Appendix 1

Further considerations and limitations

3D Slicer volumetry results

Slicer provides two different types of volumetry results: labelmap volumes and closed surface volumes. The developers of the application have reported minimal differences between the two methods of volume calculation, as long as the method is used consistently (28). They likely reflect different internal algorithms of Slicer. In our study, we chose labelmap volumes, as they were the default output. We generated a closed surface 3D representation of BV, ICV, and SV for visual inspection to detect segmentation errors. We also reviewed segmentations in orthogonal planes but did not use Closed Surface statistics for any of our calculations.

Intracranial volume with 3D Slicer

If high resolution non-contrast CT is not available, a modernCTA can be used for the described method of ICV segmentation but may require more manual editing due to the hyperdense cerebral vessels that can be "mistaken" as a bone by Slicer due to almost similar Hounsfield unit value or CT density (discussed in Instructions for segmentation using 3D Slicer). Of note, the module Wrap Solidify takes more time to process CTAs (minutes) than non-contrast CT scans (seconds).

Importantly, if the CT scan was done with a gantry tilt, it will provide inaccurate ICV segmentations, due to distortion of the sagittal plane image. Thus, 0-degree gantry tilt CT scans or CTA must be used for this method on the current Slicer versions.

FreeSurfer limitations

FreeSurfer was unable to process T1 MRI scans of 4 subjects (10.2%) and these were excluded from the study. Of note, one BV segmentation on FreeSurfer was not anatomically accurate in one patient as the clivus was segmented as brainstem. *Table S1* shows FreeSurfer processing errors.

For FreeSurfer version 5.2 and later, the brainstem is not included in the Morphometry Stats and Global Measure of Volume, because the volume of brainstem changes depends upon the field of view of the volume acquisition (29). In this study, we added the brainstem volume in the aseg.stats output file from FreeSurfer, in efforts to counteract a false negative skew of the data.

According to Gronenschild *et al.*, it is important to use the same version of FreeSurfer and the same OS and workstation (30). Thus, all segmentations in this study were created using the same workstation and the same version of FreeSurfer and Slicer. Though the scans in our study were obtained on different MRI scanners, it has been shown that FreeSurfer total brain volume measurements are consistent across MRI field strengths (31), but it is important to note that there was variation among T1 quality in our study. FreeSurfer does recommend good quality T1-weighted MRI scans with slice intervals no larger than 1.5 mm (24).

Table S1 FreeSurfer processing errors

Error case	Scan quality	Problem with T1 MRI	FreeSurfer error log	Acceptable Slicer T1 segmentation? (+/–/?)
1	Axial 2D 3 mm	Defect in T1 MRI (missing part of left temporal lobe)	"Topological defect"[†]	–/?
				Defect apparently interfered with skull stripping and output a white brain mask
2	Sagittal 3D 1.1 mm	Left hemisphere defect (possible massive infarct invading ventricle)	"Topological defect"[†]	–/?
				Skull stripping removed part of occipital lobe
3	Axial 2D 3 mm	Likely movement artifact, and right temporal lobe displaced in coronal plane	"Talairach failed!"	+
4	Axial 2D 6 mm	Unable to import MRI correctly into FreeSurfer	"Talairach failed"	+/?
				Segmentation appeared blocky: likely attributable to slice thickness
5	Axial 2D 3 mm	Clivus segmented as cerebellum	N/A	+

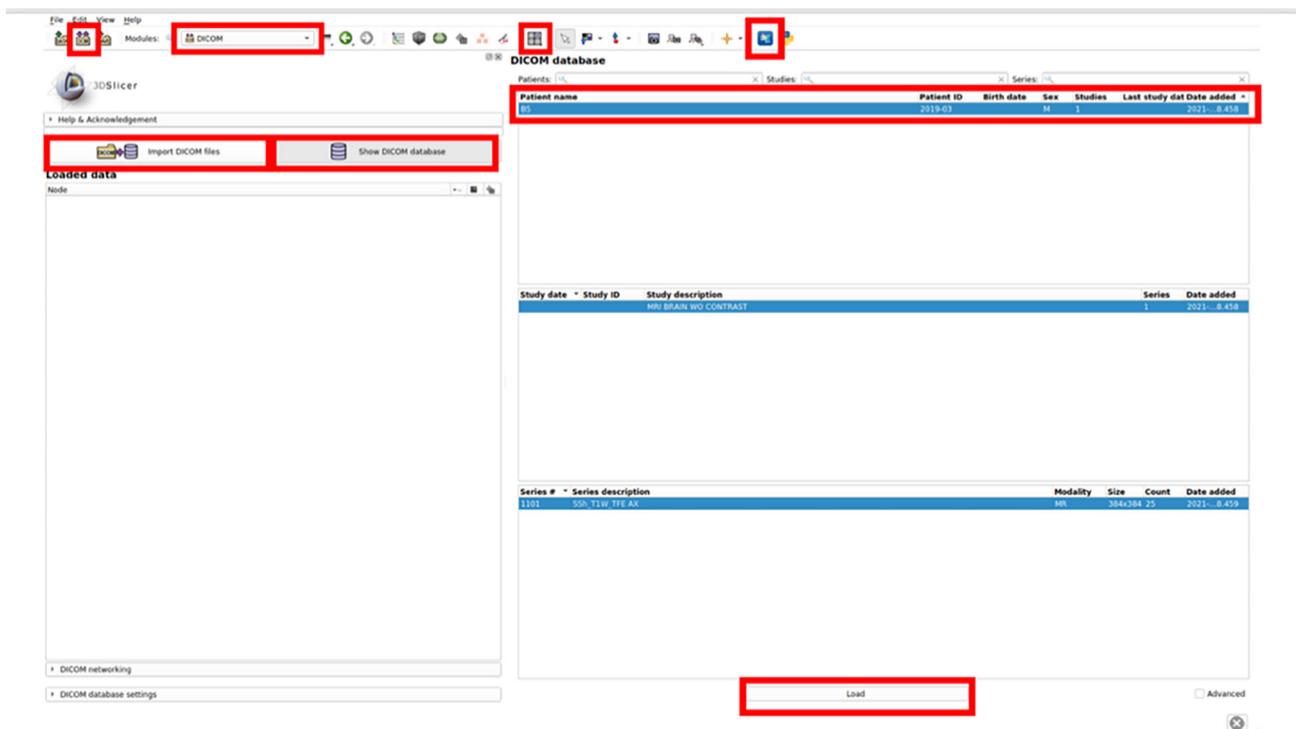[†], often happens when cerebellum or dura are not removed from wm.mgz.

