



Estimation of the density of veins from susceptibility-weighted imaging by using Mamdani fuzzy-type rule-based system. Investigating the neurovascular coupling in migraine

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ABSTRACT

Background and purpose: An impaired neurovascular coupling has been described as a possible player in neurodegeneration and cognitive decline. Migraine is a recurrent and incapacitating disorder that starts early in life and has shown neurovascular coupling abnormalities. Despite its high prevalence, the physiology and underlying mechanisms are poorly understood. In this context, new biomarkers from magnetic resonance imaging (MRI) are needed to bring new knowledge into the field. The aim of this study was to determine the vein density from Susceptibility-Weighted Imaging (SWI) MRI, in subjects with migraine and healthy controls; and to assess whether it relates to Resting-State functional MRI (RS-fMRI).

Materials and methods: The cohort included 30 healthy controls and 70 subjects with migraine (26 episodic, 44 chronic) who underwent a brain 3.0 T MRI. Clinical characteristics were also collected. Maps of density of veins were generated based on a Mamdani Fuzzy-Type Rule-Based System from the SWI MRI. Mean values of vein density were obtained in grey (GM) and white matter (WM) Freesurfer lobar parcellations. The Amplitude of Low-Frequency Fluctuations (ALFF) image was calculated for the RS-fMRI, and the mean values over the parcellated GM lobes were estimated. Differences between groups were assessed through analysis of variance (age, sex, education and anxiety as covariates; $p < 0.05$), followed by post-hoc comparisons. Associations were run between clinical and MRI-derived variables.

Results: When comparing the density of veins in GM, no differences between groups were found, neither associations with clinical variables. The density of veins was significantly higher in the WM of the occipital lobe for subjects with chronic migraine compared to controls (30%, $p < 0.05$). WM vein density in either frontal, temporal or cingulate regions was associated with clinical variables such as headache days, disability scores, and cognitive impairment (r between 0.25 and 0.41; $p < 0.05$). Mean values of ALFF did not differ significantly between controls and subjects with migraine. Strong significant associations between vein density and ALFF measures were obtained in most GM lobes for healthy subjects (r between 0.50 and 0.67; $p < 0.05$), instead, vein density in WM was significantly associated with ALFF for subjects with migraine (r between 0.32 and 0.58; $p < 0.05$).

Conclusions: Results point towards an increase in vein density in subjects with migraine, when compared to healthy controls. In addition, the association between GM vein density and ALFF found in healthy subjects was lost in migraine. Taken together, these results support the idea of abnormalities in the neurovascular coupling in migraine. Quantitative SWI MRI indicators in migraine might be an interesting target that may contribute to its comprehension.

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1. Introduction

Migraine is a common, recurrent, incapacitating neurovascular disorder, characterized by attacks of severe headache and dysfunction of the autonomic nervous system. Migraine prevalence is around 14%, being three times more common in women than men. It is the second cause of years lived with disability worldwide and the first among women under 50 years of age [Steiner et al., 2020]. Migraine pathophysiology is still not fully understood. Vasculature seems to play a role in migraine genesis and maintenance [Goadsby et al., 2002], as well as veins [Jacobs et al., 2016], giving the grounds to the described “dysfunction” of the neurovascular coupling [Hoffman et al., 2019; Fabjan et al., 2015]. This neurovascular coupling concept is described as the interplay between neural activity changes and corresponding vascular changes to alter cerebral blood flow, in order to maintain the metabolic support [Iadecola et al., 2017]. The role of veins has been largely discussed in migraine. Traditionally, a meningeal arterial vasodilation was considered the main trigger to an attack, although controversial results have obscured their primary role [Jacobs et al., 2016]. Nevertheless, the role of veins independently of dilation have not been fully investigated so far, in part due to the limitations of the available imaging techniques.

Susceptibility-weighted imaging (SWI) is a magnetic resonance imaging (MRI) sequence that measures iron deposition, allowing to generate maps of veins after combining their magnitude and phase maps. Recently, a study has been published in migraine to assess the vein asymmetry between hemispheres with MRI acquired at 1.5 T [Breiding et al., 2020]. The main limitation of this approach is that veins were quantified on the minimum intensity projection map, which allows to enhance structures that present a low intensity value by projecting the voxel with the lowest attenuation value over a given volume onto a two-dimensional image. This map is artificial and does not hold a one-to-one correspondence with the original SWI or 3D T1-weighted MRI, as a result of projecting the image. Alternative approaches have been developed that overcome this limitation that use the *veselness* measure [Frangi et al., 1998]. The filter employed by this method over the image accentuates the contrast between tubular objects and the background, thus enhancing elongated blood vessels while suppressing other anatomical features and noise. This method was applied on SWI acquired at 1.5 T MRI, where veins were assessed only in healthy subjects [Vigneau-Roy et al., 2014]. This study also outlined that both timing and amplitude of resting-state functional MRI (RS fMRI) signals are influenced by the underlying vasculature. RS fMRI paradigm has been used to assess brain function through MRI [Glover et al., 2011; Fox et al., 2010], and their signal is typically parametrized through the Amplitude of the Low-Frequency Fluctuations (ALFF).

