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Histological Quantification of Astrocytosis after Cerebral Infarction: A Systematic Review

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Abstract

Accurate histological quantification of astrocytosis after cerebral infarction is needed as this process may affect, and be affected by, many potential restorative treatments under investigation. We performed a systematic review to determine the most reliable histological method reported for measurement of post-infarction astrocytosis. We found reports of multiple techniques to quantify various parameters of immunohistochemical staining for the astrocyte marker glial fibrillary acidic protein on photomicrographs with several software packages. We found no studies directly comparing techniques. We conclude that the reported methods seem reasonable, but the descriptions were often insufficiently detailed to allow for replication, and the lack of comparison data makes the best method unclear. Further research is needed to optimize the analysis of this important tissue outcome after cerebral infarction.

Keywords

Glial Scar; Astrocytosis; Stroke; Histological Quantification; Measurement

Background

After cerebral infarction reactive astrocytes proliferate, migrate, and form a glial scar around the injured brain tissue. Some authors make a chronological distinction with the terms astrocytosis and astrogliosis, referring to the early proliferation and migration of astrocytes with the former, and the chronic accumulation of astrocyte scar tissue with the latter, but these terms are often used interchangeably as well, as well will do for this review. The processes involved in astrocytosis may have effects on stroke recovery that are negative, positive, or both.[1] Negative aspects may include inhibition of axonal growth through the formation of a physical barrier as well as the secretion of factors inhibitory to axon growth cones. Positive features may include reestablishment of structural support for cellular elements and blood vessels, reconstitution of the blood-brain barrier, and the restoration of normal extracellular fluid homeostasis.[2] Accurate quantification of astrocytosis after cerebral infarction is needed as these important recovery-related processes may affect, and be affected by, many of the potential restorative treatments that are currently under investigation for stroke. We therefore sought to determine the available evidence supporting the most reliable histological method reported for measurement of astrocytosis/gliosis at any time point after cerebral infarction.

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Methods

We searched PubMed, Academic Search Premier, and Google Scholar in April 2012 with the search terms: stroke AND astrocytosis AND astrogliosis AND gliosis AND glial scar AND glial fibrillary acid protein AND measure*. We included full articles in English published prior to April 2012 of unique experimental data that described a histological method for quantification of astrocytosis after cerebral infarction. We excluded abstracts and reports citing methods described in previous publications if no modifications were described. Titles, abstracts, or full articles were reviewed to determine if each search result matched our selection criteria. We also reviewed the references of the selected articles and review articles found by our search for additional matching articles.

Results

All the reports we found that matched our selection criteria used software to quantify aspects of images taken from brain sections immunostained for the astrocyte marker glial fibrillary acidic protein (GFAP).[3–11] We found variability in the reported section thickness, section interval used for immunostaining, image acquisition hardware and software, magnification and number of images acquired per brain section, the exact location of image acquisition relative to the infarct or anatomic landmarks, image analysis software, and the specifics of the image analysis method.

Schabitz et al[3] prepared 50 um serial sections from an unstated area of rat brains up to six weeks after cerebral infarction from photothrombotic ischemia. An unstated number and interval of sections were immunostained for GFAP. An unstated number, location, and magnification of images were obtained using an unidentified light microscope and a DMC Polaroid camera. Imaging Research AIS software was used: “For semiquantitative analysis of GFAP expression, the total area with reactive gliosis and consecutive increased optical density surrounding the ischemic lesion was automatically determined with the AIS software.” The specifics of this method were not stated, but area measurements of the glial scar were produced.

Li et al[4] prepared six um serial sections from about one mm anterior to one mm posterior to bregma of rat brains up to four months after cerebral infarction from MCAO. Every 10th section was immunostained for GFAP and vimentin (another astrocyte marker). An unstated number, location, and magnification of images were taken with an unstated microscope. Image J software was used, and the “thickness of scar was measured and averaged from the 3, 6, 9, and 12 o’clock positions around and perpendicular to the edge of cavitation to the end of the parallel fibers stained by both GFAP+ and vimentin+ on each section”. Representative images showed how they decided where to start and stop the length measurement for the glial scar. The number of sections measured per animal was not stated.

Yu et al[5] prepared eight um serial sections from 1.8 to 2.0 mm caudal to bregma of mice brains 10 weeks after cerebral infarction from MCAO, and every 20th section was immunostained for GFAP. An unstated number, location, and magnification of images were taken with an unstated microscope, and an unstated method was used to measure glial scar thickness.

