SEX-SPECIFIC VASCULAR SCORE: A NOVEL PERFUSION BIOMARKER FROM SUPERVOXEL ANALYSIS OF 3D PCASL MRI

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ABSTRACT

We propose a novel framework that leverages 3D pseudo-continuous arterial spin labeling (3D pCASL) MRI to compute sex-specific vascular scores that quantify cerebrovascular health and potential disease susceptibility. The brain is parcellated into spatially contiguous regions of homogeneous perfusion using supervoxel clustering, capturing both microvascular and macrovascular contributions. Mean cerebral blood flow (CBF) values are extracted from 186 cognitively healthy participants and used to train a custom convolutional neural network, achieving 95 percent accuracy in sex classification. This highlights robust, sex-specific perfusion patterns across the brain. Additionally, regional CBF variations and age-related effects are systematically evaluated within male and female cohorts. The proposed vascular risk-scoring framework enhances understanding of normative brain perfusion and aging, and may facilitate early detection and personalized interventions for neurodegenerative diseases such as Alzheimer's.

Index Terms— 3D pCASL MRI, CBF, sex-specific perfusion patterns, age-based perfusion changes, vascular score, cognitively healthy

1. INTRODUCTION

Arterial Spin Labeling (ASL) is a non-invasive Magnetic Resonance Imaging (MRI) technique designed to quantitatively assess cerebral blood flow (CBF) by magnetically labeling endogenous arterial blood water protons. Unlike conventional perfusion imaging methods, ASL does not require exogenous contrast agents or ionizing radiation, thereby offering a safe and repeatable approach for evaluating cerebral perfusion. The technique involves the selective inversion of magnetization in arterial blood proximal to the imaging region [1], followed by acquisition of labeled and control images. The difference in signal intensity between these images reflects the delivery of magnetically tagged blood to brain tissue, enabling quantitative measurement of regional CBF.

The fundamental process of ASL MRI involves three key steps: (i) Magnetic Labeling of Blood: A brief radiofrequency pulse is applied to arterial blood proximal to the brain, magnetically tagging the water protons within the blood without the need for contrast agents. (ii) Inversion and Delivery: The magnetically labeled blood flows into the cerebral microvasculature, delivering the inverted magnetization to brain tissue, thereby altering the local MR signal in proportion to regional perfusion. (iii) Image Acquisition and Subtraction: Two sets of images are acquired—a labeled (tagged) image with magnetically tagged blood and a control image without labeling. The voxel-wise subtraction of these paired images isolates the perfusion-weighted signal, enabling quantitative mapping of CBF [1].

ASL is primarily utilized to measure regional CBF, a critical biomarker in the diagnosis and characterization of numerous neurological disorders [2]. ASL enables the identification of ischemic or infarcted brain tissue in stroke patients, providing essential information regarding tissue viability and therapeutic planning. Furthermore, ASL is valuable in detecting and monitoring neurodegenerative conditions such as Alzheimer's disease, where alterations in cerebral perfusion often precede structural abnormalities detectable by conventional MRI. Additionally, ASL contributes to the evaluation of brain tumors by delineating perfusion heterogeneity and is increasingly applied in epilepsy to localize epileptogenic foci through perfusion abnormalities.

Arterial Spin Labeling (ASL) offers several notable advantages over traditional CBF measurement techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) [3]. These advantages include:

- Non-invasive: ASL does not require the injection of radioactive tracers or contrast agents, thereby reducing patient risk and discomfort.
- Quantitative: ASL enables absolute quantification of CBF, allowing for accurate evaluation of neurological conditions such as stroke, dementia, and brain tumors.
- Repeatable: Due to its non-invasive nature, ASL can be safely repeated on the same individual, making it well-suited for longitudinal studies and monitoring treatment responses.
- Radiation-free: In contrast to PET and SPECT, ASL uses only magnetic fields and radiofrequency pulses, avoiding

exposure to ionizing radiation.

Among ASL variants, 3D Pseudo-Continuous ASL (pCASL) is the most widely adopted in both clinical and research contexts. This technique achieves an optimal balance between sensitivity [1], safety, and practical implementation, outperforming alternatives such as Continuous ASL (CASL), Pulsed ASL (PASL), and EPI-based ASL. 3D CBF maps generated via 3D pCASL provide detailed volumetric representations of cerebral perfusion, capturing spatial distribution and relative blood flow magnitude throughout the brain. These maps are indispensable for clinical diagnostics and research applications [4], offering critical insights into brain health and function.

CBF refers to the delivery rate of oxygen-rich blood to brain tissue and serves as a crucial indicator of neural activity and structural health [5]. As it reflects the brain's metabolic demands and vascular integrity, alterations in CBF can signal the presence of neurological disorders [6], such as stroke, dementia, or traumatic brain injury. Reduced or asymmetrical flow may indicate ischemia or impaired autoregulation, while elevated perfusion can be associated with inflammation or hyperemia. Because of these associations, CBF is increasingly used in clinical settings to detect early pathological changes, guide therapeutic decisions, and monitor disease progression or recovery following intervention. In the context of healthy aging, CBF can act as a sensitive biomarker of neurovascular function, as subtle declines in perfusion may precede cognitive decline or structural brain changes. Tracking CBF across the lifespan can help distinguish normal aging trajectories from early signs of neurodegeneration, thereby supporting timely preventive or therapeutic strategies.

Since 3D pCASL is primarily a structural and functional imaging technique for quantifying CBF [4], it has been widely employed to investigate sex-related differences in brain physiology and pathology [7]. Such differences in cerebral perfusion have been documented across various neurological and psychiatric disorders. The high spatial resolution and comprehensive brain coverage of 3D pCASL enable sensitive detection and mapping of these perfusion disparities through the following mechanisms:

- Sex-Related Baseline Differences in CBF: Studies have shown that there can be sex-based differences [7, 8] in the baseline CBF. On average, women tend to have slightly higher CBF than men. This difference has been attributed to a variety of factors, including hormonal differences (e.g., estrogen's effects on vasodilation) and metabolic differences between males and females.
- Age-Related Changes in CBF: Some studies have suggested that the rate of decline in CBF with aging may differ between men and women [8, 7]. Women may show a more pronounced decline in CBF with age, especially in certain brain regions, such as the hippocampus, which is

vital for memory and is often affected in Alzheimer's disease. Using 3D pCASL, it's possible to observe these age-related changes in a more comprehensive manner, capturing perfusion changes in both the cortex and subcortical regions over time. Studies have shown a greater perfusion in healthy children and teenagers [9, 10]. As age increases, the perfusion rate decreases.
- Sex Differences in Neurological Diseases: 3D pCASL is a valuable tool to investigate how sex influences the pathophysiology of neurological diseases. For example, in Alzheimer's disease, women tend to exhibit greater reductions in CBF compared to men [11], particularly in the parietal and temporal lobes, which are commonly affected in Alzheimer's. This could reflect sex-specific differences in disease progression or pathophysiology [3]. In Parkinson's disease, while the disease itself affects both sexes, men tend to have more severe motor symptoms at diagnosis. Differences in perfusion patterns detected via 3D pCASL could provide insights into how sex-related characteristics influence the progression of motor and non-motor symptoms.

Perfusion rates can be assessed by quantifying CBF maps derived from 3D pCASL MRI using computational algorithms. The quantification process employs a model-based approach to translate ASL signal differences into physiologically meaningful units. After acquiring paired labeled (tag) and control images, their subtraction yields a perfusion-weighted image reflecting the net inflow of magnetically labeled arterial blood into brain tissue during the post-labeling delay (PLD).

To obtain absolute CBF values, the perfusion-weighted signal difference is normalized by a separately acquired M0 image, which represents the equilibrium longitudinal magnetization of brain tissue. The M0 image, typically acquired via a proton density-weighted sequence with long repetition time (TR) and minimal T1 weighting, corrects for coil sensitivity variations, scanner gain, and other system factors, enabling accurate scaling of the ASL signal. Utilization of 3D acquisition sequences, such as 3D GRASE (Gradient and Spin Echo) or 3D fast spin echo, enhances signal-to-noise ratio (SNR) and spatial coverage, facilitating robust whole-brain CBF quantification.

Quantification based on the Buxton general kinetic model [12], incorporates critical parameters including labeling duration, PLD, labeling efficiency, blood–brain partition coefficient, and T1 relaxation time of arterial blood. This model assumes instantaneous delivery and retention of labeled spins within the imaging voxel and often applies a single-compartment framework, disregarding arterial transit time dispersion. Post-processing corrections—such as for partial volume effects, patient motion, and magnetic field inhomogeneities—are routinely applied to enhance accuracy. The final CBF map provides a three-dimensional represen-

tation of perfusion, typically expressed in mL/100g/min, serving as a non-invasive quantitative biomarker for cerebral vascular health and pathology.