In this context, the working hypothesis was that the neurovascular coupling (or uncoupling) in migraine can be further explored by combining SWI and RS fMRI measures, and by assessing their relationship. Since there is a prevailing scarcity of methods to measure quantitatively the actual density of veins from SWI MRI, the first aim of the study was to implement a method based on the Mamdani Fuzzy Rule-Based System, part of the so-called artificial intelligence techniques [Zadeh 1973; Mamdani et al., 1975]. The proposed method should be able to integrate different characteristics derived from the SWI MRI, such as *veselness* or linearity, to define a voxel as a vein. The Mamdani Fuzzy Rule-Based System provides a powerful framework to incorporate expert knowledge into the decision-making process, and it generates a meaningful output even in the presence of uncertain or missing information. It employs natural language to describe the input and output variables, which can be more easily interpreted. This approach would allow to take into account different features derived from the SWI MRI in order to classify a voxel as belonging to a vein. The second aim of the study would be to relate the density of veins with the RS fMRI measures, in healthy subjects and subjects with migraine.

2. Material and methods

2.1. Subjects

The cohort consisted of right-handed adult patients with migraine ($n = 70$) and sex/age-matched healthy controls ($n = 30$). The study was approved by the local ethical committee and all subjects participating signed informed consent. The inclusion criteria for migraine patients were: diagnosis of chronic migraine or episodic migraine according to the International Classification of Headache Disorders – 3 criteria [Headache Classification Committee of the IHS, 2018] currently not taking any preventive medication. Participants were excluded if: (1) they had relevant neurological or psychiatric comorbidities as well as other chronic pain syndromes, (2) if they were taking drugs with central nervous system effect (antidepressants, neuroleptics, benzodiazepines, antiepileptic, etc...) or (3) if they had incidental relevant structural abnormalities in brain MRI. Healthy controls with a history of migraine in a first degree relative were also excluded. A neurologist specialized in headache confirmed the correct diagnosis (migraine or healthy control). All participants had at least one month electronic diary to further support the neurologist's interview. Demographic, clinical as well as patient-reported measures of anxiety, depression and disability were collected. Specifically: age, sex, years of academic education and the presence of anxiety (BAI) and depression symptoms, being this last two from the Beck Anxiety and Depression Inventory [Beck et al., 1961; Beck et al., 1988]. Moreover, patients with migraine also reported: evolution (years from migraine diagnosis), aura, monthly headache days (MHD), baseline frequency [days/month] of migraine, baseline frequency [days/month] of analgesics consumption, Migraine Disability Assessment Test (MIDAS) [Stewart et al., 2000], Headache Impact Test-6 (HIT6) [Shin et al., 2008], Migraine-Specific Quality of Life (MSQ) Questionnaire [Jhingran et al., 1998], and a baseline subjective measure of cognitive impairment (Mig-SCog) [Gil-Gouveia et al., 2011]. **Data availability statement:** The data and code generated is available after submission of a formal project and approval of the corresponding data transfer agreement between the institutions.

2.2. Image acquisition

Images were acquired on a 3 T whole-body MR scanner (Tim Trio, Siemens, Erlangen, Germany) with a 12-channel head coil. The parameters used for the sagittal 3D T1-weighted gradient-echo (MPRAGE) sequence were: Time of Repetition (TR) = 2300 ms, Time of Echo (TE) = 2.98 ms, voxel size $1.0 \times 1.0 \times 1.0 \text{ mm}^3$, flip angle (FA) 9° . The parameters for the axial SWI were: TR = 2300 ms, TE1 = 6.08 ms, TE2 = 24.60 ms, voxel size $0.7 \times 0.7 \times 3.0 \text{ mm}^3$, FA 15° . Lastly, acquisition parameters for the axial RS fMRI were: TR = 2700 ms, TE = 35 ms, voxel size $3.6 \times 3.6 \times 3.0 \text{ mm}^3$, Flip angle = 90° , 130 volumes. The subjects were instructed to remain still, close their eyes, and not fall asleep. MRI images were reviewed by an experienced neuroradiologist (AR), assessing microbleeds, cavernomas, calcifications or any other incidental finding.

2.3. MRI preprocessing

A schematic representation of the analysis pipeline to obtain the vein maps from the SWI images can be seen in Fig. 1. Summarising, the goal was to mask out the extracerebral tissue, and tissue surrounding the basal ganglia and thalamus by using both the 3D T1-weighted image and the SWI. First, the bias field correction was applied by using the N4 algorithm [Tustison et al., 2010]. From the 3D T1-weighted MRI, the corresponding grey (GM) and white matter (WM) masks were segmented with Statistical Parametric Mapping (SPM) [Friston et al., 1995]. These masks were then applied to the SWI map and entered separately into the Mamdani FRBS algorithm [Aymerich et al., 2016]. The 3D T1-weighted MRI was also parcellated with Freesurfer [Dale