Figure S1 3D Slicer GUI of DICOM database. Relevant icons are highlighted in red. GUI, graphical user interface; DICOM, digital imaging and communications in medicine.

The current FreeSurfer version unable to export segmentations to be further used on modern surgical navigation systems; at the time of this writing, the authors were unable to find an additional postprocessing solution for this. However, FreeSurfer and volBrain segmentations can be uploaded into Slicer for visual inspection in 3-dimensions (see below), with the exception of FreeSurfer ICV segmentations.

Instructions for segmentation using 3D Slicer

Getting started and loading a DICOM image
1. Using the Extensions Manager (*Figure S1*), Install 3D *Slicer* extensions: *SegmentEditorExtraEffects*, *SurfaceWrapSolidify*, and *SwissSkullStripper* (20).
2. Click the *DCM* icon in the top left of the GUI (*Figure S1*).
3. Click *Import DICOM files* (*Figure S1*).
4. Find the DCM files of interest in the local file system.
5. Click *Import*.
6. Click *Show DICOM database* (*Figure S1*). The DICOM database should be visible instead of the Viewer on the

right side of the screen.
7. In the DICOM database, click the correct patient under *Patient Name* (*Figure S1*) and click *Load* (bottom of *Figure S1*).
8. Most of the modules for these instructions will be in the *Modules* drop-down menu in the application menu at the top of *Figure S1*.
9. The view can be adjusted in the *Layout Toolbar* in the application menu.
   a. It is recommended to use the *Four-Up* view in this study.
10. More information can be found on navigating the GUI on the 3D Slicer documentation page (32).

Brain volume segmentation using T1 MRI
- The skull must first be removed via skull stripping.
- *3D Slicer* version 4.11.0 needs additional installation of *Swiss Skull Stripper* (for instructions on how to install, see above).
- Load T1 MRI DICOM file.
- Select *Swiss Skull Stripper* module within the *Segmentation* tab under the *Module Drop Down List*
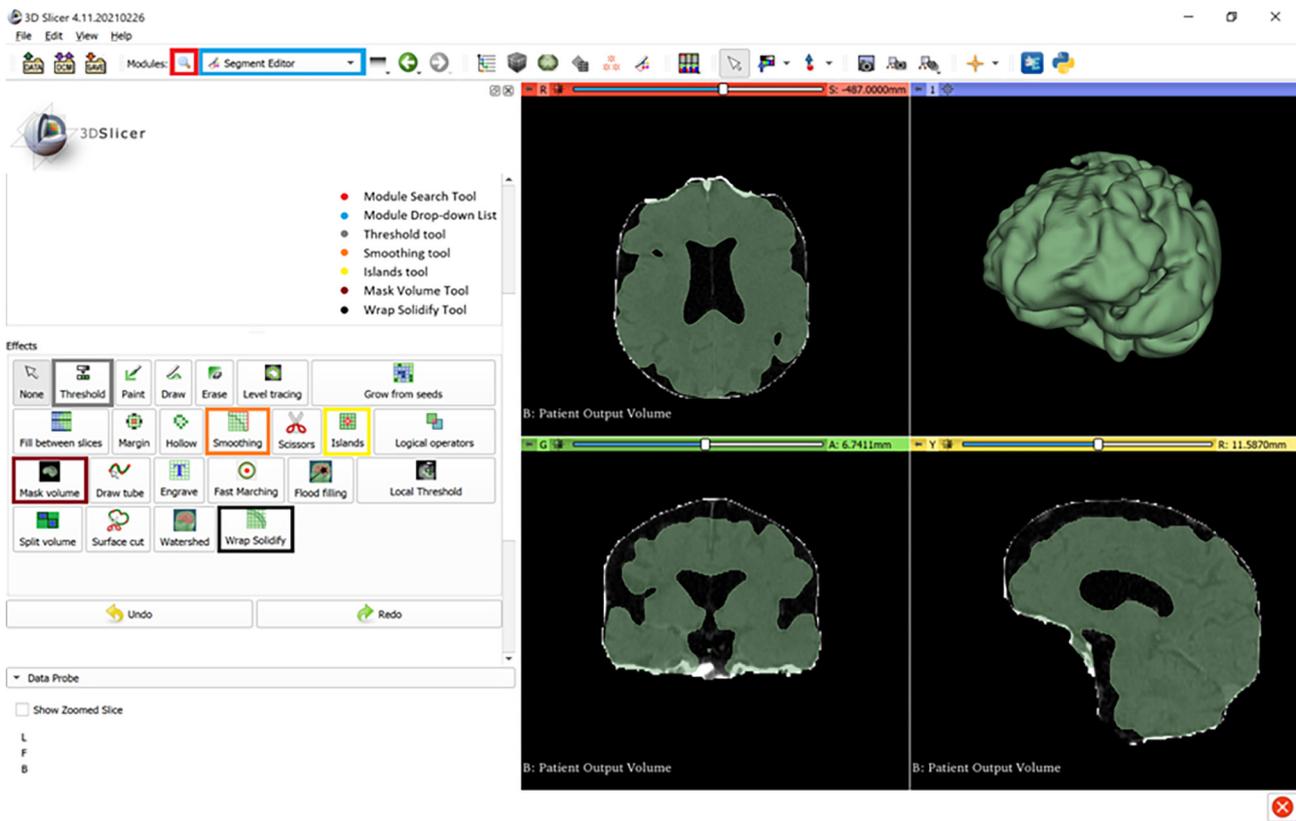
Figure S2 3D Slicer graphical user interface of Segment Editor module. Relevant icons are highlighted in color.

