INTRODUCTION

Alzheimer’s disease (AD) and other neurodegenerative disorders are associated with brain atrophy. Consistent with histologic findings, significant medial temporal lobe or hippocampal atrophy can be detected by structural magnetic resonance imaging (MRI). MRI measurements of brain volumes assess regional brain atrophy and quantify neurodegeneration, thereby enabling the detection of patients with the risk of rapid clinical deterioration.

Software packages available for volumetric brain analysis include the FSL, voxel-based morphometry, FreeSurfer (FS), and NeuroQuant (NQ). NQ was originally designed for the quantification of brain atrophy in AD, and is a fully-automated software approved by the United States Federal Drug Administration for cross-sectional brain volume measurement.
NQ uses high-resolution three-dimensional (3D) T1-weighted volumetric images to automatically provide segmentation-based measurements of cortical and subcortical volumes. NQ also provides normative percentiles of regional brain atrophy by comparing the measured volumes to a normative database adjusted for age, sex, and intracranial volume (ICV). NQ and FS use similar segmentation methods; however, NQ utilizes a different probabilistic atlas, independent codebase, intensity normalization, and gradient distortion correction method to accommodate the scanner-specific acquisition-level differences. Although NQ was introduced clinically for brain atrophy measurement, FS is still regarded as a reference standard for brain volumetry and has been used extensively in research.

In the era of big data, both clinicians and researchers are becoming increasingly aware of the reproducibility problems between different software. Volumetric results can be affected not only by the use of different software, but also by image acquisition conditions such as slice thickness. Previous studies have shown good inter-method reliability of volumetric measurements by NQ and FS for most brain regions, including the hippocampus. However, the effect of 3D T1 slice thickness on brain volumetry has not yet been investigated.

We hypothesized that different slice thicknesses in T1 volume imaging sequence might affect the inter-method reliabilities of different software depending on the structure. We reasoned that this evaluation would be more appropriately tested in participants with clinical mild cognitive impairment (MCI), as both normal healthy controls and persons with advanced stage of AD are the extremes of the spectrum in the context of clinical practice. Accordingly, this study aimed to examine the effect of slice thickness in T1 volume imaging sequence on the inter-method reliability and volumetry of NQ and FS in patients with MCI.

**MATERIALS AND METHODS**

**Study population**
The Institutional Review Board approved this study and waived the requirement for informed consent (IRB number: 2019-08-034, IRB institution: Konkuk University Medical Center) due to the retrospective nature of the study. Patients who underwent brain MRI and were subsequently diagnosed with MCI (n=102) between September 2016 and December 2017 at our memory clinic were considered. MCI was diagnosed according to the operational criteria of Petersen, et al. Patients with insufficient MRI or clinical data were excluded. Two groups were identified based on the type of MR protocol. The case-control matching procedure was used to select 40 age-matched patients from each group. Patients in Group 1 (n=40; female:male=26:14; mean age=71.6±7.0 years; age range=57–85 years) had 1.2 mm thick sagittal T1-weighted MRI, and those in Group 2 (n=40; female:male=25:15; mean age=72.2±6.8 years; age range=57–81 years) had 1 mm thick sagittal T1-weighted MRI.

**Image acquisition**
Routine MRI protocols were obtained with a 3T MR scanner (Discovery MR750+; GE Healthcare, Waukesha, WI, USA) for the following sequences: axial and sagittal T1-weighted inversion recovery imaging [repetition time (TR)/echo time (TE)=2468/12; inversion time=920 ms; section thickness 5 mm; matrix 512×224]; axial T2-weighted fast spin-echo imaging (TR/effective TE=4000/106; section thickness 5 mm; matrix 384×384); axial fluid-attenuated inversion recovery imaging (TR/TE=11000/105; inversion time=2600 ms; section thickness 5 mm; matrix 384×224); and axial T2-weighted gradient-recalled echo imaging (TR/TE=550/17; section thickness 5 mm; matrix 384×224; flip angle 15°). The sagittal T1-weighted volumetric fast spoiled gradient-recalled echo was either TR/TE=5.692/2.36; section thickness 1.2 mm; matrix 192×192; flip angle 8°; field of view (FOV) 240×240 mm for Group 1, or TR/TE=8.224/3.192; section thickness 1 mm; matrix 256×256; flip angle 12°; FOV 250×250 mm for Group 2.