Buchold et al[6] prepared 25 um serial sections of an unstated number and location of rat brains up to 28 days after cerebral infarction from MCA electrocoagulation plus bilateral common carotid artery occlusion. Every 20th section was immunostained for GFAP. An unstated number and magnification of images were taken with an unstated microscope. “Images of the stained sections were taken to cover the entire scar area by using the scanning option of the Nikon image analysis software, LUCIA. The scar area was then

calculated as the mean integrated density of the GFAP-immunopositive scar structure. Integration of the resulting partially integrated densities gave the total specific volume occupied by scar fibers (made up mainly of astrocytic extensions).” An unstated formula was applied to derive a glial scar volume.

Shen et al[7] prepared six um serial sections from about one mm anterior to one mm posterior to bregma of rat brains up to four months after cerebral infarction from MCAO. Every 10th section was immunostained for GFAP. An unstated microscope was used to take images at unstated locations and magnifications. Image J software was used: “The thickness of scar was measured and averaged from five fields of view along the ischemic boundary zone within the ipsilateral striatum.” Representative images showed how they decided where to start and stop the length measurement for the glial scar. They did not state the location of measurement, or the number of sections measured per animal.

Yasuhara et al[8] prepared serial sections of an unstated number, thickness, and location of rat brains 14 days after cerebral infarction from MCAO, and an unstated number and interval of sections were immunostained for GFAP. “Using an NIH imaging system, the glial scar was calculated by capturing images using AxioPhot (Zeiss) at 1.6-fold magnification. The damaged area was selected according to the morphology of the cells based on glial infiltration, which clearly delineated the ischemic core from the ischemic penumbra. The mean area of damage of 5–6 sections per coronal slice was calculated using the following formula: C=D/(A–B) to reveal the total infarct area per brain... Calculation of glial scar area using the formula C=D/(A–B), with total hemisphere area (A), lateral ventricle (B), glial scar (C), and penumbra (D).” The authors defined the penumbra as the space immediately outside the glial scar, but did not state how they determined where to draw their demarcation lines.

Zhong et al[9] prepared 50 um serial sections of an unstated number and location of mouse brains two weeks after cerebral infarction from photothrombotic ischemia. An unstated number and interval of sections were immunostained for GFAP. An unstated number, location, and magnification of images were obtained with an unstated microscope. “For GFAP quantification, 3 areas around the peri-infarct cortex (2 medial and 1 lateral to the infarct core) per section were randomly chosen and photographed. GFAP fluorescent signals were then analyzed with Image J software.” No further method details were provided, but the “ratio of OD periinfarction/contralateral” was presented for “reactive astrocytosis”.

Titova et al [10] prepared 30 um serial sections from an unstated area of rat brains 28 days after cerebral infarction from MCAO, and immunostained every tenth section for GFAP and aquaporin 4 (AQP4). An unstated number, location, and magnification of images were taken with an Olympus BX41 epifluorescent microscope. “Sections were then scanned using an infrared scanner and fluorescence from GFAP- and AQP4-immunoreactivity was quantified (Odyssey-system, LI-COR Biotechnology) from the same five FOVs”, which referred to “a visual field of 0.0357 mm2 at ×400 magnification from both hemispheres in two slides per rat”. The authors then stated that “Fluorescence signal intensity was reported as integrated-intensities (a.u)... The glial scar area was calculated from two histological brain sections per animal from immunolabeled anti-GFAP sections... GFAP immunofluorescence image ROIs automatically calculated the scar area using customized software. A threshold was applied where the signal intensity higher normal tissues provided a binary mask of the scar. The mask underwent computational morphological closing operations. The area of the binary mask was then calculated.” No further method details were provided, but the “Raw Integrated Intensities, A.U.” was presented for “reactive astrogliosis”.

Manwani et al[11] prepared 30 um serial sections of an unstated number and location of mouse brains 30 days after cerebral infarction from MCAO. An unstated number and interval of sections were immunostained for GFAP. Four 20X images were taken at unstated locations with an unstated microscope. The software package MacBiophotones Image J was used to count GFAP+ cells with an unstated method. “The signal was detected and images acquired with immunofluorescence confocal microscopy using Zeiss image acquisition software... Brain slices were taken at the same distance from bregma to ensure comparison of similar structures. Four 20X fields/animal... were analyzed in the penumbral area of the infarct. GFAP... positive cells were counted using MacBiophotonics ImageJ software. The total number of cells was averaged across the four fields of view for each animal. The average number of cells/field of view was used for statistical analysis as described previously.” No further method details were provided.

We found no studies directly comparing different methods.

Discussion

Accurate histological quantification of astrocytosis after cerebral infarction is necessary for a reliable assessment of the interaction of this tissue response to restorative treatments. Our review found reports of multiple histological techniques to quantify post-infarction astrocytosis, but wide variability in the methods used, and no direct comparisons. Most of the articles matching our selection criteria contained a method description of insufficient detail to allow for replication.