menu (*Figure S2*).

- The *Input Patient Volume* should be the T1 MRI DICOM.
- Click the *Patient Output Volume* drop-down menu.
  a. Choose "*Create New Labelmap Volume*".
  b. The *Patient Mask Label* should be listed as the same name as the one created above (i.e. "*Patient Output Volume*").
- Click *Apply*. This will create a multicolored label map of the brain.
  a. Note: sometimes skull stripping contains errors (*Figure S3*).
- Click the *Volumes* module.
- Change *Active Volume* to the volume named above (i.e., "*Patient Output Volume*").
- Choose *Gray* from the *Lookup Table* under the *Display* tab. This will create an output volume with intensity contrast between the brain and ventricles.
- Change module to *Segment Editor*. Under *Master volume*, select *Patient Output Volume*.
- Click *Add* Add

- Click the box next to the *Segmentation* drop-down menu to align slice views to the segmentation.
- Under *Effects*, select *Threshold*, and tweak the threshold intensity range to include all white and gray matter of brain, but not so broad that it includes any part of the ventricles. (Note: skull stripping is often imperfect (*Figure S3*), and areas of skull, dura, optic nerve, or posterior eye may appear outside the brain. Imperfect skull stripping can be manually removed using the scissor tool, the eraser tool (select sphere brush, in 3D plane), and smoothing effect (select *opening remove extrusions*) in the *Segment Editor* module. There are, occasionally, some hyperintensities noted in the ventricles which can also be manually excluded (see *Figure S2* for relevant segmentation effects).
- Select the *Segment Statistics* module under *Quantification*. Select the segmentation that was previously created and click *Apply*.
- The brain volume will be found under *Volume* (calculated by the *Labelmap Statistics* plugin) (28).
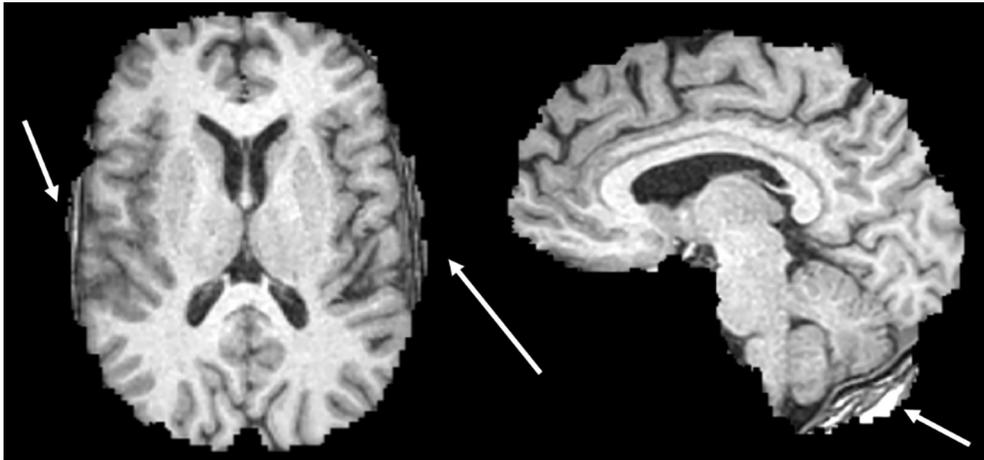
Figure S3 Skull strip errors in 3D Slicer.