**Volumetric analyses**
The sagittal T1-weighted volumetric images were analyzed with automated segmentation methods. The brain MRI data of each MCI patient were uploaded to the tool's server. The steps of NQ image processing were as follows: stripping the brain of scalp, skull, and meninges; inflating the brain to a spherical shape; mapping the spherical brain to a common spherical space shared with the Talairach atlas coordinates; identification of segmented brain regions; and deflation of the brain back to its original shape. The brain volume was corrected for the head size difference using division by ICV, and was expressed as a percentage. The results were saved in the NQ database. When a patient's brain region fell below the 5th normative percentile, it was classified as abnormally small. The automated tool also provided an age-related atrophy report, with absolute and relative volumes as a percentage of the ICV for hippocampi, lateral ventricles, and inferior lateral ventricles. The total duration for NQ image processing ranged from 10 to 15 minutes.

The FreeSurfer 6.0.0 (http://surfer.nmr.mgh.harvard.edu, Harvard University, Boston, MA, USA) uses a template-driven approach for volumetric- and surface-based segmentation, as previously described. The steps involved in FS image processing were as follows: motion correction, removal of non-brain tissue, automated Talairach transformation, segmentation of the subcortical structures, intensity normalization, tessellation of the gray matter (GM) white matter (WM) boundary, automated topology correction, surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class, surface inflation, parcellation of the cerebral cortex into units...
based on gyrus and sulcus structure, and finally, creation of a variety of surface-based data. The variable “Brain Segmentation Volume Without Ventricles from Surf,” which excludes the brainstem, was used as the FS estimate for brain volume. The variable “Total Gray Matter Volume” was used as the estimate GM volume. The WM volume was obtained by summing “cerebral WM,” “cerebellar WM,” “brainstem,” and “corpus callosum” FS variables. It is notable that FS specifically segments WM hypointensities. The brain, GM, and WM volumes were divided by the “Estimated Total Intracranial Volume” for normalization.21

Statistical analysis
The inter-method reliability was assessed by calculating the intraclass correlation coefficient (ICCs) between NQ volumes and FS volumes using MedCalc, version 19.0.5 (MedCalc Software, Ostend, Belgium). The ICC model was based on two-way mixed effects, absolute agreement, and average measures. The guidelines used for ICC interpretation were as follows:22 ICC >0.9 excellent reliability, 0.75≤ICC<0.9 good reliability, 0.5≤ICC<0.75 moderate reliability, and ICC<0.5 poor reliability.

As a secondary approach, Pearson’s r values were also calculated. Effect size (ESCohen’s d) was used to document the magnitude of differences between the two techniques without any implication of causality. The guidelines used to interpret effect size (ES) values were as follows: small, d=0.2; medium, d=0.5; and large, d=0.8.23

Ethical approval
All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent
The requirement for informed consent was waived due to the retrospective nature of this study.

RESULTS

Basic characteristics of the patients
Table 1 shows the clinicodemographic characteristics of the study patients. Age, sex ratio, WM hyperintensities, and medial temporal lobar atrophy were not significantly different between the two groups. Clinical Dementia Rating and Mini-Mental State Examination (MMSE) scores of the available subjects were significantly different between the groups (p=0.032, <0.001, respectively).

Inter-method reliability
Overall, volume measures by NQ and FS showed a strong posi-
We found that the volume measurements between NQ and FS were excellently correlated, regardless of the slice thickness used. Furthermore, our results showed that systematically measured volumes by NQ were slightly higher than those by FS, and the inter-method reliability was slightly higher for 1 mm thick slices compared to 1.2 mm thick slices.

Neurodegenerative disorders are reliably associated with the patterns of progressive neural atrophy that can be quantified by MRI post-processing techniques. In clinical practice, brain volumetry can characterize disease processes, identify the risk for rapid clinical deterioration, and predict prognosis by providing objective and quantitative evidence. However, different MRI parameters influence the results of volumetric measures. Therefore, in many clinical trials using volumetry as the outcome measure, it is recommended to use the suggested MR sequence and to require careful consideration while interpreting the data using existing methods.

