Reproducibility Between Brain Uptake Ratio Using Anatomic Standardization and Patlak-Plot Methods

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The Patlak-plot and conventional methods of determining brain uptake ratio (BUR) have some problems with reproducibility. We formulated a method of determining BUR using anatomic standardization (BUR-AS) in a statistical parametric mapping algorithm to improve reproducibility. The objective of this study was to demonstrate the inter- and intraoperator reproducibility of mean cerebral blood flow as determined using BUR-AS in comparison to the conventional-BUR (BUR-C) and Patlak-plot methods. **Methods:** The images of 30 patients who underwent brain perfusion SPECT were retrospectively used in this study. The images were reconstructed using ordered-subset expectation maximization and processed using an automatic quantitative analysis for cerebral blood flow of ECD tool. The mean SPECT count was calculated from axial basal ganglia slices of the normal side (slices 31–40) drawn using a 3-dimensional stereotactic region-of-interest template after anatomic standardization. The mean cerebral blood flow was calculated from the mean SPECT count. Reproducibility was evaluated using coefficient of variation and Bland–Altman plotting. **Results:** For both inter- and intraoperator reproducibility, the BUR-AS method had the lowest coefficient of variation and smallest error range about the Bland–Altman plot. Mean CBF obtained using the BUR-AS method had the highest reproducibility. **Conclusion:** Compared with the Patlak-plot and BUR-C methods, the BUR-AS method provides greater inter- and intraoperator reproducibility of cerebral blood flow measurement.

Key Words: brain perfusion SPECT; Patlak plot method; brain uptake ratio; reproducibility; cerebral blood flow (CBF)

Several different techniques have been applied for measurement of cerebral blood flow (CBF): xenon-enhanced CT, perfusion CT using contrast agents, MR imaging using arterial spin labeling, SPECT, and PET (1–3). Perfusion CT and xenon-enhanced CT are not in common use, because the former is an invasive technique and the latter is available in only a few institutions. The MR imaging technique for this application requires a high magnetic field and proprietary software. It has not yet become a general examination. On the other hand, nuclear medicine examinations for the quantitative measurement of CBF have been widely performed using SPECT with ^{99m}Tc-hexamethylpropyleneamine oxime, ^{99m}Tc-L,L-ethylcysteinate dimer (^{99m}Tc-ECD), and N-isopropyl-p-iodoamphetamine (^{123}I-IMP) and using PET with ^{15}O-labeling agents. The PET technique using ^{15}O-labeling agents is more accurate than the 3 SPECT agents. However, PET has some limitations in that it cannot be performed without a cyclotron and synthesis device. In addition, the quantitative measurement of SPECT counts is more accurate using ^{123}I-IMP than using other agents; however, ^{123}I-IMP SPECT has the drawback of requiring arterial blood sampling. Conversely, quantitative analysis using ^{99m}Tc-labeling agents is noninvasive and does not require arterial blood sampling.

For quantitative analysis of CBF using ^{99m}Tc-ECD, the Patlak-plot and brain-uptake-ratio (BUR) methods have been reported (4–10). The Patlak-plot method is performed in quantitative analysis to calculate mean and regional CBF using SPECT data and radionuclide angiography ranges from the vertex to the aortic arch. In addition, the BUR method is applied in quantitative analysis to calculate mean and regional CBF using SPECT data and dynamic thorax data, where mean CBF is calculated from dynamic data before the Lassen process and regional CBF is calculated from SPECT data after the Lassen process (4–6,10).

It has been reported that the Patlak-plot method has mean and regional CBF variations due to differences in intra- and interoperator reproducibility in such steps as the drawing of regions of interest (ROIs) over the aortic arch, brain, and normal basal ganglia; the determination of brain perfusion index; the setting of axes for SPECT image reconstruction; and the selection of slices for normal basal ganglia. Improvements have been proposed (4,10–14).