Intracranial volume segmentation using CT/CTA

- These instructions are heavily derived from the methods outlined by Lassoan on GitHub at https://lassoan.github.io/SlicerSegmentationRecipes/CTSkullStripping (33).
- Load non-contrast CT or CT angiogram DICOM file. Of note, current Slicer versions require CT scans acquired with 0-degree gantry tilt.
- Choose "*Segment Editor*" module → click "*Add*" button to create a new segment. (be sure the *Master Volume* is the CT scan.
- Select "*Threshold*" effect and set intensity range of bone (from approximately 300 to maximum value) → click *Apply* button.
- Remove small spots of segmentation left in the brain by using the *Islands* effect (choose *Keep largest island* option) → click *Apply* button.
    1. Note: CT angiogram may have hyperdense vessels with contrast in the intracranial space. In some instances, these vessels are not removed by the *Islands* effect. These can be manually removed using the *Eraser* tool (also choose *sphere brush* and *erase in* 3D). Be careful not to erase the segmented skull.
- Select *Wrap Solidify* effect
- Set *Region* to *Largest cavity*; enable *Split cavities* and set *Cavity Size* slider to 8 mm → click *Apply* button. This may take several minutes.
- The resulting segmentation should resemble *Figure S4A*. However, sometimes the segmentation has errors resembling *Figure S4B*.

- If this happens, undo the *Wrap Solidify* step by clicking *undo*.
- There are 2 options to fix this segmentation error.
    1. If the thresholding step did not include all of the skull margins, this is your problem. Adjust the thresholding intensity range to include all of the skull.
    2. If option 1 does not work, use the *paint* tool to paint the sinuses (click *Sphere brush* and *Edit in* 3D views) (*Figure S4C*). Note: This works best in the sagittal view. Then re-do the *Wrap Solidify* step.
- Select *Scissor* effect and delete segmentation below foramen magnum plane (this works best in the sagittal view.
- Calculate volume using *Segment Statistics* module (28).

Stroke volume segmentation using DWI B1000 MRI

- Load B1000 DWI MRI.
- Choose *Segment Editor* module and create new segmentation.
- Tweak the threshold intensity range to include the entire area of hyperintensity that indicates infarcted tissue.
- Click *Apply*.
- Calculate volume using *Segment Statistics* module.
- Results are seen in *Figure S5*.

Instructions for segmentation using FreeSurfer

Getting started

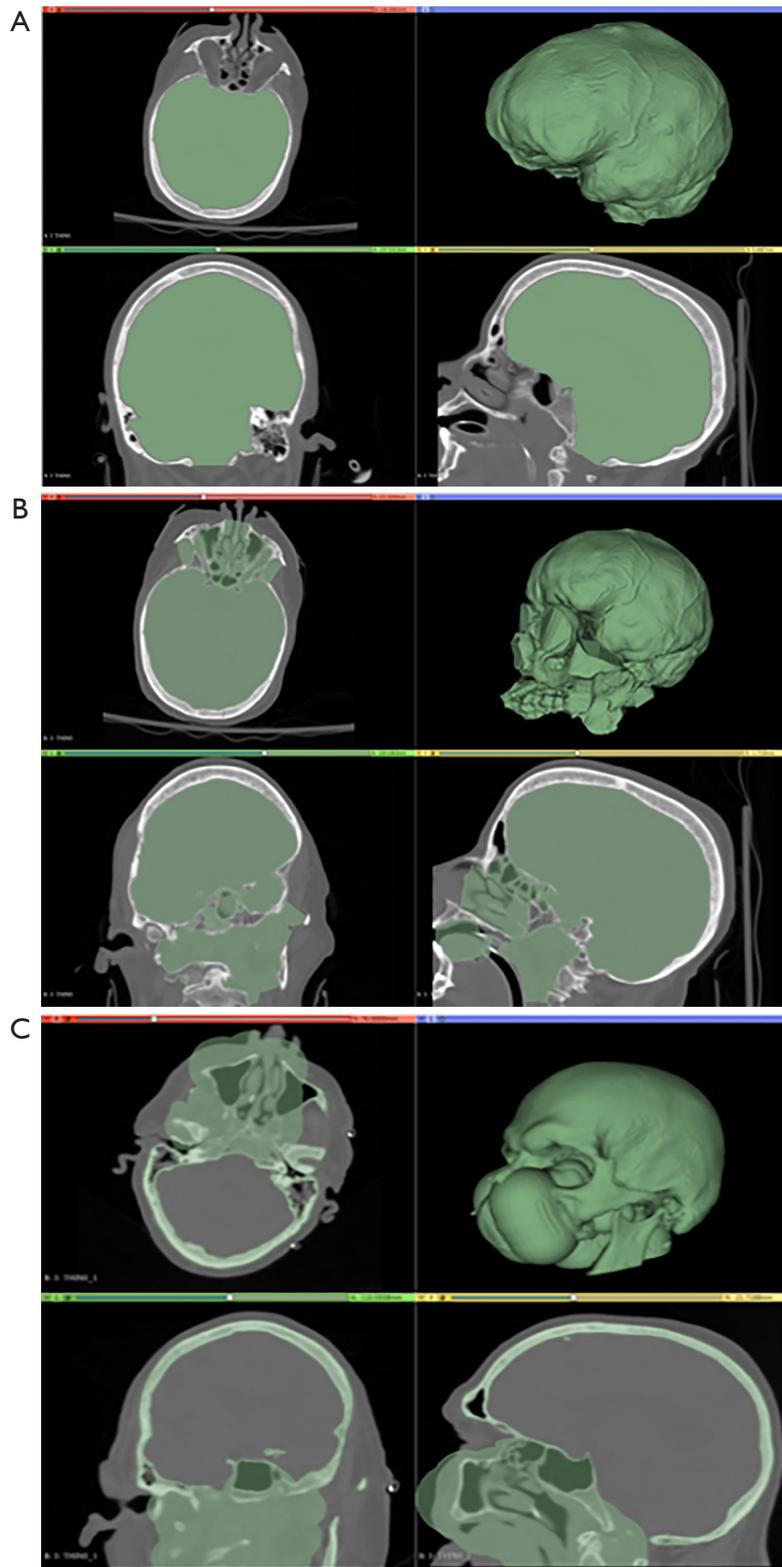- Brain volumes and intracranial volumes are calculated using T1 MRI scans on *FreeSurfer* version 7.1.1.

