Clinical Validation of Automatable Gaussian Normalized CBV in Brain Tumor Analysis: Superior Reproducibility and Slightly Better Association with Survival than Current Standard Manual Normal Appearing White Matter Normalization[^1][^2]

**Abstract**

**PURPOSE:** To validate Gaussian normalized cerebral blood volume (GN-nCBV) by association with overall survival (OS) in newly diagnosed glioblastoma patients and compare this association with current standard white matter normalized cerebral blood volume (WN-nCBV). **METHODS:** We retrieved spin-echo echo-planar dynamic susceptibility contrast MRI acquired after maximal resection and prior to radiation therapy between 2006 and 2011 in 51 adult patients (28 male, 23 female; age 23-87 years) with newly diagnosed glioblastoma. Software code was developed in-house to perform Gaussian normalization of CBV to the standard deviation of the whole brain CBV. Three expert readers manually selected regions of interest in tumor and normal-appearing white matter on CBV maps. Receiver operating characteristics (ROC) curves associating nCBV with 15-month OS were calculated for both GN-nCBV and WN-nCBV. Reproducibility and interoperator variability were compared using within-subject coefficient of variation (wCV) and intraclass correlation coefficients (ICCs). **RESULTS:** GN-nCBV ICC (≥0.82) and wCV (≤21%) were superior to WN-nCBV ICC (0.54-0.55) and wCV (≥46%). The area under the ROC curve analysis demonstrated both GN-nCBV and WN-nCBV to be good predictors of OS, but GN-nCBV was consistently superior, although the difference was not statistically significant. **CONCLUSION:** GN-nCBV has a slightly better association with clinical gold standard OS than conventional WM-nCBV in our glioblastoma patient cohort. This equivalent or superior validity, combined with the advantages of higher reproducibility, lower interoperator variability, and easier automation, makes GN-nCBV superior to WM-nCBV for clinical and research use in glioma patients. We recommend widespread adoption and incorporation of GN-nCBV into commercial dynamic susceptibility contrast processing software.

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[^3]: These authors contributed equally to this work.

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Introduction
Dynamic susceptibility contrast (DSC) MRI estimates of brain tumor cerebral blood volume (CBV) reflect tumor vascularity and neangiogenesis [1], are predictive of glioma grade and survival [2–9], and aid in assessment of treatment response [10,11] and differentiation of pseudoprogression from true tumor progression [12,13]. DSC detects the transient decrease in signal intensity (ΔSI) on continuously acquired echo-planar T2 or T2*–weighted images caused by passage of bolus gadolinium contrast through the brain capillaries. Integrating the area under the transverse relaxation difference (ΔR2 or ΔR2*) curve derived from the ΔSI curve yields the CBV for each voxel [14].

In addition to the number, size, and distribution of vessels within each voxel, CBV estimates vary with intravascular concentration, dispersion, delay, flow rate, choice of acquisition parameters [gradient vs spin-echo, repetition time (TR), echo time (TE), flip angle, contrast agent, contrast dose, leakage-reduction preload etc.] and choice of postprocessing algorithm that may or may not include gamma-variate fitting [15], baseline subtraction [16], and leakage correction [10,17]. To compensate, white matter normalization (WN) is typically performed whereby tumor CBV is divided by the mean CBV in a region of interest (ROI) selected manually in the contralateral normal-appearing white matter (NAWM), yielding a unitless “normalized CBV” (nCBV) ratio [14,17–20].

The few published studies suggest substantial coefficient of variation (CV) in NAWM ROI CBV measurements including test-retest CV of 12%–14% in healthy volunteers [21] and an interscanner CV of 25%–30% in glioma patients [22]. The roughly 20% variation in NAWM measurements is likely responsible for a substantial part of the intra- and interobserver CV of 30%–41% reported in white matter normalized brain lesion nCBV [18]. Methods proposed to reduce this variation in CBV estimates include standardization [23], Z-score normalization [22], and Gaussian normalization (GN) [22]. In GN, the tumor ROI CBV is normalized to the standard deviation (SD) of CBV throughout the whole brain rather than ROI measurements of NAWM CBV. GN eliminates completely the subjectivity of NAWM ROI selection, reduces operator time, makes automation simpler, and has been reported to provide the lowest CV in NAWM and the highest ROI selection, reduces operator time, makes automation simpler, and has been reported to provide the lowest CV in NAWM (SD) of CBV throughout the whole brain rather than ROI measurements of NAWM CBV. GN eliminates completely the subjectivity of NAWM ROI selection, reduces operator time, makes automation simpler, and has been reported to provide the lowest CV in NAWM and the highest ROI selection, reduces operator time, makes automation simpler, and has been reported to provide the lowest CV in NAWM and the highest ROI selection, reduces operator time, makes automation simpler, and has been reported to provide the lowest CV in NAWM.