In standard workflows, CBF quantification from 3D pCASL images is typically performed using various neuroimaging toolkits such as BASIL, ASLtbx, ExploreASL, or ASL-MRICloud. These pipelines implement the Buxton model and automate preprocessing steps like motion correction, M0 calibration, and registration. Voxel-wise CBF values are often averaged over ROIs or anatomical atlases, with optional partial volume correction using structural MRI. While these methods are robust and interpretable, they may miss finer regional patterns in perfusion, which can be better captured by advanced approaches like supervoxel-based clustering and deep learning.

Building on the capability of 3D pCASL to capture subtle perfusion differences, we propose a novel framework for the analysis of 3D CBF maps acquired via dual post-labeling delay pCASL (DP-pCASL). Our method employs supervoxel generation and clustering algorithms to segment brain volume into regions of homogeneous perfusion, characterized by mean intensity values extracted from each supervoxel cluster. This approach allows for robust regional characterization of cerebral perfusion heterogeneity.

Using these supervoxel features, investigate perfusion differences between cognitively normal male and female participants through a customized deep learning classifier. Given extensive research on aging and neurodegenerative disorders [1, 3, 11], an automated, deep learning-assisted sex classification system based on CBF maps could streamline diagnostic workflows, reduce clinician workload, and allow for more personalized assessments.

Deep learning models, with their ability to learn complex feature representations [13], offer promising avenues for a rapid and accurate classification of brain perfusion patterns [14, 15]. Identifying sex-specific cerebral perfusion profiles is critical, as sex differences may influence cognitive function, neuroplasticity, and disease susceptibility. Furthermore, many neuroimaging studies currently overlook sex as a confounding variable in CBF analysis [16]. By leveraging 3D CBF maps for prediction of sex, this work aims to deepen understanding of neurovascular differences and their impact on brain function and therapeutic outcomes, ultimately improving the precision of neuroimaging-based diagnostics and research.

2. RELATED WORKS

Quantitative analysis of CBF is a critical approach to elucidate sex-specific neurophysiological differences [17]. While a gradual decline in CBF is a normal part of the aging process for both sexes [7, 18], women generally exhibit higher global CBF than men throughout life, likely due to hormonal influences such as estrogen, which promotes vascular health and perfusion. However, the rate and regional patterns of decline may differ: men often show more pronounced reductions in CBF with age, whereas women may experience region-specific changes, particularly post-menopause when estrogen levels drop sharply. These sex-specific trajectories have implications for vulnerability to neurological diseases; for instance, women are at a higher risk for AD, potentially linked to abrupt hormonal shifts and associated cerebrovascular changes. Advanced imaging techniques such as ASL MRI enable non-invasive tracking of these patterns [19, 20], offering insights into sex-based differences in brain metabolism, vascular aging, and cognitive outcomes. Recognizing these distinctions enhances our understanding of healthy aging and supports the development of more tailored preventive and therapeutic strategies.

Researchers commonly use software tools like BASIL and ExploreASL to quantify CBF maps. BASIL, part of the FM-RIB Software Library (FSL), uses a Bayesian model based on the Buxton kinetic model for voxel-wise estimation of CBF and arterial transit time (ATT) [21]. It supports both single- and multi-delay ASL data but may overestimate perfusion in highly vascularized regions like gray matter due to macrovascular signal contributions, potentially misrepresenting true tissue perfusion. ExploreASL, an open-source MATLAB pipeline, also uses the Buxton model [22] and includes automated preprocessing steps such as motion correction, normalization, and segmentation. However, it does not estimate ATT and offers limited flexibility for complex acquisition protocols like multi-delay or vessel-encoded ASL.

In light of these limitations, there is a need for simpler, more efficient, and automated techniques that can capture the spatial heterogeneity of perfusion and facilitate interpretable comparisons. To address this, we propose a method that employs the SLIC algorithm for supervoxel generation and clustering of CBF maps. This approach segments the brain into spatially coherent regions with similar intensity profiles, allowing for extraction of mean intensities from both high- and low-perfusion areas. These features are then used to study perfusion distribution and identify sex-specific patterns using a deep learning model. The goal is to determine which regions contribute most significantly to overall perfusion in male and female participants and how these patterns differ.

Several studies [23] have explored innovative approaches to CBF quantification beyond conventional modeling. Okell et al. (2013) introduced a vessel-encoded pseudocontinuous ASL (VEPCASL) technique that incorporates multiple post-labeling delays (PLDs) to estimate CBF in voxels receiving blood from multiple feeding arteries [24]. Their method showed improved precision over traditional pCASL in simulations and in healthy volunteers, although it exhib-

ited a trade-off with reduced signal-to-noise ratio (SNR), potentially limiting image quality. Dual-PLD pCASL (DP-pCASL), another recent advancement, allows for estimation of both CBF and water exchange rates across the blood–brain barrier (BBB), using diffusion-sensitized gradients [25, 26]. This makes DP-pCASL particularly suitable for examining microvascular dynamics and measuring arterial transit effects, making it a promising tool for evaluating sex-related differences in perfusion.

There is growing evidence that cerebral perfusion varies by sex due to hormonal, anatomical, and metabolic factors. For instance, women generally exhibit higher regional CBF than men, especially in the prefrontal cortex, temporal lobes, and posterior cingulate cortex. These differences are likely influenced by estrogen, which has vasodilatory effects and plays a role in cerebral autoregulation [11]. Liu et al. (2012) examined CBF across multiple brain regions in 15 male and 20 female cognitively normal adults aged 23 to 84 years [8]. Their findings showed that women had 11–15% higher CBF in global gray matter, posterior cingulate cortex (PCC), and the precuneus. However, the study had limitations, including small sample size and lack of control for biological confounders such as caffeine intake, blood pressure, or diabetes, all of which may influence CBF.

Perfusion changes with age have also been studied extensively. Haller et al. (2016) analyzed 3D CBF maps in a population of 44 healthy individuals aged 4–78 and reported marked age-related declines in perfusion [1]. Children exhibited the highest gray matter CBF (97 mL/100g/min), which decreased significantly during adolescence and further declined into adulthood. On average, adults had 40% lower gray matter and 23% lower white matter perfusion than children. This age-related trend supports the need for techniques that can capture subtle spatial variations in perfusion and distinguish between normal and pathological aging.

Other studies have used DP-pCASL to assess BBB permeability and whole-brain perfusion in elderly populations at risk for cardiovascular diseases. Shao et al. (2024) evaluated 16 older adults and reported a mean whole-brain CBF of 43.3 mL/100g/min using intraclass correlation coefficient (ICC) analysis [7]. Although the study confirmed DP-pCASL's reliability, it was limited by a small dataset and did not fully explore correlations with vascular risk factors or cognitive performance, pointing to avenues for future research.

Functional ASL imaging has also been applied to investigate how perfusion changes during cognitive tasks differ by sex. Research suggests that women often display more bilateral activation patterns, while men exhibit more lateralized responses during tasks involving memory, language, or spatial reasoning [10]. These patterns are likely associated with differences in CBF and neurovascular coupling. 3D pCASL is particularly well suited for these investigations because it allows measurement of perfusion during rest or task conditions without contrast agents or ionizing radiation.

The study conducted by Mishra et al. (2022) is focused on detecting AD by analyzing 3D structural MRI scans through a feature extraction and classification approach. Initially, brain images are divided into anatomically defined regions using an atlas-based segmentation method. To pinpoint the regions most affected by AD, a ranking mechanism is applied to evaluate their significance. Once the most relevant areas are identified, supervoxel-based volumetric features are extracted specifically from these regions. These features are then combined to represent the structural abnormalities associated with AD [27] more effectively. The authors have used the support vector machine (SVM) classifier and obtained an accuracy of 90.11%.

In this study, we aim to predict sex from 3D CBF maps and analyze the differences in perfusion pattern between male and female participants. Our approach combines a novel quantification technique based on supervoxel clustering, a deep learning classifier trained on mean perfusion features from each cluster, and statistical analysis to identify discriminative regions of interest (ROIs). This integrated framework enables the model to learn complex spatial patterns in the distribution of CBF, potentially revealing sex-specific markers of cognitive function and brain health. By providing an interpretable, data-driven method for assessing sex differences in brain perfusion, our work contributes to advancing personalized neuroimaging and precision diagnostics.