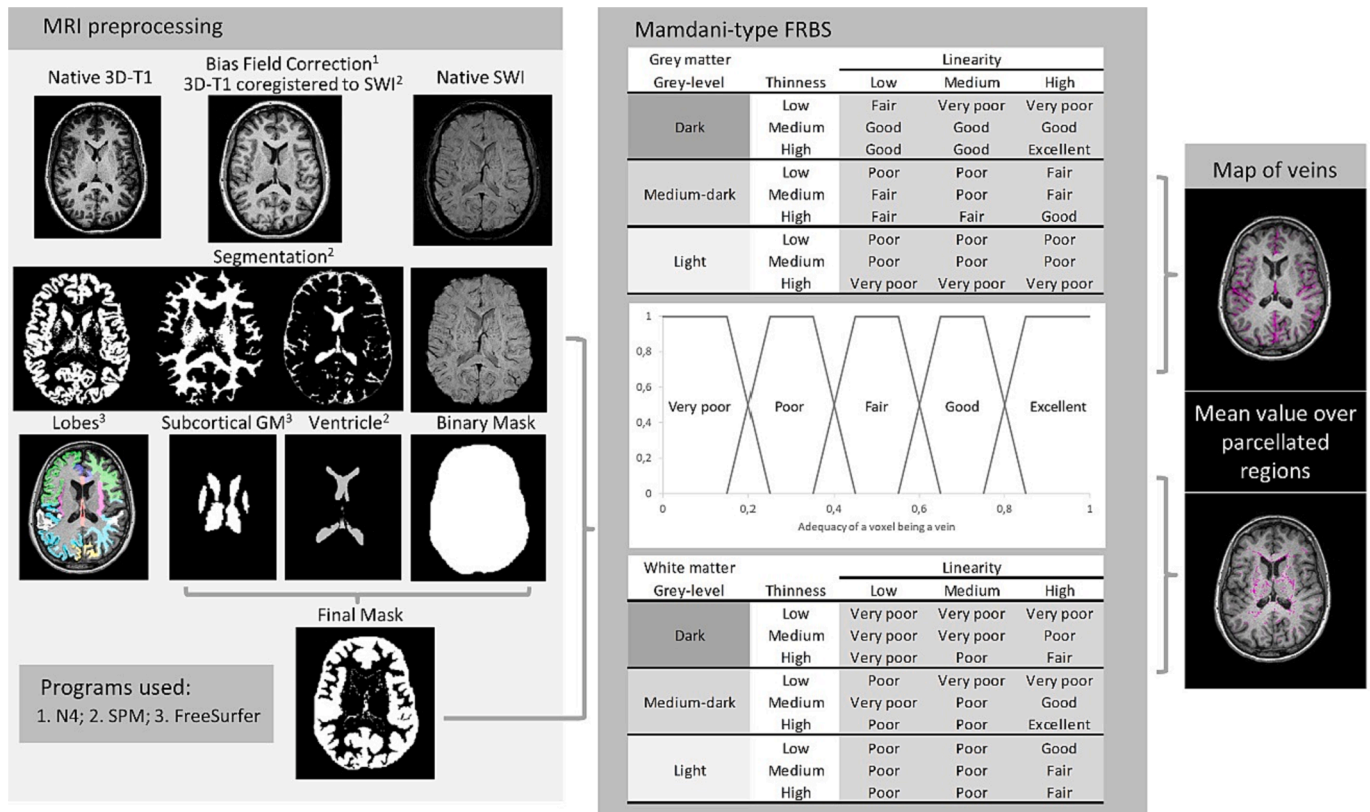


Fig. 1. Analysis pipeline for the determination of veins in the brain from susceptibility weighted images (SWI) MRI. Acquired images were preprocessed and entered into the Mamdani-type Fuzzy Rule-Based System (FRBS) that generates the corresponding vein maps. Mean values over parcellated regions were calculated. Abbreviations: SWI: susceptibility-weighted images; MRI: magnetic resonance imaging; FRBS: Fuzzy Rule-Based System; GM: grey matter.

et al., 1999] to generate a mask corresponding to each brain lobe (frontal, cingulate, insula, parietal, temporal and occipital) and the corpus callosum. Fig. 1 includes the information regarding which tool was used in each step.

2.4. Mamdani FRBSs

Mamdani FRBS allow to take decisions on the intermediate degrees of a premise through specified gradual membership functions. Briefly, in the current context, after the initial extraction of the external structures to the brain parenchyma and the application of WM and GM masks to the SWI map, the adequacy of a voxel being a vessel was then based on the combination of three features of the image that were considered to represent cerebral veins: grey-level intensity (dark, medium-dark, light), thinness (low, medium, high), and linearity (low, medium, high). The operators selected to evaluate these features were, respectively, for grey-level intensity the grey-level of the pixel object to study, for the thinness the third highest difference between the central and surrounding pixels within a 5x5 raster window centered on the pixel object to study, and for the linearity the maximum of 4 directional 3x3 kernels centered on this pixel that enable the detection of horizontal, vertical, or oblique (+45° and -45°) single pixel wide lines. The inference was based on the decision table rules defined for its segmentation in WM and GM as can be seen in Fig. 1. Then, the defuzzification to obtain the adequacy of a voxel being a vein was defined from a trapezoidal response function with five categories: very poor, poor, fair, good, excellent (Fig. 1) and the application of the center of gravity as a defuzzification method. Finally, only those voxels with an adequacy higher than 0.58 were considered veins. This value produced vein maps that showed the largest resemble to the actual brain vasculature and showed a relatively lower number of artifacts, as reviewed by an experienced neuroradiologist (AR). A complete description of the different stages can be found in [Aymerich et al.,

2016; Magdalena 2015].