Although GN decreases CV in NAWM, its effect on tumor nCBV estimates has not been studied in detail. Whether glioma GN-nCBV is as valid as current standard WM-nCBV remains to be established [24]. To address this, we compared GN-nCBV association with OS of NAWM CBV. GN eliminates completely the subjectivity of NAWM ROI selection, reduces operator time, makes automation simpler, and has been reported to provide the lowest CV in NAWM and the highest tumor contrast for glioblastoma (GBM) [21].

Methods
Human Subjects
The study was in compliance with the Health Insurance Portability and Accountability Act and approved by our institutional review board. Informed consent was waived for this retrospective study.

For this study, 51 patients were retrieved from a database of adult glioma patients newly diagnosed between 2006 and 2011 (28 men, 23 women; age mean: 56.6 years and range: 23–87 years). All patients had WHO grade IV glioblastoma, known survival, and SE-EPI DSC acquired after maximal surgical resection and before radiation therapy.

Data Acquisition
Thirty-five patients underwent 1.5-T MRI and 16 patients underwent 3-T MRI on whole body MRI scanners (GE Medical Systems, Milwaukee, WI). Axial DSC was performed utilizing a series of SE-EPI images (scan parameters: 1900-2000 milliseconds TR/80 milliseconds TE, 128*128 matrix size; 10-mm slice thickness; 40 time points) acquired 10 seconds prior to, during, and after intravenous administration of gadopentetate dimeglumine (Magnevist, Bayer Healthcare) with a power injector at a rate of 4 ml/s, followed by 20-ml saline flush. Double-dose (0.2 mmol/kg) contrast was used if acquisition was performed with 1.5 T, while one and half dose (0.15 mmol/kg) was used for 3 T, with a maximum 30 ml.

Image Analysis
The dynamic source images were visually inspected for to exclude datasets with substantial patient motion. No substantially motion-degraded scans were detected. DSC analysis was performed using the Func tool software package on Advantage Window workstation (GE Medical Systems). A lower threshold was manually adjusted to remove the background noise. All pixels with lower intensity than the threshold were removed. The remaining pixels define the brain volume used to calculate whole brain SD for GN. The beginning and end of the bolus passage were defined on the time-intensity curve to set the integration range for calculation of CBV maps.

Three readers (clinical radiologists with 15, 5, and 5 years of experience, respectively) independently selected tumor and NAWM ROIs directly on the resulting CBV maps. Each reader picked three tumor ROIs on the high CBV spots and another three ROIs on the contralateral NAWM. The radius of these ROIs was 1-2 image pixels (2-4 mm). Because it has been previously shown that the maximum or mean CBV of several tumor ROIs generates better intra- and interobserver reproducibility than a single ROI and that the maximum is slightly better than the mean [18], we calculated both the maximum and the mean of the three tumor ROIs. For NAWM, the mean of the three ROIs was calculated and used for normalization of the measured tumor CBV values.

To assess the influence of variation in ROI size on NAWM estimates, one of the three readers (reader 2) picked one large ROI (radius = 10 mm) on NAWM for each patient, and the mean CBV inside the ROI was recorded for normalization. This second evaluation was performed more than 2 months later than the first evaluation to avoid recall bias. A fourth reader (with clinical experience of 14 years) picked a single large ROI directly on the resulting CBV maps. Each reader picked three tumor ROIs on the high CBV spots and another three ROIs on the contralateral NAWM, yielding a unitless “normalized CBV” (nCBV) ratio [14,17–20]. Methods proposed to reduce this variation in CBV estimates include standardization [23], Z-score normalization [22], and Gaussian normalization (GN) [22]. In GN, the tumor ROI CBV is normalized to the standard deviation (SD) of CBV throughout the whole brain rather than ROI measurements of NAWM CBV. GN eliminates completely the subjectivity of NAWM ROI selection, reduces operator time, makes automation simpler, and has been reported to provide the lowest CV in NAWM and the highest tumor contrast for glioblastoma (GBM) [21].

Although GN decreases CV in NAWM, its effect on tumor nCBV estimates has not been studied in detail. Whether glioma GN-nCBV is as valid as current standard WM-nCBV remains to be established [24]. To address this, we compared GN-nCBV association with OS of the measured tumor CBV values.

