DIAGNOSTIC NEURORADIOLOGY

Cerebral bone subtraction CT angiography using 80 kVp and sinogram-affirmed iterative reconstruction: contrast medium and radiation dose reduction with improvement of image quality

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Abstract

Introduction The purpose of this study was to evaluate the feasibility of a contrast medium (CM), radiation dose reduction protocol for cerebral bone-subtraction CT angiography (BSCTA) using 80-kVp and sinogram-affirmed iterative reconstruction (SAFIRE).

Methods Seventy-five patients who had undergone BSCTA under the 120- $(n = 37)$ or the 80-kVp protocol $(n = 38)$ were included. CM was 370 mgI/kg for the 120-kVp and 296 mgI/ kg for the 80-kVp protocol; the 120- and the 80-kVp images were reconstructed with filtered back-projection (FBP) and SAFIRE, respectively. We compared effective dose (ED), CT attenuation, image noise, and contrast-to-noise ratio (CNR) of two protocols. We also scored arterial contrast, sharpness, depiction of small arteries, visibility near skull base/clip, and overall image quality on a four-point scale.

Results ED was 62% lower at 80- than 120-kVp (0.59 ± 0.06) vs 1.56 ± 0.13 mSv, $p < 0.01$). CT attenuation of the internal carotid artery (ICA) and middle cerebral artery (MCA) was significantly higher on 80- than 120-kVp (ICA: 557.4 ± 105.7 vs 370.0 ± 59.3 Hounsfield units (HU), $p < 0.01$; MCA: 551.9 \pm 107.9 vs 364.6 \pm 62.2 HU, p < 0.01). The CNR was also significantly higher on 80- than 120-kVp (ICA: 46.2 ± 10.2 vs 36.9 ± 7.6 , $p < 0.01$; MCA: 45.7 ± 10.0 vs 35.7 ± 9.0 , $p < 0.01$). Visibility near skull base and clip was not significantly different ($p = 0.45$). The other subjective

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scores were higher with the 80- than the 120-kVp protocol $(p < 0.05)$.

Conclusion The 80-kVp acquisition with SAFIRE yields better image quality for BSCTA and substantial reduction in the radiation and CM dose compared to the 120-kVp with FBP protocol.

Keywords Angiography . Computed tomography . Image processing . Radiation protection . Intracranial aneurysm

Introduction

Technical advances have rendered cerebral CT angiography (CTA) an accurate and useful imaging modality for the evaluation of intracranial aneurysms [\[1](#page-6-0)]. Although 3D digital subtraction angiography (3D-DSA) remains the reference standard [[2\]](#page-6-0), it is time consuming, technically demanding, invasive, and has a 0.12–0.5% rate of permanent neurologic complication [[3](#page-6-0)–[5](#page-6-0)]. As a non-invasive imaging modality, MR angiography (MRA) is more expensive and less available than CTA, requires long scanning times (which could cause motion artifacts, especially in settings of acute subarachnoid hemorrhage (SAH)), and has limitations in the evaluation of clipped aneurysms because of susceptibility artifacts [\[6](#page-6-0), [7](#page-6-0)]. On the other hand, cerebral bone subtraction CTA (BSCTA) is fast, minimally invasive, widely available, and its diagnostic performance is comparable to 3D-DSA for the detection of intracranial aneurysms, even lesions adjacent to the skull base that can be missed by non-subtraction CTA (NSCTA) [[8](#page-6-0)–[10](#page-6-0)]. In addition, compared to NSCTA, it improves the accuracy for detecting aneurysm remnants after clipping surgery [[11](#page-6-0)]. Consequently, BSCTA might be considered a first-line imaging modality for the diagnosis, treatment planning, and

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follow-up of intracranial aneurysms, especially in patients with lesions near the skull base and clip.

