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RESEARCH ARTICLE

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Effect of vitamin D supplementation on cerebral blood flow in male patients with adrenoleukodystrophy

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Abstract

One-third of boys with X-linked adrenoleukodystrophy (ALD) develop inflammatory demyelinating lesions, typically at the splenium. These lesions share similarities with multiple sclerosis, including cerebral hypoperfusion and links to vitamin D insufficiency. We hypothesized that increasing vitamin D levels would increase cerebral blood flow (CBF) in ALD boys. We conducted an exploratory analysis of vitamin D supplementation and CBF using all available data from participants enrolled in a recent single-arm interventional study of vitamin D supplementation in boys with ALD. We measured whole brain and splenium CBF using arterial spin labeling (ASL) from three study time points (baseline, 6 months, and 12 months). We used linear generalized estimating equations to evaluate CBF changes between time points and to test for an association between CBF and vitamin D. ASL data were available for 16 participants, aged 2–22 years. Mean vitamin D levels increased by 72.7% ($p < .001$) after 6 months and 88.6% ($p < .01$) after 12 months. Relative to baseline measures, mean CBF of the whole brain (6 months: +2.5%, $p = .57$; 12 months: +6.1%, $p = .18$) and splenium (6 months: +1.2%, $p = .80$; 12 months: +7.4%, $p = .058$) were not significantly changed. Vitamin D levels were positively correlated with CBF in the splenium (slope = .59, $p < .001$). In this exploratory analysis, we observed a correlation between vitamin D levels and splenial CBF in ALD boys. We confirm the feasibility of measuring CBF in this brain region and population, but further work is needed to establish a causal role for vitamin D in modulating CBF.

KEYWORDS
adrenoleukodystrophy, arterial spin labeling, cerebral blood flow, perfusion, vitamin D

1 | INTRODUCTION

X-linked adrenoleukodystrophy (ALD) is a rare genetic disorder linked to a mutation in ABCD1 gene that results in impaired metabolism of very long chains of fatty acid in the brain, blood, and adrenal tissues (van Haren et al., 2019). Among boys and men with ALD, approximately two-thirds develop inflammatory demyelinating brain lesions; most lesions originate at the splenium of the corpus callosum (Engelen et al., 2012). Although demyelinating brain lesions in ALD share many similarities with multiple sclerosis (MS), cerebral

Abbreviations: ALD, adrenoleukodystrophy; ASL, arterial spin labeling; CBF, cerebral blood flow; GM, gray matter; MRI, magnetic resonance imaging; MS, multiple sclerosis; PCASL, Pseudo-continuous arterial spin labeling; PLD, post-labeling delay; RF, Radio frequency; ROI, region of interest; TR, Repetition time; WM, white matter.

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ALD lesions do not respond to MS therapies but enlarge in volume unless hematopoietic stem cell transplantation is needed (Mahmood et al., 2007). Epidemiologic evidence from MS suggests that vitamin D insufficiency causes the development of brain lesions while vitamin D supplementation may confer a reduction in brain lesions and inflammatory markers, such as cerebral blood flow (CBF) (Lauer et al., 2017). Furthermore, a previous study suggested that vitamin D rise improved CBF in patients with MS (Müller et al., 2019).

Arterial spin labeling (ASL) is a quantitative magnetic resonance imaging (MRI) technique that enables the measurement of CBF using magnetically labeled blood water (Alsop et al., 2015). Since the magnetically labeled blood water serves as the endogenous tracer for ASL, the procedure is non-invasive and radiation-free and enables fully quantitative CBF measurement, making ASL a favorable method for assessing CBF in longitudinal studies where multiple measurements are needed. For example, the correlation between CBF measured by ASL and cognition was shown to be predictive of general fluid cognition for elderly individuals in a 4-year longitudinal study (De Vis et al., 2018). A consensus paper on ASL has recommended the use of the pseudo-continuous ASL (PCASL) technique with a single post-labeling delay (PLD) as the clinical application for CBF quantification due to its high robustness and reproducibility (Alsop et al., 2015; Mutsaerts et al., 2015; Zhao et al., 2017). CBF can be computed by either scaling the ASL difference data or fitting the general kinetic model to the ASL difference data (Alsop et al., 2015; Buxton et al., 1998). Reduced CBF has been reported in the white matter (WM) of patients with MS compared with healthy subjects using ASL techniques (Steen et al., 2013). In relapsing–remitting MS, reduced CBF was also observed using single-PLD PCASL before tissue atrophy occurred in several cortical and deep gray matter (GM) regions including the thalamus, putamen, and hippocampus (Debernard et al., 2014). The link between vitamin D deficiency and reduced CBF in patients with WM lesions led to our central hypothesis that ASL can potentially examine CBF changes in ALD patients undertaking oral vitamin D supplementation.

In this work, we hypothesized that vitamin D supplementation in male ALD patients would increase CBF of the whole brain and splenium. Female patients were not included because they are rarely affected by cerebral ALD (Loes et al., 2003). ASL data were collected from 16 male ALD patients in three sessions separated by 6 months as part of a pilot study of vitamin D supplementation. CBF was quantified using the general kinetic model and compared on a whole brain and regional basis.

Significance

X-linked adrenoleukodystrophy (ALD) is a rare genetic disorder linked to a mutation in the ABCD1 gene that results in impaired cerebral blood flow (CBF) and lesions in the white matter of the brain. ALD shares similarities with multiple sclerosis, including links to vitamin D deficiency and lesion-associated hypoperfusion. We investigated the impact of taking vitamin D supplements on CBF in 16 male patients with ALD using arterial spin labeling MRI. Results showed a positive correlation between vitamin D levels and CBF.