2.5. Parcellation of veins

Once the maps of veins were generated, the mean value over the parcellations done by Freesurfer on the 3D T1-weighted MRI (frontal, cingulate, insula, parietal, temporal, occipital lobes as well as corpus callosum) was obtained. The regions included for the frontal lobe were: rostral middle frontal, superior frontal, caudal middle frontal, pars opercularis, pars orbitalis, pars triangularis, precentral, paracentral and frontal pole; for the parietal lobe: superior parietal, inferior parietal, supramarginal, postcentral and precuneus; for the temporal lobe: superior temporal, middle temporal, inferior temporal, banks of superior temporal sulcus, fusiform, transverse temporal, entorhinal, parahippocampal and temporal pole; for the occipital lobe: lateral orbito-frontal, lingual, cuneus and pericalcarine; for the cingulate: rostral anterior cingulate and caudal anterior cingulate; and insula. The corpus callosum (CC) included: posterior, mid posterior, central, mid anterior, anterior and fornix. All the mentioned parcellations, except for the CC, were subdivided into GM and WM, and into left-hemisphere (LH) and right (RH).

2.6. Resting-state connectivity analysis

RS fMRI images were analyzed according to the default pipeline included in the CONN toolbox [Whitfield-Gabrieli et al., 2012] which performs the following steps: functional realignment and unwarp; slice-timing correction; outlier identification; direct segmentation and normalization; and functional smoothing. Then, the default denoising pipeline was implemented to remove the remaining noise from the blood-oxygen-level-dependent (BOLD) signal. It consisted of two steps: linear regression of potential confounding effects in the BOLD signal

through the WM and cerebro-spinal fluid measured time-courses, and temporal band-pass filtering. With this last step, temporal frequencies below 0.008 Hz or above 0.09 Hz were removed from the BOLD signal to focus on slow-frequency fluctuation. As an indicator of the regional intensity of spontaneous fluctuations in the BOLD signal, the ALFF was used. ALFF maps represent a measure of BOLD signal power within the frequency band of interest, previously mentioned. Finally, the mean values of ALFF over the parcellated ROIs were obtained, just for the GM regions.

2.7. Statistical analysis

The Shapiro-Wilk test was used to confirm the parametric/non-parametric distribution of the sample. Clinical and vein density characteristics were compared across groups by means of univariate analysis of variance with age, sex, education, and the severity of anxiety as covariates, followed by post-hoc comparisons. The correction by multiple comparisons was performed by using the Benjamini-Hochberg test, with a false discovery rate of 10% and considering 13 comparisons (6 right and 6 left lobes, plus the corpus callosum). In order to rule out that the differences in the density of veins between groups were not driven by the parcellated volumes, a two-sample *t*-test was performed. The ALFF characteristics were compared the same way. To measure the degree of association between MRI-derived measures in subjects with migraine and clinical characteristics, partial correlations were run with age, sex, education, and severity of anxiety as control variables. The association between vein density and ALFF measures was also assessed with partial

correlations. For all statistical analyses, the level of significance was set at $p < 0.05$ (two-tailed). All comparisons and assessments were performed with the program Statistical Package for the Social Sciences (IBM, version 28).

3. Results

All cases could be analyzed through the proposed pipeline. The demographic and clinical characteristics of all subjects are summarized in Table 1. A representative example of the generated vein maps can be seen in Fig. 2. The volumes of the parcellated lobes, obtained after parcellation of the 3D T1-weighted MRI, did not differ significantly between groups. The density of veins in GM showed a non-parametric distribution, their analysis was performed considering so.

3.1. Distribution of veins in the brain

The density of veins in GM was considerably higher than in WM. In GM, the occipital was the region presenting the highest density, followed by the insula, cingulate, parietal and temporal. The region showing the lowest density was the frontal (Table 1). In WM, the CC showed the highest density followed by the insula, occipital and then the rest of the regions according to the distribution in GM (Table 1). The order of the regions was the same for all groups. The density of veins was systematically higher for subjects with migraine compared to healthy subjects, although no significant differences were found in GM. In WM, there was a significant increase in the density of veins for subjects with

Table 1

Demographic and clinical variables of the cohorts analyzed; and the adjusted mean [lower–upper bound] for the estimated density of veins in the grey and white matter portions of the parcellated brain lobes. Differences between groups were assessed through an univariate analysis of variance, with age, sex, education and anxiety severity as covariates, followed by post-hoc comparisons. The *p*-value reported corresponds to the effect of group. Significant differences are outlined in bold; trends ($0.065 < p < 0.05$) are outlined in italics. Abbreviations: HC: healthy controls; R: right; L: left; H: hemisphere; MHD: monthly headache days; d/m: days per month; N/A: not applicable.