However, BSCTA involves substantial radiation exposure because it requires two consecutive (pre- and post-contrast enhanced) scans and the injection of iodinated contrast medium (CM). To minimize the biological effect of ionizing radiation, the radiation dose should be as low as possible without impairing diagnostic performance [[12](#page-6-0)–[14](#page-6-0)]. Especially in patients with unruptured aneurysms or SAH, the cumulative radiation exposure from CT is of concern because these patients tend to be relatively young and may need to be followed long term [\[15](#page-6-0)–[17](#page-6-0)]. A reduction in the CM dose may be also desirable to decrease the risk of CM-induced nephropathy (CIN) in patients with renal dysfunction [\[18](#page-6-0), [19](#page-6-0)], because they may have to receive additional intra-arterial CM administrations for subsequent invasive DSA examination and endovascular therapy.

Low tube voltage and iterative reconstruction (IR) techniques effectively reduce the radiation and the CM dose, because the radiation dose is proportional to the square of the tube voltage and the attenuation of iodine CM is increased at lower tube voltages since the mean photon energy of the X-ray is closer to the iodine K-edge (33 keV). Although the image noise increases because the X-ray spectrum is less penetrable, it can be lowered by using IR instead of filtered back-projection (FBP) algorithms. The usefulness of these techniques for various diagnostic tasks has been documented [[20](#page-6-0), [21\]](#page-6-0).

Several studies indicated the usefulness of the low tube voltage technique for cerebral CTA [[22](#page-6-0)–[28](#page-7-0)]. Among these, only one study [[27\]](#page-6-0) combined a low tube voltage with IR technique to reduce the radiation dose at NSCTA. However, our search of the literature found no reports that examined the feasibility of combining a low tube voltage with an IR to obtain a reduction in not only the radiation but also the CM dose. In addition, the image quality of subtraction images acquired at low tube voltage with the use of IR also remains to be assessed. Therefore, the purpose of this study was to evaluate the image quality yielded by a radiation and CM dose reduction protocol using 80 kVp and sinogram-affirmed iterative reconstruction (SAFIRE; Siemens Healthineers, Forchheim, Germany), a second-generation IR algorithm, for cerebral bone subtraction CTA.

Materials and methods

Patients

examination was performed for the suspicion, ruling out, or follow-up of intracranial aneurysms as the standard of care in our institution between November 2014 and October 2015. The standard 120-kVp protocol ($n = 44$) was performed until April 2015, and the 80-kVp protocol $(n = 46)$ was started from May 2015 for the purpose of radiation dose reduction, because national diagnostic reference levels were enacted in 2015 in our country. We excluded patients with severe motion artifacts $(n = 3)$ and patients scanned using CM injection protocols or image reconstruction that differed from our routine 120- or 80 kVp protocols ($n = 12$). Consequently, a total of 75 patients $(120 \text{ kVp}, n = 37; 80 \text{ kVp}, n = 38)$ were included in this study.

Image acquisition and reconstruction for each protocol

All scans were performed on a 128-slice single-source MDCT scanner (Definition AS+; Siemens Healthineers, Forchheim, Germany) and under both protocols; the tube current was modulated by automated exposure control (CARE Dose 4D; Siemens Healthineers, Forchheim, Germany). The 120-kVp scans were performed with 350 quality reference mAs, and the acquired images were reconstructed with FBP (H30f). The 80-kVp scans were performed using automated tube voltage selection (CARE kV; Siemens Healthineers, Germany), which employs reference value of 120 kV and 350 mAs for CTA setting (slider bar 11). That system automatically selected 80 kVp and 469 quality reference mAs for all patients. The acquired 80-kVp images were reconstructed with SAFIRE at strength level 3. We selected this medium IR strength level according to the vendor's recommendation.

Under the 120-kVp protocol, the patients received a CM dose of 370 mgI/kg body weight (BW) over 12 s. We delivered a CM dose of 296 mgI/kg BW over 10 s for the 80-kVp protocol to minimize venous enhancement [[24\]](#page-6-0). This was followed by a 30 ml saline flush performed at the CM injection rate. In all examinations, iopamidol 370 mgI/ml (Iopamiron 370; Nihon Bayer, Tokyo, Japan) was injected via a 20-gauge catheter inserted into an antecubital vein with a power injector (Dual Shot GX-7; Nemoto Kyorindo, Tokyo, Japan). A bolus-tracking technique was used to time the start of scanning after CM injection. A region of interest (ROI) was placed in the right internal carotid artery (ICA). Scanning began 4 s after attenuation reached the pre-defined threshold of 100 Hounsfield units (HU).