2 | METHODS

2.1 | Experimental design

The study data were collected as part of a registered Phase I study of vitamin D supplantation in boys and young men with ALD (Clinicaltrials.gov identifier: NCT02595489). The study was performed in compliance with the regulations of the institutional review board of our institution (Stanford IRB Protocol #30805). All participants' parents/guardians provided written informed consent. Assent was also collected for participants 7 years of age and older. Participants were eligible for enrollment if they were males with a molecular diagnosis of X-linked ALD between the age of 1.5 and 25 years, without evidence of gadolinium-enhancing cerebral demyelination, and had baseline 25-hydroxyvitamin D level less than or equal to 60 ng/mL. Participants were excluded if they had a history of liver, kidney, or thyroid disease, or had contraindication to completing a brain MRI every 6 months. Enrollment was limited to male participants because the clinical standard of care limits brain MRI surveillance to males with ALD because of their unique susceptibility to brain lesion formation; brain lesions in women with *ABCD1* mutations are exceedingly rare (Loes et al., 2003). Each participant received sublingually dissolvable vitamin D tablets (Continental Vitamin Company, Vernon, California, USA). Drug compliance was examined using quarterly compliance questionnaires and by analyzing plasma 25-hydroxyvitamin D levels. The dosing regimens and primary study outcomes were described previously by the senior author of this work (Haren et al., 2022). In short, all participants were assigned to daily oral vitamin D supplementation with a sublingually dissolvable tablet with an assigned daily dose ranging from 1000 IU daily to 4000 IU daily depending on the body weight and phase of enrollment. Supplementation was continued for 12 months. Serum levels of 25-hydroxyvitamin D were measured every 3 months. Vitamin D levels were measured by serum 25-hydroxyvitamin D using blood samples collected from each participant.

Imaging data were collected from participants using a 3T MRI system (Discovery MR750, GE Healthcare, Waukesha, WI, USA). All participants' parents/guardians provided written informed consent. Assent was also collected for participants 7 years of age and older. Perfusion analysis was collected for participants enrolled at our institution. All participants received a presymptomatic molecular diagnosis of ALD; five participants (31%) were originally diagnosed via statewide newborn screening; the remaining 11 (69%) had been screened for ALD due to a positive family history. Eight (50%) participants had adrenal insufficiency at enrollment; their steroid regimens remained unchanged

throughout the study period. A ninth participant (participant 13) was diagnosed with adrenal insufficiency after the 6-month study visit and started on daily steroid supplements. All participants had normal developmental histories and normal neurologic exams at enrollment and throughout the study period. The full description of the procedure can be found in our previous work (Haren et al., 2022). The procedures were conducted according to the Declaration of Helsinki, and the data were collected between November 2016 and February 2020. Each subject received MRI scans at the baseline, month 6, and month 12 of the experiment to screen for the appearance of cerebral ALD lesions as part of the routine procedure. In each MRI session, ASL data were acquired using the parameters described in Table 1, and structural T1-weighted data were also collected using a fast spoiled gradient echo T1-weighted scan (Ellingson et al., 2015).

2.2 | Cerebral blood flow quantification

The ASL difference data were obtained by subtracting the label and control data. For each voxel, the relative CBF was computed by fitting the general kinetic model to the ASL difference data. This model was implemented using the spatially regularized variational Bayesian method implemented in the FSL tool BASIL (Chappell et al., 2009; Groves et al., 2009). Since the general kinetic model has been widely used to analyze ASL data, we have adopted it in our analysis to quantify CBF. Although single-delay ASL was used in our imaging protocol, there were three number of excitations (NEX = 3 in Table 1) in the ASL sequence such that three repeats were performed and used for model-fitting. The parameter values used in CBF quantification are listed in Table 2. Since the repetition time (TR) of the proton density data was less than 5 seconds, its signal intensity was corrected to obtain the proton density when all the spins were fully relaxed. This was achieved by dividing the original proton density data by $1 - e^{-TR/T_{1,Tissue}}$, to estimate the equilibrium magnetization of the tissue, assuming $T_{1,Tissue}$ to be 1300 ms for the whole brain (Alsop et al., 2015). The corrected proton density data was then used to calculate the equilibrium magnetization of the arterial blood for calibrating the CBF data, assuming a blood–brain partition coefficient (λ) of .9 (Zhao et al., 2021). The partial volume effects on the edge of the calibration image were corrected using an erosion and extrapolation technique (Zhao et al., 2017). Finally, the CBF maps in absolute units (mL/100 g/min) were computed using the relative CBF and the calibration data.

A whole brain mask for group analysis was created by extracting the brain from the T1-weighted template in the Montreal Neurological Institute (MNI)152-2 mm space using the FSL tool BET (Smith, 2002). The region of splenium was extracted from the Oxford-GSK-Imanova Structural–anatomical Striatal Atlas (Tziortzi et al., 2011). A rigid-body transformation between the CBF and T1-weighted structural data of each subject was created using the FSL tool FLIRT and considering boundary-based registration (Greve & Fischl, 2009; Jenkinson & Smith, 2001). For each subject, a second transformation matrix and warp image between the T1-weighted structural data and the (MNI)152-2 mm standard space were created using the non-linear registration tool FNIRT (Woolrich et al., 2009). Finally, the CBF data of each subject in each session were transformed to the MNI-152 standard space by combining the rigid-body transformation matrix and the non-linear transformation matrix and warp using the tool APPLYWARP (Smith et al., 2004). The mean CBF of the full brain and splenium was computed for each subject. All CBF images were smoothed using a Gaussian filter of 3 mm FWHM for statistical analysis.

TABLE 1 Pseudocontinuous ASL MRI data collection parameters.

Parameter	Unit	Value
Labeling pulse shape		Hanning
Labeling pulse duration	ms	.5
Labeling pulse spacing	ms	1.22
RF pulse strength	Gauss	.014
Mean gradient strength	mT/m	.7
Maximal gradient strength	mT/m	7
Flip angle	degrees	23
Bolus duration	ms	1450
TR/TE	ms	4854/10.7
TR of proton density data	ms	2000
PLD	ms	2025
NEX		3
Acquisition matrix		8 spirals × 512 sampling points
Number of slices		36
FOV	cm^3	24
Acquisition voxel size	mm	3.73 × 3.73 × 4
Reconstruction voxel size	mm	1.875 × 1.875 × 4
Number of background suppression pulses		5
Scan duration	min	4:13

TABLE 2 ASL MRI data quantification parameters.

