classifier has been applied in the recently launched Stroke-SOLES (protocol: Wilson et al 2022; manuscript in preparation). This free, online database systematically collects, synthesises, and displays all experimental evidence in animal models of focal cerebral ischaemia by integrating a series of automated tools (https://camarades.shinyapps.io/STROKE-SOLES/). While it was not developed in time to utilise in the current project, we will be using Stroke-SOLES in future systematic reviews, where it is expected to substantially reduce the amount of time spent searching for and screening potentially relevant articles. For searching for more specific studies within Stroke-SOLES, literature related to aged and comorbidities can be identified by applying the RegEx developed for our systematic review of the effect of age and comorbidities, in vitro (Part 4, described above). We plan to validate Stroke-SOLES against traditional search strategy approaches for study identification in our next systematic review.

Protocol:

Wilson, E., Cruz, F. A., Ghanawi, J., Liao, J., McCann, S., Macleod, M. R., & Sena, E. (2022, February 5). Systematic Online Living Evidence Summary (SOLES) of Preclinical In Vivo Focal Cerebral Ischaemia Research. https://doi.org/10.17605/OSF.IO/EK6DF

Conference abstract:

Wilson, E., Cruz, F., Ghanawi, J., Liao, J., McCann, S., Macleod, M., & Sena, E. Developing a living evidence summary of preclinical in vivo focal cerebral ischaemia research. European Stroke Journal. 2021. 6(1_SUPPL). Poster. Available at: https://osf.io/5g39a

Conference presentation:

McCann, S. How to unlock preclinical studies' data treasury to find promising new drugs for cerebrovascular disease. European Stroke Organisation Conference (ESOC); Speaker in: Session 2 Technology / Multi-discipline / Beyond medicine - reduce, reuse, recycle – Go green to improve efficiency and reduce waste in stroke research. Lyon, France, 05 May 2022.

Budget description

The finance plan from the initial grant proposal was carefully evaluated in the beginning of the project phase. All positions were needed and the requested costs spent accordingly. Only the requested budget for open access fees could not be used as the publication are still awaiting submission.

Necessity and appropriateness of the project work performed

In the beginning of the project phase, the decision was made to split the overall project into several sub-projects separating the studies that look at in vitro models from in vivo models and further on separating the comorbidities diabetes and obesity as metabolic diseases from arterial hypertension and ageing. In our intermediate report, we argued that the decision was made to better validate the data on the different subfields. This iterative approach enabled us to improve our quality control throughout the course of the overall project. Further, we were able to consecutively and effectively make use of our student assistant, who otherwise would have the majority of workload in the beginning of the project phase with limited use at the end of the project. While all requested personnel were necessary and appropriate, the dual task of quality control and reviewing for our

postdoctoral researcher limited effectiveness which had to be compensated by other members of the working group.

Use of results and concrete plans for future

Dissemination

To date, we have published six protocols associated with this project, explicitly documenting each study's design and analysis plan, in order to minimise bias, help reduce duplication of efforts, and enhance the prospects for collaboration. To further increase transparency and dissemination, two sub-projects have been registered with PROSPERO, the Prospective Register of Systematic Reviews (https://www.crd.york.ac.uk/prospero/).

Despite interruption due to the COVID-19 pandemic, we were able to present preliminary results from three sub-projects at four international conferences. Together with our co-applicants, we have published the results of our in vitro classifier (Wilson et al 2023; open access). We have begun preparing manuscripts for the remaining sub-projects and will publish the results of each review in open access journals, in addition to making them available in a free, online SOLES (see below). Raw data exported from SyRF will be deposited in a repository (e.g., OSF) and accompanied by a data descriptor article to facilitate reuse by the research community. Code will be shared on GitHub to enable others to test the analytic reproducibility of our findings.

Exploitation

In the context of StrokeCoMorb, we reached out to Prof. Patrick Lyden of the University of Southern California, the project lead of the Stroke Preclinical Assessment Network (SPAN; https://spannetwork.org/), to explore possible avenues for collaboration with primary preclinical stroke researchers. We met several times, including at the 'Brain & Brain PET' conference in June 2022, where we presented some preliminary results from StrokeCoMorb.

The SPAN is a network of laboratories that conducts late-stage multicentre preclinical stroke studies evaluating putative cerebroprotectants. It represents the first attempt by the stroke community to evaluate the efficacy of multiple interventions concurrently, using a study design that includes essential elements of rigour typically found in clinical trials.

Having just completed testing six interventions in SPAN 1.0, which involved over 2600 animals, the network are now looking to design SPAN 2.0. In addition to rigour associated with internal validity, external validity will be of utmost importance for the translational focus of SPAN. This presents the opportunity to apply the results of our systematic reviews to help inform optimal study designs related to modelling stroke patient characteristics. We plan to work with the SPAN to integrate our evidence related to the factors affecting stroke outcomes in aged and comorbid animals to help ensure SPAN results are as generalisable as possible to clinical populations.