BSCTA image data were obtained by subtracting nonenhanced image data from the CTA image data using a 3D workstation (Synapse Vincent, Fuji Film Medicals) equipped with bone subtraction software. The scanning parameters and image reconstruction algorithms for non-enhanced CT scans were the same as for enhanced CT scans to reduce potential misregistration artifacts [\[29](#page-7-0)]. The detailed scanning and reconstruction parameters for each protocol are shown in Table [1](#page-2-0).

Table 1 Scanning parameters for each protocol

QRM quality reference mAs, FBP filtered back-projection, SAFIRE sinogram-affirmed iterative reconstruction

Radiation dose measurement

The volume CT dose index (CTDI_{vol}) and dose length product (DLP) were recorded for each protocol. The estimated effective dose (ED) was calculated by multiplying the DLP by the conversion factor 0.0021 mSv/(mGy×cm) [\[30](#page-7-0)].

Quantitative image analysis

A board-certified radiologist with 9 years of neuroimaging experience performed quantitative image analysis. On axial source images, we placed circular ROIs in the bilateral ICA at the cavernous and in the middle cerebral arteries (MCA) at the M1 segments and in the thalamus. Attempts were made to select ROIs measuring approximately 3.0 and 0.5 mm^2 in the ICA and the MCA, respectively, carefully avoiding the vessel wall or partial volume effects. An ROI of approximately 30 mm2 was placed in the thalamus. Visible blood vessels and focal lesions were carefully excluded from the ROI measurements. To minimize bias from single measurements and to ensure data consistency, the average of the two values was calculated. Image noise was defined as the standard deviation (SD) of attenuation in the thalamus. The contrast-to-noise ratio (CNR) was calculated with the formula

$$
CNR_{\text{ICA}} = (CT
$$
 attenuation_{ICA} – CT attenuation_{thalamus})
/
 $/$
 $CNR_{\text{MCA}} = (CT$ attenuation_{MACA} – CT attenuation_{thalamus})
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Qualitative image analysis

Two board-certified radiologists with 10 and 8 years of neuroimaging experience, respectively, independently graded the image quality yielded by each protocol. They used multiplanar reconstruction (MPR), slab maximum intensity projection (slab-MIP), and volume-rendered (VR) images. The CT data sets were randomized, and the readers were blinded to the acquisition parameters. On a four-point subjective scale, they independently graded arterial contrast $(1 =$ unacceptable for diagnosis, $2 =$ suboptimal but still diagnostic. $3 =$ average, $4 =$ excellent). Vascular sharpness was assessed by evaluating vessel wall sharpness $(1 = blurry, 2 = poorer$ than average, $3 =$ average, $4 =$ sharpest). Visualization of arteries adjacent to the skull base—and, in patients who had undergone clipping surgery, visualization around the clip was also graded $(1 = \text{unacceptable because of severe venous})$ enhancement or artifacts, $2 =$ venous enhancement or artifacts present and partially interfering with depiction, $3 =$ venous enhancement or artifacts present without interfering with depiction, 4 = no venous enhancement or artifact). In addition, they scored the depiction of small arteries $(1 = no$ depiction, 2 $=$ faint depiction, 3 $=$ average depiction, 4 $=$ excellent depiction). Lastly, the overall image quality was graded $(1 = \text{unac-}$ ceptable for diagnosis, $2 =$ suboptimal but still diagnostic, $3 =$ average, 4 = excellent). A training session was provided to both readers before image review to familiarize them with the scoring system. Interobserver disagreement was resolved by the two readers' consensus during joint reading to determine the final score.

