Brain arteriovenous malformations (AVMs) are vascular deformities characterized by an arteriovenous shunt draining blood from arterial feeders via a vascular nidus into a cerebral vein.\(^1,2\) AVMs are of particular concern because of the lifelong risk of hemorrhage. Bleeding from an AVM results in high morbidity (23%–40.5%)\(^3,4\) and mortality (12%–66.7%).\(^5,7\) Unruptured AVMs may come to clinical attention by causing other symptoms, such as epilepsy, progressive neurological deficits, headaches, or as an incidental finding in cranial imaging performed for unrelated reasons.\(^8,9\) The pathophysiology behind the development of AVMs and their risk of rupture is not yet fully understood.\(^10\) Inflammation is known to play a crucial role in various vascular diseases. This feasibility study was conducted to investigate the expression of enzymes (cyclooxygenase 2 [COX-2] and NLRP3 [NOD-, LRR-, and pyrin domain–containing protein 3]) in the AVM nidus that are essential in their inflammatory pathways and to explore how these influence the pathophysiology of AVMs.

**OBJECTIVE** The pathophysiology of development, growth, and rupture of arteriovenous malformations (AVMs) is only partially understood. However, inflammation is known to play an essential role in many vascular diseases. This feasibility study was conducted to investigate the expression of enzymes (cyclooxygenase 2 [COX-2] and NLRP3 [NOD-, LRR-, and pyrin domain–containing protein 3]) in the AVM nidus that are essential in their inflammatory pathways and to explore how these influence the pathophysiology of AVMs.

**METHODS** The study group comprised 21 patients with partially thrombosed AVMs. The cohort included 8 ruptured and 13 unruptured AVMs, which had all been treated microsurgically. The formaldehyde-fixed and paraffin-embedded samples were immunohistochemically stained with a monoclonal antibody against COX-2 and NLRP3 (COX-2 clone: CX-294; NLRP3: ab214185). The authors correlated MRI and clinical data with immunohistochemistry, using the Trainable Weka Segmentation algorithm for analysis.

**RESULTS** The median AVM volume was 2240 mm\(^3\). The proportion of NLRP3-positive cells was significantly higher (26.23%–83.95%), compared to COX-2 positive cells (0.25%–14.94%, \(p < 0.0001\)). Ruptured AVMs had no higher expression of NLRP3 (\(p = 0.39\)) or COX-2 (\(p = 0.44\)) compared to nonruptured AVMs. Moreover, no patient characteristics could be reported that showed significant correlations to the enzyme expression.

**CONCLUSIONS** NLRP3 consistently showed an approximately 10-fold higher expression level than COX-2, making the inflammatory process in AVMs appear to be mainly associated with ischemic (NLRP3)–driven rather than with mechanical (COX-2)–driven inflammatory pathways. No direct associations between NLRP3 and COX-2 expression and radiological, standard histopathological, or patient characteristics were found in this cohort.

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**KEYWORDS** arteriovenous malformation; AVM; inflammation; cerebral hemorrhage

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**ABBREVIATIONS** AVM = arteriovenous malformation; COX-2 = cyclooxygenase 2; IHC = immunohistochemical; NLRP3 = NOD-, LRR-, and pyrin domain–containing protein 3.


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tion of polymorphism in interleukin-6 and interleukin-1β that have been connected to AVM growth and rupture.\textsuperscript{14,15} Interleukin-1β is, in turn, part of the priming process of the pyroptosis enzyme NOD-, LRR-, and pyrin domain–containing protein 3 (NLRP3).\textsuperscript{16} In contrast, interleukin-6 is shown to induce the transcription of cyclooxygenase-2 (COX-2).\textsuperscript{17} COX-2 is proven as a critical enzyme in the endovascular inflammatory process.\textsuperscript{18–20} The biochemistry of COX-2 is interlinked with NLRP3 through a direct and indirect inhibition.\textsuperscript{18} NLRP3 has been linked to autoimmune diseases but also more recently to an inflammatory response to cerebral ischemia.\textsuperscript{21,22} COX-2 is expressed as a direct response to local physical stress in cerebral vessels.\textsuperscript{23} Considering this, a study on COX-2 expression in AVMs has already been conducted.\textsuperscript{24} However, only a marginal expression of COX-2 could be detected. In the present study we aimed to determine the expression and correlation between COX-2 and NLRP3 in the AVM nidus. Our purpose was to identify whether the inflammatory response in the AVM nidus is more likely to be driven by physical or ischemic factors, and to assess how it is connected to its characteristics and clinical course.