Parameter	Unit	Value
CBF prior	mL/100 g/min	$(0, 10^{-12})$[a]
Tissue T1	ms	1300
Arterial blood T1	ms	1650
PLD	ms	2025
Bolus duration	ms	1450
Blood-water partition coefficient		.9
Labeling efficiency	ms	.85

[a]Values indicate the mean and standard deviation of the Gaussian prior used in the model-fitting process in BASIL.

2.3 | Statistical analysis

We used a generalized estimating equations (GEEs) approach to estimate the change in vitamin D and CBF over the course of the study. For each outcome, we used a log-linear regression model, with fixed effects for each of the three time points, and an exchangeable correlation structure to account for repeat measurements at the patient level. Specifically, we fit four statistical models. The first model fits the log of the vitamin D level to fixed effects representing the three time points. The second model fits the log of the CBF to fixed effects representing both the three time points and whether the CBF corresponds to the whole brain or to the splenium; the latter fixed effect term allows us to compare splenium with whole brain CBF. The last two models are used to determine the association between vitamin D levels and CBF; we fit a linear model that averages over the three time points, with the same patient-level correlation structure. With these models, we report the association with both the slope of the linear fit, and the marginal R^2 (as described by Zheng (2000)), which can be interpreted as a measure of goodness of fit.

To account for the multiple hypotheses being tested, we apply a Bonferroni adjustment to the significance threshold. There are five primary outcomes under consideration: the effect of vitamin D supplements on vitamin D levels, whole brain/splenium CBF, and the association between vitamin D levels and whole brain/splenium CBF. As such, our adjusted threshold for significance is $p = .01$. Statistical analyses were conducted in Python version 3.8.5, and the GEE model was fit using the statsmodels package.

3 | RESULTS

3.1 | Summary of subject conditions

Table 3 shows the summary of participant information and experimental parameters. We recruited 16 individuals with ALD with an average age of 8.2 years (range: 1.8–22 years, all males) and a mean vitamin D level of 49 ng/mL. Each patient received 3 MRI scans (baseline, after 182 ± 10 days, and after 363 ± 20 days).

TABLE 3 Summary of patient information and experimental parameters.

Parameter	Unit	Value range	Mean	SD
Number of participants		16		
Age	years	1.8–22	8.2	6.3
Participants with ALD brain lesions or abnormal neurological findings		0 (0%)		
Participants with concurrent diagnosis of adrenal insufficiency		8 (50%)		
Time between scanning sessions	days	154–216	182	15.2
Vitamin D level, baseline visit	ng/mL	19–42	29.1	6.1
Vitamin D level, 6-month visit	ng/mL	32–73	52.6	9.8
Vitamin D level, 12-month visit	ng/mL	36–95	56.6	11.2

3.2 | Cerebral blood flow quantification

Figure 1 shows the CBF and vitamin D levels of four example subjects. Overall, the CBF level of each participant showed different variations after the subject took the vitamin D supplement for a year. Using the baseline (month 0) CBF as the reference, the whole brain CBF of subject 1 increased steadily in months 6 and 12; the CBF of subject 2 peaked in month 6 but decreased slightly in month 12; the CBF of subject 3 decreased by 11% in month 6 before increasing by 8% in month 12; the CBF of subject 4 decreased in both months 6 and 12. Figure 2 shows the mean CBF of the whole cohort in the three scanning sessions. Although CBF showed significant contrast between GM and WM regions, the GEE regression model detected no significant change in mean CBF, as will be shown in Figure 4.

3.3 | Impact of vitamin D on cerebral blood flow

Figure 3 shows the vitamin D level of each participant at months 0, 6, and 12. Using the value at month 0 as the reference, the vitamin D level of the cohort increased significantly by 72.7% [53.4%, 94.5%] ($p < .001$) after 6 months and 88.6% [64.6%, 116.1%] ($p < .001$) after 12 months. The largest variation was recorded in subject 7 (280%). The vitamin D level of all participants can be found in Table 4.

Figure 4 shows the mean CBF of the whole brain and splenium and the GEE model results. In all three sessions, the mean CBF of the whole brain was significantly higher than in the splenium: 14.0% [7.3%, 21.0%] in month 0; 15.4% [7.7%, 23.8%] in month 6; 12.6% [6.2%, 19.2%] in month 12 ($p < .001$ at all 3 time points).

Figure 5 shows the correlation between the mean CBF and vitamin D level of each participant, averaged over the three scanning sessions using a GEE regression model. While vitamin D levels did not correlate with whole brain CBF ($p = .11$), they did correlate with the CBF in splenium ($p < .001$). Specifically, an increase of 10 ng/mL in vitamin D levels was associated with an increased CBF in the splenium of 5.9 ± 2.8 mL/100 g/min ($p < .001$). Despite this, while the study resulted in a significant change in vitamin D, we did not detect a corresponding significant change in splenium CBF.

Figure 6 shows the whole brain and splenium CBF in each participant at months 0, 6, and 12. No harmonized CBF changes were observed in the whole brain and splenium.