Statistical analysis

All numeric values are reported as the mean \pm SD. To compare the patient age, BW, the CM amount and injection rate, the ED, CT attenuation, image noise, and CNR of the two protocols, we used the unpaired Student's t test after normal distribution was confirmed with Kolmogorov–Smirnov test. We compared the male/female ratio and the ratio of patients with and without clipped aneurysm using the chi-squared test. The visual scores assigned to the 120- and the 80-kVp images were compared with the Mann–Whitney U test. Differences of $p < 0.05$ were considered statistically significant. The scale for the kappa coefficients was less than $0.20 =$ poor, $0.21 - 0.40$ $=$ fair, $0.41-0.60$ = moderate, $0.61-0.80$ = substantial, and $0.81-1.00$ = near perfect. Statistical analyses were performed with statistical software (R, version 2.6.1; [www.r-project.](http://www.r-project.org/) [org/\)](http://www.r-project.org/).

Results

Patient characteristics and CM and radiation doses

As shown in Table [2,](#page-3-0) there was no significant difference in the characteristics of the patients scanned with the two protocols $(p = 0.32{\text -}0.56)$. The indications for CTA in the 120-kVp group were suspicion of aneurysmal rupture due to nontraumatic SAH confirmed by non-enhanced CT $(n = 12)$,

Data are shown as the mean \pm standard deviation

N/A not applicable, AN aneurysm

suspicion of aneurysms on MRA $(n=7)$, oculomotor paralysis $(n = 1)$, and follow up of known untreated aneurysm $(n = 8)$ and clipped aneurysm $(n = 9)$. The indications in the 80-kVp group were suspicion of aneurysmal rupture due to non-traumatic SAH confirmed by non-enhanced CT $(n = 11)$, suspicion of aneurysms on MRA $(n = 5)$, and follow up of known untreated aneurysm $(n = 9)$ and clipped aneurysm ($n = 13$). The 120-kVp protocol identified a total of nine clipped aneurysms in 9 patients (ICA, $n = 4$; MCA, $n = 3$; anterior communicating artery (AcomA), $n = 2$); the 80kVp protocol identified a total of 14 (ICA, $n = 5$; MCA, $n = 4$; AcomA, $n = 5$) in 13 patients. By referring to the surgery records, we confirmed that all clips were made from titanium (Sugita clip, Mizuho, Tokyo, Japan). The radiation dose and the total iodine load of the 80-kVp protocol were significantly lower than those of the 120-kVp protocol (ED: 0.59 ± 0.06 vs 1.56 ± 0.13 mSv, $p < 0.01$; CM volume: 42.5 ± 7.7 vs 54.9 ± 11.5 ml, $p < 0.01$) (Fig. 1). The CM injection rate was not significantly different between the two protocols $(4.2 \pm 0.8 \text{ m}$ /s for 80 kVp vs $4.6 \pm 1.0 \text{ m}$ /s for 120 kVp, $p = 0.11$).

Quantitative image analysis

Table 3 summarizes the result of quantitative analysis. Attenuation of the ICA and MCA was significantly higher under the 80- than the 120-kVp protocol (ICA: 557.4 \pm 105.7 vs 370.0 \pm 59.3 HU, $p < 0.01$; MCA: 551.9 \pm 107.9 vs 364.6 \pm 62.2 HU, $p < 0.01$). While image noise was significantly higher on the 80- than the 120-kVp images (11.1 \pm 1.2 vs 9.3 \pm 1.1 HU, p < 0.01), the CNR of the ICA and the MCAwas significantly higher on the 80- than the 120-kVp images (ICA: 46.2 ± 10.2 vs 36.9 ± 7.6 , $p < 0.01$; MCA: 45.7 ± 10.0 vs 35.7 ± 9.0 , $p < 0.01$).

Qualitative image analysis

The visibility of arteries adjacent to the skull base and clips was not significantly different between the two protocols $(p = 0.45)$. Higher subjective scores were assigned to the 80-

Fig. 1 Box-and-whisker plot showing the mean contrast medium (CM) dose (a) and the effective dose (ED) (b) for each protocol. The CM dose was significantly lower with the 80- than the 120-kVp protocol $(42.5 \pm 7.7 \text{ vs } 54.9 \pm 11.5 \text{ ml}, p < 0.01)$ as was the ED $(0.59 \pm 0.06 \text{ vs } 1.5 \pm 1.5 \text{ ml}, p < 0.01)$ 1.56 ± 0.13 mSv, $p < 0.01$)

than the 120-kVp images ($p < 0.05$). There was moderate to substantial interobserver agreement for arterial contrast, vascular sharpness, visibility of arteries near the skull base and clip, depiction of small arteries, and overall image quality $(kappa = 0.70, 0.58, 0.61, 0.64, and 0.63, respectively)$ (Table [4](#page-4-0)).