**Methods**

**Study Cohort**

All patients with brain AVM tissue samples harvested during microsurgical resection between 2015 and 2019 were eligible for this study. The study protocol was authorized by the local ethics advisory board. All patients or their relatives gave written informed consent before inclusion in the study. The study was conducted according to the principles of the Declaration of Helsinki and was compliant with the Health Insurance Portability and Accountability Act.

**Clinical Data Collection**

Demographic, radiographic, and clinical parameters of the patients were prospectively collected and stored in the clinical digital documentation system. Data included radiological images and reports; pathological reports; and clinical data on preexisting conditions, medications, comorbidities, and other risk factors. In addition, a structured telephone interview was performed before the initiation of the study to obtain an up-to-date clinical outcome as well as information regarding persisting AVM-associated epilepsy or recurring AVM.

**Immunohistochemical Staining**

AVM wall sections (1 μm) were deparaffinized and rehydrated through descending alcohol series and cleaned with distilled water. Sections were then immersed in boiling citrate buffer (pH 6.0) in a 700-W microwave oven for 15 minutes, followed by immersion in 3% hydrogen peroxide in distilled water to block the activity of the endogenous peroxidase. The tissues received immunohistochemical (IHC) staining with the first antibody (COX-2: 1:50 dilution, clone CX-294 [DAKO]; NLRP3: 1:200 dilution, ab214185 [Abcam]). After overnight incubation (> 1 hour), sections were washed in phosphate-buffered saline, incubated with the biotinylated secondary antibody (1:200 dilution, Vector Laboratories; COX-2: BA-9200; NLRP3: BA-1000), and further supplemented with a horseradish peroxidase/streptavidin conjugate. Slides were then incubated with 3’3’-diaminobenzidine as substrate for precisely 5 minutes to visualize positively immunostained cells. Finally, all sections were counterstained with hematoxylin. To avoid false-positive results due to the unspecific binding of the secondary antibody, a negative control was used in each staining passage. Furthermore, to ensure that the antibodies detected the specific protein, we used human adrenal parenchyma as positive control tissue for COX-2 and non–small cell lung cancer for NLRP3 (Fig. 1). Additional standard histopathological stains, such as H&E, Perls Prussian blue, and Elastica van Gieson were used, and specimens were collected within the clinical routine. All staining protocols are available on request.

**Histological Image Analysis**

Histopathological slides were digitized using an Aperio Brightfield slide scanner (Leica Microsystems) at ×20 and ×40 magnification. For further evaluation and comparability, the same 5 areas were selected in the COX-2 and NLRP3 slides, using the CaseViewer software package (version 2.3 RTM; https://www.3dhistech.com/software-downloads). Semiquantitative analysis was performed with the Fiji software package (version 2.0.0-rc-69/1.52p; https://imagej.net/Fiji/Downloads) using the microscopy pixel classification machine learning tool Trainable Weka Segmentation (doi:10.1093/bioinformatics/btx180). Every picture containing at least 16 million pixels was divided into 3 groups using 5 variables for each group to train the algorithm to differentiate between positive IHC signal, normal tissue area, and nuclei. To quantify the enzyme expression of COX-2 and NLRP3, we have calculated the coverage of IHC-positive cells as a percentage correlative to the whole image area. Every single image evaluated by the algorithm was rechecked for plausibility by an experienced team member. Thus, we established a 2-factor analysis and fallback level for our immunohistochemistry. The mean of the positive cell coverage for all 5 regions was calculated as an estimate of the enzyme expression across the entire AVM (Table 1).