3. METHODOLOGY

3.1. Participants

In this study, a publicly available dataset from OpenNeuro comprising 186 cognitively healthy participants was utilized. The cohort includes 89 males and 97 females, with ages ranging from 8 to 92 years. They are of white/Caucasian, Latinx, African American, and Asian origin.

Each participant underwent diffusion-prepared, pseudo-continuous arterial spin labeling (DP-pCASL) magnetic resonance imaging, from which three-dimensional cerebral blood flow (CBF) maps were generated. These maps were analyzed to investigate sex classification, under the hypothesis that regional variations in mean perfusion intensity may follow sex-specific trajectories across the lifespan.

3.2. MR data acquisition

The 3D CBF images acquired using diffusion-prepared pseudo-continuous arterial spin labeling (DP-pCASL) were obtained from the OpenNeuro repository. These data were originally collected by Shao et al. (2024) as part of their investigation into the functional integrity of the blood-brain

barrier and its variation across age and sex. Imaging was conducted on 3T Siemens Prisma scanners using either 32- or 64-channel head coils [7]. DP-pCASL acquisition parameters included a spatial resolution of $3.5 \times 3.5 \times 8$ mm^3, repetition time (TR) of 4.2 s, echo time (TE) of 36.2 ms, and a field of view (FOV) of 224 mm, with 12 slices and an additional 10% oversampling. ASL was performed with a labeling duration of 1.5 s. For a post-labeling delay (PLD) of 0.9 s, measurements were acquired at b = 0 and 14 s/mm^2 across 15 repetitions. For a PLD of 1.8 s, measurements were obtained at b = 0 and 50 s/mm^2 over 20 repetitions. The total scan time was approximately 10 minutes. T1-weighted structural images were acquired using 3D magnetization-prepared rapid gradient echo (MPRAGE) sequences to enable segmentation and coregistration. MPRAGE parameters included a TR of 1.6 s, inversion time (TI) of 0.95 s, TE of 3 ms, and isotropic spatial resolution of 1 mm^3, with a total acquisition time of approximately 6 minutes. Slight variations in these parameters occurred across participating sites.

3.3. Proposed technique

Figure 1 and Figure 2 depict the architecture of the proposed pipeline for classification of participants into males and females using cerebral perfusion imaging. The workflow consists of multiple stages designed to extract and model biologically meaningful features from 3D DP-pCASL CBF maps.

The pipeline begins with data loading and preprocessing, where raw 3D CBF maps are spatially normalized and intensity standardized to ensure inter-participant comparability. Following this, a supervoxel-based clustering technique is applied to segment the brain volume into spatially coherent regions. These supervoxels serve as data-driven regions of interest (ROIs) that preserve anatomical and functional locality while reducing dimensionality.

Within each supervoxel, mean CBF intensity is computed, resulting in a feature vector that characterizes regional cerebral perfusion for each volunteer. These features are then used to train a deep learning model—specifically, a neural network tailored for classification—designed to distinguish between male and female participants based on perfusion profiles.

After training, the model is evaluated on held-out data to assess classification performance using standard metrics such as accuracy, sensitivity, specificity, and area under the ROC curve (AUC). Finally, the pipeline includes an analysis module that investigates group-level differences in regional perfusion. This includes comparisons between male and female volunteers as well as age-stratified subgroups, allowing for the exploration of how perfusion patterns evolve across the lifespan and vary by sex.

This comprehensive approach not only aims to achieve accurate classification of sex but also to enhance our understanding of the neurobiological differences reflected in CBF patterns.
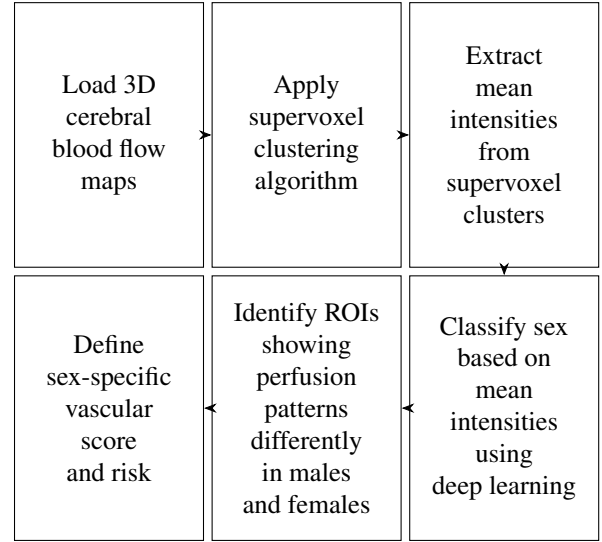

Fig. 1. Proposed framework to obtain sex-based perfusion differences using 3D CBF maps

The key contributions of this study are as follows:

- Development of a novel CBF analysis approach - the supervoxel clustering method - that enables simplified and robust analysis of perfusion data;
- Design and implementation of a deep learning-based sex classification framework that demonstrates high predictive accuracy using 3D DP-pCASL CBF maps;
- Empirical characterization of sex-related differences in cerebral perfusion patterns across the brain;
- Investigation of age-related variations in regional CBF, highlighting the interaction between aging and perfusion dynamics.
- Designing a concept called, "Vascular Score" to identify the risk of acquiring vascular diseases in males and females.

3.3.1. Loading of 3D CBF maps

In this study, our aim is to predict sex based on three-dimensional CBF maps obtained from diffusion-prepared pseudo-continuous arterial spin labeling (DP-pCASL) MRI, using a deep learning-based approach and analyze the variation in perfusion patterns between men and women as they age. The analysis begins with the loading of CBF data stored in NIfTI (Neuroimaging Informatics Technology Initiative) format, where each file represents a volumetric brain scan. These 3D CBF maps undergo preprocessing, including intensity normalization, to standardize the data across participants and ensure compatibility with the neural network input requirements. This step is essential for reducing
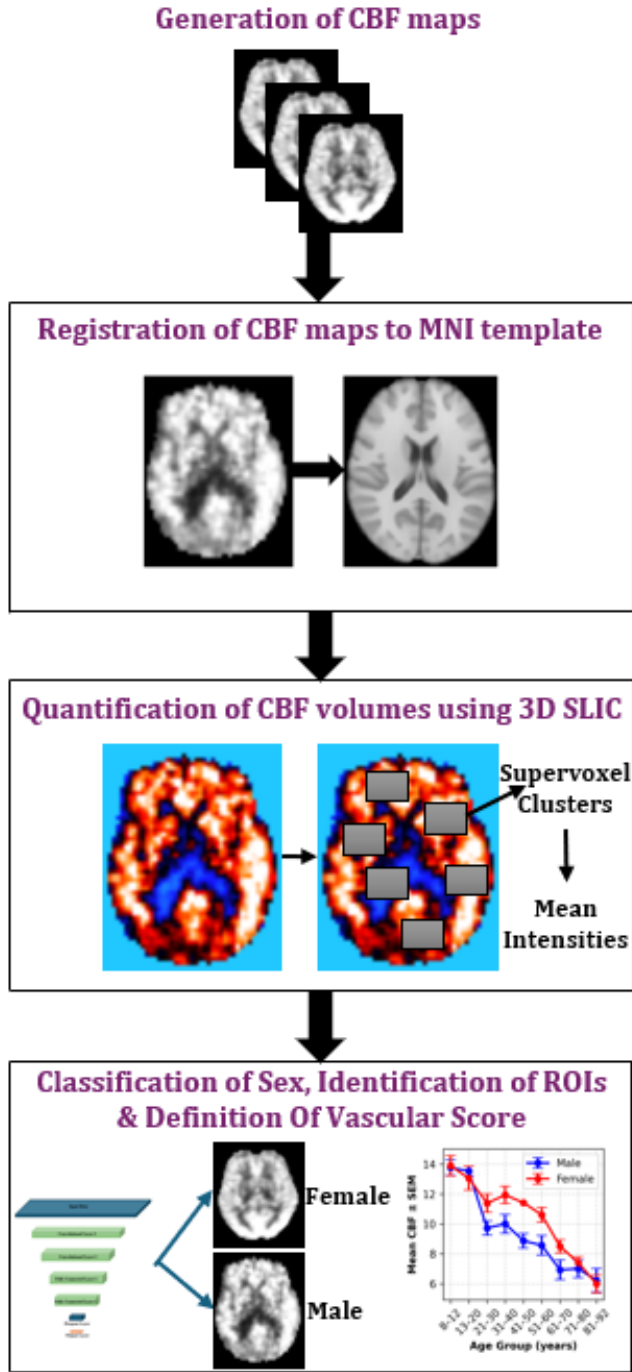
Fig. 2. Different stages of the proposed workflow: Stage 1—Generation of 3D CBF maps; Stage 2-Registration of CBF maps to MNI space; Stage 3—Quantification of CBF maps using 3D SLIC; Stage 4—Classification of sex, identification of significant ROIs, statistical validation and definition of vascular score denoting cerebrovascular risk

inter-participant variability and enabling the model to learn consistent patterns associated with sex-specific perfusion characteristics.