Figure S4 Intracranial volume considerations. (A) Intracranial volume segmentation using Slicer. (B) Segmentation error due to Slicer difficulty recognizing margins of the intracranial space (best seen on sagittal view). (C) Correction of segmentation error (B) using the paint tool.
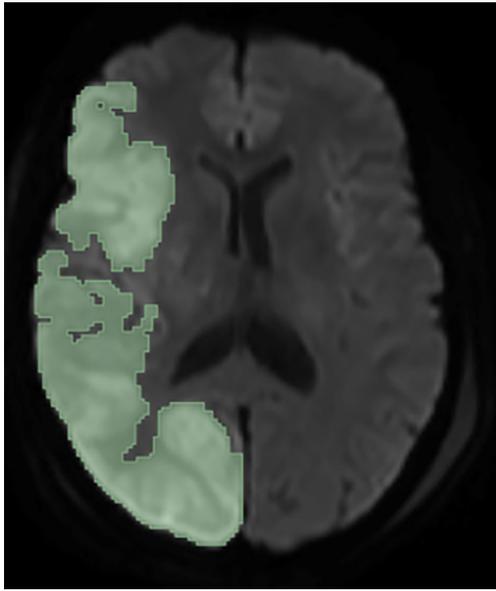
Figure S5 SV segmentation using DWI B1000 MRI. SV, stroke volume; DWI, diffusion weighted imaging; MRI, magnetic resonance imaging.

- Of note: FreeSurfer only processes T1 MRI scans of the brain.
- Open a terminal or command line and set up an environment using the following commands (can copy & paste).
  - o export FREESURFER_HOME=$HOME/FreeSurfer
    export SUBJECTS_DIR=$FREESURFER_HOME/subjects
    source $FREESURFER_HOME/SetUpFreeSurfer.sh
- Additional Information on installation: https://surfer.nmr.mgh.harvard.edu/fswiki/Installation
- Additional Information on setup: https://surfer.nmr.mgh.harvard.edu/fswiki/SetupConfiguration
- After *FreeSurfer* (FreeSurfer) is downloaded and installed on a workstation, move all subject DICOM folders to the *freesurfer/subjects* directory.
  - o These instructions assume that DICOM files are stored in a separate folder specifically named for each patient (*patientname*)

Brain volume and intracranial volume segmentation using T1 MRI scans
- Within a command line, navigate to the *freesurfer/subjects* directory and locate the directory with the T1

DICOM files of interest (i.e., *patientname*).
  - o Ensure that your terminal is *not* in the *patientname* directory, but the *subjects* directory.
- Type:
  - o dcmunpack -src patientname
  - o *FreeSurfer* will then interrogate the directory to find the anatomical DICOM file (*anatomical.dcm*)
- Copy the *anatomical.dcm* file.
- Within the command line, navigate into the *patientname* directory.
- Type the following command:
  - o recon-all -i anatomical.dcm -subject newdirectory -all
  - o *FreeSurfer* will create a new directory in *freesurfer/subjects* in which all of the output data will be stored.
  - o *newdirectory* can be named anything of your choosing.
- *FreeSurfer* will run recon-all. This may take several hours.
- Volume data can be found in the *aseg.stats* output file under the *stats* directory in the *newdirectory*. The volume is reported in mm^3, which can be converted to cm^3 or mL.
- The *BrainSegVolNotVent*, provides the brain segmentation volume without the ventricles (BV). Note: the aseg.stats file also provides a *SupratentorialVolNotVent* value for the brain tissue above the tentorium cerebelli, excluding the ventricles. The *EstimatedTotalIntraCranialVol* provides the volume of the entire intracranial space.
- The FreeSurfer wiki states that for version 5.2 and later, the brainstem is not included in the global measures of volume because the amount of brainstem changes depending upon the field of view of the volume acquisition (29).
- You can measure the volume of the brainstem and add it to the *BrainSegVolNotVent*.
- Type:
  - o mri_segstats --ctab-default --seg aseg.mgz --id 16 --sum brainstem.dat
- This command will provide the volume of the brainstem.

