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Quantitative Analysis of Blood to CSF Exchange over 24 Hours Following Intravenous Contrast MRI

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ABSTRACT

Background: Impairment of the glymphatic system and brain solute clearance is associated with aging, traumatic brain injury, and neurodegenerative pathology, motivating efforts to quantify glymphatic transport in humans. Intrathecal contrast-enhanced MRI directly probes CSF–ISF exchange but has limited research applicability. Intravenous gadolinium-based contrast agents (GBCAs) are known to enter intracranial CSF, suggesting a complementary, less invasive strategy to probe brain fluid transport.

Methods: Hourly MRI data was acquired over a 24-hour period following IV GBCA administration in seven healthy adults. Quantitative T1 mapping was analyzed using linear mixed-effects models (LMMs) to test the effect of time across vascular, CSF, and brain compartments. A heavily T2-weighted inversion recovery sequence was included to improve identification of low concentration of GBCA in fluid compartments. Finally, pharmacokinetic compartment models were used to estimate transport between blood, CSF, and brain interstitial compartments.

Results: Quantitative MRI following intravenous GBCA administration indicates significant transport between the blood and subarachnoid space (SAS) ($p < 0.001$), with evidence for the involvement of the cerebral veins on hT2w-IR. In contrast, transport between the blood and ventricular CSF is comparatively limited ($p = 0.923$). Kinetic modeling further suggests that the SAS is not a single well-mixed compartment but best represented by both mobile regions and “sluggish” compartments.

Conclusions: Finally, exchange of GBCA into the brain interstitial space was not reliably detected, indicating that IV GBCA–based MRI is unlikely to provide a sensitive or specific

23 measure of glymphatic CSF–ISF exchange. Together, these findings help refine the role of IV
24 contrast MRI studies for quantifying solute transport in the human CNS and highlight important
25 constraints on its use for assessing glymphatic function in humans.

26 **Keywords:** brain clearance, MRI, Blood-CSF-barrier, IV Contrast

LIST OF ABBREVIATIONS

Aβ	– amyloid beta
ANTS	– Advanced Normalization Tools
BB	– black-blood
BCSFB	– blood CSF barrier
BDF	– backward differentiation formula
CBV	– cortical bridging vein
ChP	– choroid plexus
CNR	– contrast-to-noise ratio
CSF	– cerebrospinal fluid
GBCA	– gadolinium-based contrast agent
GM	– gray matter
hT2W-IR	– heavily T2-weighted inversion recovery
ICP-MS	– inductively coupled plasma–mass spectrometry
ISF	– interstitial fluid
IT	– intrathecal
IV	– intravenous
LV	– lateral ventricles
LMM	– linear mixed-effects model
ODE	– ordinary differential equation
PD	– proton density
PS	– permeability–surface area product
ROI	– region of interest
SAS	– subarachnoid space
SS	– superior sagittal sinus
SyMRI	– Synthetic Magnetic Resonance Imaging
T1	– longitudinal relaxation time
T1W	– T1-weighted
T2	– transverse relaxation time
T2W	– T2-weighted
TR / TE / TI	– repetition time / echo time / inversion time

WM – white matter

1 BACKGROUND

2 The glymphatic system is a brain-wide pathway for cerebrospinal fluid (CSF)–to–interstitial
3 fluid (ISF) exchange that supports the transport and clearance of solutes, including amyloid b
4 ($A\beta$) and tau, which are implicated in neurodegenerative conditions such as Alzheimer’s disease
5 (1–4). Glymphatic function is impaired under conditions that are risk factors for
6 neurodegenerative disease, including aging (5), traumatic brain injury (6,7) and chronic sleep
7 disruption (8), while impairment of glymphatic function is sufficient to promote the development
8 of $A\beta$ (9,10) and tau pathology (7,11–13). Based on these findings, impairment of glymphatic
9 function is proposed as a key driver of neurodegenerative disease pathogenesis (14,15),
10 motivating ongoing studies to measure glymphatic function in the human brain and under
11 different clinical conditions. Intrathecal (IT) contrast-enhanced MRI has emerged as a direct
12 approach for defining CSF–ISF exchange in the human brain (16–18) but this approach requires
13 lumbar puncture and the off-label use of gadolinium-based contrast agents (GBCAs), creating
14 barriers to widespread implementation of this method.

15 Evidence that intravenously (IV) administered GBCAs enter intracranial CSF is well
16 established. Both inductively coupled plasma–mass spectrometry (ICP-MS) analyses of CSF
17 (19,20) and MRI studies (21,22) have demonstrated measurable GBCA within CSF
18 compartments and identified potential sites of exchange including the subarachnoid space (SAS)
19 (23–25), ventricles (25,26), surrounding the optic nerves (27,28), and near cortical veins (29,30).
20 These findings support the view that the brain, blood, CSF, ISF, and cranial lymphatic pathways
21 form an interconnected, and potentially bidirectional, transport network (31). Intrathecal
22 approaches primarily assess solute efflux out of the CSF – whether into the brain interstitium, to
23 the descending cervical lymphatics, or to the blood - whereas quantifying contrast influx into the

CSF is confounded by preexisting contrast. Intravenous contrast administration, however, offers a complementary means to probe brain fluid and solute transport between the blood, CSF, and other compartments.

While the noted prior studies have utilized IV GBCA MRI to evaluate cranial solute transport, many relied on qualitative imaging methods, underscoring the need for quantitative approaches to more accurately characterize transport kinetics. Furthermore, limited temporal sampling often only included a few early or delayed post-contrast timepoints. Finally, because GBCA is simultaneously present in both vascular and CSF compartments, the choice of a single MRI modality strongly influences the relative contribution of each compartment to the measured signal and constrains our ability to distinguish between them.

To address these gaps, we acquired hourly MRI data over a full 24-hour post-contrast period with quantitative T1 mapping. Regular, extended sampling ensures that both early and delayed transport processes are captured. This quantitative approach overcomes the challenges associated with qualitative assessments including changes in scanner stability over time and the biphasic behavior of T1-weighted signal at low concentrations (32). The data were fitted pharmacokinetic compartment models to better understand potential transport pathways between blood, CSF, and the brain interstitial compartments, and to quantify transport rates between them. In the final two participants, a heavily T2W inversion recovery sequence was added to isolate fluid signals, in brain regions that were otherwise difficult to identify on T1 mapping.

METHODS

Participants

Seven healthy adults (female = 2, age range = 23-57 years) participated in this study. The study was approved by the IRB at the University of Washington and adhered to principles outlined in Declaration of Helsinki. Exclusion criteria included contraindications for MRI or GBCA administration; estimated glomerular filtration rate < 30 ml/min/1.73m²; diagnosis of cancer, neurological disorders or psychiatric illnesses; or presence of a known sleep disorder.

MRI Acquisition

All participants were scanned on a 3T MRI system (Ingenia Elition X, Philips Healthcare). After four participants, the system was upgraded to an Ingenia MR7700; internal testing indicated no impact on image acquisition or derived measures. Each participant underwent up to 24 MRI scans: one baseline scan, one scan 20 minutes after IV GBCA administration (gadobutrol, 0.1 mmol/kg; Gadovist, Bayer Pharmaceuticals), and hourly scans from 1 to 22 hours post-contrast. Baseline imaging was completed between 7:00 and 9:00 a.m.

At each post-contrast time point, at least one MRI sequence was acquired along with a corresponding baseline image. The MRI protocol was modified for the final two participants based on observations from earlier sessions indicating detectable GBCA in CSF, with the goal of confirming CSF enhancement while minimizing vascular signal contribution. Participants exited the scanner between scans and were not instructed to remain awake; however, opportunities for sleep were limited by scan frequency. One participant did not complete all 24 time points due to technical issues with the scanner (**Table 1**).