3.3.2. Quantification of CBF

To ensure anatomical consistency across the volunteers and enable group-level analysis, all CBF maps are first spatially registered to the MNI152 standard brain template (Montreal Neurological Institute). This registration step facilitates the alignment of individual brain images into a common space, allowing for voxel-wise comparisons and identification of perfusion patterns in anatomically corresponding regions. Following spatial normalization, a perfusion quantification technique is applied, consisting of several key steps designed to extract and summarize regional CBF characteristics. The details of this quantification process are outlined below.

Supervoxel clustering

Supervoxel clustering is employed to segment each three-dimensional CBF map into spatially contiguous and intensity-homogeneous regions, referred to as supervoxels. This technique extends the well-established concept of superpixels from two-dimensional image processing to volumetric neuroimaging data, enabling structured analysis of complex anatomical patterns. In this study, the Simple Linear Iterative Clustering (SLIC) algorithm is utilized, which applies a modified k-means clustering approach in a combined feature space defined by voxel spatial coordinates (x, y, z) and intensity values. Through iterative refinement, SLIC produces compact and approximately uniform clusters that respect intrinsic anatomical boundaries while preserving perfusion contrast.

Supervoxel clustering has emerged as a powerful technique in medical imaging analysis, offering a structured approach to capture spatial and functional heterogeneity within volumetric data. Its application enhances the accuracy and reliability of perfusion assessment by leveraging voxel similarity and spatial coherence. The key advantages of supervoxel clustering in medical data analysis include:

- Enhanced Spatial Coherence: Supervoxel clustering aggregates voxels exhibiting similar signal characteristics, thereby preserving spatial contiguity and enabling the identification of biologically relevant regional perfusion patterns. This grouping minimizes the influence of random spatial noise and enhances interpretability of functional heterogeneity within tissue.
- Noise Suppression: By averaging voxel-level measurements within each supervoxel, the technique effectively improves the signal-to-noise ratio (SNR). This noise reduction facilitates the detection of subtle perfusion changes that may be critical for early diagnosis and monitoring of pathological conditions.

- Improved Regional Segmentation: Supervoxel clustering aids in the precise delineation of functional subregions characterized by distinct perfusion properties, such as differentiating ischemic tissue from normally perfused regions. This refined segmentation enhances the accuracy of quantitative assessments and supports targeted therapeutic interventions.

Applied to CBF maps generated from 3D pseudo-continuous arterial spin labeling (pCASL) MRI, supervoxel clustering serves to reduce data complexity and suppress voxel-level noise—both common limitations in arterial spin labeling data—by aggregating voxels with similar perfusion properties into coherent regions. The brain volume of each participant is segmented into 100 supervoxels and the mean CBF intensity is calculated within each supervoxel, resulting in a regionalized perfusion profile. This process is applied uniformly across all participants, generating standardized feature representations for both male and female participants.

These supervoxels are treated as data-driven regions of interest (ROIs), enabling a biologically meaningful and computationally efficient quantification of regional cerebral perfusion. By transforming high-dimensional voxel-wise data into a compact set of interpretable features, this approach facilitates robust inter-participant comparisons and statistical analyses. Importantly, the extracted supervoxel-wise mean intensities serve as input features for downstream machine learning models, including sex classification tasks. Furthermore, this framework allows for systematic investigation of sex- and age-related variations in perfusion by supporting region-wise group comparisons and exploratory mapping of perfusion heterogeneity across the brain.

Extraction of mean intensities of supervoxel clusters

The primary objective of this step is to extract spatially localized cerebral perfusion features that enable meaningful comparisons both across individual participants and between male and female groups. To achieve this, the Simple Linear Iterative Clustering (SLIC) algorithm is employed to partition each normalized 3D CBF map into a fixed number of supervoxels—compact, spatially contiguous regions characterized by homogeneous intensity values. This data-driven parcellation approach offers a more flexible and anatomically agnostic alternative to traditional atlas-based region definitions, capturing localized perfusion variations with higher sensitivity.

For each participant, the CBF volume is segmented into 100 supervoxels, denoted as "Cluster 1" through "Cluster 100." Within each supervoxel, the mean intensity is computed, resulting in a 100-dimensional feature vector that characterizes the participant's regional perfusion profile. These intensity features are subsequently associated with the participant's sex (male or female) and grouped accordingly to facilitate sex-specific analysis of cerebral perfusion patterns.

This procedure ensures that the same number of features is extracted from each participant, enabling standardized input to downstream machine learning models. Importantly, all supervoxels, including those with zero or near-zero mean intensities, are retained. This inclusion guarantees consistent feature dimensionality across participants, which is critical for supervised classification tasks and statistical comparisons.

Overall, this supervoxel-based quantification framework provides a scalable, reproducible, and interpretable method for capturing localized perfusion characteristics from CBF maps, thereby supporting fine-grained analysis of physiological variability related to sex and other demographic factors.

Extraction of mean intensities of the neighboring voxels

To capture the spatial context of cerebral perfusion beyond discrete regions of interest (ROIs), we quantified the average CBF intensity within the immediate neighborhoods surrounding the supervoxel clusters identified via SLIC segmentation. While conventional neuroimaging analyses often focus exclusively on ROI-centric metrics, the perfusion characteristics of adjacent tissue provide critical insights into the vascular microenvironment, particularly in studies of cerebral aging and vascular health where interactions between microvasculature and macrovasculature may be pivotal.

For each ROI, defined by its bounding box and centroid coordinates, concentric neighborhoods were delineated by applying increasing margins at radial distances of 0.2 mm, 0.5 mm, 1 mm, and 5 mm from the centroid (assuming isotropic voxel resolution of 1 mm^3). A masking procedure was implemented to isolate these peri-regional zones while excluding the core ROI itself, ensuring that measured intensities reflect the perfusion of the immediately adjacent tissue rather than the central cluster. Mean CBF intensities within these surrounding regions were computed individually for all ROIs and participants, yielding spatially resolved perfusion metrics at multiple scales.

Aggregating these neighborhood perfusion values by sex enabled direct comparisons of peri-regional blood flow patterns between male and female participants. This approach is grounded in the hypothesis that sex-specific differences in cerebral microvasculature and macrovasculature—such as capillary density, vessel diameter, and autoregulatory capacity—may manifest not only within focal perfusion hotspots but also in their surrounding vascular territories. By extending analysis into these perivascular zones, we aim to capture subtle sex-related variations in vascular architecture and function that influence local hemodynamics.

Our analyses revealed two notable trends:

- A monotonic decrease in mean neighborhood perfusion intensity as a function of distance from the ROI centroid, consistent with the expected spatial gradient

from macrovascular inflow regions to the surrounding microvascular beds.

- Slightly elevated mean neighborhood intensities in females compared to males across all distances, suggesting sex-dependent differences in perfusion extending beyond primary ROIs.

These findings underscore the utility of neighborhood intensity metrics as potential imaging biomarkers sensitive to early vascular alterations. Changes in perivascular perfusion can precede measurable deficits within the core regions, providing a window into the pathophysiological progression of cerebrovascular and neurodegenerative disorders. In addition, the incorporation of multiple spatial scales in three-dimensional neighborhood analyses improves the sensitivity and specificity of perfusion characterization, facilitating a more comprehensive understanding of the interaction between macrovascular supply and microvascular tissue perfusion.

Figure 3 illustrates the variation in perfusion intensity at the four prescribed distances from the centroid of supervoxel clusters, highlighting the spatial heterogeneity of CBF in the peri-regional environment.
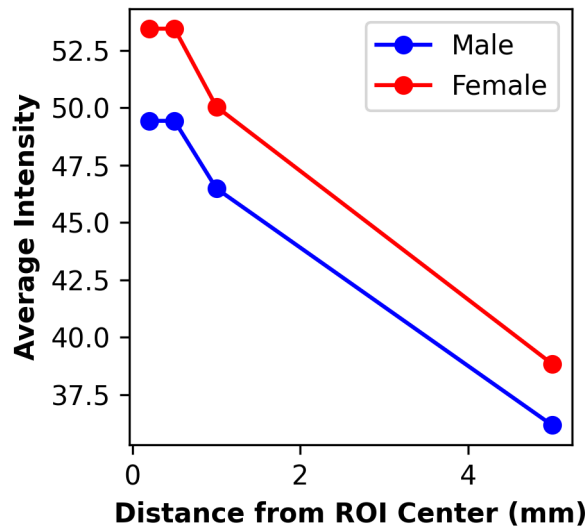

Fig. 3. Variation in the average intensity at different distances from the centre of the supervoxel regions

3.4. Classification of participants into male and female groups using Convolutional Neural Network (CNN)

In this study, we implemented a deep learning framework to classify participant sex based on regional CBF features extracted from three-dimensional pCASL MRI data. The input features to the model consisted of supervoxel-based mean intensity values derived from CBF maps. This data-driven parcellation enables the model to capture subtle and spatially distributed perfusion patterns potentially associated with sex differences.