Representative cases are shown in Figs. [2,](#page-4-0) [3,](#page-5-0) and [4.](#page-5-0) Figure [2](#page-4-0) shows the case of a 71-year-old woman with a left MCA aneurysm who underwent BSCTA under both the 120 kVp and the 80-kVp protocols after 6 months. Although the radiation and the CM doses of the 80-kVp scans were substantially lower than those in the 120-kVp protocol (CTDI $_{vol}$:

Table 3 Quantitative image analysis

	120 kVp	80 kVp	p value
ICA attenuation	370.0 ± 59.3	557.4 ± 105.7	< 0.001
MCA attenuation	364.6 ± 62.2	551.9 ± 107.9	< 0.001
Image noise	9.3 ± 1.1	11.1 ± 1.2	< 0.001
CNR of ICA	36.9 ± 7.6	46.2 ± 10.2	< 0.001
CNR of MCA	35.7 ± 9.0	45.7 ± 10.0	< 0.001

Data are shown as the mean \pm standard deviation

CNR contrast-to-noise ratio

Table 4 Qualitative image analysis

Data are shown as the median (interquartile range)

14.8 vs 41.8 mGy; CM dose: 51 vs 65 ml), the image quality of the 80-kVp scan was better than that of the 120-kVp scan. Figure [3](#page-5-0) shows the 80-kVp images of a 73-year-old woman who had undergone clipping of a right ICA aneurysm (CTDI_{vol}: 16.4 mGy; CM: 38 ml). Arteries around the skull base and clip were obscured on the non-subtraction images. They were clearly depicted on subtraction images without severe clip-induced artifacts or confounding contrast enhancement of the cavernous sinus. Figure [4](#page-5-0) shows the 80-kVp images of an 81-year-old woman with a clipped left MCA aneurysm (CTDI_{vol}: 16.2 mGy; CM: 32 ml). The ICA at the level of the skull base and the aneurysm remnant at the left MCA, obscured on the non-subtraction image, were clearly depicted on the subtraction image.

Fig. 2 A 71-year-old woman underwent bone subtraction CTA under the 120-kVp (CTDI_{vol}: 41.8 mGy, Iopamiron 370; 65 ml) and the 80-kVp protocols (CTDIvol: 14.8 mGy, Iopamiron 370; 51 ml) at the 6-month interval. Subtraction MIP (a) and volume rendering (b) images of the 120-kVp (left) and the 80-kVp (right) protocols. Compared to the 120 kVp imaging, the 80-kVp protocol allowed for a 64.6% reduction in the radiation exposure and a 20% reduction in the amount of contrast medium while improving the arterial enhancement and overall image quality

Discussion

Our study demonstrates that under the 80-kVp with SAFIRE protocol, both the radiation and the CM dose were substantially reduced at cerebral BSCTA and that the image quality was better than on scans obtained with the standard 120-kVp with FBP protocol.

Several prior studies [\[22](#page-6-0)–[28](#page-7-0)] applied low tube voltage techniques and investigated strategies to reduce the radiation or the CM dose at NSCTA. Luo et al. who used 80 kVp obtained a substantial radiation and CM dose reduction compared to a 120-kVp protocol [[26](#page-6-0)]. They reported that at 80 kVp, the CNR of arteries was significantly lower than at 120 kVp because of the higher image noise (15 \pm 2 vs 8 \pm 1 HU). On the other hand, Chen et al. [\[27](#page-6-0)] applied IR to NSCTA and reported that compared to a 120 kVp with FBP protocol, at 70 kVp with SAFIRE protocol using the same amount of CM (60 ml), they obtained a decrease in the radiation dose while maintaining the CNR. Despite these suggestive findings, there has been no available literature on the reduction of both the radiation and the CM dose obtained by combining 80 kVp and SAFIRE for cerebral BSCTA.