Mean and regional CBF variations due to the differences in intra- and inter- operator reproducibility have also been reported for the BUR method. Variability has been reported in such steps as the drawing of ROIs over the aortic arch and the normal basal ganglia, the γ-function fitting process for time–activity curves, the setting of axes for SPECT image reconstruction, and the selection of slices for normal basal ganglia. Improvements have also been proposed for this method (4,10,11,15–17). These quantitative analysis errors may decrease diagnostic accuracy during follow-up and when determining the need for treatment adaptation. Therefore, achieving a reproducible CBF is important to maintaining diagnostic accuracy.

Takaki et al. reported that regional CBF reproducibility was improved by the use of SPECT images anatomically standardized with a statistical parametric mapping algorithm to automate the selection of slice ranges and basal ganglia ROIs in the Lassen process (12). However, anatomic standardization has not been applied to mean SPECT count, which is needed to calculate mean CBF with the BUR method. We defined BUR with anatomic standardization using the statistical parametric mapping algorithm (BUR-AS), which then analyzed mean SPECT count. The BUR-AS method is expected to improve the inter- and intraoperator reproducibility of mean CBF measurements. The objective of this study was to demonstrate this reproducibility for the BUR-AS method in comparison to the conventional BUR (BUR-C) and Patlak-plot methods.

MATERIALS AND METHODS

Subjects

Images for 30 patients (11 men and 19 women; age range, 25–88 y; mean age, 71 y) who underwent brain perfusion ^{99m}Tc-ECD SPECT in 2013 were retrospectively used in this study. These subjects had encephalosis ($n = 1$), Parkinson disease ($n = 6$), moyamoya disease ($n = 2$), degenerative disease ($n = 3$), Alzheimer disease ($n = 4$), dementia with Lewy bodies ($n = 2$), frontotemporal dementia ($n = 1$), internal carotid artery stenosis ($n = 2$), internal artery stenosis occlusion ($n = 3$), dementia ($n = 4$), and cerebral infarction ($n = 1$). One subject had a normal brain. Permission for this study was obtained from the hospital ethics committee.

Acquisition Protocols

^{99m}Tc-ECD imaging was performed using a dual-head SPECT scanner (Infinia3; GE Healthcare). After a bolus injection of 600 MBq of ^{99m}Tc-ECD into the right brachial vein, radionuclide angiography from the vertex to the aortic arch was performed for 2 min (1 s per frame; matrix, 128 × 128; zoom factor, 1.0; pixel size, 4.42 mm) using 1 of the 2 detectors equipped with a low-energy high-resolution parallel-hole collimator and a 140-keV ± 10% energy window. The SPECT study was performed using the same collimators and energy window. Projection data were acquired with a 64 × 64 matrix (zoom factor, 2.0; pixel size, 4.42 mm) continuously over 360° in 4° steps for 5 rotations at 4 min per rotation.

Patlak-Plot Method

The Patlak-plot method used manual processing with Xeleris (version 3.0; GE Healthcare). ROIs were drawn manually over the aortic arch and bilaterally over the brain hemispheres on sequential radionuclide angiography images, and a time–activity curve was generated. Then, we determined the brain perfusion index and calculated mean CBF using regression Equation 1 based on the ^{133}Xe method (18,19):

$$\text{mean CBF} = 2.59 \times \text{BPI} + 19.8. \qquad \text{Eq. 1}$$

BUR Methods

The BUR-C method used manual processing with Xeleris. A time–activity curve to calculate the area under the curve (AUC) was obtained by manually drawing an ROI over the aortic arch on radionuclide angiography images. The time–activity curve was fitted with the γ-function. The AUC was divided by the ROI area and converted to counts/cm^2. SPECT images were reconstructed using the manual axis setting with ordered-subset expectation maximization. Reconstruction used 6 subsets, 8 iterations, and a Butterworth filter (order, 8; cutoff frequency, 0.49 cycle/cm). Attenuation was