Table 1. Demographics of Participants

Participant	Age	Sex	Blood Collection	MRI Sequences			
				3D QALAS	MPRAGE	hT2W-IR	BB
Cohort 1 (n = 5)	23-44	2 F, 3 M	5	5 ^a	1	0	0
Cohort 2 (n = 2)	50-57	2 M	2	2 ^b	2	2	1

^a One participant only completed up to 13 hour-post GBCA due to scanner technical issues

^b Cohort 2 completed all sequences at baseline, but 3D QALAS/MPRAGE were collected at even hours and hT2W-IR and BB at odd hours

Imaging Sequences

Quantitative T1 mapping was acquired using 3D-QALAS (33), an interleaved Look–Locker sequence comprising five equally spaced segmented fast gradient echo acquisitions preceded by T2 preparation and inversion recovery modules. Whole-brain quantitative T1, T2, and proton density maps were generated using standalone software (SyntheticMR v0.61.0). Acquisition parameters were TR/TE = 5.1/2.2 ms; FOV = 288 × 288 × 175 mm³; resolution = 0.86 × 0.86 × 1.0 mm³; echo train length = 150; flip angle = 4°; compressed SENSE factor = 2; scan time = 6:30 min.

T1-weighted (T1W) images were acquired using a 3D fast gradient echo sequence (TR/TE = 12/4.5 ms; TI = 1000 ms; FOV = 256 × 240 × 166 mm³; isotropic resolution = 0.8 mm³; CS factor = 3.5; scan time = 3:28 min). Black-blood (BB) imaging was performed using 3D BrainVIEW Black Blood, a T1-weighted turbo spin echo sequence (TR/TE = 700/35 ms; FOV = 200 × 250 × 160 mm³; acquisition resolution = 0.75 mm³ isotropic; reconstructed resolution = 0.49 × 0.49 × 0.375 mm³; compressed SENSE factor = 6; scan time = 3:34 min).

Both T1 mapping and MPRAGE contrasts reflect signal contributions from CSF, tissue, and blood compartments. To enhance sensitivity to CSF, a heavily T2-weighted inversion recovery

(hT2w-IR) 3D turbo spin echo sequence (21,34) was used, which is primarily sensitive to protons with long T2 relaxation times. The inversion recovery pulse inverts the CSF signal; gadolinium-induced T1 shortening shifts the CSF null point, increasing sensitivity to low gadolinium concentrations within cranial fluid spaces. Acquisition parameters were TR/TE = 15000/580 ms; TI = 2700 ms; echo train length = 119; flip angle = 140°; FOV = 192 × 168 × 144 mm³; resolution = 0.5 × 0.5 × 1.0 mm³; CS factor = 5; scan time = 8:45 min. Real images preserving the polarity of longitudinal magnetization following inversion were analyzed to maximize dynamic signal range.

Blood Collection

GBCA was administered by nursing staff outside the scanner suite via an intravenous catheter placed in the medial cubital, cephalic, or basilic vein. Blood samples (1.5 mL) were collected from the same catheter at 1, 2, 4, and 10 hours post-injection, after which the catheter was removed. One participant did not complete the 10-hour blood collection. Whole blood samples were frozen at -40 °C within 10 minutes of collection and later analyzed in batches using inductively coupled plasma–mass spectrometry (ICP-MS).

Phantom

A fluid phantom was constructed using 50 mL conical tubes filled with deionized water and serial dilutions of gadobutrol, yielding concentrations ranging from 0 to 1 mM. Identical 3D-QALAS and hT2w-IR acquisitions were performed at room temperature. The phantom experiments were conducted to assess the sensitivity of each sequence to low GBCA concentrations.

Image Analysis

Quantitative T1, T2 and PD maps were generated from 3D-QALAS acquisitions using SyntheticMR software (v0.61.0). Synthetic T1W and T2W images and gray matter (GM), white matter (WM), and CSF probability masks were also reconstructed for image segmentation and registration. Baseline synthetic T1W (SyT1W) images were segmented using FreeSurfer recon-all (v.8). Resulting segmentations were transformed back to each participant's native space and individual ROIs were extracted, including cortical GM, cerebral WM, superior sagittal sinus (SS), and lateral ventricles (LV).

GM, WM, and CSF probability maps from SyMRI were thresholded at 95% and used to refine the FreeSurfer-derived ROIs. Choroid plexus (ChP) masks were manual segmented; to exclude ChP borders from the LV, ChP masks were dilated and subtracted from LV ROIs. A cortical subarachnoid space (SAS) ROI was created by subtracting the ventricular, cisternal and cerebellar CSF from the thresholded CSF mask. Based on recently described openings in the arachnoid barrier surrounding cortical bridging veins (30), manual cortical bridging vein (CBV) ROIs were drawn on baseline SyT2W images for each participant. To define CSF and GM regions adjacent to CBVs, CBV masks were mean-dilated and intersected with the corresponding tissue probability maps. ROIs for contrast-to-noise ratio (CNR) measurements on hT2w-IR post-contrast images were manually delineated in regions demonstrating enhancement.

All images were registered using linear registration with dense sampling in ANTsRegistration (Advanced Normalization Tools, v2.1). Post-contrast images were registered to their corresponding baseline images. Baseline images from each sequence were then registered to the corresponding T1 maps, and the resulting transforms were applied to all remaining time points.

To visualize the T1 change after GBCA administration, the difference of log transformed T1 measurements was calculated as:

$$\Delta \log[T1] = \frac{T1_{pre} - T1_{post}}{T1_{pre}} \quad (1)$$

GBCA concentration was calculated using the following equation

$$[c] \cdot r1 = \frac{1}{T1_{pre}} - \frac{1}{T1_{post}} \quad (2)$$

where $r1 = 5 \text{ [L mmol}^{-1}\text{s}^{-1}\text{]}$, the relativity of Gadovist in plasma (35).

For each participant, voxel-wise values within each ROI were averaged, and 95% confidence intervals were estimated using 5,000 bootstrap resamples. For non-quantitative hT2w-IR images, contrast-to-noise ratio (CNR) was calculated as:

$$CNR = \frac{Signal_{ROI} - Signal_{corpus\ collosum}}{\sigma_{corpus\ collosum}} \quad (3)$$

The corpus callosum was used as a reference region because, at TE = 580 ms, its signal is expected to be largely attenuated.

All statistical analyses were performed in R (version 4.4.1). To symmetrically measure change over time, T1 measurements were log-transformed prior to analysis. Longitudinal changes in T1 were assessed for each ROI using linear mixed-effects models (LMMs) with time modeled as a categorical fixed effect and participant included as a random intercept to account for repeated measures (lme4 package). The significance of the fixed effect of time was evaluated using Type III analysis of variance with Kenward–Roger–adjusted degrees of freedom (lmerTest package). For ROIs showing a significant main effect of time, post hoc comparisons were performed using Dunnett-adjusted tests comparing each post-contrast time point to baseline,

based on estimated marginal means (emmeans package). Statistical significance was defined as adjusted $p < 0.05$.

Compartment Modeling

Compartmental models were developed to describe contrast movement between intracranial ROIs, informed by prior literature, and contrast MRI data. Models were fitted to MRI-derived concentrations to estimate transport parameters between compartments. The following ROIs were included: sagittal sinus (blood), lateral ventricles, cortical subarachnoid space (SAS), and combined gray and white matter.