In this study, the volume measurements by NQ and FS showed excellent inter-method reliability for 20 brain regions (ICC=0.72–0.96), except for the total ICV, putamen, pallidum, and thalamus. Our finding was consistent with the findings of Ochs, et al., which reported good-to-excellent inter-method reliability between NQ and FS in 60 subjects for all brain regions except the pallidum and cerebellar WM. Our ICCs between the two volumetric analyses were comparable to the

**DISCUSSION**

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**Table 2. Correlation of Volume Measurements between NeuroQuant and Freesurfer**

| Region                  | Left   |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-------------------------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                         | ES     | r     | ICC   | 95% CI | ES     | r     | ICC   | 95% CI | ES     | r     | ICC   | 95% CI |
| Total intracranial      | 0.05   | 0.36  | 0.45  | 0.15–0.65 | -0.08  | 0.90  | 0.94  | 0.91–0.96 | -0.08  | 0.90  | 0.94  | 0.91–0.96 |
| Whole brain volume      | -0.56  | 0.87  | 0.86  | 0.22–0.95 | -0.52  | 0.85  | 0.86  | 0.37–0.95 |
| Cortical gray matter    | -0.46  | 0.83  | 0.86  | 0.53–0.94 | -0.56  | 0.87  | 0.86  | 0.22–0.95 |
| White matter            | -0.20  | 0.78  | 0.86  | 0.77–0.91 |
| Hippocampus             | 0.51   | 0.83  | 0.84  | 0.43–0.93 | 0.53   | 0.89  | 0.87  | 0.25–0.96 |
| Amygdala                | -0.96  | 0.82  | 0.72  | -0.20–0.91 | 0.13   | 0.84  | 0.90  | 0.85–0.94 |
| Caudate                 | 0.75   | 0.84  | 0.79  | -0.08–0.93 | 0.71   | 0.83  | 0.79  | 0.02–0.93 |
| Putamen                 | -2.56  | 0.68  | 0.26  | -0.10–0.63 | -2.25  | 0.72  | 0.29  | -0.16–0.57 |
| Pallidum                | 6.31   | 0.19  | -0.02 | -0.06–0.07 | 6.35   | -0.25 | -0.02 | -0.06–0.05 |
| Thalamus                | -1.21  | 0.71  | 0.58  | -0.22–0.84 | -1.30  | 0.84  | 0.62  | -0.17–0.88 |
| Cerebellum              | 0.26   | 0.94  | 0.96  | 0.64–0.98 | 0.28   | 0.92  | 0.94  | 0.81–0.97 |

ES, effect size; r, Pearson correlation coefficient; ICC, intraclass correlation coefficient; CI, confidence interval.

**Fig. 2. Percentages of volumes measured by NQ and FS, (NQ–FS)/FS×100%. NQ, NeuroQuant; FS, Freesurfer.**
reliability between human raters (0.73 to 0.85). 24

The poor reliability and very large ES by NQ versus FS measurement of the pallidum was notable and could be explained by the similar intensities of the pallidum and WM in T1-weighted MRIs.17 This finding also corroborated the previous observation by Ochs, et al.13 It is known that FS calculates the volume by including WM between the pallidum and the neighboring putamen; in contrast, NQ uses color mapping images.13 Furthermore, colored segmentation maps of NQ are smoothed and overlaid onto the original grayscale image, whereas the colored map of FS is neither smoothed nor overlaid onto a grayscale image, which might further exacerbate differences in the volume measurements. Fischl, et al.17 reported statistically indistinguishable results between automated FS segmentation and manual segmentation of deep brain structures, which has made FS a status of the gold standard for volumetric measurement. Therefore, we recommend careful interpretation while determining the pallidum volumes by NQ.

We also observed larger volumes by NQ compared to FS in several brain locations, including the whole brain (0.78%), cortical GM (5.34%), and WM (2.68%). A mean ES difference of +0.40 was determined for the individually measured regions. These results were similar to the reports of Ochs, et al.,13 where the whole brain parenchyma volume by NQ was 6.5% larger than that reported by FS, with a mean ES difference of +0.40 for individually measured regions. However, the whole brain volume showed excellent ICC; however, the total ICV showed weaker ICC (0.4–0.6), which was not in line with the previous study.13 We speculate this may have originated from the fundamental errors owned by the software, as ICV is calculated and estimated based on each calculation formula.