Variables			HC	Episodic	Chronic	p-value
N (%female)			30 (80%)	26 (85%)	44 (81%)	0.384
Age			39.17 (8.70)	41.62 (7.50)	40.50 (9.50)	0.305
Education			15.40 (3.50)	13.31 (3.34)	12.91 (3.14)	0.309
Evolution			N/A	24.85 (8.85)	25.64 (10.17)	0.370
Aura (%)			N/A	10 (38%)	11 (25%)	N/A
Monthly headache days			N/A	11.08 (2.13)	19.39 (3.25)	<0.001
Anxiety severity			4.13 (5.70)	8.92 (7.18)	13.45 (11.00)	0.033
Depression severity			3.50 (4.74)	7.77 (7.57)	9.25 (7.64)	0.217
Analgesic (days/month)			N/A	9.96 (4.13)	12.34 (5.08)	0.023
Pain Laterality			N/A	2.58 (1.03)	2.73 (1.09)	0.285
Vein density grey matter	Frontal	LH	4.57[4.22–4.92]	4.75[4.47–5.04]	4.77[4.42–5.13]	0.652
		RH	4.66[4.33–4.99]	4.88[4.61–5.15]	4.95[4.62–5.28]	0.421
	Parietal	LH	5.98[5.59–6.36]	6.02[5.71–6.33]	6.25[5.86–6.64]	0.575
		RH	6.44[6.01–6.88]	6.62[6.27–6.97]	6.65[6.21–7.08]	0.759
	Temporal	LH	4.93[4.55–5.32]	5.27[4.96–5.58]	5.29[4.90–5.67]	0.315
		RH	5.38[5.04–5.73]	5.58[5.30–5.86]	5.61[5.26–5.96]	0.584
	Occipital	LH	10.04[9.44–10.63]	9.70[9.22–10.18]	10.30[9.70–10.90]	0.344
		RH	10.51[9.94–11.09]	10.82[10.35–11.28]	10.44[9.86–11.02]	0.586
	Cingulate	LH	6.27[5.36–7.17]	6.52[5.79–7.26]	6.43[5.52–7.34]	0.908
		RH	6.24[5.37–7.11]	6.37[5.66–7.08]	6.67[5.79–7.55]	0.785
	Insula	LH	7.60[6.83–8.37]	7.64[7.01–8.27]	7.69[6.91–8.47]	0.987
		RH	8.03[7.21–8.86]	8.24[7.57–8.91]	8.37[7.53–9.20]	0.843
Vein density white matter	Frontal	LH	0.51[0.36–0.66]	0.65[0.53–0.77]	0.77[0.62–0.92]	0.062
		RH	0.48[0.34–0.62]	0.67[0.56–0.78]	0.68[0.54–0.82]	0.056
	Parietal	LH	0.96[0.76–1.16]	1.10[0.94–1.25]	1.24[1.04–1.44]	0.144
		RH	1.06[0.83–1.28]	1.34[1.16–1.52]	1.32[1.09–1.55]	0.111
	Temporal	LH	1.65[1.30–2.01]	1.64[1.35–1.93]	1.96[1.60–2.32]	0.372
		RH	1.84[1.47–2.20]	2.10[1.8–2.39]	2.32[1.95–2.69]	0.182
	Occipital	LH	2.10[1.79–2.40]	2.26[2.01–2.50]	2.65[2.34–2.96]	0.039
		RH	1.93[1.69–2.17]	2.03[1.84–2.23]	2.45[2.20–2.69]	0.009*
	Cingulate	LH	0.91[0.68–1.14]	1.09[0.90–1.28]	1.28[1.04–1.51]	0.095
		RH	1.23[1.01–1.46]	1.39[1.20–1.57]	1.50[1.27–1.73]	0.246
	Insula	LH	3.23[2.75–3.71]	3.05[2.66–3.44]	3.59[3.11–4.07]	0.271
		RH	2.96[2.44–3.49]	3.13[2.71–3.56]	3.53[3.00–4.06]	0.311
	Corpus callosum		5.54[4.93–6.16]	5.30[4.80–5.80]	5.79[5.16–6.41]	0.526

*significant after Benjamini-Hochberg correction.