Viewing results of recon-all in FreeView
- Navigate to */subjects/patientname/newdirectory/mri*
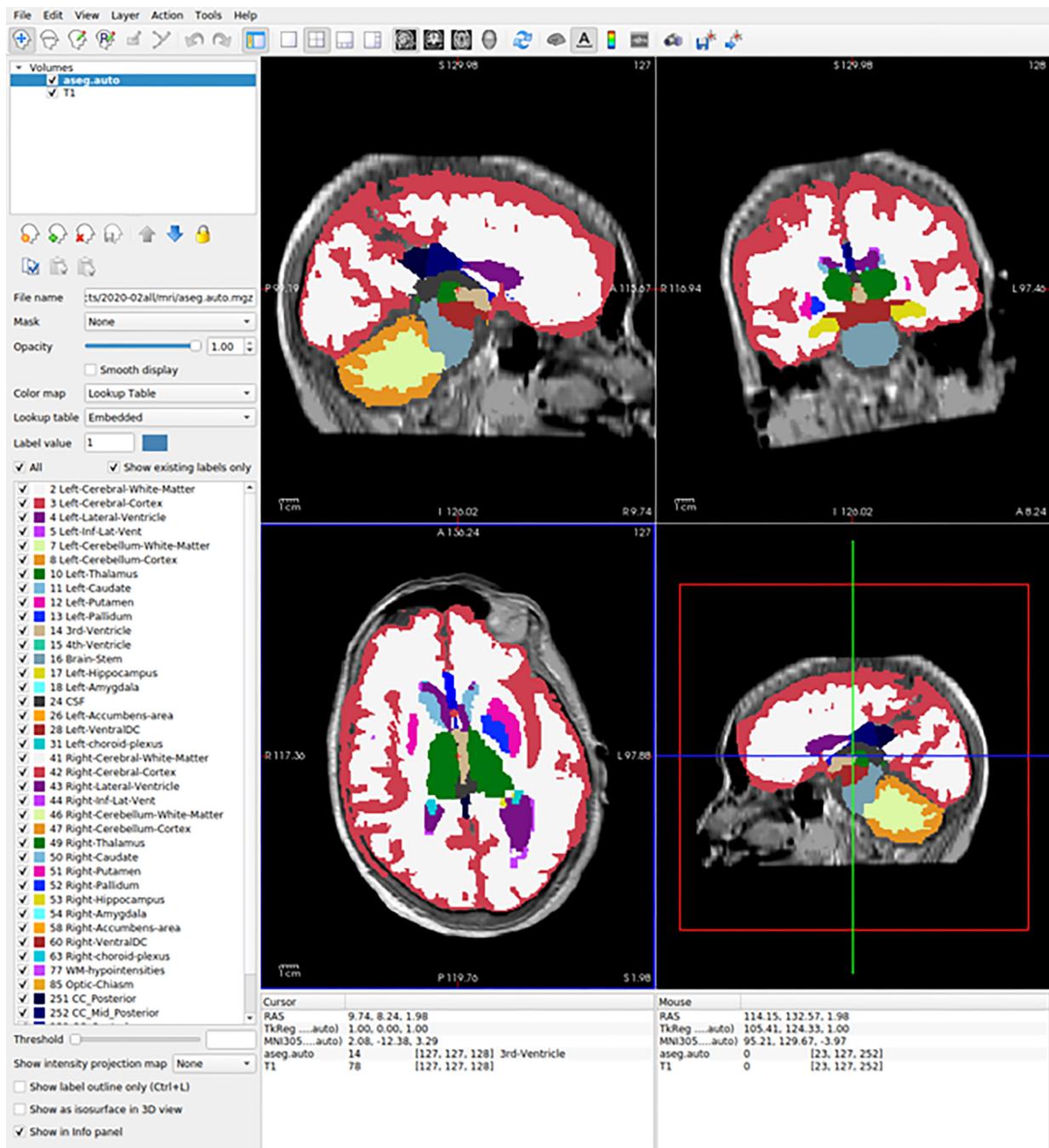 - o "*patientname*" in the above line being the earlier directory name chosen for patient name.

Figure S6 GUI of FreeView. GUI, graphical user interface.

o "*new directory*" is the name of the output directory chosen previously.

- Type the following command: *freeview T1.mgz aseg.auto.mgz*
  o This will load the original T1 MRI and the brain segmentation in FreeView (*Figure S6*) for visual

inspection.

Visualization of volBrain and FreeSurfer segmentation results in 3D Slicer

- volBrain allows the user to download the.nii.gz file. This can be uploaded as "Data" into Slicer. One can
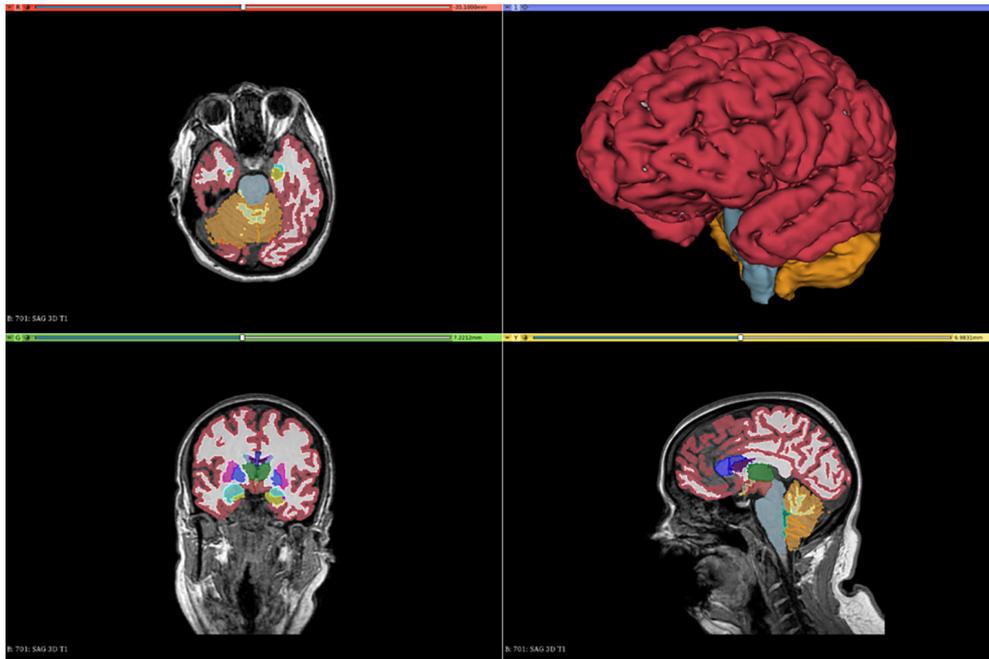
Figure S7 3D representation of FreeSurfer segmentation in 3D Slicer viewer.