Figure 4 illustrates representative CBF maps from female and male participants, demonstrating distinct regional perfusion patterns that reflect inherent biological variability. These perfusion disparities constitute salient features leveraged by the Convolutional Neural Network (CNN) to enable robust sex classification.

The CNN architecture was specifically tailored to exploit the spatial relationships inherent in the supervoxel feature vectors. Initial convolutional layers were employed to learn localized patterns of perfusion heterogeneity, preserving spatial contiguity across clusters. These layers were followed by fully connected layers designed to integrate the learned features and perform nonlinear transformations culminating in a binary classification output corresponding to biological sex.

To robustly evaluate model performance and minimize bias arising from dataset imbalances, we employed stratified 5-fold cross-validation, ensuring representative distributions of male and female participants in each fold. Throughout training, performance metrics including loss and classification accuracy were systematically tracked. This approach enabled us to assess the discriminative power of supervoxel-based CBF features in delineating sex-specific cerebral perfusion signatures. The classification results provide insights into the feasibility of employing advanced deep learning techniques on physiological imaging data for sex prediction, with potential implications for personalized diagnostics and understanding sex-dependent brain function.
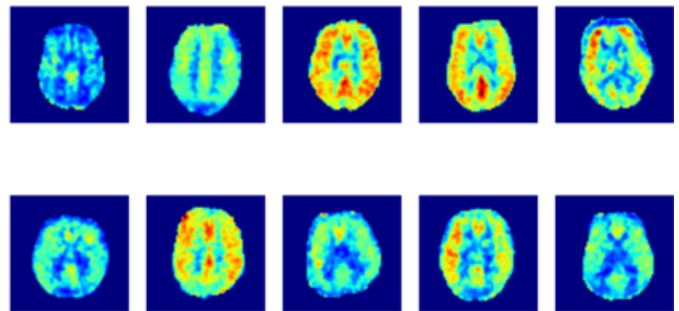

Fig. 4. Colored 3D CBF maps where the upper row represents five female participants and the lower row represents five male participants; the bright red colored regions indicate increased perfusion whereas, the dark blue colored regions indicate reduced perfusion, demonstrating variability in regional cerebral perfusion patterns in both sexes

The 3D CBF maps shown in Figure 4 generated from five female and five male participants serve as visual representations of the sex-related variability in regional brain perfusion.

These maps reveal pronounced spatial heterogeneities, with specific brain regions exhibiting differential perfusion intensities between males and females. In female participants, certain cortical and subcortical areas display consistently higher CBF values, which may reflect underlying neurovascular or hormonal influences. In contrast, male participants tend to show lower perfusion in corresponding regions, highlighting a pattern that persists across multiple individuals. These inter-individual and inter-sex differences in perfusion patterns are not uniformly distributed but are instead localized to particular regions.

By capturing and learning from the intricate regional patterns in the 3D CBF maps, the CNN is able to effectively differentiate between male and female brains based on their perfusion profiles. These findings not only support the feasibility of using machine learning approaches for perfusion imaging-based sex classification, but also underscore the broader relevance of CBF as a biomarker for exploring sex-based neurophysiological differences.

3.5. Age-related variation in cerebral perfusion

CBF is known to exhibit a characteristic decline with advancing age, reflecting physiological alterations in neurovascular coupling, vascular compliance, and cerebral metabolism [28]. The highest CBF values are typically observed in pediatric populations, reflecting elevated metabolic demands during brain development. As individuals transition into adolescence and adulthood, cerebral perfusion gradually decreases, with a more pronounced decline observed in older adults, which may be indicative of age-associated vascular and neurodegenerative changes.
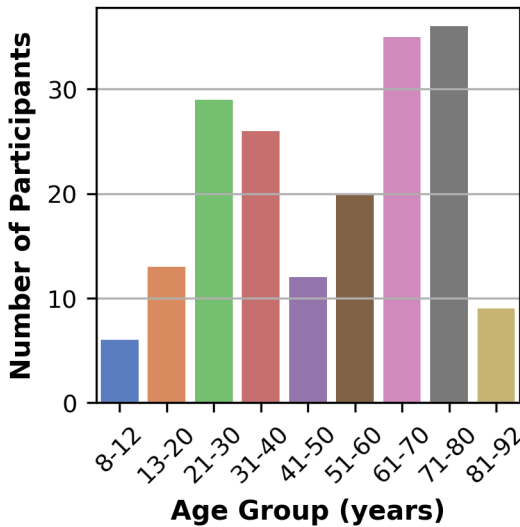

Fig. 5. Distribution of participants in each age group

Figure 5 illustrates the distribution of participants across the

defined age groups, providing a visual representation of the demographic composition of the study sample.

To quantitatively characterize age-related perfusion dynamics, we computed the mean of the mean intensity values derived from supervoxel clusters, analyzed as a function of participant age and stratified by sex. This analysis facilitates the investigation of potential sex-specific differences in CBF trajectories, which may reflect the underlying vascular aging mechanisms. Figure 6 illustrates these trends, demonstrating a gradual decline in CBF with increasing age for both sexes. Across the age span, females exhibited higher average intensity values compared to males, indicating relatively preserved perfusion. These findings may partially explain the observed sex differences in vascular aging and longevity.
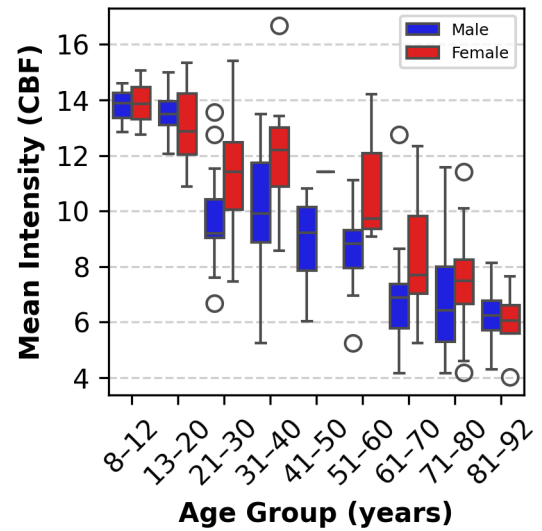

Fig. 6. Effect of age on perfusion in males and females

Cerebral perfusion patterns exhibit age- and sex-related variations across the lifespan. In childhood (ages 8–12), perfusion intensities are comparable between males and females, reflecting similar stages of neurodevelopment. During adolescence (13–20 years), females demonstrate slightly higher perfusion values than males. This difference becomes more pronounced in early adulthood (21–30 years), where females consistently exhibit elevated CBF relative to their male counterparts. In middle age (31–50 years), this trend persists, with females showing greater perfusion across brain regions. Notably, in the 51–70 year age range, females continue to demonstrate higher CBF levels than males. However, in older adults (71–92 years), sex-related differences diminish, and perfusion rates converge between males and females. Overall, these findings suggest a gradual decline in perfusion with age for both sexes, with females generally maintaining higher CBF throughout most of the lifespan. The observed patterns are consistent with previous literature [7, 28, 20, 18] that re-

ports sex-dependent changes in cerebral perfusion, which can arise from differences in hormonal influences, cardiovascular risk profiles, and cerebrovascular reserve capacity. Importantly, the supervoxel-based approach facilitates regional specificity in age-related perfusion analyzes, allowing the detection of localized vulnerability or resilience within the vascular architecture of the brain.

These findings contribute to a growing body of evidence underscoring the importance of considering both age and sex as key biological variables in cerebral perfusion studies. This nuanced understanding can inform the development of gender-specific perfusion biomarkers for the early detection of cerebrovascular pathology and guide personalized therapeutic interventions.

4. RESULTS

4.1. CNN-based classification

Using supervoxel mean intensity values extracted from MRI-derived 3D pCASL CBF maps, the CNN model was trained to classify a cohort of 186 cognitively normal participants by sex. The aggregation of perfusion intensities within spatially contiguous supervoxel clusters provides a compact and biologically meaningful representation of regional cerebral hemodynamics. The trained CNN achieved a classification accuracy of 95%, underscoring the discriminative power of localized perfusion patterns to differentiate male and female brains.