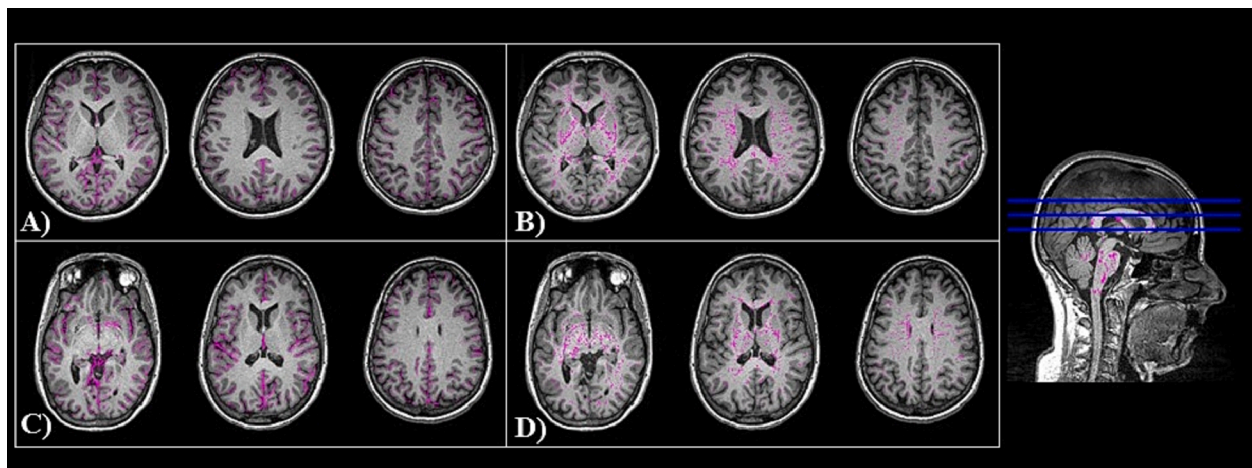


Fig. 2. Representative example of vein maps obtained through the proposed methodology. Left columns (panel A and C) represent grey matter maps, and right panels (B and D), white matter maps. The upper row belongs to a healthy subject and the lower row, to a subject with migraine.

chronic migraine compared to healthy in the occipital WM (post-hoc left hemisphere $p = 0.039$; right hemisphere $p = 0.009$) and a trend in the frontal (Table 1). The differences in the right occipital lobe remained significant after correcting for multiple comparisons with the Benjamini-Hochberg test. Although the differences were not significant, the group of subjects with episodic migraine presented mean values of vein density that felt between healthy and chronic migraine.

3.2. Association between vein density and clinical variables

Regarding the associations between GM vein density and the clinical variables, a weak but significant negative association was found between the HIT-6 score and the right insula ($\rho = -0.294$; $p = 0.017$), and a trend was found in the left temporal ($\rho = -0.229$; $p = 0.065$). When assessing the density of veins in WM, more regions showed a significant positive association with the clinical scores, although it was also weak (ρ between 0.25 and 0.40) (Table 2). The density of veins in the frontal and cingulate WM regions were the ones that showed the largest number of significant associations with clinical variables.

3.3. Association vein density with ALFF

ALFF mean values were higher in the insula, followed by the temporal, occipital, cingulate, parietal and frontal. When comparing ALFF mean values between healthy subjects and subjects with migraine, there were no significant differences (Table 3). Regarding the associations

between ALFF and the density of veins in GM, 8 out of 12 of the regions considered showed moderate to strong associations in healthy subjects (ρ above 0.5), while in subjects with migraine, just the temporal lobe showed significant associations, although it was weak (Table 4). As an additional solely exploratory analysis, and since differences in vein density between healthy subjects and subjects with migraine were found in WM regions, the association between the density of veins in WM and ALFF (in GM) was also assessed. For healthy subjects, associations between WM vein density and ALFF were still significant in most of the regions although they were weak to moderate. In subjects with migraine, all of the regions showed a significant association, and it ranged also between weak and moderate (Table 4). When assessing the episodic and the chronic groups separately, the episodic group showed moderate to strong associations, while for the chronic group, those were moderate.

4. Discussion

In the present study, we generated quantitative maps of density of veins in healthy subjects and subjects with migraine from 3.0 T SWI MRI. The proposed methodology is based on the maps generated from the SWI MRI in conjunction with a Mamdani-Fuzzy algorithm. The distribution of veins in the brain lobes has been quantified, as well as their associations with clinical characteristics and ALFF measures from the RS fMRI. The group of subjects with migraine has also been subdivided into episodic and chronic, and their differences have been assessed compared to

Table 2

Associations between the density of veins in white matter portions of the brain lobes with clinical variables for the subjects with migraine (ρ , p -values legend at the bottom of the table). Abbreviations: MHD: monthly headache days; MIDAS: migraine disability assessment test; MSQ: migraine-specific quality of life; Mig-SCog: migraine-specific cognitive impairment; L: left; R: right; H: hemisphere.

White matter	Evolution	MHD	MIDAS	HIT6	MSQ	Mig-SCog
LH	0.004	0.205	0.400**	0.241	0.205	0.295*
RH	0.082	0.302*	0.324**	0.262*	0.171	0.170
LH	0.618**	0.155	0.218	0.281*	0.191	0.214
RH	0.147	0.247*	0.197	0.218	0.163	0.198
LH	0.129	0.062	0.340**	0.082	0.214	0.366*
RH	0.209	0.195	0.357**	0.186	0.241*	0.275*
LH	0.089	0.203	0.281	0.314*	0.268*	0.025
RH	-0.016	0.091	0.067	0.079	0.102	0.106
LH	0.204	0.173	0.189	0.280	*0.268	*0.025
RH	0.103	0.155	0.248*	0.264*	0.378**	0.097
LH	-0.001	-0.057	0.203	0.043	0.133	0.176
RH	0.108	0.047	0.187	0.174	0.232	0.028
Corpus callosum	0.151	-0.103	0.135	-0.054	0.011	0.094*

*0.05 < p < 0.01; **0.01 < p < 0.001.