FIGURE 1. The 12 ROIs standardized using 3DSRT: callosomarginal, precentral, central, parietal, angular, temporal, posterior cerebral, pericallosal, lenticular nuclear, thalamic, hippocampal, and cerebellar.
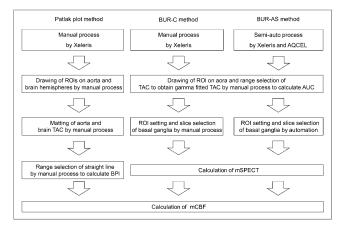
FIGURE 2. Flowchart for Patlak-plot, BUR-C, and BUR-AS methods. Patlak and BUR-C include manual processing. BUR-AS includes manual processing for aortic ROI and time–activity curve range and automatic processing for basal ganglia ROI and slice selection. mCBF and mSPECT = mean CBF and SPECT counts, respectively; TAC = time–activity curve.

corrected using the Chang method (attenuation coefficient, 0.09 cm^{-1}; threshold, 13%) (20); however, scatter correction was not performed. Basal ganglia slices were selected from transverse images of the manual axis setting, and ROIs to calculate the mean SPECT count were drawn manually on the normal side. Mean SPECT count was calculated using Equation 2:

$$\text{mean SPECT count} = \frac{\text{SPECT count in ROI of normal side}}{\text{ROI area (pixels)}}.$$

Eq. 2

The BUR-AS method used semiautomatic processing with Xeleris and AQCEL software (automatic quantitative analysis for cerebral blood flow of ECD tool; Fujifilm RI pharma Co., Ltd.) (12,13,21). AUC was converted to counts/cm^2 using the same process as for the BUR-C method.

The SPECT images were reconstructed by ordered-subset expectation maximization using AQCEL. Image reconstruction and attenuation correction were performed as for the BUR-C method; scatter correction was omitted. Slices 31–40 of the basal ganglia were selected after anatomic standardization (Fig. 1) (12). The ROIs were automatically set on standardized image slices using a 3-dimensional stereotactic ROI template (3DSRT; Fujifilm RI pharma Co., Ltd.). The ROIs comprised 12 segments (callosomarginal, precentral, central, parietal, angular, temporal, posterior cerebral, pericallosal, lenticular nucleus, thalamus, hippocampus, and cerebellum) (22). The mean SPECT count was calculated from the 3DSRT ROIs of slices 31–40 of the normal-side basal ganglia.

Mean BUR-C and BUR-AS were calculated as in Equation 3 using the AUC-converted count/cm^2 and the mean SPECT count:

$$\text{mean BUR} = \frac{\text{mean SPECT} \times A}{\text{AUC} - \text{converted count/cm}^2},$$

Eq. 3

where A is a cross-calibration factor. The BUR-C and BUR-AS mean CBFs were calculated using regression Equation 4 based on the ^{123}I-IMP microsphere method (9):

$$\text{mean CBF} = 13.2 \times \text{mean BUR}^{0.513}.$$

Eq. 4

Figure 2 is a flowchart of the Patlak-plot and BUR-AS methods.

Reproducibility Evaluation

The Patlak, BUR-C, and BUR-AS mean CBFs were analyzed by 3 radiologic technologists. Inter- and intraoperator reproducibility was estimated using the coefficient of variation (CV) and Bland–Altman plotting, with mean CBF obtained for each of the 3 technologists. To estimate intraoperator reproducibility, the technologists were analyzed 3 times at intervals of more than 1 mo.

Statistical Analysis

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University), which is a graphic user interface for R (version 2.13.0; The R Foundation for Statistical Computing) (23). The mean CBF CVs were analyzed using the Kruskal–Wallis test, and multiple comparisons among the 3 methods were done with post hoc Steel methodology (the nonparametric analog comparable to the Dunnett test). In all analyses, a P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Interoperator Reproducibility