Conventional NAWM ROIs are selected to exclude tumor, but whole brain SD used in GN includes tumor. To examine whether the presence of variable amounts of tumor introduces significant variation between patients in whole brain SD, we selected 10 patients with the largest volume of enhancing tumor in this cohort. One investigator manually contoured the ROIs slice by slice on both contrast-enhanced pGd-T1WI and fluid-attenuated inversion recovery T2WI...
small ROI measurements were used in building mixed-effects models. After the two sets of ROIs were copied to the aligned CBV maps, the volume of enhancing and nonenhancing tumor in (FLAIR-T2). The percentage change in SD resulting from exclusion of the tumor ROI was calculated as:

\[ SD \text{ change} = \frac{(\sigma_{CBV \text{ Whole Brain enhancing}} - \sigma_{CBV \text{ Whole Brain nonenhancing}})}{\sigma_{CBV \text{ Whole Brain}}} \times 100\% \] (2A)

Or

\[ SD \text{ change} = \frac{(\sigma_{CBV \text{ Whole Brain FLAIR nonenhancing}} - \sigma_{CBV \text{ Whole Brain}})}{\sigma_{CBV \text{ Whole Brain}}} \times 100\% \] (2B)

Because the ROI size subanalysis demonstrated that the small NAWM ROI mean CBV was similar to the large NAWM ROI CBV (see results section), only small ROI NAWM estimates were used for nCBV normalization. Twelve nCBVs in total were generated: 

\[ nCBV_{\text{mean}} = \frac{\text{tumor } CBV_{\text{mean}}}{\text{NAWM } CBV_{\text{mean}}}, \quad nCBV_{\text{mean}} = \frac{\text{tumor } CBV_{\text{max}}}{\text{NAWM } CBV_{\text{max}}}, \quad nCBV_{\text{mean}} = \frac{\text{tumor } CBV_{\text{mean}}}{\text{NAWM } CBV_{\text{mean}}}, \quad nCBV_{\text{max}} = \frac{\text{tumor } CBV_{\text{max}}}{\text{NAWM } CBV_{\text{max}}}, \text{ where } i = \text{reader 1,2,3.} \]

**Statistical Analysis**

Tumor CBV_{\text{mean}}, Tumor CBV_{\text{max}}, NAWM CBV_{\text{mean}} inside the selected ROIs, nCBV_{\text{mean}} and nCBV_{\text{max}}, measurements were summarized by means and SDs for i = reader 1,2,3. Pairwise comparisons among readers in these outcome measurements were summarized by mean differences and corresponding standard errors (SEs) and assessed by paired t tests, separately. For reader 2 who selected both NAWM large ROIs and small ROIs, a paired t test was also used to evaluate the effect of ROI size. Mixed-effects models were built for each outcome variable with readers as fixed effects to evaluate differences among the three readers and subjects as random effects to account for correlation among measurements from the three readers on the same subject. Only the small ROI measurements were used in building mixed-effects models.

The between-subject standard deviation (bSD), within-subject standard deviation (wSD), repeatability coefficient (RC), within-subject coefficient of variation (wCV), intraclass correlation coefficient (ICC), and concordance correlation coefficient (CCC) were calculated based on variance components of the aforementioned mixed-effects models [25] to compare repeatability across readers.

Bland-Altman plots provided an intuitive methodology using the concept of limits of agreement for assessing agreement between two readers. Lastly, we calculated the receiver operating characteristics (ROC) curves to associate nCBV measurements with 15-month overall survival (OS) based on the reported 12-15 month median post-operative OS in GBM patients [26,27]. We evaluated the strength of association by the estimated area under the ROC curve (AUC). A P ≤ .05 was considered to be statistically significant. Statistics were computed using Stata (Stata v14, StataCorp., College Station, TX), with the exception that CCC was calculated in R-package [28].