**Radiological Data Collection and Measurements**

Four-vessel digital subtraction angiography (DSA) was performed using a Philips Allura angiography suite (Philips Healthcare) capable of 3D rotational DSA MRI was acquired with various MR systems ranging from 1-T up to 3-T magnetic field strength (Siemens, GE, Philips, and Toshiba). Pulse sequences additionally included diffusion-weighted imaging and time of flight in all AVM cases. Image evaluation (histology and MRI) was performed by two experienced neurovascular surgeons (K.H.W., M.D.O.) and an MD student (J.R.) using the Horos DICOM viewer (version 3.3.5; https://horosproject.org/). To obtain the AVM nidus volume, the maximum diameters of the AVMs were measured in the multiplanar reconstruction of time-of-flight MRI data. In the frontal and sagittal planes, the AVM dimensions were measured horizontally, whereas in the transverse plane, the dimension was meas-
sured vertically. The volume calculation was performed by multiplication of all 3 values and the factor 0.5.

Statistical Analysis

Statistics were performed using the R and RStudio software packages (R version 3.6.0, https://cran.r-project.org/bin/windows/base/; and RStudio version 1.2.1335, https://www.rstudio.com/products/rstudio/download/) with these additional packages: GGPlot2, GGExtra, Readxl, and fBasic. For categorical variables, proportions were calculated and a 2-sided t-test was used. For continuous variables, median values with interquartile ranges (between the 25th and 75th percentiles) or mean values with SD were reported for nonnormally and normally distributed continuous data. The Spearman correlation was applied for the complete cohort. The significance level α was set at 0.05, and the CI was set at 0.95%.

Results

Study Cohort

A total of 21 patients (7 male) with ruptured (n = 8) and unruptured (n = 13) AVMs were included in the study. Nine AVMs were partly embolized before microsurgical extirpation. Ages ranged between 14 and 78 years (mean 37.86, median 35, SD ± 17.51 years) at the time of surgery. No deaths occurred during the study period.

In all AVMs, signs of fibrosis and degeneration of the vessel wall structure were present. Moreover, in 81%, elastic fibers were lost in the AVM nidus; and in 52%, iron de-

FIG. 1. Positive and negative control for primary antibodies. IHC staining of intracranial AVMs is displayed in B and D (images in columns 1 and 3: original magnification ×4; images in columns 2 and 4: original magnification ×40). A: Adrenal tissue was used to validate the staining process for the COX-2 antibody. A strong signal is depicted in the positive control (A1 and A2), whereas the negative control (A3 and A4) without the first antibody shows no signal. B: Example of COX-2 staining in AVMs, with (B1 and B2) and without (B3 and B4) the first antibody. C: Non–small cell lung carcinoma tissue was used to validate the NLRP3 staining process. A powerful signal is shown in the positive control (C1 and C2), whereas the negative control (C3 and C4) without the first antibody shows no signal. D: Example of NLRP3 staining in AVMs, with (D1 and D2) and without (D3 and D4) the first antibody.
position in the wall structures was reported. Immigration of inflammatory cells was detected in 10% of the cases. The median radiological volume of the AVM nidus was 2240 mm$^3$ (interquartile range 924–8372 mm$^3$).

AVM and Its Association With NLRP3

Expression of NLRP3 in the nidus samples of AVMs varied between 26.23% and 83.95% (mean 55.19%, median 53.73%, SD ± 12.57%; Fig. 2). The size of AVMs in relation to the nidus volume showed no association with increased or decreased expression of NLRP3 (p = 0.33). Moreover, there was no correlation between pathological characteristics, like loss of elastic fibers (p = 0.86), iron deposition (p = 0.89), or even cellular inflammation signs (p = 0.35). Even patient characteristics like age (p = 0.91), body mass index (p = 0.38), hypertension (p = 0.38), acetylsalicylic acid intake (p = 0.56), smoking (p = 0.89), or alcohol intake (p = 0.36) showed no connection. Neither radiological intervention for embolization of the AVM (p = 0.16), nor ruptured AVMs (p = 0.39), nor clinical severity of the bleeding degrees according to Hunt and Hess (p = 0.33) showed increased NLRP3 expression (Table 1).

AVM and Its Association With COX-2

The COX-2 expression in AVMs ranged between 0.25% and 14.94% (mean 4.71%, median 3.65%, SD ± 3.57%). Again, no association between AVM volume and increased COX-2 expression could be reported (p = 0.99; Supplemental Fig. 1).