Table 3

Adjusted mean values [lower–upper bound] of Amplitude of Low-Frequency Fluctuations (ALFF) in the parcellated grey matter regions for healthy controls (HC) and subjects with migraine. The p-value reported corresponds to the effect of group. Abbreviations: L: left; R: right; H: hemisphere.

ALFF		HC	Episodic	Chronic	p-value
Frontal	LH	0.22 [0.2–0.23]	0.22 [0.21–0.23]	0.23 [0.21–0.24]	0.530
	RH	0.21 [0.2–0.23]	0.22 [0.21–0.23]	0.23 [0.21–0.24]	0.404
Parietal	LH	0.22 [0.2–0.24]	0.22 [0.21–0.23]	0.23 [0.21–0.25]	0.662
	RH	0.22 [0.2–0.24]	0.23 [0.21–0.24]	0.23 [0.22–0.25]	0.832
Temporal	LH	0.26 [0.25–0.28]	0.26 [0.25–0.27]	0.26 [0.25–0.28]	0.399
	RH	0.26 [0.25–0.27]	0.26 [0.25–0.27]	0.27 [0.26–0.28]	0.798
Occipital	LH	0.24 [0.23–0.26]	0.24 [0.23–0.26]	0.25 [0.23–0.27]	0.786
	RH	0.25 [0.23–0.26]	0.25 [0.23–0.26]	0.25 [0.24–0.27]	0.494
Cingulate	LH	0.24 [0.23–0.26]	0.24 [0.23–0.25]	0.25 [0.24–0.27]	0.184
	RH	0.24 [0.22–0.26]	0.24 [0.23–0.25]	0.26 [0.24–0.27]	0.850
Insula	LH	0.29 [0.27–0.30]	0.28 [0.27–0.30]	0.29 [0.27–0.31]	0.850
	RH	0.29 [0.27–0.30]	0.29 [0.28–0.31]	0.29 [0.28–0.31]	0.721

Table 4

Associations between the Amplitude of Low-Frequency Fluctuations (ALFF) and the density of veins (rho, p-values legend at the bottom of the table). Abbreviations: HC: healthy controls; L: left; R: right; H: hemisphere.

RHO ALFF-veins			HC	Migraine	Episodic	Chronic
Grey matter	Frontal	LH	0.586**	0.209	0.228	0.230
		RH	0.570**	0.182	0.424*	0.149
	Parietal	LH	0.542**	0.222	0.431*	0.175
		RH	0.555**	0.114	0.380	0.079
	Temporal	LH	0.671**	0.381**	0.396	0.386*
		RH	0.707**	0.357**	0.511*	0.256
	Occipital	LH	0.689**	0.224	0.163	0.263
		RH	0.205	0.003	0.375	0.041
	Cingulate	LH	0.232	0.045	0.200	0.148
		RH	0.240	0.172	0.481*	0.088
	Insula	LH	0.515**	0.143	0.428	*0.760
		RH	0.347	0.041	0.337	0.153
White matter	Frontal	LH	0.554**	0.455**	0.708**	0.352*
		RH	0.204	0.405**	0.734**	0.290
	Parietal	LH	0.406*	0.478**	0.644**	0.371*
		RH	0.549**	0.507**	0.633*	0.415*
	Temporal	LH	0.580**	0.526**	0.554**	0.470**
		RH	0.717**	0.516**	0.552**	0.549**
	Occipital	LH	0.418*	0.368**	0.640**	0.309
		RH	0.331	0.409**	0.530**	0.364*
	Cingulate	LH	0.257	0.414**	0.778**	0.362*
		RH	0.431*	0.579**	0.698**	0.509**
	Insula	LH	0.539**	0.330*	0.276	0.332*
		RH	0.557**	0.316*	0.559*	0.444**

*0.05 < p < 0.01; **0.01 < p < 0.001.

healthy subjects.

Previous studies in migraine assessing the density of veins were performed on images acquired on a 1.5 T magnet and were based on using the minimum intensity projection map. In addition, no quantitative values of the density of veins were reported and healthy subjects were not included [Breiding et al., 2020]. The fact of working on the minimum intensity projection map, an artificial image, implies the no correspondance with the 3D T1-weighted image, used to parcellate the

mean values over selected regions. Alternative approaches to estimate veins require the acquisition of additional sequences, such as time of flight angiography or working in the normalized Montreal Neurological Institute space [Bernier et al., 2018], which implies additional transformations of the images with the consequent interpolations. We designed an approach that worked in the native space and did not require additional transformations of the SWI image, since it is a sequence with a relatively low signal to noise ratio. By comparing the results obtained with the published results, the top regions sort of agree (insula, temporal and occipital). Regarding the density in WM, it has been reported to be approximately 4 times lower than in GM, as obtained in our results. Just to add the fact that the density on the WM CC was comparable to those of some GM regions. The CC density of veins reflects the contribution of the atrial, septal and subependymal veins, which may contribute to their high density [Wolfram-Gabel et al., 1992].