4 | DISCUSSION

In this work, we used ASL MRI to examine changes in CBF among boys and young men with ALD who were receiving daily oral vitamin

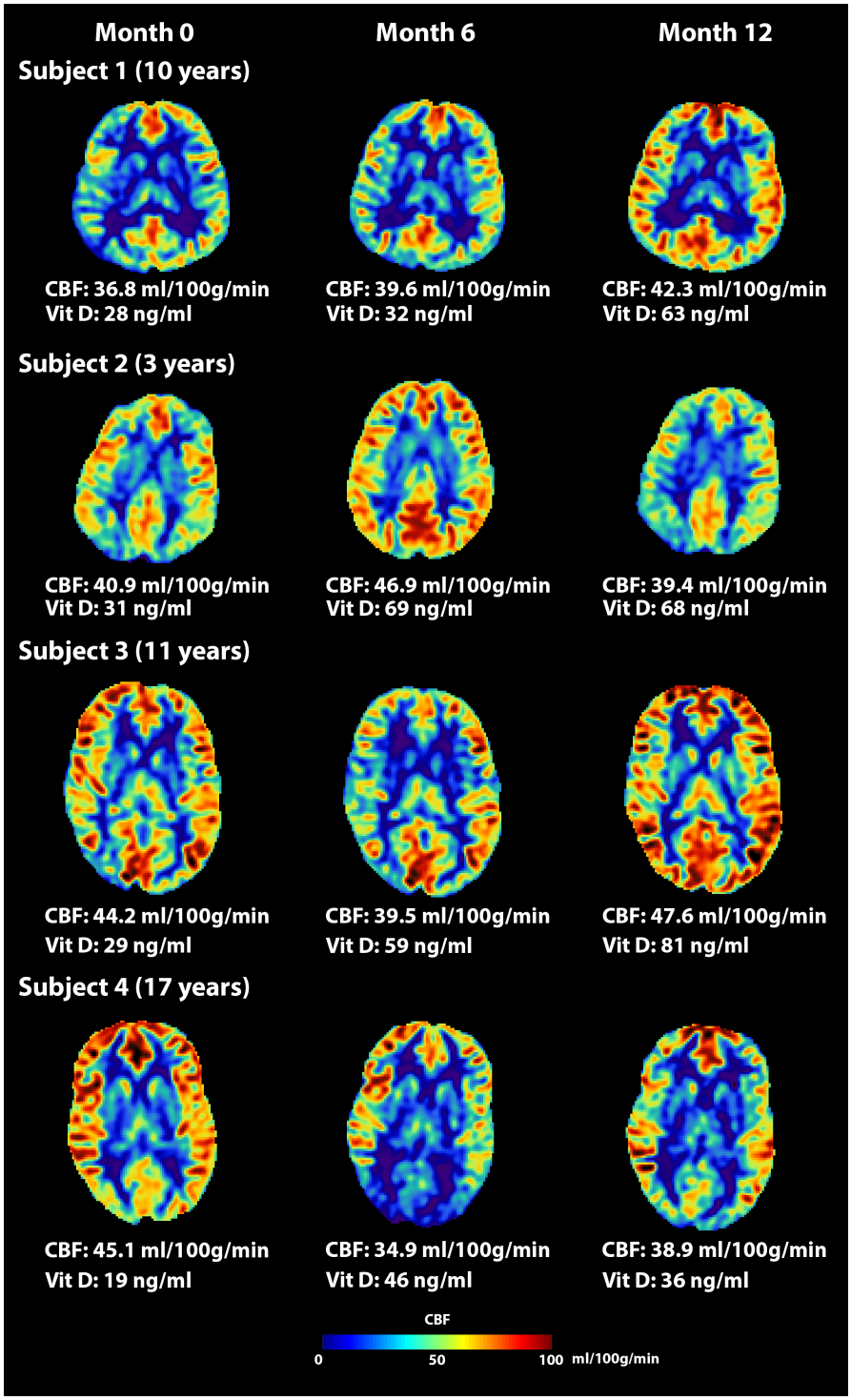

FIGURE 1 Cerebral blood flow (CBF) maps and vitamin D levels of four example subjects. The mean CBF of each subject showed different variations after the subject took the vitamin D supplement for 12 months.

D supplementation over a 12-month study period. We found that current methodologies were effective for quantifying CBF in the whole brain and splenium of boys and young men with ALD. We also observed a correlation between serum 25-hydroxyvitamin D levels and brain perfusion. The correlation was strongest for perfusion of the splenium, which is the region at highest risk for hypoperfusion and associated inflammatory lesion formation in ALD boys.

4.1 | Cerebral blood flow quantification in adrenoleukodystrophy patients

This is the first study that examined the perfusion change of boys and men with ALD using ASL. Although ASL has been widely applied in many longitudinal studies in adults (De Vis et al., 2018; Mutsaerts et al., 2014; Steketee et al., 2015), its application in pediatric imaging