But smoking in general (p = 0.025) and an increased number of pack-years (p = 0.038) showed correlations to higher COX-2 expression. Nevertheless, standard histopathological data, the remaining patient characteristics, and radiological interventions or features had no linked association to the COX-2 expression altogether (Table 1).

**Relationship Between the Expressed Inflammatory Enzymes in AVMs**

Overall, COX-2 is expressed at only approximately 10% of the strength of NLRP3 (p < 0.0001). For example, ruptured AVMs expressed on average only 3.76% COX-2, whereas NLRP3 was expressed on average 57.29%. Moreover, patients with diagnosed hypertonia expressed a mean of 4.81% COX-2 and 62.01% of NLRP3. In all categories, we thus have an absolute difference between the COX-2 and NLRP3 expression of 50.64 percentage points. A direct correlation between increased NLRP3 and concomitant increased COX-2 expression could not be shown (p = 0.42). This expression scheme and characteristic are found in all study variables (Table 1).

**Discussion**

This study aimed to assess the influence of inflammatory pathways and their histopathological markers (COX-2 and NLRP3) in the development and rupture of AVMs. In line with an earlier publication, we identified a low but consistent expression of COX-2. As a novel finding, we were able to report a very strong expression of NLRP3 in the AVM nidus. This expression pattern hints to an ische-
matically rather than a mechanically driven inflammatory pathway in AVMs.

The biochemistry of the inflammatory enzymes COX-2 and NLRP3 are interlinked. NLRP3 shows a variety of activation and regulation factors. Especially in cerebral pathophysiology, ischemia and postischemic reorganization have been identified as the main drivers of this enzyme. In comparison, the activation of COX-2 in the cerebral vessels is most likely due to sheer stress and inflammatory cells. However, a regulatory effect of COX-2 on the protein output of the NLRP3 inflammasome has already been demonstrated. Moreover, the catalytic product of COX-2, prostaglandin E2, inhibits the NLRP3 inflammasome directly. This connection leads to the assumption of an anticipatory relationship between these two enzymes.

In their study, Keränen et al. have already reported a comparably low COX-2 expression in the AVM nidus. Furthermore, they suggested that the pathophysiology of AVMs might be in line with the pathophysiology of cerebral aneurysms, leading to small aneurysms in the AVM nidus. These might in turn be the driving force behind a rupture event. However, our results show a very low COX-2 expression, indicating that this enzyme might not be solely responsible for an inflammatory remodeling of the vascular wall.

In our series, the overall enzyme expression of NLRP3 was consistently strong, whereas the COX-2 expression was significantly lower. Given the known impact of inflammation on AVMs, this significantly different expression hints at the noteworthy influence of ischemia on the inflammatory process in AVMs. This result contrasts

FIG. 2. COX-2 and NLRP3 expressions in AVMs. IHC staining (images in columns 1 and 3: original magnification ×40; images in columns 2 and 4: original magnification ×40) with COX-2 and NLRP3 antibodies of AVMs. In all AVMs, an average of 55.19% of cells are positive for NLRP3. At the same time, only an average of 4.71% of cells are positive for COX-2. A: The mean COX-2 (A1 and A2) in this AVM is 18.08%, whereas the NLRP3 (A3 and A4) expression is 62.99%. At 630 mm³, this AVM is one of the smallest 30% of the cohort. B: Of these 3 AVMs, however, in the largest (924 mm³), enzyme expressions of COX-2 (9.03%, B1 and B2) and NLRP3 (63.38%, B3 and B4) place very close to the average. C: One of the smallest 10% of the included AVMs (490 mm³), this one has the second highest expression of NLRP3 (73.33%, C3 and C4). In turn, COX-2 shows average enzyme expression (4.3%, C1 and C2).
with the findings of histopathological studies of other intracranial vascular pathologies (i.e., aneurysms), in which mechanical stress seems to be the main driving force. Ischemia in connection with AVMs has been linked to clinical symptoms induced by these lesions, identifying the size of the nidus as the main factor causing ischemia in the adjacent brain tissue. However, in our cohort the expression of NLRP3 in the nidus itself did not correlate with its size. In addition, the consistently high expression of NLRP3 shows that the pyroptosis process in the AVM wall structure is widely activated. Thus, this pyroptosis marker may indicate that a constant remodeling process occurs in all AVMs. Interestingly, we could not demonstrate the anticipated relationship between COX-2 and NLRP3 expression.