We have also worked with the Cell Cycle and Brain Ischaemia group at Charité – Universitätsmedizin Berlin's Center for Stroke Research, headed by Prof. Christoph Harms. The group has an interest in modelling ageing and stroke and recently completed a series of preclinical animal experiments where they noted possible sex differences in their results. We subsequently completed a sub-analysis of our systematic review dataset of studies involving aged animals of both sexes and provided this as background to help inform the next steps in the group's experimental plan.

This has resulted in a combined PhD position (partly funded by the DFG, see below) involving a project that will incorporate a preclinical systematic review of sex differences in ischaemic stroke

followed by laboratory-based experiments applying the evidence from the systematic review to inform experimental study design (candidate co-supervised by McCann and Harms, Jan 2023 – Dec 2025). This model of systematically reviewing the available literature before embarking on new preclinical experiments is one that we are promoting through other projects in our group and will have the added benefit of providing a blueprint for how such an approach can work in practice.

Ongoing plans

The preliminary results from the in vivo sections of this project helped inform a successful Deutsche Forschungsgemeinschaft (DFG) research grant (project number 504323693; McCann is principal investigator), "Systematic review and meta-analysis of sex differences in preclinical ischaemic stroke research". This project will extend our analysis of factors relevant to the external validity of preclinical ischaemic stroke studies (funded Jan 2023 – Dec 2025). Stroke occurs in both males and females; however, the most commonly used models of stroke are male rodents. This male bias in preclinical research is of particular concern given the well-established sexual dimorphism in clinical stroke. In this review we will identify and synthesise all preclinical ischaemic stroke research investigating sex differences. We will integrate and evaluate the evidence on mechanisms of sexual dimorphism in ischaemic cell damage and repair and responses to stroke treatments.

Of particular relevance, the work programme of this project includes the development of a SOLES of factors assumed to affect the external validity of preclinical stroke research. In addition to including the effects of sex, we will include the results of our reviews on the effects of advanced age and the comorbidities examined in the current project: diabetes, obesity, metabolic syndrome, and hypertension. This SOLES will share most backend processes with our in vivo Stroke-SOLES (https://camarades.shinyapps.io/STROKE-SOLES/) but differs in that it specifically identifies studies relevant to external validity, and includes in vitro and non-intervention studies. We will use automated tagging of relevant studies with criteria of external validity related to ageing and comorbidities. To accommodate these features, the user interface will be designed separately but linked to our Stroke-SOLES. The SOLES dashboard format allows users to visualize and interrogate the data, and download relevant results. It provides a bridge to enable in vivo researchers and other stakeholders to leverage emerging data science tools to quickly and accurately identify literature relevant to external validity in preclinical stroke research.

We anticipate our SOLES will be particularly useful for multiple groups, including: i) preclinical researchers, to plan future experiments, ii) systematic reviewers, to answer research questions additional to those we address, iii) other methodologists e.g. statisticians or data scientists who require large datasets to develop models or tools, iv) translational or clinical researchers, who may pursue treatments with sufficient evidence to clinical trial, and v) peer reviewers who will benefit from the ability to gain a rapid overview when reviewing grants or manuscripts.

Our work on the systematic review of in vitro studies has also contributed to a successful collaborative grant "Improving the credibility and utility of in vitro research" funded by the Medical Research Council (UK) under the "Better Methods, Better Research" programme (grant ref: MR/W029898/1; Sena is principal investigator, McCann is a co-investigator). Frameworks for the systematic review of animal and clinical research are well-established, and their use is associated with improvements in the quality of experimental design and reporting. No such framework exists for in vitro research and the tools and infrastructure to identify, synthesise and appraise in vitro research are not yet developed. Research is incremental, and if findings from in vitro studies are flawed, this represents an important source of waste in basic biomedical research that can also lead to waste in later research stages.

In this new project (Nov 2022-Oct 2025), we will develop a framework for the systematic review of in vitro studies, including tools to systematically identify in vitro studies (e.g., through our classifier) and to assess study quality. This will include a Delphi process, involving stakeholders from across the spectrum of in vitro research, to establish the factors important for the validity of in vitro studies. Based on the results of this Delphi process, we will develop a RoB tool for systematic reviews of in vitro research. This will lead to the development of a community-led consensus-based tool that is far more robust than would be possible through an individual systematic review. By developing this infrastructure, we will provide the research community and associated stakeholders with the key tools to develop robust evidence to guide improvements in the design, conduct and reporting of in vitro research.