We found variability in the reported brain area sectioned, section thickness, and section interval used for immunostaining. These differences are likely from the varying test species as well as lab specific methods for immunohistochemical tests that were performed in each study. Nearly every study lacked a thorough enough description in technique to replicate the exact sequence, number, and anatomical location of each section used for immunostaining. A detailed description of the brain location, number, and interval of sections stained is needed if any further integration of glial scar area to obtain total scar volume is to be performed.

Across all studies the immunostaining of GFAP appears to be the most accepted procedure for recognizing astrocytes. [3–11]This is an essential first step in order to view the glial scar in any manner that would be useful for its direct measurement. GFAP immunostaining appears to be the most commonly used method to prepare images for analysis. Two studies evaluated other astrocyte markers, vimentin and AQP4, in addition to GFAP, without explicitly comparing measurement results between the different markers to determine if they added value. [4, 10] Only some of the studies specified the hardware and software used for preparing images for analysis.[3, 6–11] This information would be helpful for replication, but is likely less important than the other specifics of the methods used for quantification as most modern equipment likely produces acceptable image quality for analysis. The computer imaging software used for quantitative analysis appeared to be diverse as well. Packages of the free image software ImageJ appear to be the most often used, but by no means the only program used for measurements. [6,11] This is also probably a less important variable, as most software packages likely perform similarly for simple image analysis tasks like these. There appears to be no technique used to measure the glial scar thus far that has been proven superior in accuracy or consistency to any other method. This is a result of several factors, including the incompleteness of most technique descriptions as well as the lack of comparative data between methods. While several studies reported exact glial scar measurements, the methods used were either not clearly defined enough for replication, or missing completely. The methods used to determine the amount of scarring and astrocytosis

are also widely ranging. Several studies reported the amount of glial scar formation as a direct measurement of the number of GFAP+ astrocytes present in the infarcted area. [9,11] Counting reactive astrocytes can be challenging due to extensive process thickening, elongation, ramification, and overlap, which potentially adds variability to the results. Other studies used a geometry-based approach using computer software to outline or measure thickness of the scar itself. [8] A primary problem of this approach is the subjectively involved in deciding where to demarcate the line separating areas of astrocytosis from normal brain tissue, particularly early when there is often an extensive area of gradually increasing astrocytosis approaching the lesion. These two methods need to be directly compared for accuracy and consistency in future experiments in order to determine if one is superior to the other in terms of reliability within and between investigators performing these manual measurements. Another interesting parameter with respect to the measurement of acute reactive astrocytosis versus long term astrogliosis is the timeframe of the observed measurements. Several studies reported measurements within 30 days of infarction, quantifying the initial proliferation and migration of reactive astrocytosis. [6, 8–11] Other studies took measurements at 30 days or more following infarction, which quantifies a more chronic accumulation of scar tissue. Future studies may focus on the specific timing of the measurements made in order to provide congruency between studies, as different measurement methods might perform optimally at different phases of astrocytosis/gliosis. Preliminarily, a comparison of the accuracy and consistency of different methods at each stage of the astrocytosis process must be conducted, particularly those pertaining to the actual direct measurement of the glial scar. Once a single method at each stage is conclusively shown to be better than other comparable methods, a detailed and replicable description of the process is needed to allow comparison between future studies.

We did not find any previous systematic reviews of histological quantification of astrocytosis after cerebral infarction, but our review has limitations. It is likely that we did not find applicable articles due to incomplete indexing of the databases we used. We think this is likely because all of our selected articles included the information of interest in their methods section, but the study itself was focused on another scientific question, such as the evaluation of a neuroprotectant intervention. With the depth and breadth of our search, however, we feel confident that a definitive comparison study of available methods has not been reported.

Conclusion

While the published methods all seem reasonable, the reported technique descriptions are often insufficiently detailed to allow for replication. The lack of comparative data makes statements about the superiority of any particular method impossible, and further research is clearly needed to optimize the analysis of this important experimental outcome. A comprehensive study directly comparing several of the leading candidates for the measurement of the glial scar presented here is needed to determine the most accurate and consistent method during all phases of astrocytosis/gliosis after cerebral infarction. When such a technique is determined to be consistently optimal, a detailed and replicable description is needed, in order for future studies regarding the glial scar and its potentially negative or positive effects to be compared adequately. We recommend that subsequent reports include the following key pieces of information to allow replication: brain area sectioned, brain section thickness, brain section interval used for immunostaining, image acquisition hardware and software used, magnification and number of images acquired per brain section, the exact location of image acquisition relative to the infarct or anatomic landmarks, image analysis software used, and the specifics of the image analysis method.

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