All of the included AVMs showed preoperative evidence of partially thrombosed sections. On one hand, this occlusion might have contributed to the expression levels of ischemia factors, such as NLRP3. On the other hand, only small, circumscribed areas of the AVMs were occluded in our series. At the same time, a strong NLRP3 expression was found in all analyzed samples from different parts of the AVM. Therefore, the fact of partial thrombosis of the AVMs does not seem to show a substantial impact on the NLRP3 expression levels in our study. Nevertheless, further investigations of local inflammation burden in AVM, including the samples with and without endovascular pretreatment, are required to clarify the inflammatory background of AVM genesis.

Furthermore, radiological intervention by embolization of the AVM is most likely to result in local ischemia. Our results do not show higher NLRP3 levels in cases with presurgical embolization. The ischemia-induced inflammation accompanies the process of AVM formation from the beginning, apparently reaching expression levels that cannot be further exceeded by another ischemia factor (like embolization). At least, further subdivision of the ischemic inflammatory response was not quantifiable by our histological examinations. Taking these aspects into account, we consider ischemia to be a greater driving force for growth and rupture than COX-2–driven inflammation, because it is strongly expressed in all AVMs regardless of their size. Most likely due to the small sample size, we did not detect a substantial variation in both enzymes’ expression and could not ascribe increased expression to a clinical or radiological characteristic. Nevertheless, our results suggest a relevant role of ischemically rather than mechanistically induced inflammatory pathways in the pathophysiology of AVMs, although a direct correlation of the intensity of this inflammation with clinical or radiographic factors was not present in our cohort. Ultimately, the missing correlations between inflammation and rupture risk as well as inflammation and clinical characteristics in AVMs are in line with the literature.

**Limitations**

All our samples were from surgically treated patients, which may introduce selection bias to our results that cannot be avoided. Moreover, the small sample size, which remains reasonable in the post-ARUBA (A Randomized Trial of Unruptured Brain Arteriovenous Malformations) era, limits the statistical power of this study. Nevertheless, our results show very consistent expression of the enzymes COX-2 and NLRP3, making the results valuable despite the limitations mentioned above.

**Conclusions**

The expression rate of the inflammatory enzymes NLRP3 and COX-2 in AVMs showed a significant difference. This study identified a dominance of NLRP3, suggesting a mainly ischemic influence on inflammatory pathways in AVMs. The expression of inflammatory markers showed no correlation to standard histopathological stains, or to radiological or patient characteristics in this cohort. The influence of inflammatory pathways on the development and clinical course of AVMs remains unclear. To better understand the role of ischemia in AVMs, further histological and molecular investigation of enzymes in the up- and downstream pathways of the NLRP3 inflammasome are warranted. Therefore, we strongly advocate establishing a multicenter registry of histopathological samples and corresponding radiological and clinical data to further investigate AVM pathophysiology in a larger cohort.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Darkwah Oppong, Wrede. Acquisition of data: all authors. Analysis and interpretation of data: Rodemerk. Drafting the article: Rodemerk, Darkwah Oppong, Wrede. Critically revising the article: Darkwah Oppong, Junker, Deuschl, Zhu, Jabbarli, Wrede. Reviewed submitted version of manuscript: Rodemerk, Darkwah Oppong, Dammann, Uerschels, Jabbarli, Sure, Wrede. Approved the final version of the manuscript on behalf of all authors: Rodemerk. Statistical analysis: Rodemerk. Administrative/technical/material support: Junker, Zhu. Study supervision: Darkwah Oppong, Wrede.

Supplemental Information

Online-Only Content

Supplemental material is available online. Supplemental Fig. 1. https://thejns.org/doi/suppl/10.3171/2022.4.FOCUS2210.

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