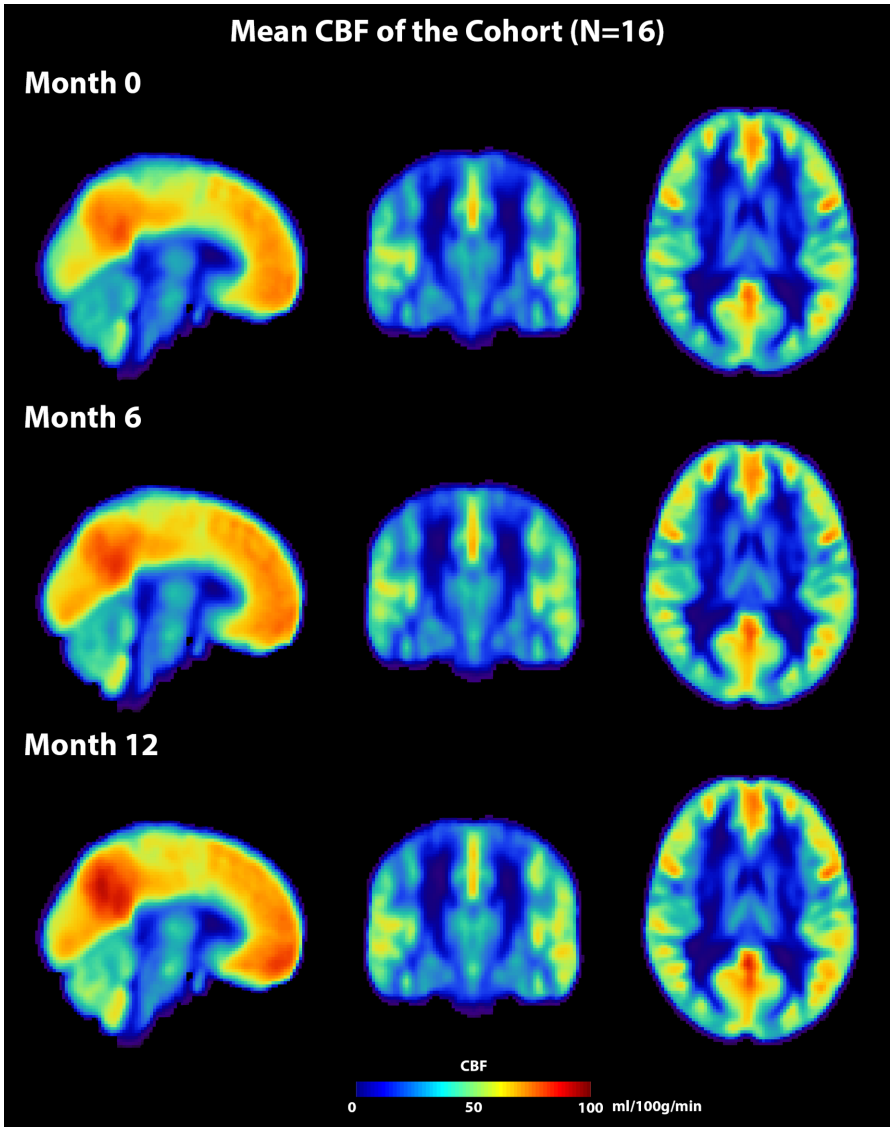

FIGURE 2 Mean cerebral blood flow (CBF) of the group at baseline (month 0), month 6, and month 12. Overall, no significant change in CBF is seen between baseline and month 6 and between baseline and month 12.

was challenging due to brain development, motion artifact, and the lack of a brain template (Proisy et al., 2016). In this work, we observed that the range of the whole brain CBF was between 23 and 51 mL/100 g/min for our cohort of 1.8 to 22 years, which was within the range of a previous study that investigated the CBF (20–97 mL/100 g/min) in subjects between 4 and 20 years using continuous ASL (Biagi et al., 2007). The results obtained in this work demonstrated that ASL can be applied to investigate CBF in males with ALD and quantitative CBF measurements can be obtained non-invasively. We also reported that the CBF in the splenium was significantly lower than in the whole brain, which was also in agreement with previous observations that the WM CBF (such as in the splenium) is generally lower than the GM CBF (Clement et al., 2017; Petersen et al., 2010). Therefore, the results here demonstrated that it is feasible to apply ASL in studying the CBF of males with ALD.

Comparing the CBF during each scanning session, although a slight increase in CBF of the whole brain was found after the participants had taken vitamin D for a year (median CBF of the cohort at month 0: 37 mL/100 g/min; month 6: 38 mL/100 g/min; month 12: 39 mL/100 g/min in Figure 4), no statistical significance was achieved in these comparisons. This may be explained by two reasons: (1) The accuracy of the selected ASL technique (PCASL) is sensitive to the flow velocity of the arterial blood, and the scanning parameters were not optimized for the current cohort. Several studies have reported that the flow velocity of the arterial blood of participants was higher than those in healthy adults, and the resulting CBF can be underestimated by 8% without a correct measurement of the flow velocity (Gevers et al., 2012; Makki et al., 2019). A possible solution to improve the accuracy of CBF quantification would be to use a pulsed ASL labeling technique, which is insensitive to flow velocity variations, though this would also reduce the signal-to-noise ratio of the ASL data. (2) A potential vitamin D–induced change in CBF may be confounded by the natural CBF variation during brain development. It has been shown that CBF is very low (20 mL/100 g/

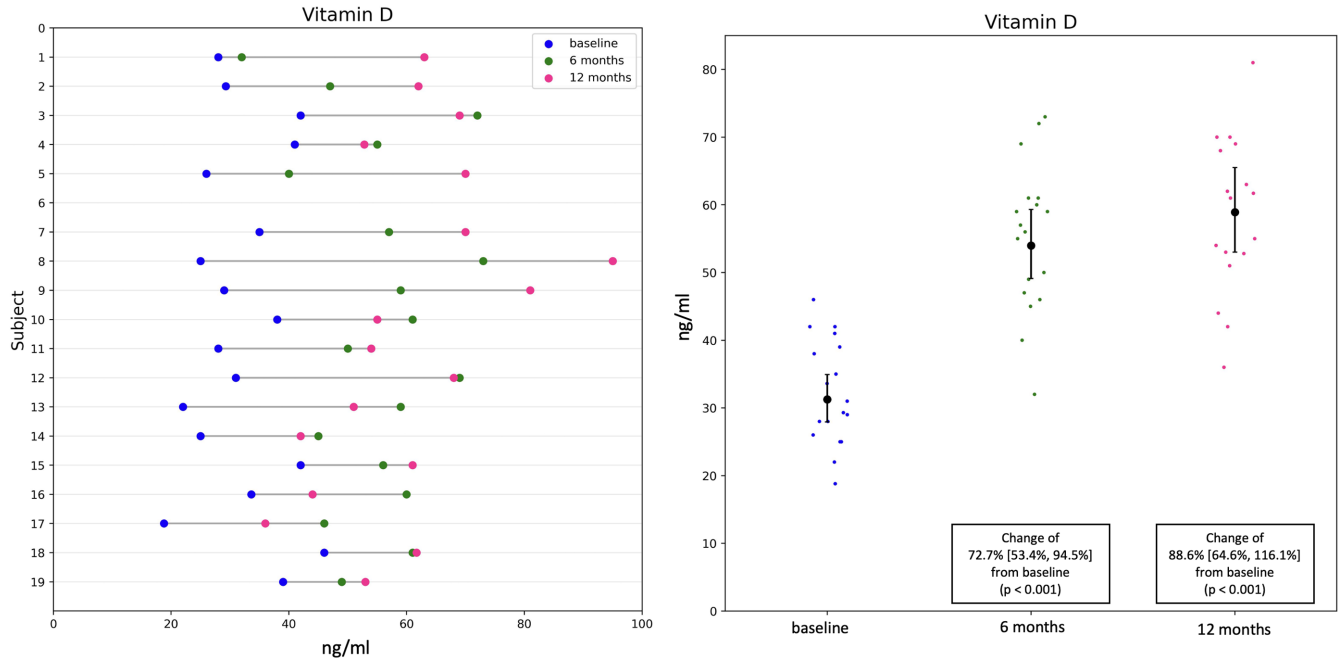

FIGURE 3 Vitamin D level of each patient at months 0, 6, and 12. The increase in vitamin D level varies in each subject. Overall, vitamin D level increased by 72.7% ($p < .001$) after 6 months and 88.6% ($p < .001$) after 12 months. The largest cerebral blood flow (CBF) increase occurred in subject 7 (280% after 12 months).