Interestingly, different slice thickness (1.2 mm vs. 1 mm) did not affect the final volumetric results, although the ICCs improved slightly with thinner image slices. Furthermore, regardless of the slice thickness used, the volume measurements were consistently higher with NQ as compared to FS.

We opine that our observation of slightly improved ICCs with thinner image slices would have many implications in the near future. Currently, many software vendors recommend the use of rather thick slices (1.2 mm) for clinical practice, instead of using 1 mm, which is a norm of research community.25 To clarify these recommendations, we used the ES to compare the mean difference between the two groups in a standardized manner.26 For instance, while the overall reliability was excellent, the ES of hippocampal measurement with 1.2 mm thick slices was larger (ES=0.64) compared to using 1 mm thick slices (ES=0.40–0.45), which means that the use of 1.2 mm slice thickness is prone to a bigger difference in volume measurement when a volumetry software is switched to another software. Therefore, we recommend careful interpretation of the results of volume measurements using a slice thickness of 1.2 mm, instead of 1.0 mm, in both FS and NQ.

In the context of clinical practice, speed is a major advantage of NQ over FS. NQ saves time by abandoning the intensive com-

| Region                        | With 1 mm slice thickness | With 1.2 mm slice thickness |
|-------------------------------|---------------------------|-----------------------------|
|                              | ES  | r  | ICC | 95% CI    | ES  | r  | ICC | 95% CI    |
| Total intracranial volume     | -0.14 | 0.43 | 0.61 | 0.26–0.79 | 0.15 | 0.37 | 0.41 | -0.12–0.69 |
| Whole brain volume            | -0.19 | 0.96 | 0.97 | 0.91–0.99 | 0.00 | 0.84 | 0.91 | 0.83–0.95 |
| Lt cortical gray matter       | -0.37 | 0.91 | 0.92 | 0.64–0.97 | -0.57 | 0.75 | 0.79 | 0.33–0.91 |
| Rt cortical gray matter       | -0.56 | 0.92 | 0.89 | 0.00–0.97 | -0.57 | 0.81 | 0.82 | 0.27–0.94 |
| Cortical gray matter          | -0.47 | 0.92 | 0.91 | 0.27–0.97 | -0.57 | 0.78 | 0.81 | 0.30–0.92 |
| White matter                  | -0.51 | 0.94 | 0.90 | 0.10–0.97 | -0.02 | 0.66 | 0.78 | 0.59–0.89 |
| Lt hippocampus                | 0.40 | 0.88 | 0.87 | 0.62–0.95 | 0.64 | 0.80 | 0.80 | 0.16–0.93 |
| Rt hippocampus                | 0.45 | 0.92 | 0.91 | 0.38–0.97 | 0.64 | 0.84 | 0.82 | 0.09–0.94 |
| Lt amygdala                   | -1.00 | 0.89 | 0.75 | -0.16–0.93 | -0.93 | 0.79 | 0.68 | -0.16–0.88 |
| Rt amygdala                   | 0.04 | 0.85 | 0.90 | 0.82–0.95 | 0.25 | 0.85 | 0.90 | 0.79–0.95 |
| Lt caudate                    | 0.73 | 0.84 | 0.78 | -0.18–0.94 | 0.77 | 0.85 | 0.78 | 0.11–0.93 |
| Rt caudate                    | 0.64 | 0.87 | 0.84 | 0.03–0.95 | 0.82 | 0.75 | 0.69 | -0.06–0.88 |
| Lt putamen                    | -2.34 | 0.73 | 0.26 | -0.07–0.64 | -2.57 | 0.69 | 0.27 | -0.09–0.65 |
| Rt putamen                    | -2.30 | 0.79 | 0.32 | -0.10–0.70 | -2.27 | 0.78 | 0.36 | -0.08–0.74 |
| Lt pallidum                   | 7.14 | 0.06 | 0.00 | -0.02–0.04 | 6.25 | 0.31 | 0.02 | -0.06–0.06 |
| Rt pallidum                   | 7.28 | 0.06 | 0.00 | -0.02–0.04 | 6.28 | 0.33 | 0.02 | -0.06–0.07 |
| Lt thalamus                   | -1.05 | 0.76 | 0.66 | -0.21–0.89 | -1.42 | 0.65 | 0.47 | -0.23–0.78 |
| Rt thalamus                   | -1.34 | 0.84 | 0.60 | -0.16–0.88 | -1.35 | 0.82 | 0.59 | -0.17–0.87 |
| Lt cerebellum                 | 0.23 | 0.94 | 0.96 | 0.86–0.98 | 0.27 | 0.95 | 0.96 | 0.78–0.98 |
| Rt cerebellum                 | 0.23 | 0.90 | 0.93 | 0.83–0.97 | 0.33 | 0.94 | 0.93 | 0.67–0.98 |