Each compartment was assumed to be well mixed, with transport between adjacent compartments described by idealized mechanisms:

1. *Transport without a barrier.* For compartments connected without a physical barrier (e.g., CSF flow from ventricles to SAS), the molar rate of contrast movement is given by $Q \cdot C$, where Q is the volumetric flow rate and C = contrast concentration.
2. *Transport across a barrier with bulk flow.* For bulk flow across a permeable membrane where contrast may be selectively rejected, the molar rate is $K \cdot Q \cdot C$, where K is the partition coefficient representing fraction of contrast agent passing through the membrane.
3. *Transport across a barrier without bulk flow.* For movement driven by a concentration difference (e.g., blood to SAS across a bridging vein vascular wall), transport is described by the permeability–surface area (PS) product with molar rate $PS \cdot \Delta C$. Here, $PS = k \cdot SA$, where the k is the mass transfer coefficient and SA the surface area of the exchange barrier. PS is useful when the exact surface area is unknown.

A mass balance is performed on each compartment according to:

$$\Delta \frac{\text{moles}}{\text{time}} = \sum \text{molar rate in} - \sum \text{molar rate out} \quad (4)$$

For example, the concentration dynamics within a generic compartment C , which 1) receives contrast from compartment A via bulk flow without a barrier, 2) receives contrast from compartment B across a membrane without bulk flow, and 3) loses to compartment D across a membrane with bulk flow are described by:

$$\Delta \frac{\text{moles}}{\text{time}} = V_B \cdot \frac{dC_B}{dt} = Q_{AB} \cdot C_A + K_{CB} \cdot Q_{CB} \cdot C_C + PS_{DB} \cdot (C_D - C_B) \quad (5)$$

where V is the compartment volume, C is the contrast concentration, Q is the volumetric flow rate, and subscripts denote the compartments.

A system of ordinary differential equations (ODEs) was derived from such mass balances for all compartments, based on their interconnections. The system of equations contains “fixed” parameters from the literature and “fitted” parameters estimated by fitting the model to the CE-MRI data. Fixed parameter values and references are provided in **Table S1**. Notably, reported CSF volumes and flow rates vary, corresponding to cranial CSF residence times of 6–10 hours.

The system of ODEs was solved using the LSODA algorithm, which automatically switches between the Adams-Bashforth-Moulton method and Backward Differentiation Formula (BDF) methods, as implemented in Python (v3.13.5, SciPy v1.15.3). Transport parameters were estimated using least-squares nonlinear regression in combination with the ODE solver. Sensitivity of the fitted parameters to variations in CSF residence time was assessed with a Monte Carlo analysis (Supplemental Data).

RESULTS

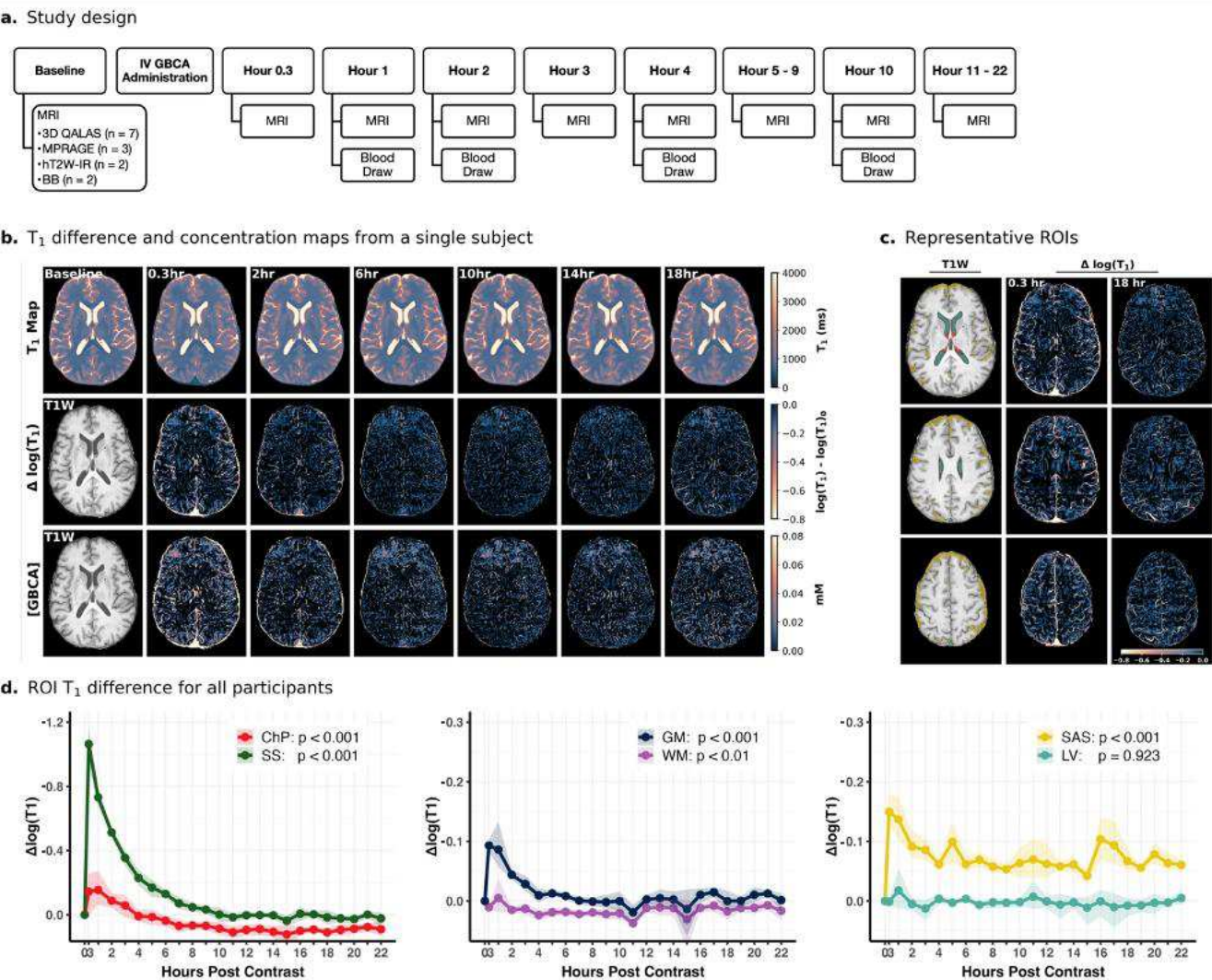
Phantom Validation of T_1 mapping

The sensitivity of the 3D QALAS sequence to low concentrations of gadobutrol was evaluated using a custom phantom with expected T_1 values between ~280 and 3000 ms. Measured T_1 values decreased with increasing gadobutrol concentration (**Supplementary Figure 1a and b**). The relaxation rate ($R_1=1/\Delta T_1$) was strongly correlated with gadobutrol concentration ($R^2 = 0.968$) and the measured relaxivity was $r_1 = 3.45 \text{ mM}^{-1}\text{s}^{-1}$ (**Supplementary Figure 1c**). Variability increased at long T_1 (>2500 ms), as reflected by the standard deviations.

GBCA Distribution in CSF Compartments Detected with Quantitative T_1 Mapping

Quantitative T_1 maps (ms), log-transformed difference ($\Delta \log[T_1]$), gadolinium concentration image, and ROI-based measurements are shown in **Figure 1b-d**. Significant main effects of time (**Table 2**) were observed in the superior sagittal sinus (SS) ($p < 0.001$), choroid plexus (ChP; $p < 0.001$), cortical gray matter (GM; $p < 0.001$), and cerebral white matter (WM; $p = 0.001$). While time was significant in the cerebral WM, the estimated mean differences were positive indicating increases rather than GBCA-related T_1 decreases. A significant effect of time was also observed in the cortical subarachnoid space (SAS; $p < 0.001$), whereas no effect was found in lateral ventricles (LV; $p = 0.92$). Full quantitative values, including estimated GBCA concentrations, are provided in **Tables S4 and S5**.