Performance metrics obtained through 5-fold stratified cross-validation further substantiate the robustness and generalizability of the model. Across all folds, CNN consistently demonstrated high accuracy, precision, recall, and F1 scores as shown in Table 1. In particular, precision and recall values were well balanced for both sexes, indicating minimal classification bias and confirming the model's ability to generalize across heterogeneous participant data.

These results suggest that supervoxel-based CBF features effectively encapsulate subtle yet physiologically relevant sex differences in cerebral perfusion. The CNN architecture used efficiently exploits spatial hierarchies within the perfusion data, making it a robust and reliable approach for binary classification tasks based on neuroimaging biomarkers.

Table 1. Prediction using CNN model

	Precision	Recall	F1-Score
Female	0.94	0.97	0.95
Male	0.97	0.93	0.95
Accuracy			0.95

4.2. Comparing the results from other classifiers

Initially, classification of the 3D CBF maps was performed using several conventional machine learning algorithms, including Logistic Regression (Linear), Support Vector Machine (SVM-using Radial Basis Function kernel), Random Forest Classifier (RFC) and XGBoost Classifier (XGBC). These classifiers were selected based on their capabilities to model linear and nonlinear relationships, capture complex feature interactions, and efficiently process both structured and unstructured imaging data.

Table 2 presents the classification accuracies achieved by the alternative models evaluated. Ensemble-based approaches, such as the RFC and the XGBC, demonstrated improvements in robustness and predictive performance compared to simpler baseline models. However, their accuracy remained inferior to that of the convolutional neural network (CNN)-based method. This performance gap highlights the efficacy of deep learning architectures, which inherently capture spatially localized features and learn hierarchical representations from volumetric neuroimaging data.

Table 2. Prediction consistency across classifiers

Classifier	Accuracy
Logistic Regression (Linear)	0.79
SVM (Radial Basis Function kernel)	0.87
RF Ensemble	0.82
Ensemble Boosting	0.89

These findings affirm that classification into male and female based on CBF maps derived from pCASL MRI is feasible. Given the non-invasive nature and physiological relevance of ASL imaging, utilizing CBF maps for sex classification offers promising potential for advancing personalized neuroimaging biomarkers and understanding sex-specific cerebral perfusion differences.

4.3. Statistical Analysis

To systematically characterize sex-related differences in cerebral perfusion, we performed a rigorous statistical analysis on the supervoxel-derived mean intensity features extracted from the 3D CBF maps. Specifically, a one-way Analysis of Variance (ANOVA) was conducted independently for each supervoxel cluster to evaluate whether the mean CBF intensities significantly differ between male and female cohorts. This univariate approach enables the identification of spatially localized perfusion patterns that may contribute to sex-specific cerebral hemodynamics.

The ANOVA test assesses the null hypothesis that the mean perfusion values in each supervoxel do not differ between sexes, against the alternative hypothesis of a statistically significant difference. Prior to analysis, assumptions of ho-

mogeneity of variance and approximate normality of residuals were evaluated to validate the applicability of ANOVA. Where necessary, data transformations or nonparametric alternatives could be considered, though this was not required in the present study due to adherence to statistical assumptions.

For each supervoxel feature, the ANOVA yielded an F-statistic and an associated p-value, quantifying the probability that observed group differences arose by random chance. The features were ranked in ascending order of p-values to prioritize those regions exhibiting the most pronounced sex-related perfusion disparities. To account for multiple comparisons inherent in testing numerous supervoxels, appropriate corrections such as the Bonferroni or False Discovery Rate (FDR) methods were considered; however, results presented here focus on uncorrected p-values with emphasis on features surpassing stringent significance thresholds ($p < 0.05$).

Notably, 17 supervoxel clusters exhibited p-values below 0.05, reflecting robust and reproducible sex-dependent variations in regional CBF. These statistically significant clusters not only corroborate previously reported sex differences in cerebral perfusion but also pinpoint specific anatomical regions warranting further neurophysiological and clinical exploration. Furthermore, these discriminative features serve as valuable inputs for subsequent machine learning classifiers, potentially enhancing model interpretability and predictive accuracy in classification tasks.

4.4. Identification of Regions of Interest (ROIs)

To ascribe biological and anatomical relevance to the statistically significant supervoxel clusters derived from CBF maps, we employed an integrative approach combining data-driven image segmentation with atlas-based anatomical labeling. While the Simple Linear Iterative Clustering (SLIC) algorithm partitions the volumetric CBF images into supervoxels—spatially contiguous voxel clusters exhibiting homogeneous perfusion intensities—these clusters lack intrinsic neuroanatomical annotation as they are defined solely by image intensity and spatial proximity.

To contextualize these supervoxel clusters within a neuroanatomical framework, we co-registered the segmented CBF maps to a standardized brain space and aligned them with the Brainnetome atlas, a high-resolution parcellation atlas offering comprehensive anatomical delineations mapped onto the Montreal Neurological Institute (MNI) template. This atlas provides well-characterized cortical and subcortical region labels, facilitating meaningful interpretation of perfusion patterns in relation to known brain structures.

The assignment of supervoxels to anatomical ROIs was operationalized by a frequently-occurring supervoxel-labeling scheme: for each supervoxel, the constituent voxels were overlaid onto the Brainnetome atlas, and the frequency distribution of ROI labels within the cluster volume (obtained from statistical analysis) was computed. The anatomical label most frequently represented within the supervoxel was then attributed to as its corresponding ROI. This strategy ensures that each supervoxel is robustly mapped to a singular anatomical region, minimizing ambiguity from partial volume effects or spatial overlap.

This enabled quantification of perfusion metrics within anatomically defined structures. Subsequently, the dataset was stratified by biological sex, allowing for group-wise statistical comparisons using independent two-sample t-tests to identify ROIs exhibiting significant sex-dependent differences in regional perfusion.

Table 3. Top 6 anatomical ROIs that help differentiate females from males based on perfusion changes

ROIs
Medial Area 6
Medial Area 10
Area V5
Occipital Polar Cortex
Ventral Dysgranular & Granular Insula
Dorsal Dysgranular Insula

Table 3 summarizes the top six anatomical regions demonstrating statistically significant differences ($p < 0.05$) in mean CBF intensity between male and female participants.

The identified regions—Medial Area 6, Medial Area 10, Area V5, Occipital Polar Cortex, Ventral Dysgranular and Granular Insula, and Dorsal Dysgranular Insula—demonstrate marked sex-related variations in regional perfusion, highlighting their relevance as discriminative features in sex classification models based on CBF data.

Medial Area 6, located within the premotor and supplementary motor cortices of the frontal lobe, plays a central role in motor planning and cognitive control. Enhanced perfusion observed in females may reflect increased functional integration of motor and executive processes. Medial Area 10, part of the anterior prefrontal cortex, is implicated in higher-order cognitive functions such as prospective memory, social cognition, and multitasking; sex-based differences in this region may underpin reported behavioral distinctions in these domains.

Area V5 (also known as MT) and the Occipital Polar Cortex, both integral to visual motion processing and early-stage visual perception, respectively, exhibit greater perfusion in females, potentially corresponding to documented sex differences in visual attention and perceptual sensitivity.

The Ventral Dysgranular and Granular Insula and the Dorsal Dysgranular Insula—key subregions of the insular cor-

tex—show robust sex-related perfusion asymmetries. These areas are core components of the salience network, involved in interoceptive awareness, emotion regulation, and cognitive flexibility. Increased CBF in these regions among females may relate to sex-specific patterns in affective processing and adaptive control mechanisms.

Collectively, these perfusion differences likely reflect underlying neurobiological sex distinctions and underscore the functional significance of these regions in data-driven models for sex classification using CBF metrics.

5. DEFINING A VASCULAR SCORE BASED ON AGE- AND SEX-SPECIFIC MEAN CBF INTENSITY

The observed age-dependent decline in mean CBF intensity is distinctly modulated by sex, as shown in Figure 7. Our analysis reveals that females consistently exhibit higher mean CBF intensities across all age groups compared to males, consistent with known physiological differences in cerebral perfusion. These patterns highlight the necessity of using sex-specific reference ranges when assessing individual cerebrovascular health.

To quantitatively describe vascular health and potential risk, we define a "Vascular Score (VS)" based on whether an individual's mean CBF lies within a normative, age- and sex-specific range derived from a healthy population. Rather than creating a separate or normalized score, VS is inferred directly from each group's mean ± standard deviation (STD) values, which reflect typical variation in healthy cerebral perfusion.