ES, effect size; r, Pearson correlation coefficient; ICC, intraclass correlation coefficient; CI, confidence interval; Lt, left; Rt, right.
putation process of FS for parcellation of the cerebral cortex. As a result, NQ outputs the overall volumes of cerebral GM and WM, whereas FS calculates the individual volume and thickness measurements of virtually every cerebral gyrus.\textsuperscript{13} NQ processing takes approximately 10 minutes in a conventional desktop computer, and the only input is the study to be segmented. NQ can interact directly with a PACS server, or can be configured as a remotely hosted web server. The final report provides the volumes of structures in cubic centimeters and the ICVs as percentage. A normative range is provided for the hippocampus, lateral ventricle, and temporal horn of the lateral ventricle, based on previously segmented healthy subjects aged 50–95 years.\textsuperscript{1} In contrast, FS analysis takes approximately 8 hours with a 2.4 GHz Macintosh computer, and requires knowledge of UNIX programming for analysis.\textsuperscript{13} In our study, NQ took 5–10 minutes while FS took 4–6 hours for analysis.

Since the results of the analysis can be affected by the MRI scanner setting, MRI software, NQ and FS software, and computer hardware, it is ideal to use the same hardware and software for comparison purposes.\textsuperscript{27} In this study, the effect of hardware or software was controlled by using the same volumetric software programs and computer hardware for all patients.

The current study had several limitations. First, as the study used a small sample of patient data from a single tertiary referral hospital, there is potential for selection bias. Second, the MMSE results between two groups differ significantly, which might affect the difference in volume. Third, the repeatability of different MR sequences in the same scanner was not considered. Moreover, different MR scanning parameters might affect the volume measurements in different ways. Lastly, this study lacked biomarkers or neuropsychological assessments; therefore, patient factors, such as disease severity, could have affected the results between groups.

In conclusion, NQ and FS showed excellent inter-method reliability in volumetric measurements of all brain regions, except pallidum, in patients with MCI. The slice thickness might affect the inter-method reliability of volumetric measures, albeit to a very small degree, with thinner slices providing better reliability than thicker slices. The study outcomes could improve the precise interpretation of automated volume measurements in clinical practice. Future studies are warranted to examine specific measures as biological markers in patients with cognitive impairment.

ACKNOWLEDGEMENTS

This study was supported by a 2017 Clinical Practice Guideline Research Fund grant from the Korean Society of Radiology.

AUTHOR CONTRIBUTIONS

Conceptualization: Younghiee Yim and Won-Jin Moon. Data curation: Yeonsil Moon and Hong Jun Jeon. Formal analysis: Younghiee Yim, Yeonsil Moon, and Hong Jun Jeon. Funding acquisition: Won-Jin Moon. Investigation: all authors. Methodology: Younghee Yim, Won-Jin Moon, Ji Young Lee, and Ji Eun Park. Project administration: Younghee Yim. Resources: Yeonsil Moon and Hong Jun Jeon. Software: Younghee Yim. Supervision: Won-Jin Moon. Validation: Ji Young Lee, Se Won Oh, Mi Sun Chung, and Ji Eun Park. Visualization: Younghiee Yim and Won-Jin Moon. Writing—original draft: Younghiee Yim and Won-Jin Moon. Writing—review & editing: Younghiee Yim, Ji Young Lee, Se Won Oh, Mi Sun Chung, Ji Eun Park, and Yeonsil Moon. Approval of final manuscript: all authors.

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