TABLE 4 Vitamin D level (ng/mL) in each patient.

	Month 0	Month 3	Month 6	Month 9	Month 12
Patient 1	28	23	32	55	63
Patient 2	26.6	29.3	47	50.7	62
Patient 3	42	70	72	73	69
Patient 4	41	54.4	55	107	48
Patient 5	26	49	40	74	70
Patient 6	35	56	57	72	70
Patient 7	25	74	73	74	95
Patient 8	29	59.2	59	78.7	81
Patient 9	38	64	61	87.3	55
Patient 10	28	66	50	50	54
Patient 11	31	64	69	76	68
Patient 12	22	60.6	59	NA	51
Patient 13	25	38	45	NA	42
Patient 14	42	60	56	53	61
Patient 15	33.6	45.3	60	42.5	44
Patient 16	18.8	37.1	46	33.9	36

Note: The measurement in Patients 12 and 13 was unavailable in month 9.

min) in neonates and increases rapidly in the first 24 months of life to as high as 80 mL/100 g/min and continues to rise steadily until peaking at 120 mL/100 g/min between 5 and 10 years. Afterward, the CBF levels decrease continuously during adolescence until leveling off between 30 and 70 mL/100 g/min at 30 years (Chiron et al., 1992; Hales et al., 2014; Wintermark et al., 2004). Only one participant was prescribed a new medication that might be expected to alter CBF. After the 6-month study visit, participant 13 was started on daily hydrocortisone for new onset adrenal insufficiency; his CBF in both whole brain and splenium was decreased at 12 months compared with the 6-month visit. The lack of a control group in this study made it difficult to separate the CBF variation due to vitamin D effect from the natural development of the brain. In essence, ASL allows non-invasive and quantitative CBF measurements in absolute units (mL/100 g/min), enabling the direct assessment of CBF and vascular hemodynamics in longitudinal studies without the need for radiation and contrast agents. Although the whole brain and splenium CBF changes were insignificant, our experimental design may be applied to similar studies investigating the impact of medical and/or surgical treatments on CBF.

From the CBF results, we can conclude that although the current ASL technique achieved quantitative CBF measurement to facilitate group comparison for boys and men with ALD, its accuracy can be improved by optimizing the scanning parameters for this cohort and a reference CBF of an age-matched group without receiving vitamin D would benefit the interpretation of results.

4.2 | Relationship between vitamin D and cerebral blood flow levels

In this study, we hypothesized that taking vitamin D supplements increased CBF for ALD patients based on the previous observation that exposure to vitamin D, which resembles the structure of steroids, generally improves CBF (Lang et al., 2015). The results