The underlying principle is as follows: individuals whose mean CBF falls within or above the mean ± STD range for their age and sex are considered to have a normal or healthy vascular profile. In contrast, mean CBF values below this range are indicative of vascular risk, suggesting reduced perfusion that may signal impaired autoregulation or emerging vascular pathology.

This framework enables a biologically grounded interpretation of perfusion health using routinely available CBF measurements. By leveraging simple descriptive statistics, it provides a clinically interpretable threshold for identifying individuals at elevated risk for cerebrovascular dysfunction without requiring complex normalization or machine learning–based classification.

5.1. Participant-specific vascular score

To operationalize this concept, we compute group-level statistics from the healthy cohort. For each age group a and sex $S \in \{M, F\}$, we determine the group's mean CBF value μ_a^S and standard deviation σ_a^S. These values define the normal perfusion range:

$$\left[\mu_a^S - \sigma_a^S, \ \mu_a^S + \sigma_a^S\right]$$

An individual's mean CBF is then evaluated relative to this range. If it falls below the lower bound $\mu_a^S - \sigma_a^S$, the individual is considered to be at vascular risk, as their perfusion lies outside the expected healthy range. This lower bound acts as a biologically informed threshold for potential concern.

Unlike traditional percentile-based or z-score cutoffs, this approach directly visualizes and interprets variation in vascular health within the context of group-level variability. Importantly, the method accommodates known age-related and sex-specific physiological differences in CBF without imposing artificial standardization.

5.2. Age- and sex-stratified patterns in vascular health

Figure 7 displays mean CBF values ± standard deviation (STD) across age groups, separated by gender. The mean values represent the average perfusion intensity for each group, while the STD error bars capture inter-individual variability within each category. This visualization allows for identification of critical trends in cerebral perfusion associated with aging and sex.
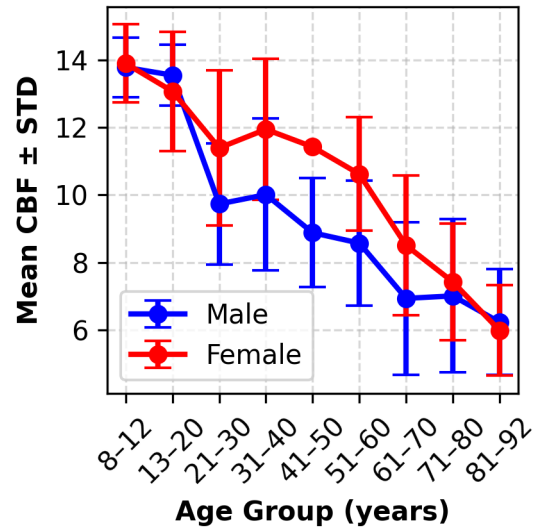

Fig. 7. Mean cerebral blood flow (CBF) intensity plotted across age groups, shown separately for females (red) and males (blue). Error bars indicate standard deviation, representing the normal variability range within each group. This figure defines the normative bounds used to infer vascular score (VS) and identify vascular risk.

We observe that in early life (ages 8–20), both males and females tend to have high and consistent VS, indicating robust cerebral perfusion. However, between ages 21–40, sex-

based divergence begins, with males showing slightly reduced scores. This difference becomes more pronounced in mid-to-late adulthood (41–80), where male VS declines more steeply than female VS, suggesting greater vulnerability to vascular aging. In the oldest age group (81–92), both sexes show significant decline, a marker of increased cerebrovascular risk.

Individuals whose CBF values fall below the lower bound of these error bars (i.e., one standard deviation below the group mean) are flagged as having low vascular scores, indicating increased risk. In this model, the lower limit of the error bar serves as the operational threshold for vascular risk.

$$
\text{VS} = \begin{cases} \text{Normal}, & \text{if mean CBF} \geq \mu_a^S - \sigma_a^S \\ \text{At Risk}, & \text{if mean CBF} < \mu_a^S - \sigma_a^S \end{cases}
$$

Here, the VS is not a continuous numeric index but a categorical classification based on an individual's mean cerebral blood flow (CBF) relative to their age- and sex-specific reference group. Specifically, μ_a^S represents the group-level mean CBF for individuals of age group a and sex $S \in \{M, F\}$, and σ_a^S is the corresponding standard deviation. If an individual's mean CBF lies below the threshold $\mu_a^S - \sigma_a^S$, they are classified as *At Risk*, indicating potentially reduced perfusion and elevated vascular risk. Otherwise, they are considered *Normal*, reflecting perfusion within the expected physiological range.

5.3. Biological and clinical implications

This simplified definition of the vascular score offers a practical and interpretable biomarker of cerebral perfusion status. It does not require individualized normalization or complex modeling, yet still captures meaningful age- and sex-specific patterns in vascular health. By identifying individuals whose perfusion falls below a biologically derived normative range, it enables early detection of potential cerebrovascular compromise.

Such a score may be useful in preventive neurology, especially when integrated with longitudinal imaging or cognitive assessments. Additionally, it offers a scalable, population-level approach to stratify vascular health risk and track changes over time. This is a simplified model and could be extended in future work to include continuous risk scores or integration with other biomarkers.

5.4. Summary of contributions

The proposed framework introduces a statistical, interpretable definition of vascular score (VS) derived from age- and sex-specific mean CBF values and their standard deviations. It is designed to translate perfusion data from routine ASL MRI into a functional indicator of cerebrovascular health with the following advantages:

- Simple and interpretable: based on direct group statistics
- Personalized: accounts for both age and sex
- Clinically useful: identifies individuals below normative perfusion ranges
- Early-stage biomarker: detects perfusion decline before symptoms
- Suited for routine ASL MRI and longitudinal monitoring

6. DISCUSSION

6.1. Comparison with State-of-the-Art methods

Several prior studies have explored sex classification and investigated sex-related differences in the brain utilizing various neuroimaging modalities and analytical techniques.

Ebel et al. (2022) developed a customized convolutional neural network, BraiNN, for sex classification from structural MRI data. Their model achieved an accuracy of 86% when evaluated on the same cohort and 73% accuracy in cross-cohort prediction [29]. This study employed multivariate analysis of gray matter features, adjusting for total brain volume, to assess sex classification performance. Notably, the robustness of machine learning classifiers was evaluated in the context of generalizability across independent cohorts. However, limitations included the absence of sex-specific biological measurements beyond self-reported sex and reliance on coarse anatomical regions of interest, which may have constrained model performance.

Sen et al. (2019) proposed a sex classification framework based on task-evoked functional MRI data from 475 healthy volunteers obtained from the Human Connectome Project (HCP) database. Functional connectivity features were derived via Pearson correlation coefficients of time-series data from anatomically defined brain regions. Using Partial Least Squares (PLS) regression coupled with feature selection, the authors reported a classification accuracy of 88% distinguishing males and females, particularly for emotion task paradigms [30]. They suggested further work to enhance interpretability through spatial mapping of differential activations and refining signal extraction techniques.

More recently, Shao et al. (2024) utilized dynamic pseudo-continuous arterial spin labeling (DP-pCASL) to characterize spatiotemporal trajectories of CBF, arterial transit time (ATT), and blood-brain barrier (BBB) water exchange rates (kw) across age and sex in the cohort of 186 cognitively normal participants aged 8 to 92 years — the same dataset analyzed in the present study [7]. Their findings demonstrated significantly higher CBF and shorter ATT in females relative to age-matched males, alongside sex-dependent declines in BBB water exchange rates. Specifically, whole-brain CBF values were reported as 51.5±11.9, 43.9±10.7, and 31.9±8.5 ml/100g/min for males and 60.5±10.7, 51.2±11.7, and 39.6±10.3 ml/100g/min for females, across young,

middle-aged, and elderly groups respectively. These observations underscore the influence of sex on cerebrovascular physiology and highlight the multifactorial nature of perfusion variability, including lifestyle, healthcare access, and other social determinants, which may be further investigated through animal models for mechanistic validation.

Satterthwaite et al. (2014) demonstrated in the Philadelphia Neurodevelopmental Cohort that perfusion diverges during adolescence; CBF declines in males but stabilizes or increases in females post-puberty—particularly in default-mode and executive network hubs [31]—suggesting puberty-driven vascular modulation. These findings highlight how sex differences in perfusion not only emerge during critical developmental windows but may also carry long-term implications for brain health across the lifespan, influencing differential risk profiles for both cardiovascular and neurodegenerative diseases.