then visualize the original T1 MRI and segmentation.
- By clicking the "eye" icon, one can view "crisp_mmni" volume. Click the eye icon to view the volume, then right click the eye icon and click "Show in 3D views as volume rendering". This will show a volume of the brain with CSF on the outside in the 3D viewer (this will represent the intracranial space).
- Additionally, one can threshold the "crisp_mmni" volume in Segment Editor to see the brain without the CSF (use segmentation editor).
- One can also load FreeSurfer results into Slicer by loading the "mri" result folder as "data". Right click the eye icon next to the "brain" volume. Then right click the eye icon and click "Show in 3D views as volume rendering" to see the segmentation in 3D.
- We also utilized the tools fs2dicom, dcmqi, and docker to export FreeSurfer segmentation to a DICOM file and upload into Slicer for 3D visualization (*Figure S7*) (*Figure 2A,2B* in main text).
  - o This would be a difficult step for the user, and we recommend simply loading the mri result folder as data as mentioned above. More information on exporting FreeSurfer segmentation to Slicer can be found at the following links:
  - o https://github.com/QIICR/dcmqi/

  - o https://github.com/corticometrics/fs2dicom
- Exporting FreeSurfer segmentation as a DICOM file for manual editing of errors proved untenable as the DICOM file was unable to be utilized with other segmentation software.

Stroke volume analysis

Here we compare SV calculations between Slicer and manual StealthViz segmentations using B1000 scans. SV was manually segmented on Medtronic StealthViz software v. 1.3.0.26 by senior author (VP). Instructions for using StealthViz is beyond the scope of this study as it is not a free, open-source software. Our SV calculations were made using a simple thresholding tool in Slicer. Manual segmentations via StealthViz were our gold standards for SV calculations, because in addition to a thresholding tool, a manual slice-by-slice correction was also used. StealthViz is our gold standard segmentation method against which we are comparing Slicer. SV correlation between Slicer and StealthViz showed good agreement and a strong correlation with R^2 =0.8892 (see *Figure S8* and *Table S2*). All 39 subjects were included for SV analysis (demographics included in *Table S3*).

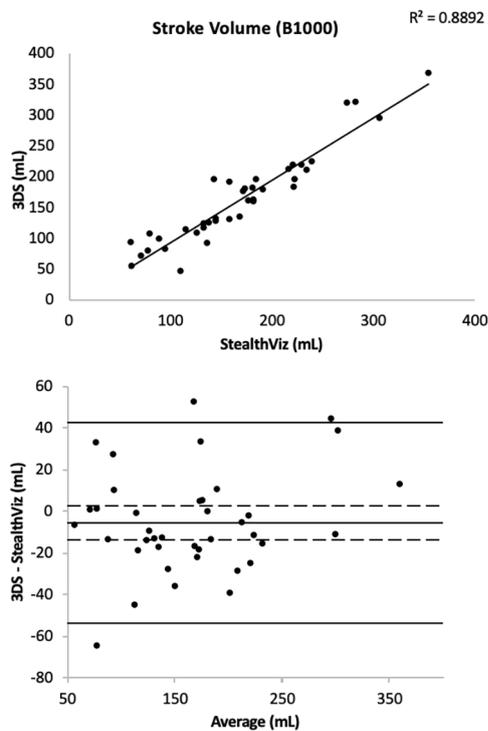FreeSurfer is unable to process B1000 scans. Thus, if a

Figure S8 SV correlation between StealthViz and 3D Slicer (R^2 =0.8892). SV, stroke volume.

Table S2 Demographics of subject population in SV analysis

Gender	Number of subjects (percentage of total)	Average age ± standard deviation (years)
Male	17 (43.5%)	61.41±16.06
Female	22 (56.4%)	61.18±17.55

SV, stroke volume.

Table S3 SV analysis

Comparison	R^2	95% CI	P value
SV			
Slicer *vs.* StealthViz	0.8892	0.8086 to 0.9228	<0.0001

SV, stroke volume; CI, confidence interval; Slicer, 3D Slicer.

free software is to be used, we recommend using Slicer for SV calculation (see above for instructions).