**Results**

Summary statistics of reader-specific Tumor CBV_{\text{mean}}, Tumor CBV_{\text{max}}, and NAWM CBV_{\text{mean}} measurements are listed in Table 1 and plotted in Figure 1. All three measured parameters using small ROIs showed

| Reader | ROI Size | Tumor CBV_{\text{mean}} | Tumor CBV_{\text{max}} | NAWM CBV_{\text{mean}} |
|--------|----------|--------------------------|------------------------|------------------------|
| 1‡      | Small (2-4 mm) | 125.19 ± 85.04 P < .002* | 146.61 ± 102.22 P < .001* | 31.41 ± 11.38 P = .04* |
| 2‡      | 135.95 ± 83.92 140.87 ± 86.48 172.00 ± 100.85 27.95 ± 14.95 |
| (1-2)†  | -10.76 ± 5.39 4.73 7.12 19.63 |
| (1-3)†  | -15.68 ± 4.88 5.84 8.24 21.76 |
| (2-3)‡  | -4.91 ± 4.84 7.12 9.34 21.76 |
| 2‡†     | Large (10 mm) | N/A 4.73 7.12 19.63 |
| 4‡      | N/A N/A N/A 36.37 ± 13.70 |
| (2,4)†  | N/A N/A N/A |

* Data are represented as mean ± SD.
† Data are represented as mean ± SE.
‡ Data from reader 2 in big ROIs.
¶ Differences among three readers were assessed by mixed-effects models.
§ The measurements from the two readers differed statistically (paired t tests).

Figure 1. Box plots of NAWM mean CBV measurements among different readers, varying ROI radius size, and the SDs of the brains used for normalization in GN.
statistically significant differences among readers. The differences came largely from reader 1 in Tumor CBVmean and Tumor CBVmax, and between readers 1 and 3 in NAWM CBV mean. The NAWM measurements using large ROI also showed a significant difference between readers 2 and 4. The variations in NAWM mean measurements were similar regardless of ROI size (Table 1). This is illustrated by the data from reader 2 who performed measurements using both small and large ROIs while achieving comparable means and SDs ($P = .80$). This suggests that a larger ROI radius does not offer better precision. Therefore, in order to reduce the overall number of comparisons, when calculating nCBV for testing the effects of normalization, we only used the NAWM CBVmean measured from the small ROI measurements.

![Figure 2](image1)

**Figure 2.** Conventional images and perfusion imaging CBV map demonstrating ROIs manually contoured on contrast-enhanced T1WI and FLAIR-T2WI, respectively, and copied to CBV map.

The substantially smaller variation among patients resulting from $\sigma_{\text{CBVWhole Brain}}$ used in GN compared to manually selected NAWM CBVmean used in WN is illustrated in Figure 1.

![Figure 3](image2)

**Figure 3.** Change (%) in SD of the whole brain CBV as a function of the excluded volume of contrast-enhancing tumor (A) and enhancing and nonenhancing tumor (B).

Table 2. Comparison of nCBV Measurements Derived from NAWM Normalization (WN) Versus GN

| Reader | nCBVmean | nCBVmax |
|--------|----------|---------|
| WN 1*  | 4.04 ± 2.26 | 4.69 ± 2.62 |
| WN 2*  | 4.70 ± 3.09 | 5.58 ± 3.55 |
| WN 3*  | 6.53 ± 4.95 | 7.85 ± 5.67 |
| WN (1-2)* | -0.66 ± 0.33 | -0.88 ± 0.37* |
| WN (1-3)* | -2.50 ± 0.54† | -3.16 ± 0.62† |
| WN (2-3)* | -1.83 ± 0.56† | -2.27 ± 0.63† |
| GN 1†  | 3.99 ± 1.94 | 4.73 ± 2.31 |
| GN 3†  | 4.12 ± 1.92 | 5.06 ± 2.33 |
| GN (1-2)† | -0.34 ± 0.12‡ | -0.46 ± 0.15‡ |
| GN (1-3)† | -0.47 ± 0.15‡ | -0.80 ± 0.21‡ |
| GN (2-3)† | -0.13 ± 0.16† | -0.34 ± 0.22‡ |

* Data are represented as mean ± SD.
† Data are represented as mean ± SE.
‡ nCBV measurements between two readers were significantly different (paired $t$ test).
§ Differences among three readers were assessed by mixed-effects models.

A value $<0.40$ was considered poor, values between 0.40 and 0.59 were considered fair, values between 0.60 and 0.74 were considered good, and values $>0.75$ was considered excellent.

| Reader | ICC | CCC | RC | wCV | bSD | wSD |
|--------|-----|-----|----|-----|-----|-----|
| TN 1*  | 0.93 | 0.92 | 62.60 | 16.87% | 82.10 | 22.60 |
| TN 2*  | 0.91 | 0.90 | 83.43 | 18.86% | 96.57 | 30.12 |
| TN (1-2)* | 0.66 | 0.66 | 20.94 | 25.11% | 10.66 | 7.56 |
| TN (1-3)* | 0.54 | 0.48 | 6.82 | 48.39% | 2.64 | 2.46 |
| TN (2-3)* | 0.55 | 0.48 | 7.74 | 46.28% | 3.06 | 2.80 |
| N 1†  | 0.86 | 0.85 | 1.99 | 18.49% | 1.79 | 0.72 |
| N (1-2)† | 0.82 | 0.79 | 2.74 | 21.10% | 2.08 | 0.99 |