The present study has shown that the density in veins in both GM and WM was systematically higher in subjects with migraine, and even higher for subjects with chronic migraine, although significant increases (that survived a false discovery rate of 10%), were only found in the right WM occipital lobe, when comparing episodic to healthy controls. The episodic group lied between the density of veins estimated in healthy subjects and the chronic group, suggesting an intermediate stage. Since the main difference between the two groups are the days in a month with headache, this intermediate stage for episodic was somehow expected. Further studies are needed including MRI and serum markers to highlight the possible role of angiogenic factors. Actually, several studies have reported a decrease in angiogenic factors in migraine rather than an increase [Michalak et al., 2017; Mozafarihashjin et al., 2022]. Another possible player might be the presence of WM hyperintensities, detected in subjects with migraine. These lesions are observed in WM and may trigger an inflammatory response. In the cohort analyzed, the percentage volume of WM hyperintensities to the total intracranial volume was below 0.2%, according to the Freesurfer segmentations (results not shown). Due to their low relative volume, no further analysis was carried out with this variable. A recent study in elderly healthy subjects outlined no association between WM hyperintensities and subcortical WM vein densities [Li et al., 2022]. Their proposed method was based on the minimum intensity projection map, which again is the main difference with our approach, besides the fact that WM was not parcellated the same way. Another possible explanation for the increased density of veins found in migraine might be as a compensatory mechanism to cope with unbalanced metabolic demands and/or oxygen demands, which have been described in migraine [Jacobs et al., 2016; Hoffman et al., 2019; Torres-Ferrus et al., 2021]. In our study, we found negative associations between the density of veins and clinical variables in GM, and positive associations in WM. Concretely, the density of veins in the parietal lobe was related with disease evolution; frontal and temporal lobes were related with the MIDAS and cognitive scores; and the occipital and cingulate, with headache impact and quality of life scores. Previous studies in migraine were focussed on assessing visual vein asymmetries and their association with the headache side, but mean values per region were not reported [Bernier et al., 2018]. Our study did not assess effect the laterality of the headache pain.

Regarding the association between vasculature and RS fMRI, a previous study performed in healthy subjects and a 1.5 T magnet showed positive associations between ALFF and vascular density in GM, which were in the range of the results obtained in our healthy subjects' cohort. This study made a voxel-by-voxel and global analysis, which was different than the one proposed in this study, but the results pointed towards the same direction. On the other hand, negative associations were found in WM, which was not the case in our study. Further studies are needed in this line to outline the possible discrepancies found. In the present study, no differences were found in the ALFF values between healthy and subjects with migraine. The strong associations found between ALFF and veins in GM in healthy subjects were lost in migraine;

and curiously, associations between ALFF and veins in WM were in the same range for both groups. Here the differences between the subgroups of subjects with migraine compared to healthy subjects were not as straight and systematic as with the density of veins. Actually, in the parietal lobe, the associations found in episodic patients were higher than in chronic, and in the same range as healthy subjects. It should be noted that in order to discard an effect of the vasomotor activity in RS fMRI, the ALFF was obtained again with a temporary band-pass of 0.06 as an upper limit, instead of 0.09. No differences between the generated images were found (data not shown) so the initial filter was maintained. Thus, in healthy subjects, a larger number of veins in GM translates into a larger ALFF; while in subjects with migraine, not, and it is actually related with the number of veins in WM. The loss of association between ALFF and veins in GM for subjects with migraine seem to suggest an uncoupling in the neurovascular response. Consequently, interpretation of RS fMRI results should be done with cautious.

One of the main limitations of our study is that the test–retest reliability was not provided, but it was also not included in previous studies. Also, although the field of the MRI scanner strength was higher (3 T) than in some of the mentioned studies (1.5 T), it could be improved since nowadays there are new developments regarding the gradients, which results in sequences with higher resolution. Besides the fact that studies with larger cohort sizes may help to confirm the increases found in the density of veins in migraine in the WM, since the results found in the occipital barely survived after correcting for multiple comparison. Another limitation with respect to the SWI could be the presence of magnetic field inhomogeneity artifacts at the cortical border or other conditions such as microbleeds and calcifications which appear hypointense on SWI, which could affect the vessels quantification. Several studies in migraine have focussed in aura, which has not been considered in our study due to the relatively small sample size of subjects presenting this symptom. An additional limitation of the study is that the interval of headache free period has not been taken into account in the analysis. Nevertheless, this limitation is intrinsic to the type of population studied, specially with chronic migraine.

5. Conclusion

Taken together the results in a clinical context, they seem to suggest that the density of veins in WM is the relevant player in subjects with migraine. Their measures are associated with clinical outcomes, such as evolution, cognitive impairment and disability. It also seems to be increased in chronic compared to healthy, and episodic subjects lying between. In this context, the density of veins in subjects with migraine may temptatively be considered a predictor of disease chronification. In addition, the loss of association between veins in GM and ALFF support the idea of abnormalities in the neurovascular coupling. Actually, the coupling between veins and RS fMRI seems to be articulated with an emerging role of the veins in WM, which deserves further investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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