Intracranial reserve analysis

Here we define "intracranial reserve" as the brain volume to intracranial volume ratio. Intracranial reserve can be relevant in a study involving space occupying pathology such as ischemic stroke. It in theory has prognostic value due to the ability of the skull to accommodate swelling from an ischemic stroke. Refer to *Figure S9* for the inter-method reliability for intracranial reserve. Note that the only acceptable correlation is between FreeSurfer and volBrain using 3D scans. *Table S4* shows the Pearson correlation coefficients, 95% CI, and P value for our intracranial reserve analysis.
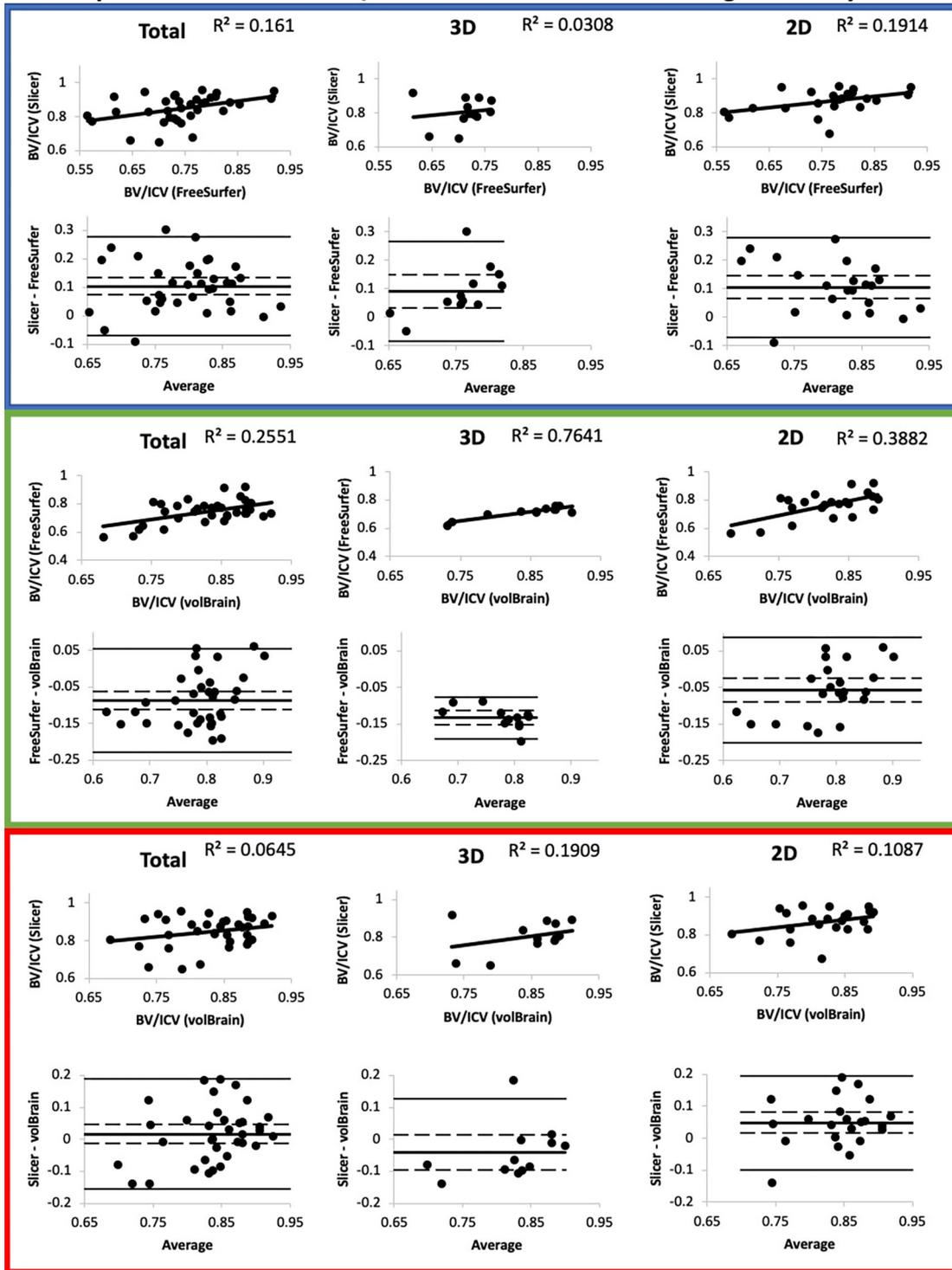
Figure S9 Intracranial reserve analysis. For each Slicer ratio, T1 was used for BV and CT was used for ICV. BV, brain volume; ICV, intracranial volume; Slicer, 3D Slicer; CT, computed tomography.

Table S4 R2 values and corresponding 95% CI assessing the association between volume estimates obtained using different imaging methods

Quality	Comparison	R^2	95% CI	P value
Ratio of BV to ICV				
All	Slicer *vs.* FreeSurfer	0.1610	0.0044 to 0.3717	0.0169
	Slicer *vs.* volBrain	0.0645	0.0000 to 0.2583	0.1409
	FreeSurfer *vs.* volBrain	0.2551	0.0408 to 0.4600	0.0020
2D	Slicer *vs.* FreeSurfer	0.1914	0.0000 to 0.4477	0.0417
	Slicer *vs.* volBrain	0.1087	0.0000 to 0.3658	0.1340
	FreeSurfer *vs.* volBrain	0.3882	0.0695 to 0.6025	0.0020
3D	Slicer *vs.* FreeSurfer	0.0308	0.0000 to 0.3310	0.5663
	Slicer *vs.* volBrain	0.1909	0.0000 to 0.5059	0.1355
	FreeSurfer *vs.* volBrain	0.7641	0.3714 to 0.8634	<0.0001

The methods being compared are described in the Comparison column of the table. P values are from tests comparing the observed R2 value to 0 (zero; no association). CI, confidence intervals; BV, brain volume; ICV, intracranial volume; Slicer, 3D Slicer.

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33. Lasso A. 3D Slicer segmentation recipes. Available online: https://lassoan.github.io/SlicerSegmentationRecipes/CTSkullStripping