* Data are represented as mean ± SD.
† Data are represented as mean ± SE.
‡ nCBV measurements between two readers were significantly different (paired $t$ test).
§ Differences among three readers were assessed by mixed-effects models.
but 1 of the 10 patients analyzed. In the outlying patient with tumor occupying 12% of the brain, the SD changed by only 2.5%-3%. This analysis shows that variation in tumor volume has minimal effect on SD even in the worst case where tumor involves a very large fraction of brain.

Table 2 summarizes the statistical analysis of the GN-ncCBV and WM-ncCBV estimates. Both nCBV\textsubscript{mean} and nCBV\textsubscript{max} measurements obtained from WN differed significantly among the three independent readers ($P < 0.001$). The difference is largely attributable to reader 3 whose measurements were significantly higher than readers 1 and 2. GN of nCBV measurements did not resolve the significant differences among the three readers for either nCBV\textsubscript{mean} or nCBV\textsubscript{max} measurements ($P = 0.004$ and $P < 0.001$, respectively) but did substantially decrease the SEs of each pairwise comparison.

Reproducibility metrics obtained for Tumor CBV\textsubscript{mean}, Tumor CBV\textsubscript{max}, and NAWM CBV\textsubscript{mean} and nCBV measurements among the three readers are listed in Table 3. Tumor CBV\textsubscript{mean}, Tumor CBV\textsubscript{max}, and nCBV measurements derived from GN all had an excellent ICC value ($\geq 0.82$) and CCC ($\geq 0.79$). NAWM CBV\textsubscript{mean} measurements had good ICC and CCC values both of 0.66. On the other hand, nCBV measurements derived from WN had a fair ICC value between 0.54 and 0.55 and CCC of 0.48. The RC of the absolute measured values on CBV maps was relatively high, with Tumor CBV\textsubscript{max} RC of 83.43 and NAWM

![Figure 4](image)

**Figure 4.** Bland-Altman scatter plots of tumor and NAWM mean CBV. The dotted lines show the 95% confidence interval. Y-axis is the difference between the two readers' measurements.

![Figure 5](image)

**Figure 5.** Bland-Altman scatter plots of manual ROI normalized and Gaussian normalized nCBV between readers. The dotted lines show the 95% confidence interval. Y-axis is the difference between the two readers' measurements.
CBVmean RC of 20.94. But because RC measures the within-subject variance, it is expected to be relatively high for high mean values. The RC of normalized nCBV was smaller using GN compared to WN, demonstrating a better reproducibility of using GN. The wCV, already scaled by the mean, can be used to compare across all measurement types. The Tumor CBVmean, Tumor CBVmax, and wCV were less than 20%, scaled by the mean, can be used to compare across all measurement types. The ICC between readers supports this, revealing excellent Tumor CBV ICC between readers (0.93) and Tumor CBVmax ICC (0.91) (Table 3) but relatively poor NAWM CBV mean ICC (0.66.). WN markedly decreases tumor CBV ICC from 0.93 to 0.54 for nCBVmean and nCBVmax, nearly identical to their published “average” CV of 30% for nCBVmax and 35% for nCBVmean. For NAWM CBV, our “average” CV of 23% accords well with the 20% in that report. As an operator-independent statistical method, GN is completely reproducible within individual patients. The σCBVWhole Brain used for GN is very robust and reliable (Figure 3). It has substantially smaller interpatient variation than NAWM CBV regardless of reader or NAWM ROI size (Figure 1), which may be an important advantage for use in clinical trial grouped analyses.

Sources of Variation

Tumor CBV measured by reader 1 was significantly different from reader 2 (P = .004) and reader 3 (P = .002) (Table 1). While neither WN nor GN eliminated interreader variation completely (Table 2), SD is much lower (1.9 vs 2.3-5.0) in the GN-nCBV data, suggesting that the variation in NAWM ROI selection is responsible for a substantial degree of interoperator variation in nCBV. Analysis of ICC between readers supports this, revealing excellent Tumor CBVmean (0.93) and Tumor CBVmax (0.91) ICC (Table 3) but relatively poor NAWM CBVmean ICC (0.66.). WN markedly decreases tumor CBV ICC from 0.93 to 0.54 for nCBVmean and 0.91 to 0.55 for nCBVmax. Normalization inevitably decreases ICC somewhat by combining measurement error from the denominator (NAWM CBVmean or σCBVWhole Brain) with that of the numerator (Tumor CBVmean or Tumor CBVmax), but this marked decrease produced by WN contrasts with the relatively slight ICC decrease (GN-nCBVmean 0.86 and GN-nCBVmax 0.82), providing further evidence that NAWM ROI variability is the primary source of nCBV variation (Table 3).