209 **Figure 1** Quantitative T_1 mapping reveals GBCA distribution following IV GBCA.



210

211 **Fig 1. a.** Diagram of study design. **b.** T_1 maps from an example subject (Cohort 2) at selected post-contrast
212 timepoints. Second row shows log-transformed T_1 differences from baseline ($\Delta \log(T_1)$); negative values
213 indicate reduced T_1 and the color scale in inverted to compare the corresponding increased gadolinium
214 concentration [GBCA], shown in the third row. **c.** Representative ROIs include the cortical subarachnoid space
215 (yellow), lateral ventricle (teal), choroid plexus (red), and superior sagittal sinus (green). **d.** ROI mean values
216 and bootstrapped 95% confidence intervals for all participants ($n = 7$) with the p-values displayed for main
217 effects of time (linear mixed models).

Table 2: Linear mixed-effects model results for longitudinal T1 changes across ROIs.

ROI	F value	df	p-value
Cerebral WM (WM)	2.44	23, 105.03	<0.01
Cortical GM (GM)	13.90	23, 105.16	<0.001
Lateral Ventricle (LV)	0.60	23, 105.18	0.923
Cortical Subarachnoid Space (SAS)	7.68	23, 105.26	<0.001
Superior Sagittal Sinus (SS)	126.74	23, 105.18	<0.001
Choroid Plexus (ChP)	9.86	23, 105.13	<0.001
Corpus Callosum (CC)	1.54	23, 105.08	0.075
Basal Ganglia (BG)	3.47	23, 105.04	<0.001
Frontal GM	12.97	23, 105.13	<0.001
Occipital GM	21.83	23, 105.26	<0.001
Parietal GM	10.98	23, 105.34	<0.001
Insula GM	7.298	23, 105.12	<0.001
Temporal GM	8.99	23, 105.15	<0.001
3rd Ventricle (3V)	1.23	23, 105.08	0.241
4th Ventricle (4V)	1.08	23, 105.68	0.374
Cortical Bridging Veins (CV)	60.97	23, 105.04	<0.001
CSF around CV	4.98	23, 105.32	<0.001
GM around CV	15.77	23, 105.36	<0.001
Optic Nerve	5.86	23, 105.14	<0.001
Trigeminal Nerve Cave	3.20	23, 105.10	<0.001

Post-hoc Dunnett-adjusted comparisons against baseline revealed compartment-specific temporal patterns (**Table S3**). In the SS, T1 reductions were immediate (0.3 h to 6 h post-contrast; all $p < 0.01$), consistent with high intravascular gadolinium concentration followed by clearance. This was mirrored by blood ICP-MS measurements (**Table 3**).

Table 3. Blood Measurements of GBCA

1hr	2hr	4hr	10hr ^a
m ± sd (mM)			
0.070 ± 0.045	0.025 ± 0.003	0.012 ± 0.004	0.001 ± 0.001

^a One participant did not complete the 10 hour collection

Early T1 reductions were also seen in cortical GM (0.3–2 h; all $p < 0.001$) and ChP (0.3–1 h; both $p < 0.001$), each of which are composed of a substantial fraction of microvasculature. Later ChP time points (11–18 h; all $p < 0.05$) also showed positive deviations, however, these are inconsistent with GBCA-induced T1 shortening. In contrast, SAS exhibited sustained T1 reductions from 0.3 h through 22 h (all $p < 0.05$ except 15hr), with the largest early effects and secondary reductions throughout the 22-hour period, indicating prolonged GBCA presence. Timing of late SAS enhancement varied between participants.

Secondary ROIs were analyzed to investigate regional effects. Cortical GM in the insula, frontal, parietal, and temporal lobes followed the total GM longitudinal profile. The occipital lobe exhibited the largest early change, likely due to partial volume contributions of the posterior sagittal and transverse sinuses (**Figure S2a**). The third and fourth ventricular ROIs (3V and 4V) were not significant at any time points; 3V displayed the highest variability (**Figure S2b**).

241 *Qualitative T1W images capture similar SAS enhancement*

242 Qualitative T1-weighted (MPRAGE) images from three participants were plotted at the same
243 timepoints as the quantitative T1 maps. Signal was normalized to the corpus callosum, which had
244 no significant effect of time in the T1 mapping data ($p = 0.07$). The $\Delta\log[\text{T1W signal}]$ closely
245 mirrored the spatial and temporal patterns observed in the quantitative maps (**Figure S3**).
246 Statistical analyses were not performed due to the limited sample size ($n = 3$).

247 *Heavily T2W Inversion Recovery Isolates GBCA in Cranial Fluid Compartments*

248 Phantom measurements confirmed that hT2W-IR can detect low GBCA concentrations in fluid
249 (**Figure 2b**). In vivo, regions of hyperintensity identified GBCA in CSF compartments,
250 highlighting potential sites of blood-to-CSF GBCA exchange or GBCA accumulation, including
251 the anterior cortical bridging veins (CBV; teal), choroid plexus (ChP; red), optic nerves (csfON;
252 blue), and trigeminal nerve cave (csfTN; red) (**Figure 2a**). This non-quantitative sequence was
253 only acquired for two participants (cohort 2), with ROI CNR for each participant shown in
254 **Figures 2c -f**. Enhancement peaks occur within the first five hours in all ROIs.

255 **Figure 2** Contrast enhancement dynamics across CSF regions on real hT_2W -IR MRI