Aanerud et al. (2017) reported that premenopausal women exhibit significantly higher global CBF—especially in temporal and parietal cortices—compared to age-matched men, with this difference diminishing post-menopause [32]. Moreover, Barnes et al. (2017) and Ivanov et al. (2017) corroborated these findings, attributing elevated female CBF and cerebrovascular reactivity to estrogen-enhanced nitric oxide and prostacyclin signaling, which confer greater vasodilatory capacity to the female neurovasculature [33, 34].

Physiological investigations reinforce this mechanistic link: estrogen promotes endothelial NO synthase (eNOS) activity and prostacyclin synthesis, reducing cerebrovascular resistance and enhancing microvascular perfusion, while testosterone and vasoconstrictive prostanoids produce the opposite effect. In addition to humoral influences, structural and developmental factors contribute to these observations [35]. Females generally display higher gray-to-white matter ratios in cortical regions [36] associated with language, emotion, and memory (e.g., inferior frontal gyrus, anterior cingulate, hippocampus), which may underlie localized perfusion enhancements captured in our supervoxel-based analysis. Furthermore, developmental ASL studies [28] suggest that puberty initiates divergent cerebral hemodynamic trajectories between sexes, with female CBF stabilizing or increasing during mid-adolescence, while male CBF continues to decline.

Current studies [28, 37, 11] substantiate the existence of sex-specific cerebral characteristics identifiable via neuroimaging modalities. Our study extends this body of work by leveraging three-dimensional pCASL-derived CBF maps and supervoxel-based regional intensity features to achieve robust sex classification in cognitively normal individuals and also define a vascular score that determines the cerebrovascular risk. The integration of data-driven perfusion features with deep learning frameworks demonstrates promising generaliz-ability and classification accuracy.

Among the regions with significant sex-related differences in cerebral perfusion, we observed that the frontal lobe, occipital lobe, and insula stand out as particularly relevant. The frontal lobe, involved in executive function and motor planning, exhibits higher perfusion in females, potentially reflecting enhanced cognitive integration. The occipital lobe, responsible for visual processing, also shows increased CBF in females, which may relate to differences in perceptual sensitivity. Notably, the insula—a key region for interoception, emotion, and salience detection—demonstrates robust sex-based asymmetries, suggesting its role in affective and self-referential processing differences between males and females.

6.2. Highlights of our study

In our study, we have observed that females exhibit higher cerebral perfusion levels than males. The sex-specific CBF levels and the ROIs identified using our proposed method are supported by the results obtained by Shao et al. (2024) in their sex-related study. Both studies show that, the regions such as the occipital lobe and the frontal lobe play a crucial role in distinguishing females from males as the perfusion levels in those areas vary significantly across both the sexes. Our supervoxel–CNN framework effectively integrates these micro- and macrovascular variations through spatially coherent intensity features, enabling 95% accuracy in sex classification. This high performance validates the sensitivity of our method in capturing biologically grounded perfusion differences driven by sex hormones, developmental trajectories, and structural brain variability. Integrating this physiological and imaging evidence, our pipeline offers a robust and interpretive sex-sensitive biomarker platform—poised to inform studies on healthy aging, hormone-related cerebrovascular changes, and early detection of sex-linked neurovascular disorders.

Here, we introduce a novel vascular score (VS) designed to quantitatively evaluate cerebral microvascular health by leveraging mean CBF intensities obtained from 3D pCASL MRI data. This score provides an individualized measure of vascular status by comparing a subject's mean CBF to age- and sex-specific normative reference values established within a healthy population. Such an approach allows the VS to serve as a sensitive biomarker for detecting subtle deviations from expected cerebrovascular function before clinical symptoms manifest.

The concept of VS that we developed points out the risk of becoming prone to various vascular diseases as the participants age. Elevated perfusion may offer a degree of protection against cardiovascular diseases, which are frequently linked to compromised vascular health [38] and reduced CBF. However, this same physiological trait may also contribute to sex-specific brain aging trajectories. As females

age, the interplay between cerebral perfusion, hormonal fluctuations—particularly post-menopause—and neurovascular mechanisms may increase their susceptibility to neurodegenerative disorders, such as AD. Our finding that females show consistently higher regional perfusion than males aligns with the established literature linking sex differences in CBF to hormonal, neurovascular, and developmental mechanisms.

Unlike traditional vascular biomarkers that often rely on structural lesions (e.g., white matter hyperintensities), or demand advanced acquisitions such as cerebrovascular reactivity (CVR) testing, the VS is derived from standard resting-state ASL data. This enables broader clinical applicability and makes it suitable for integration into routine neuroimaging protocols. Furthermore, by incorporating demographic stratification, the VS captures subtle, individualized deviations from normative perfusion, which are often missed by binary risk thresholds or global CBF cutoffs.

While the present study demonstrates the feasibility and robustness of using supervoxel-based CBF features from 3D pCASL MRI for sex classification, several limitations warrant consideration. A notable limitation of the present investigation is the relatively modest cohort size, reflecting the limited availability of clinical 3D pCASL data. Although the dataset includes a wide age range and cognitively normal participants, the sample size (n = 186) restricts statistical power and may limit the generalizability of the findings across more heterogeneous or clinical populations. Future studies involving larger, multi-center cohorts with harmonized acquisition protocols are warranted to validate and extend these findings across diverse imaging environments and demographic backgrounds. Moreover, our analysis did not account for additional covariates such as hormonal status, cognitive scores, or vascular risk factors, which are known to influence cerebral perfusion and may confound sex-related differences.

Another limitation lies in the reliance on perfusion maps derived solely from pCASL, without the integration of other imaging modalities that could provide complementary structural or functional insights. While the SLIC-based supervoxel clustering offers a data-driven approach to regional feature extraction, the anatomical interpretability of these clusters depends on post hoc mapping to atlas-defined regions, which may introduce variability due to anatomical differences across individuals. Furthermore, although 5-fold cross-validation was employed to mitigate overfitting, external validation on independent datasets is essential to fully assess model generalizability.

Looking ahead, future extensions of this work could explore multi-class classification schemes that go beyond binary sex differentiation. For example, stratifying participants into age-based categories could uncover nonlinear trends in perfusion across the lifespan and improve the characterization of developmental and degenerative trajectories. Incorporating domain adaptation and transfer learning strategies could address variability across different imaging protocols, thereby enhancing cross-scanner robustness in multicenter applications. The synergistic fusion of perfusion maps with other neuroimaging modalities, such as functional MRI or diffusion tensor imaging, may further improve classification accuracy and reveal sex-specific neurovascular signatures underlying cognition and behavior. Nonetheless, our approach may be further enhanced by incorporating additional covariates like education and socioeconomic status, which are known to modulate cerebrovascular health [7].

Targeting perfusion alterations within brain regions implicated in neurodegenerative disorders such as Alzheimer's disease could also yield clinically valuable biomarkers. Longitudinal studies examining perfusion dynamics over time may help distinguish state-related fluctuations from stable, trait-based vascular features. Additionally, the integration of perfusion imaging with functional MRI could elucidate sex-specific neurovascular responses to pharmacological or behavioral interventions, thereby advancing the development of personalized treatment strategies in neurological care. Collectively, these directions highlight the promise of CBF-derived features as biologically meaningful markers in both health and disease, supporting the broader vision of precision medicine in neuroimaging.

Future work should focus on expanding the cohort size, incorporating additional demographic and clinical covariates, and integrating multimodal imaging data to further refine and validate perfusion-based biomarkers. Ultimately, the proposed deep learning pipeline can be integrated into clinical workflows to support personalized diagnostic and therapeutic strategies tailored to individual neurovascular profiles.

7. CONCLUSION

In this study, we present a framework leveraging 3D pseudo-Continuous Arterial Spin Labeling (pCASL) MRI to quantify CBF through supervoxel clustering, enabling anatomically and physiologically meaningful segmentation of brain perfusion patterns. By extracting mean CBF intensities from spatially contiguous supervoxels in 186 cognitively normal adults, we characterized sex- and age-specific variations in cerebral perfusion. A custom convolutional neural network (CNN) trained on these supervoxel-level features demonstrated robust sex classification performance, achieving high accuracy, thereby, validating the discriminative value of regional CBF metrics as a biomarker. Using the mean intensities, we developed a novel participant-specific metric called, "Vascular score" that will reveal the risks of acquiring vascular diseases as people age.

These findings project significant regional differences in cerebral perfusion between males and females, reflecting under-

lying neurovascular and physiological variations relevant to healthy brain aging. The approach offers a sensitive and non-invasive biomarker framework that captures both microvascular and macrovascular contributions to brain perfusion. Importantly, this methodology has the potential to enhance understanding of sex-specific brain aging trajectories and facilitate early detection of neurodegenerative conditions such as Alzheimer's disease.

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