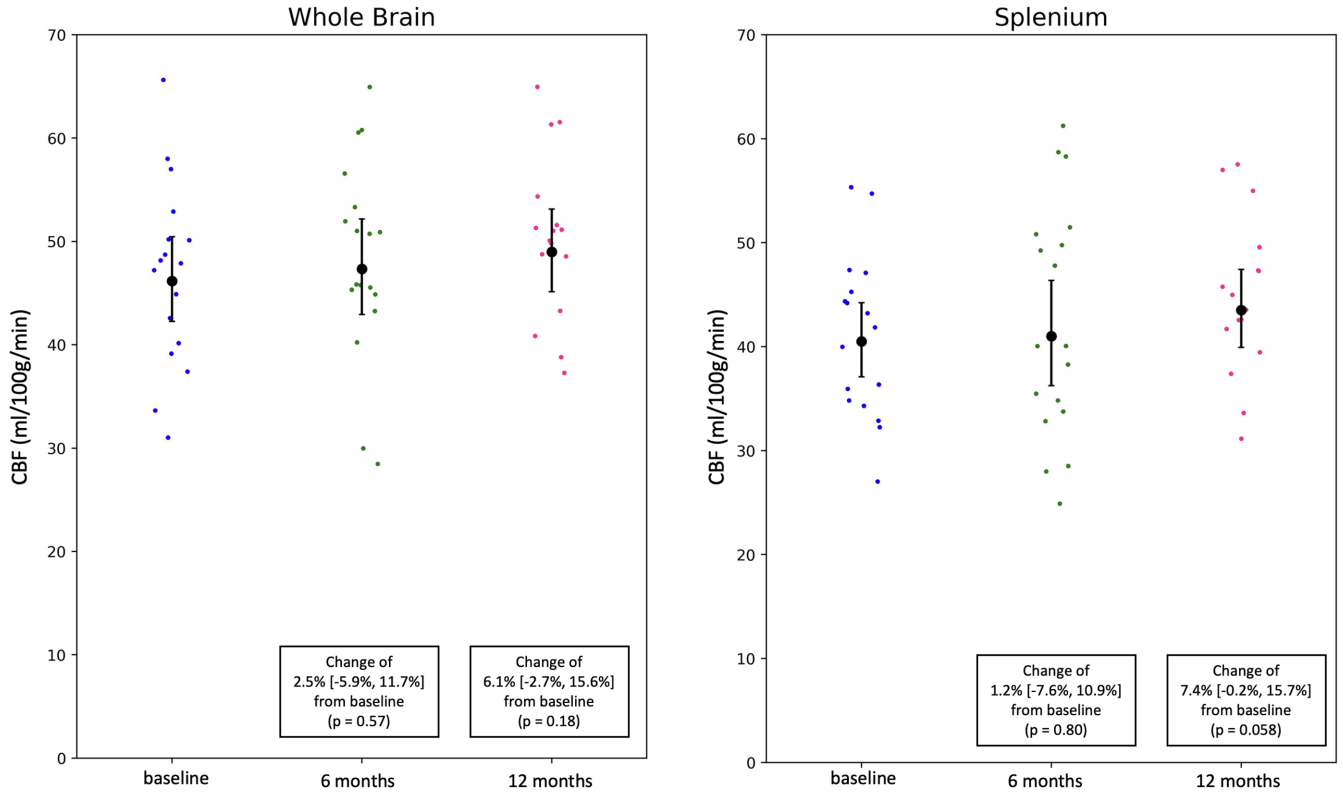

FIGURE 4 Mean cerebral blood flow (CBF) of the whole brain and the splenium. For all 3 sessions, the mean CBF of the whole brain was significantly higher than in splenium. However, no significant CBF changes were observed between the different scan sessions. Each dot represents the mean CBF of a patient at a given time point. GEE was used to compare the CBF values between different time points followed by Bonferroni correction. The *p* values for comparing CBF of whole brain between months 0 and 6 and between months 0 and 12 are .57 and .18, respectively. The p values for comparing CBF of the splenium between months 0 and 6 and between months 0 and 12 are .80 and .058, respectively.

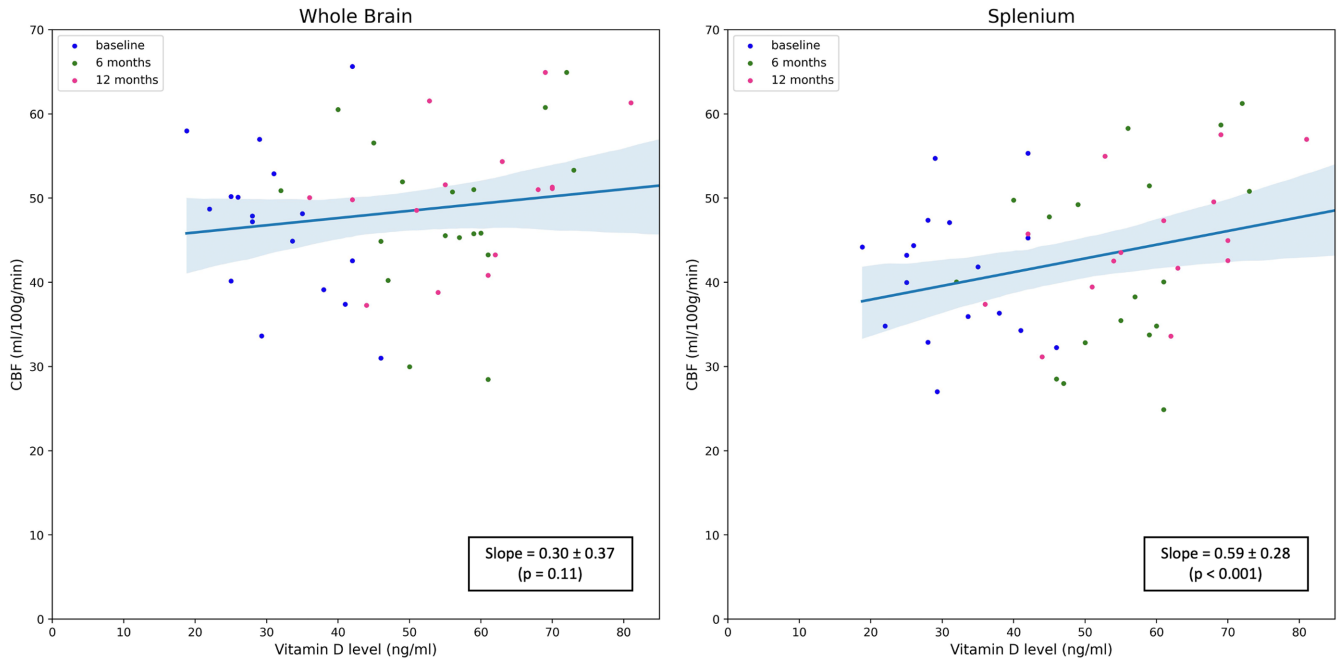

FIGURE 5 Correlation between cerebral blood flow (CBF) and vitamin D levels in the whole brain and splenium. The shaded area represents the 95% confidence interval. The statistical test results of the regression fit for the whole brain are: slope = .30, $p = .11$; the statistical results of the regression fit for splenium are slope = .59, $p < .001$.