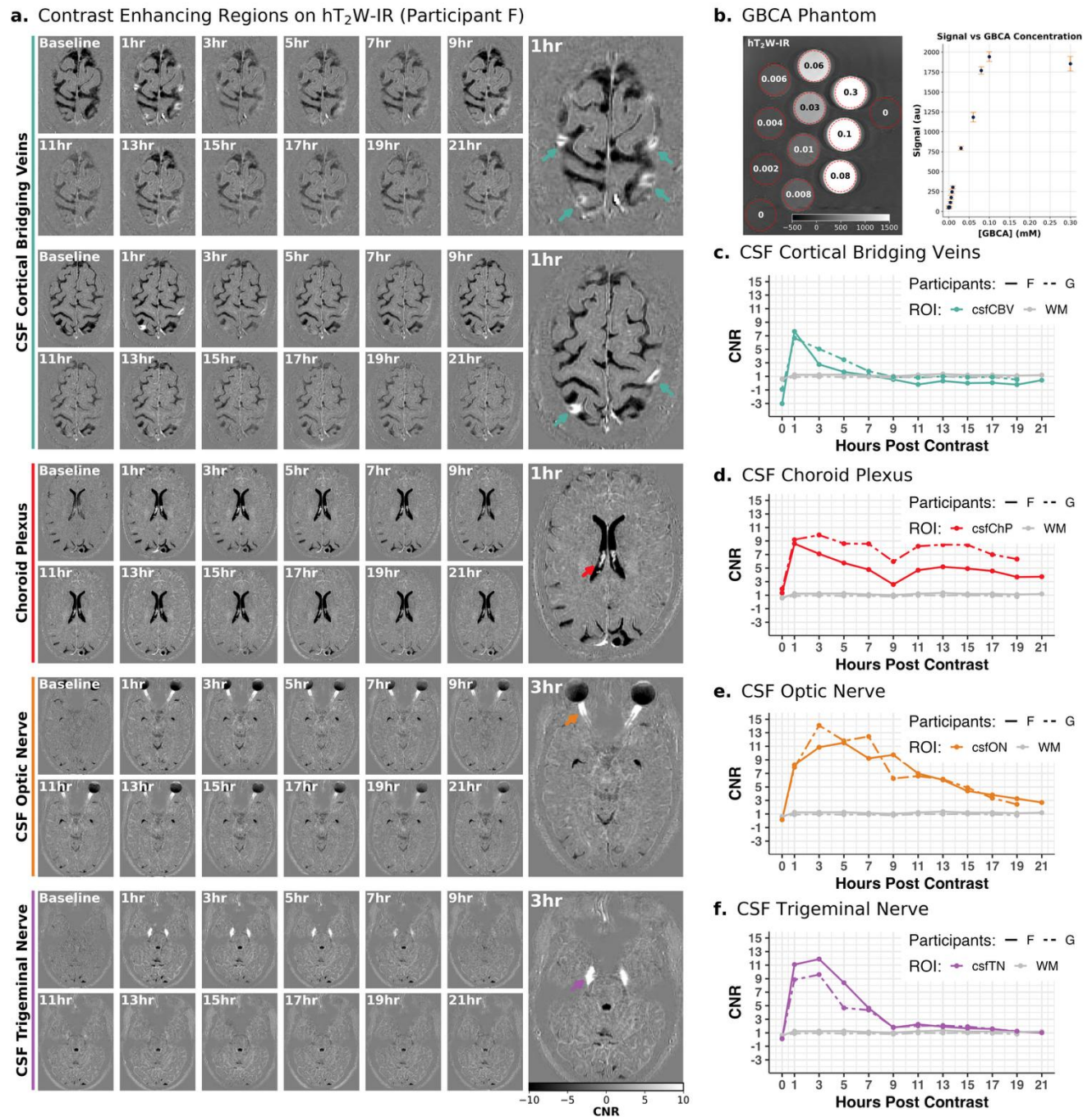


Fig 2. a. Representative axial hT_2W -IR real images showing visible enhancement at post-contrast timepoints in cortical bridging veins (teal), choroid plexus (red), optic nerve (orange), and trigeminal nerve (purple).. **b** hT_2W -IR phantom image with increasing concentrations (mM) of gadobutrol in deionized water and plot of the signal intensity for each tube. **c–f.** Time courses of contrast-to-noise ratio (CNR) in CSF ROIs for cortical bridging veins (c), choroid plexus (d), optic nerve (e), and trigeminal nerve (f). Plots show mean $\pm$ 95% confidence interval for both participants (solid = Participant F, dotted = Participant G).

Targeted T1 Map Analysis of hT2W-Identified Fluid Compartments

While hT2W clearly revealed blood-to-CSF GBCA exchange at cortical veins, long echo times prevented measurement in adjacent tissue. Regions with visible contrast enhancement with hT2W-IR were examined on quantitative T1 maps for all participants and representative ROIs are shown in **Figure 3a**, along with corresponding black blood MRI to clearly visualize the vasculature. A significant effect of time in the gray matter immediately surrounding cortical bridging veins (gmCBV; $p < 0.001$) was found, and like the total cortical GM, this was driven by the early timepoints (**Figure 3b**). Additionally, the CSF around CBVs closely matched the trends of the total SAS and significant timepoints persisted throughout the study period (**Figure 3b**).

Figure 3 Quantitative T1 map analysis of regions identified on hT2W images

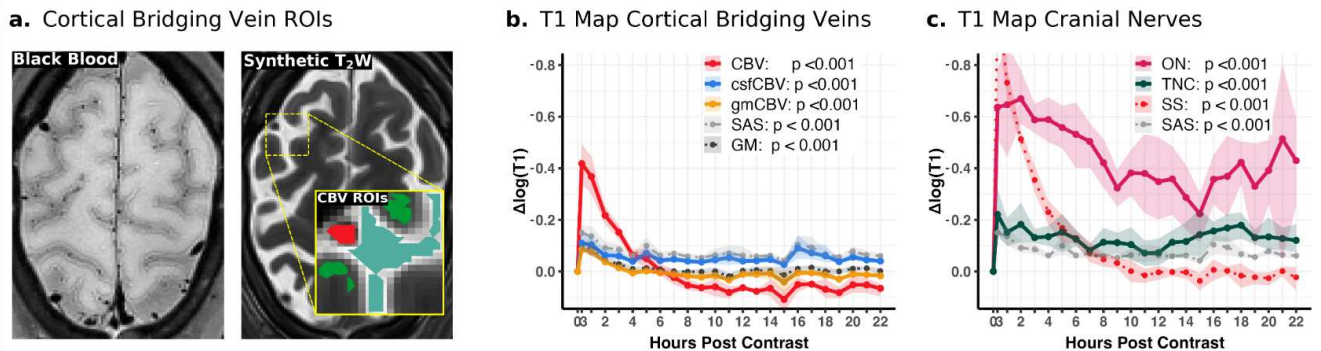


Fig 3. a. Representative image of the cortical bridging veins and manual ROIs. **b.** $\Delta\log(T1)$ in ROIs of the cortical bridging veins (CBV; red) and surrounding CSF and gray matter for all participants (csfCBV; blue and gmCBV; orange) plotted with the overall cortical gray matter (GM; gray) and subarachnoid space (SAS; light gray). $n=7$ **c.** $\Delta\log(T1)$ in the trigeminal nerve cave (TNC; green), and optic nerve (ON; pink). Both remained negative after the sagittal sinus (SS; red) returned to baseline (~ 10 hrs). $n=7$.

The fluid within the trigeminal nerve cave (TNC) was manually segmented on T1 maps (Figure 3C). At 0.3 hours, $\Delta\log[T1]$ in the TNC exceeded that in the SAS and remained elevated across subsequent timepoints. The largest changes were observed surrounding the optic nerve (ON). Due to the sequence parameters and resolution, this ROI contains contributions from both blood and fluid. Notably, at timepoints beyond 6 hours, where the SS has returned to baseline, the optic nerve remained significantly decreased compared to baseline for nearly all timepoints (Figure 3C), indicating the presence of GBCA. Results of hourly post-hoc tests are included in the Table S6.

Black Blood Imaging Contrast Enhancement in the Parasagittal Dura and Choroid Plexus

A black blood sequence was acquired at baseline in cohort 2 to localize cortical bridging veins, with additional post-contrast time points in one participant. Signal enhancement was observed in the parasagittal dura, and in the choroid plexus during early post-contrast (1–3 h), consistent with hT2W findings (Figure 4). In contrast, enhancement around cortical bridging veins appeared less pronounced.