The CVs of the Patlak, BUR-C, and BUR-AS mean CBFs were 0.039, 0.068, and 0.024, respectively (Fig. 3). CVs were significantly lower for the BUR-AS method than for the other methods (Patlak vs. BUR-AS, $P = 0.001$; BUR-C vs. BUR-AS, $P < 0.001$). Differences in mean CBF for the 3 methods are shown for each technologist in Figure 4. The average differences in Patlak, BUR-C, and BUR-AS mean CBFs among the 3 technologists were 0.9, 1.2, and −0.8, respectively. In addition, the limits of agreement were −3.5 to 5.3, −8.1 to 10.4, and −3.8 to 2.3 for Patlak, BUR-C, and BUR-AS, respectively. As a result, BUR-AS had the
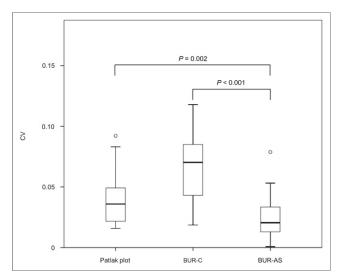

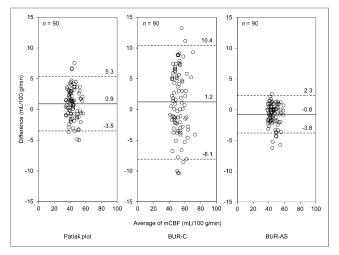**FIGURE 3.** CVs for interoperator reproducibility. CV is significantly lowest for BUR-AS.

FIGURE 4. Bland–Altman plots for interoperator reproducibility. Difference is lowest for BUR-AS.

smallest mean CBF range of the 3 methods. The case of worst reproducibility among the 3 technologists for the BUR-AS method is shown in Figure 5. The ROIs of the aortic arch, the slice selections for the dynamic images, and the range settings of time–activity curve–fitted γ-function differed among the 3 technologists.

Intraoperator Reproducibility

The CVs for the Patlak, BUR-C, and BUR-AS mean CBFs were 0.031, 0.024, and 0.010 for technologist 1; 0.028, 0.020, and 0.016 for technologist 2; and 0.033, 0.035, and 0.022 for technologist 3 (Fig. 6). The BUR-AS method had the lowest mean CBF for all technologists; however, no significant difference was shown between BUR-C and BUR-AS for technologist 2 and between Patlak and BUR-AS for technologist 3 ($P = 0.40$ and 0.057). The difference in each technologist's mean CBF is shown in Figure 7. The average differences in the Patlak, BUR-C, and BUR-AS mean CBFs among the 3 technologists were 0.3, −0.2, and −0.1, respectively. In addition, the limits of agreement were −3.7 to 4.4, −4.3 to 3.9, and −2.6 to 2.5 for the Patlak-plot, BUR-C, and BUR-AS methods, respectively. As a result, BUR-AS mean CBF had the smallest range of the 3 methods.

DISCUSSION

Many institutions have evaluated use of the Patlak-plot and BUR methods to obtain noninvasive quantitative measurements of brain perfusion. Matsuda et al. reported that the CBF obtained from the Patlak-plot method with ^{99m}Tc-hexamethylpropylene amine oxime converts to ^{133}Xe CBF using a regression expression (4). They also reported that the Patlak-plot method CBF obtained with ^{99m}Tc-ECD correlates with that obtained with ^{99m}Tc-hexamethylpropylene amine oxime (6). Miyazaki et al. reported that the CBF obtained from the BUR method using ^{99m}Tc-ECD correlates with that obtained from continuous arterial blood sampling using ^{123}I-IMP (9). In clinical studies, Kuroda et al. reported that CBF and cerebrovascular reactivity derived from quan-

titative analysis of stress and rest brain perfusion ^{99m}Tc SPECT are useful for diagnosis, staging, and treatment of carotid artery occlusion (24–26). However, Otake et al. reported interoperator differences in the manual setting of ROIs on the aortic arch and bilaterally on the brain hemispheres (11). These differences are considered to influence decisions about treatment and evaluation of revascularization therapy for cerebral ischemia. It is for this reason that we proposed and in this paper have validated the BUR-AS method, which improves inter- and intraoperator reproducibility.