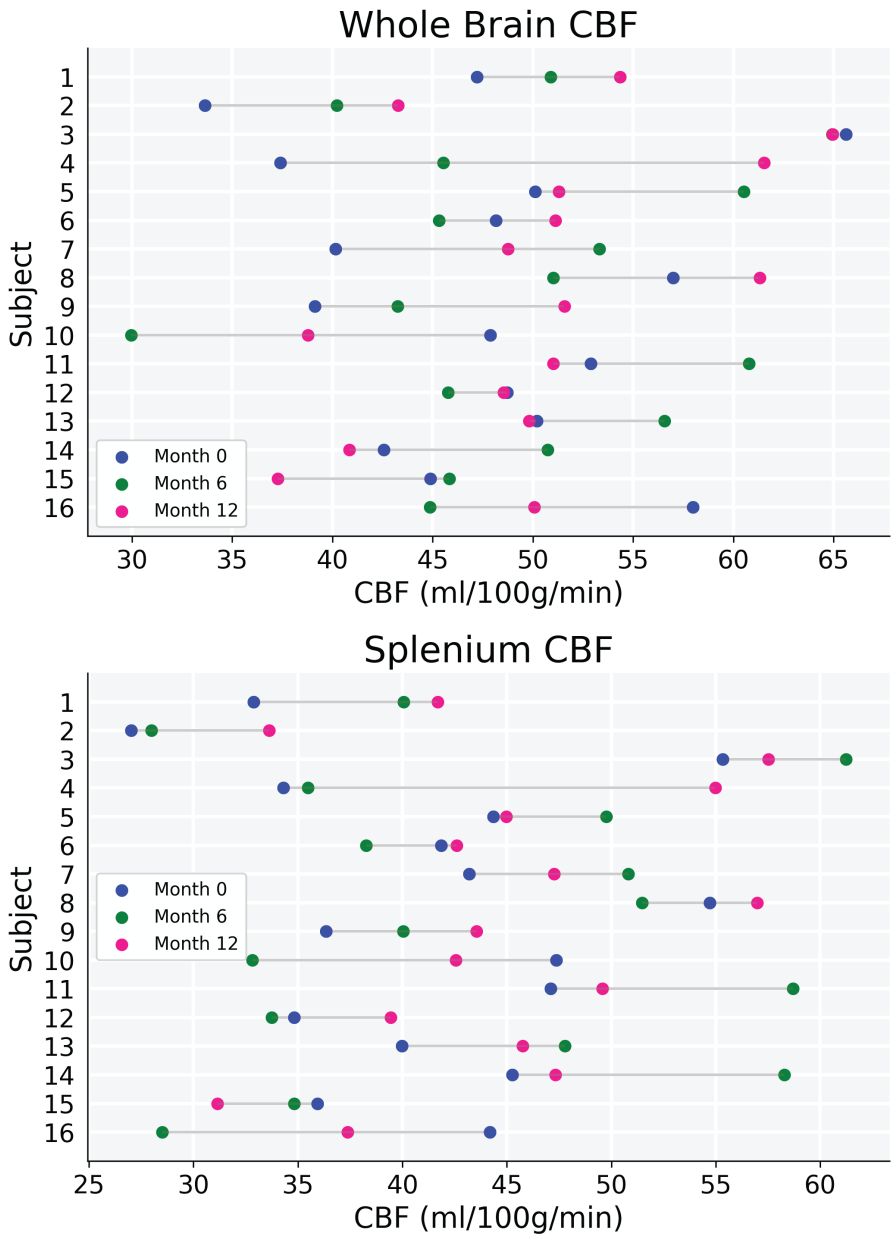

FIGURE 6 Whole brain and splenium CBF changes in each patient at months 0, 6, and 12.

showed that the increase in vitamin D level of each patient enhanced the correlation with the CBF of the whole brain and splenium after 12 months. It should be noted that the volume of the splenium region of interest (ROI) used here is 12,344 mm (Mahmood et al., 2007), which is significantly larger than the resolution of the MNI-2 mm standard space template (2 × 2 × 2 mm (Mahmood et al., 2007), or 8 mm (Mahmood et al., 2007)). In total, the splenium ROI covered 1543 voxels, which is large enough for our CBF analysis. Although our study did not include a placebo arm, we found a significant positive correlation between vitamin D levels and splenial CBF (Figure 5). Our inability to achieve a significant increase in splenial CBF between baseline and month 12 ($p = .058$; Figure 4) may be due to the small sample size.

Although there is sparse literature focusing on the effect of vitamin D on CBF. One study reported by Muller et al. demonstrated that taking vitamin D enhanced blood perfusion in adult patients with MS (Müller et al., 2019). One study showed that vitamin D deficiency was associated with a higher risk for vascular dementia and small vessel disease in elderlies (Prabhakar et al., 2015). However, the direct relationship between low vitamin D levels and the risk for neurodegeneration was not reported. Thus, a systematic study on the impact of vitamin D on CBF in patients of different age groups is needed to fill the knowledge gap.

4.3 | Limitations and future considerations

There are several limitations to this work. One is the lack of a control arm that could potentially provide the reference CBF and vitamin D levels for different age groups and include patients without

receiving vitamin D, evaluating the CBF variation of this cohort was difficult without reference values of an age-match cohort. Since ALD is a rare disease with a low prevalence (1 in 20,000 worldwide according to Raymond, et al.) (Raymond et al., 1993), our sample size was relatively small (16 patients) in this study, which limited the statistical power when analyzing the relationship between vitamin D levels and CBF as well as the investigation of the impact of ALD on CBF in different age groups. Another limitation was the reliance on a single type of ASL method. Although this technique has been recommended for clinical applications for studies of adult brains, it was not optimized for assessing vascular hemodynamics in pediatric applications. This might be partially addressed by including other ASL techniques, such as pulsed ASL with multiple PLDs, although further work using different ASL techniques on pediatric patients would be valuable to address this challenge.

5 | CONCLUSIONS

In this exploratory analysis of our existing clinical trial data, we used ASL MRI to investigate the impact of vitamin D supplementation on CBF of boys with ALD. We found a correlation between CBF and vitamin D levels. The CBF measurements of the cohort were consistent with the range published in previous studies applying different ASL techniques on subjects of the same age group. We observed a slight increase in CBF in the whole brain and splenium, although the results did not achieve pre-defined statistical thresholds. We conclude that although ASL shows viability in quantitative CBF measurements and a potential correlation between plasma vitamin D status and CBF in this pilot study, further work on parameter optimization and study template may be needed to achieve the full potential of ASL in assessing the CBF in response to vitamin D supplementation.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

AUTHOR CONTRIBUTIONS

Moss Y. Zhao: methodology, formal analysis, investigation, writing and funding acquisition. **Alex Dahlen**: methodology and formal analysis. **Norma Jimenez Ramirez**: data curation. **Michael Moseley**: supervision and funding acquisition. **Keith Van Haren**: conceptualization, supervision, and funding acquisition. **Greg Zaharchuk**: conceptualization, supervision, and funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr. Greg Zaharchuk received funding support through GE Healthcare and Bayer Healthcare and equity from Subtle Medical.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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