Figure 4 Black Blood Sequence signal enhancement in the parasagittal dura and the choroid plexus

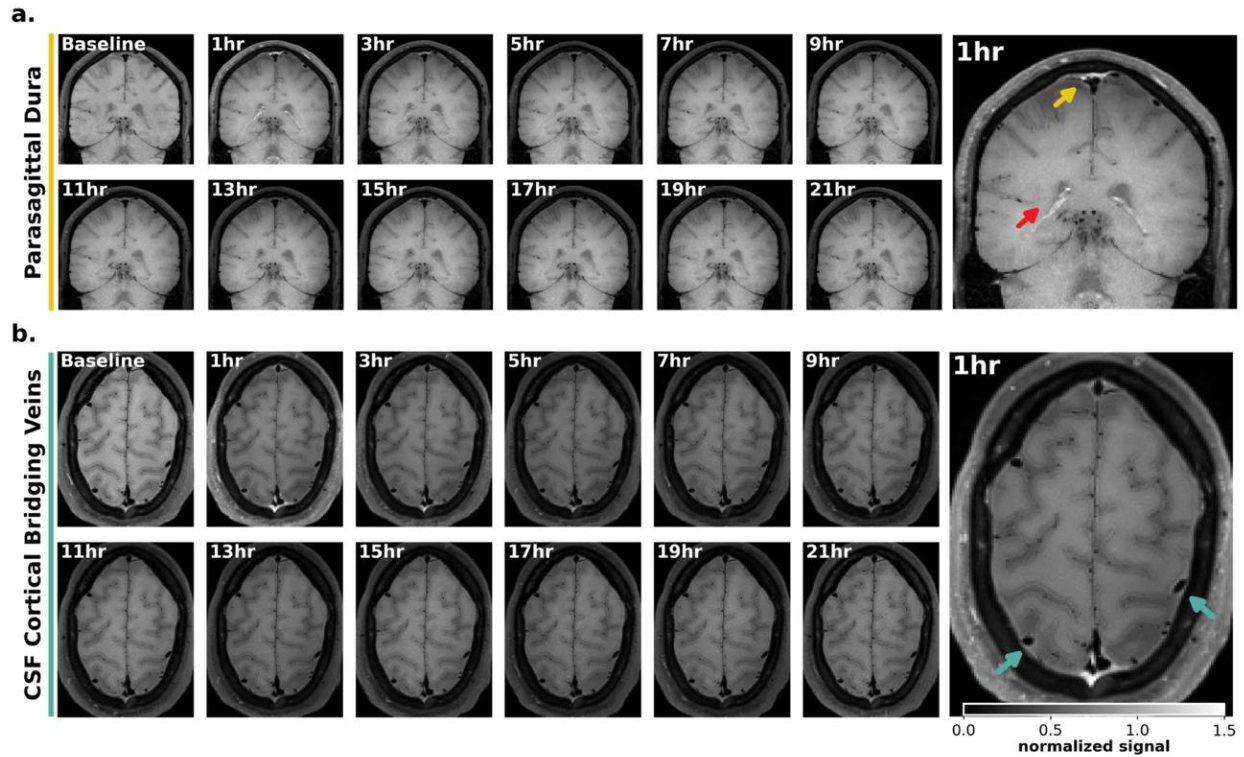


Fig 4. Black blood sequence collected in 1 participant. Images were normalized to the signal in the white matter. **a.** Signal enhancement was observed beginning at 1 hour in the parasagittal dura (yellow arrow) and the choroid plexus (red arrow). **b.** Enhancement around the cortical bridging veins (teal arrows), was less pronounced compared to hT2w.

Compartment Models and Contrast Dynamics

Quantitative T1 data informed the relevant compartments and their connectivity. No significant changes were found in the lateral ventricles (**Figure 1d**), indicating minimal contribution of the choroid plexus to contrast exchange into the CSF and $K_{CP} \approx 0$. An early increase in SAS GBCA concentration indicates direct blood-to-SAS exchange, while prolonged signal change may reflect poor mixing or stagnant regions within the SAS. At 6 hours, blood (SS) concentration has dropped below SAS concentration, eliminating the concentration difference that drives GBCA transport from the blood to the SAS, while SAS concentrations remain elevated through 22 hours. GBCA may accumulate in stagnant regions during early high-concentration periods and gradually return to the mobile SAS, maintaining the approximately constant SAS signal observed

in the experimental data. Therefore, the SAS is described as two compartments in the model – a “mobile” compartment with CSF flow (SAS) and “stagnant” compartment with negligible movement (SAS Reservoir) that enables the sustained concentrations shown in the data despite dynamic driving forces between the blood and the SAS.

GM T1 decreased immediately after contrast infusion but returned to baseline by 2 h, suggesting microvascular effects without sustained parenchymal accumulation; thus, parenchyma was excluded from the IV infusion model. The rate of IV delivery is known, and the blood elimination was fitted to a double exponential-decay model using the SS GBCA concentration ($t_{1/2} = 0.56$ hours, $t_{2/2} = 2.47$ hours, $C_0 = 0.3$ mM, $\alpha = 0.63$) (**Supplemental Data**).

In the cranial CSF circulation model (Figures 5a and b): 1) GBCA is infused into the blood, 2) CSF is produced from blood at the choroid plexus and flows into the ventricles with contrast exchange at a rate described by K_{CP} , 3) CSF moves from the ventricles into the SAS, 4) GBCA permeates from the blood directly into the SAS described by $PS_{BL,SAS}$, 5) GBCA is eliminated from the body at a rate dependent upon its concentration in the blood. GBCA exchange between the two SAS compartments is described by $PS_{SAS,Res}$ and the volumes of the compartments (V_{Res} and $V_{SAS} - V_{Res}$).

Contrast in the CSF is Described by Direct Exchange from Blood to SAS

Two different SAS compartment arrangements were tested: 1) GBCA transport between the blood and SAS CSF occurs through a sluggish SAS compartment before reaching the mobile SAS, such as with the optic nerve (Model A, **Figure 5a**), and 2) direct blood-to-mobile SAS transport and a secondary stagnant SAS reservoir, where contrast can accumulate, such as the sulci (Model B, **Figure 5b**). **Figure 5c** compares a predicted range of SAS concentration curves

334 for Models A and B for a range of reasonable transport parameters. Model A significantly
335 underpredicted early time points relative to the data, indicating a more direct blood to SAS route
336 must be the primary source (e.g., bridging veins). Model B reproduced observed concentration
337 dynamics and was used to estimate transport parameters.

338

Figure 5 Two-compartment model of a mobile SAS compartment and a sluggish reservoir