Table 4. AUC Correlating nCBV with 15-Month OS

| Reader | AUC (SE) | nCBVmax | nCBVmax |
|--------|----------|---------|---------|
|        |          | WN      | GN      | WN      | GN      |
| 1      | 0.60 (0.08) | 0.67 (0.08) | 0.62 (0.08) | 0.69 (0.08) |
|        | P = .25   | P = .03 | P = .16 | P = .01 |
| 2      | 0.67 (0.08) | 0.74 (0.08) | 0.67 (0.08) | 0.71 (0.07) |
|        | P = .03   | P = .002 | P = .02 | P = .004 |
| 3      | 0.70 (0.07) | 0.71 (0.07) | 0.72 (0.07) | 0.75 (0.07) |
|        | P = .008  | P = .003 | P = .003 | P = .001 |
| Mean   | 0.68 (0.08) | 0.73 (0.07) | 0.70 (0.07) | 0.73 (0.07) |
|        | P = .02   | P = .002 | P = .007 | P = .001 |

P values evaluate the differences of the estimated AUC to 0.5.
Possible explanations for the observed lower reproducibility of NAWM ROI measurements compared to tumor ROI measurements include larger degree of freedom in selection of NAWM ROI compared to tumor ROI and greater impact of intravoxel noise and partial volume averaged blood vessels and gray matter on lower NAWM CBV estimates compared with higher tumor CBV estimates. We varied the size of NAWM ROI to test the effect of differences in partial volume averaging and voxel SNR but detected no significant difference in NAWM mean or SE between large and small ROI (Table 1).

Weaknesses

The major weakness of our analysis is that the nCBV ROC AUC in our cohort is lower than previously reported AUC of 0.86 and 1.0 for all gliomas and pure astrocytic tumors respectively [1]. In part, this difference likely reflects the larger sample size and more heterogeneous mix of tumor histologies and treatments in our patient group. As such, 0.7 may be a more realistic estimate of the diagnostic value of nCBV in a typical mixed clinical practice. Also, we chose not to use leakage correction methods for this analysis in order to avoid introducing additional computational model complexity and/or sources of variation. This may have contributed to the lower AUCs observed.

Genetic differences between tumors were not considered in this study. Many known genetics tumor markers reflect differences in tumor biology that affect the patient’s OS, including IDH-1 mutation and MGMT methylation, among others. In addition, although all subjects received standard of care consisting of maximal resection followed by Stupp protocol temozolomide chemoradiation, many subsequently underwent different experimental treatments on trial. None of these experimental treatments has been proven to improve OS, but it is possible that variations in therapy may have affected survival in some patients. This genetic and treatment heterogeneity in our dataset may have lowered the overall AUC for nCBV association with OS in our dataset, but the observed association with OS nevertheless remains substantial. Since our analysis was designed to validate the newer more reproducible and automatable GN method of CBV analysis and compare it to the existing standard white matter normalization method, rather than to investigate the absolute strength of nCBV association with OS per se, this should not affect our conclusions. In other words, because the same test population with the identical perfusion and survival data was used for both the GN and conventional WN analyses, the heterogeneity should not introduce any bias into our comparison between these normalization methods. From a clinical translation point of view, this heterogeneous population is advantageous. Since it closely simulates a typical clinical population of patients with different tumor genetics undergoing different treatments, our results suggest that GN-nCBV should perform robustly in the clinic.

Conclusion

Both brain tumor nCBV maps produced by GN and by current standard manual NAWM ROI normalization correlate strongly with 15-month OS in our newly diagnosed GBM patients, but the GN-nCBV had a consistently stronger association and far lower interoperator and intersubject variability. This slightly better validity and superior reproducibility, combined with computational simplicity and potential for full automation, argue for implementation of fully automated GN in DSC processing software and for clinical and research use of the GN-nCBV maps produced. Implementation of GN by MRI and postprocessing software vendors is needed to allow widespread use of this technique and can be expected to improve patient care.

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