Regarding interoperator reproducibility, the BUR-AS method produced the lowest variability in mean CBF. Although all processing in the Patlak-plot and BUR-C methods was manual, the BUR-AS method used automatic processing except for the drawing of the aortic arch ROI and the fitting of the γ-function of the time–activity curve. Manual processing reduces reproducibility; the Patlak-plot and BUR-C methods had worse reproducibility than the BUR-AS method. As far as interoperator reproducibility is concerned, BUR-AS was the best of the 3 methods.

Regarding intraoperator reproducibility, the BUR-AS method produced the lowest variability in mean CBF; however, there was no significant difference from the BUR-AS method for BUR-C for technologist 2 and Patlak for technologist 3. One possible reason for the high intraoperator reproducibility of the Patlak-plot and BUR-C methods is the clear criteria that the technologists had. Another possibility is that the BUR-AS method had analysis errors due to the manual processing steps. Stressing the selection criteria would be expected to improve such problems, and automation of all processing would best improve quantitative accuracy. Variability in mean CBF was lowest with the BUR-AS method (Fig. 7), which we therefore suggest to be the best quantitative approach regarding intraoperator reproducibility.

This study had some limitations. First, selection of the aortic arch ROI and fitting of the γ-function of the time–activity curve were not automated; the BUR-AS method
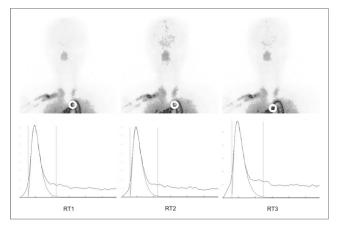

FIGURE 5. Examples of ROI setting and γ fitting by the 3 radiologic technologists (RT1, RT2, and RT3). At top are the dynamic image slices and the chosen ROI; at bottom are graphs of time–activity curves.
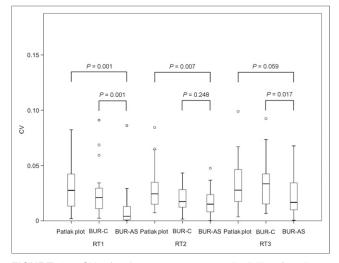
FIGURE 6. CVs for intraoperator reproducibility for the 3 radiologic technologists (RT1, RT2, and RT3). CV was lowest for BUR-AS; CV for BUR-C for RT2 and Patlak plot for RT3 did not significantly differ.

therefore had analysis errors in some cases. Odashima et al. reported improved reproducibility through automation of these functions in the BUR method (*17*). Use of their method may have improved operator reproducibility in our study. Second, our study used anterior images. Inoue et al. found that using 10° left anterior oblique images instead of anterior, along with using an ascending aorta ROI instead of aortic arch, gave a good correlation between the BUR method and ^{123}I-IMP continuous arterial blood sampling (*15*). Moreover, Ito et al. made the same alterations and also found a good correlation between the BUR method and H$_2$^{15}O PET(*16*). Acquisition of the dynamic data in the 10° left anterior oblique view allows separation of the ascending aorta from the descending aorta, allowing the ROI to easily be set on ascending aorta, and would be expected to improve the inter- and intraoperator reproducibility of the BUR-AS method.
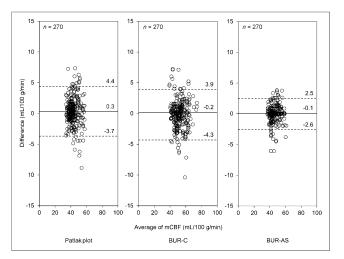

FIGURE 7. Bland–Altman plots for intraoperator reproducibility. Difference is lowest for BUR-AS.

CONCLUSION

We have demonstrated the inter- and intraoperator reproducibility of mean CBF as determined using the Patlak-plot, BUR-C, and BUR-AS methods. The BUR-AS method produced the highest reproducibility. Improvement of inter- and intraoperator reproducibility in quantitative analysis of CBF is expected to increase diagnostic accuracy during follow-up and when determining the need for treatment adaptation.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

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