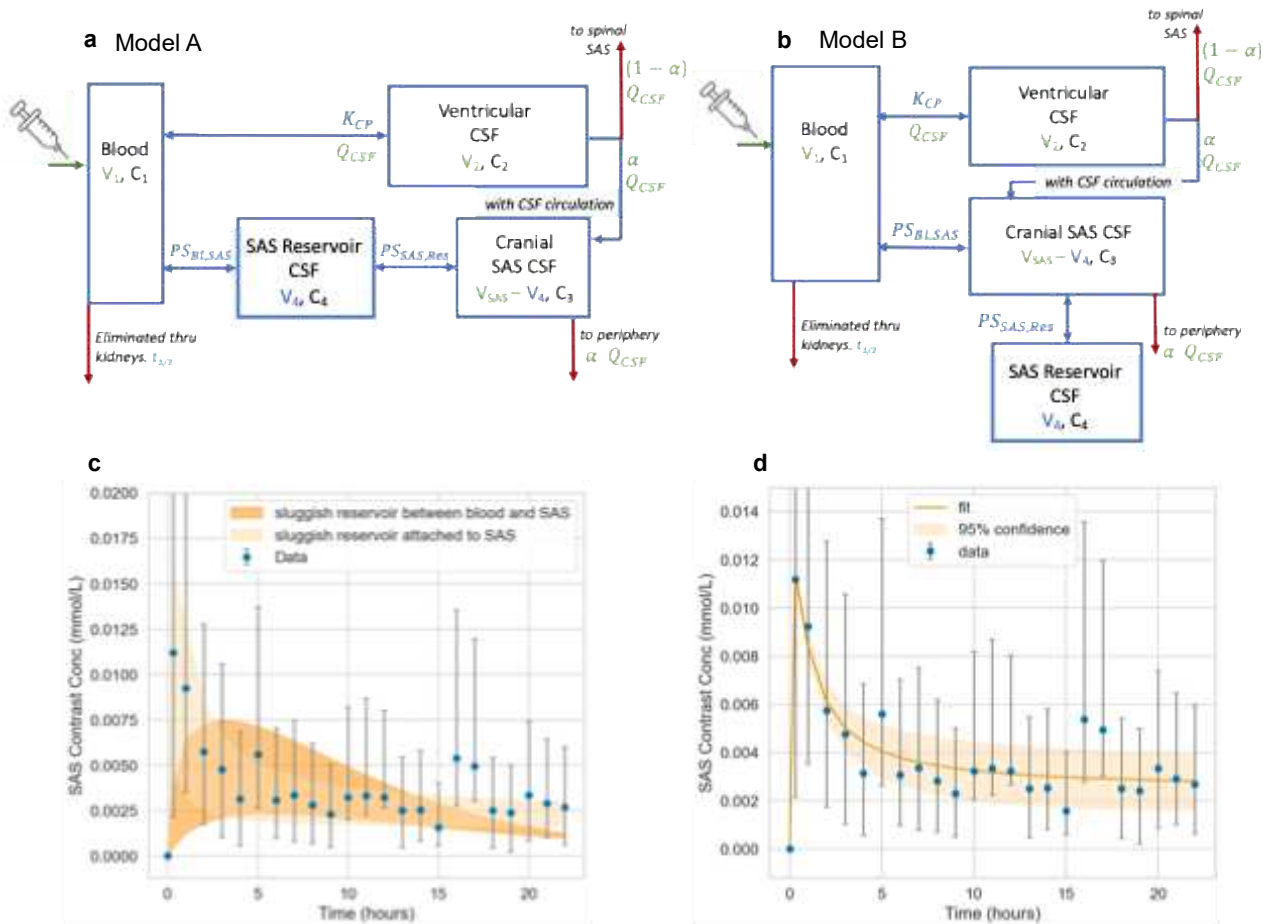


Fig 5. Four-compartment transport models of CSF circulation for IV GBCA including compartments: blood, ventricular CSF, cranial SAS CSF, and SAS reservoir. Values of “fixed” parameters (green text); experimental data (black); and model estimated parameters (blue). The half-life of blood elimination (light blue) was determined from experimental data. **a.** Schematic of model A with the sluggish reservoir between the blood. **B.** Schematic of model B with SAS reservoir. **c.** Comparison of SAS concentration predictions for model A (dark orange) and model B (light orange). Model A did not fit the high concentrations at early time points or the sustained concentration at later timepoints. **d.** Best fit of Model B to experimental data: mean across participants. Model best fit by non-linear regression with 95% confidence interval determined from Monte Carlo simulations.

Model Predictions and Transport Parameters

SAS concentration from the model was calculated by volume averaging concentration in the SAS and the SAS Reservoir compartments. Model predictions for Model B agreed well with participant-averaged SAS concentrations (**Figure 5d**). Transport parameter values from this prediction are reported in **Table 4**. The estimated GBCA transport from the blood-to-SAS was ~50x faster than SAS-to-SAS Reservoir (10^{-2} mmol/hr vs 10^{-4} mmol/hr). Between 14–15 hr, predicted transport reversed, with contrast moving from the reservoir back to SAS, consistent with prolonged low-level SAS concentrations observed up to 22 hr. Monte Carlo sensitivity analysis provided standard deviations for fitted parameters. Additional details in **Supplemental Data**.

Table 4. Results of Transport Analysis using Compartment Modeling with Monte Carlo Sensitivity Analysis

Fitted Transport Parameter*	Value**
$PS_{B1,SAS}$ cm ³ /hr	48 ± 11
$PS_{SAS,Reservoir}$ cm ³ /hr	1.01 ± 0.18
$V_{Reservoir}$	$93 \pm 0.4\%$ of V_{SAS}

* K_{CP} was set to a low value of 0.025% based on ventricular concentrations being below detectable limits.

** standard deviation determined from Monte Carlo simulations.

DISCUSSION

Using quantitative T1 mapping with regular sampling over 24 hours post-contrast, this study demonstrated rapid exchange of intravenously administered GBCA from blood to CSF, detectable as early as 20 minutes post-injection and persisting for up to 22 hours. Sustained T1 shortening was observed in the cortical subarachnoid space (SAS), consistent with the effect of GBCA, whereas no significant T1 changes were detected in the lateral ventricles. These findings support direct blood-to-SAS exchange, with routes around cortical bridging veins further supported by focal enhancement on hT2W imaging.

Although these effects are subtle and difficult to appreciate visually, quantitative imaging enables confirmation of sustained low-level GBCA in the SAS and allows estimation of transport parameters that have not previously been measured in humans. Compartmental modeling indicates an early phase of blood-to-SAS exchange followed by poor mixing within the SAS, best described by a two-compartment model consisting of a mobile SAS compartment and a relatively stagnant reservoir.

Together, our data supports the use of IV GBCA MRI to measure solute transport between the blood and CSF – which is important for our overall understanding of CSF clearance and how biomarkers in both the blood and CSF are interpreted. In contrast, CSF–brain interstitial fluid exchange was not sufficiently appreciated to support its use as a quantitative measure of glymphatic function. Therefore, future IV contrast studies would benefit from quantitative techniques optimized for CSF measurement to further investigate solute exchange

Direct Solute Exchange from the Blood to CSF at the SAS

Our experimental and computational data both support early, direct exchange of intravenously administered GBCA into SAS. Prior studies have reported the presence of IV GBCA within the SAS in both rodent models (36) and humans (23–25); however, these observations have been largely qualitative. A key strength of the present study is the quantitative T1 mapping, which enabled kinetic modeling and allowed us to confirm that the observed effects were not attributable to scanner preparation, calibration, or field inhomogeneity across longitudinal sessions (37). One prior quantitative study did measure GBCA within the SAS, with observations within 3 hours post-IV administration (25). Here, we extend these findings by demonstrating that GBCA-related signal changes within the SAS persists for up to 22 hours. Notably, our effects were observed in healthy participants. While blood–SAS exchange has been described in the context of pathologies (38), our results provide evidence that measurable solute exchange between the blood and SAS also occurs in the healthy brain. This provides necessary context for distinguishing pathological changes from normal variation and is important for future studies interpreting altered blood–CSF transport in disease states.

With hT2W-IR, we identified discrete anatomical sites of GBCA entry into the SAS that are consistent with prior work including regions surrounding cortical bridging veins (29,30), and cranial nerves (27,28,39,40). Because hT2W-IR preferentially highlights fluid compartments while largely suppressing tissue and intravascular signal at long echo times, the observed enhancement is most consistent with GBCA within CSF rather than within blood or parenchyma. Early enhancement around cortical bridging veins was suggested to represent arachnoid cuff exit points; recently identified as openings in the arachnoid barrier that permit solute exchange between the dura mater and subarachnoid space (30). While animal data suggest that the blood–brain barrier remains intact at these sites, their anatomy in humans remains incompletely

characterized Moreover, because dural blood vessels are fenestrated and known to be permeable to proteins (41), contrast could first enter the dura and subsequently exchange into the SAS. Distinguishing between direct blood–SAS exchange at the arachnoid cuff versus indirect transfer via the dura was not possible at the spatial resolution of the present study.

Temporal analysis suggests heterogeneity in exchange dynamics across identified entry sites. Enhancement near cortical bridging veins exhibited a rapid rise and fall, peaking at approximately 1 hour, consistent with quick transit from blood into the broader SAS. In contrast, CSF surrounding the optic nerve showed a rapid initial increase followed by sustained elevation for 7–9 hours, despite declining blood concentrations. This pattern is consistent with local accumulation and slower subsequent transport, rather than rapid clearance. This pattern may reflect local accumulation with slow subsequent transport, rather than rapid clearance. Importantly, enhancement alone should not be interpreted as a proxy for transport rate, as elevated signal may primarily reflect accumulation and delayed clearance. We also observed inter-individual variability in the number and distribution of cranial nerve–associated enhancement sites, which may reflect true differences in exchange pathways, anatomical variability, or temporal dynamics that could not be fully resolved with our limited sample size.

Our computational model estimates a blood-to-SAS *PS* of 48 cm³/hr for exchange routes in the SAS. For molecules with passive transport across the blood brain barrier (BBB), *PS* correlates with lipophilicity (logP) (42). Gadobutrol has a logP = -5.4 and for molecules with logP = -5, it has been reported that *PS* ranges from 0.0002-0.0005 mL/min per gram of brain tissue, corresponding to 0.01-0.03 cm³/hr. To compare these brain-wide estimates to the blood-SAS value reported here, we can assume that the blood- SAS exchange regions are much smaller than the vascular surface area of the brain parenchyma of 100–200 cm²/g. Moreover, our data

suggest that blood-to-SAS exchange occurs at specific anatomical sites, such as cortical bridging veins. If these exchange regions correspond to less than ~10 g of brain tissue in equivalent vascular surface area, the estimated *PS* would imply a 100-fold enhancement relative to BBB permeability, making the observed values physiologically plausible and indicating greater passive permeability at the blood-CSF barrier as compared to the BBB.

Our compartmental modeling data indicates poor mixing within the SAS and is best described by a two-compartment model consisting of a mobile SAS compartment and a relatively stagnant reservoir. A model of complex mixing within the cortical SAS is consistent with prior studies demonstrating substantial spatial variability in SAS flow, including regions of sluggish movement (43). The SAS extends into cortical sulci—narrow, variably shaped channels that increase resistance to transport and may promote local fluid stagnation (44). Supporting this concept, intrathecal GBCA has been detected in human CSF up to 48 hours after injection, particularly in regions associated with restricted molecular motion, such as the parasagittal dura (45). While the SAS is anatomically continuous, it is functionally heterogeneous and likely consists of multiple compartments with distinct transport properties including fast transport pathways along specific SAS routes (46,47). This is supported by observations from preclinical studies including rapid tracer movement along the margins of surface arteries rather than through bulk subarachnoid CSF (2) and elevated tracer transport rates near subarachnoid arteries (48). Similar rapid-transport pathways could exist in humans and would be consistent with the small-volume, fast-exchanging mobile SAS compartment identified in our model and the poor overall mixing between small regions of rapid transport and larger, sluggish reservoirs. Although simplified, the two-compartment SAS model employed here captures key aspects of this heterogeneity at a systems level. Improved characterization of SAS flow dynamics and

compartmental structure remains an important area for future investigation as it is not yet understood how reported age-related changes in SAS CSF volume (49) and diffusion properties (50) contribute to solute clearance from the brain and CSF.

Ventricular CSF and Brain Parenchyma

No significant decreases in T1 were detected in the ventricular CSF, although our T1 data shows GBCA within the ChP. On T1, ChP ROI contains both the vascular and stroma compartments, both of which likely contain GBCA due to the fenestrated vessels of the ChP (51,52). Corresponding enhancement was also evident on hT2W-IR imaging, confirming that contrast is present outside the vasculature. However, the absence of enhancement in the lateral ventricles argues against substantial exchange across the ChP epithelial cells that make up the BCSFB.

This finding is inconsistent with other reports of GBCA leakage across the ChP to the lateral ventricles (25,26). This may be attributed to the MRI sequence used in our study. The sequence (33) was previously optimized for brain tissue with relatively short T1 values (800–1500 ms) rather than for CSF, which has substantially longer T1 values (2000–4000 ms)(53). Consistent with this limitation, our phantom experiments demonstrated increased variability for T1 values above 2.5 s. Accurate simultaneous mapping of both tissue and CSF remains technically challenging due to this wide separation in relaxation times. Continued development of quantitative methods specifically optimized for CSF characterization (53–56) will be important for future IV GBCA studies. If contrast does exchange directly into the ventricular space, this would impact our modeling results. Using ventricular concentrations reported by Wahlin et al. for the first 3 hours post-injection (assuming similar blood concentrations) within our

compartment model framework yields an estimated $K_{CP} = 0.032$, indicating that approximately 3.2% of the contrast in the blood enters the ventricles via bulk CSF production. Substituting this value for the previously assumed $K_{CP}=0.25\%$, results in only a 2% change in the estimated permeability–surface area (PS) values reported in **Table 3**, indicating that our primary modeling conclusions are relatively insensitive to this parameter.

The absence of GBCA measured in the brain parenchyma limited both the scope of this study and the modeling approaches that could be applied. While exchange between CSF and parenchyma may contribute to SAS concentration dynamics, it could not be explicitly modeled. Detectable parenchymal enhancement—given the substantially larger tissue volume compared to CSF—would enable application of finite-element or physics-informed neural network approaches to estimate intraparenchymal transport.

Implications

Taken together, our findings indicate that blood–SAS exchange is a more prominent pathway for GBCA transport to the CSF than blood–ventricular pathway. Despite clear evidence of contrast within the choroid plexus, the absence of measurable ventricular CSF enhancement suggests that direct blood–ventricular transfer contributes minimally to overall CSF GBCA influx in healthy adults. In contrast, early and sustained GBCA signal within the SAS, along with model-derived permeability estimates, supports the presence of distinct blood–SAS exchange routes. These findings are important because IV GBCA–enhanced MRI provides a practical approach for probing solute transport pathways across blood–CSF barrier. The BCSFB is closely linked to CSF circulation and has been functionally connected to glymphatic brain clearance (57). Moreover, emerging evidence suggests that brain clearance and immune surveillance of the

central nervous system are linked at brain border regions such as the dura, which harbors dense immune cell populations, with regions surrounding cortical bridging veins providing potential dural–SAS connections (30,58). IV GBCA–enhanced MRI could be used for future investigations of both normal physiology and disease-related alterations in CNS solute transport and immune surveillance.

Limitations and Conclusions

Several limitations should be considered when interpreting these findings. The GBCA concentrations detected in CSF compartments are extremely low, approaching the sensitivity limits of current MRI methods, contributing to measurement uncertainty and inter-individual variability. In addition, the cohort spanned a wide age range, and blood–brain barrier permeability is known to change with age (59); age-stratified studies may therefore yield additional insight. Finally, site-specific dynamics remain incompletely resolved, underscoring the need for specialized imaging approaches targeting structures such as cortical veins, cranial nerves, and the choroid plexus.

Despite these limitations, our results demonstrate the utility of IV GBCA MRI for quantifying solute transport in the human CNS, specifically the BCSF barrier. A comprehensive understanding of brain clearance will require the integration of alternative methods to fully capture the complexity of solute transport across all compartments.

DECLARATIONS

Ethics approval and consent to participate: The study was approved by the Internal Review Board (IRB) at the University of Washington and adhered to principles outlined in Declaration of Helsinki. All participants provided informed consent.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors J.J.I. and S.R.L. declare the existence of financial and incentive stock options competing interests.

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538 interpretation, review and editing. S.R.L: conceptualization, imaging methodology, data
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