Our quantitative image analysis showed that the CNR was better at 80 kVp with SAFIRE than at 120 kVp with FBP and that arterial attenuation was markedly higher on the 80- than the 120-kVp images despite a 23% CM dose reduction. Although the image noise on the 80 kVp with SAFIRE scans was higher than on the 120 kVp with FBP images (11.1 vs 9.3 HU), the difference was smaller than in earlier studies that compared lower tube voltage scans without IR and the 120 kVp cerebral CTA protocols [\[23](#page-6-0)–[27\]](#page-6-0). The relatively low image noise and substantially higher arterial attenuation on the 80-kVp images resulted in a higher CNR on the 80- than the 120-kVp images. Similarly, in qualitative image analysis, the arterial contrast, vascular sharpness, depiction of small arteries, and overall image quality were significantly better on the 80- than the 120-kVp images ($p < 0.05$). These findings suggest that a further reduction in CM and radiation dose may be possible at 80 kVp with SAFIRE protocol.

In addition, we evaluated the image quality of the subtraction CTA images, which is considered clinically important because BSCTA has higher diagnostic performance than NSCTA and could replace invasive 3D-DSA for evaluation of aneurysms [[8](#page-6-0)–[11](#page-6-0)]. The visibility of arteries at the skull base on the 80- and the 120-kVp images was rated as equivalent. While higher venous enhancement in the cavernous sinus at low tube voltage may obfuscate the cavernous part of the ICA, we encountered no venous contamination preventing the visibility of the ICA, probably because we used compact CM injection in our 80-kVp protocol [[24\]](#page-6-0). Although clipinduced artifacts are generally stronger at lower than higher tube voltage settings [[31\]](#page-7-0), there was no significant difference in visibility around the clips between protocols. All clips in

Fig. 3 A 73-year-old woman who had undergone clipping of a right ICA aneurysm was scanned with the 80-kVp protocol $(iopamidol-370, 38 ml; CTDI_{vol}:$ 16.4 mGy). The upper panel shows the non-subtraction slab-MIP (left) and subtraction MIP with clip (*middle*) and without clip (right). The lower panel shows volume-rendered images of non-subtraction (left) and subtraction with clip (middle) and without clip (right). Arteries around the skull base and clip were obscure on the nonsubtraction image. They were clearly depicted on subtraction images without severe clipinduced artifacts or confounding contrast enhancement of the cavernous sinus

our series were made of titanium; they produce fewer artifacts than cobalt alloy clips [\[31](#page-7-0)], and the subtraction technique and the SAFIRE algorithm seem to further decrease these artifacts [\[31,](#page-7-0) [32\]](#page-7-0). As the number of clipped aneurysms was limited in both protocols, further studies are needed to determine the difference in clip-related artifacts between protocols.

Our study has some limitations. First, the number of enrolled patients and of identified clips was relatively small. Additional large-scale studies are needed to confirm this study's results. Second, we focused on the image quality and did not evaluate diagnostic performance with respect to specific intracranial diseases such as intracranial aneurysms or aneurysm remnants after clipping surgery. 3D-DSA as reference standard is not routinely performed at our institution; almost all patients were diagnosed and followed up on the basis of BSCTA findings. In the most recently reported study, Ni et al. demonstrated that the CTA protocol using 80 kVp with FBP detects aneurysms with the same diagnostic accuracy as 120 kVp while reducing the radiation and CM doses [[28](#page-7-0)]. As they used FBP algorithm at the 80 kVp protocol, additional investigations are needed to confirm whether the BSCTA protocol combining 80 kVp with SAFIRE improves aneurysm detection (especially at the skull base or near the clip) while reducing both the radiation and CM doses. Third, we acquired unenhanced mask CT images using the same

Fig. 4 Volume-rendered (VR) images of an 81-year-old woman with a clipped left MCA aneurysm. Scanning was with the 80-kVp protocol (iopamidol-370, 32 ml; CTDIvol, 16.2 mGy). The upper and lower rows show the right anterior oblique and inferior views, respectively, of the nonsubtraction CTA (left) and subtraction CTA with clip (*middle*) and without clip (*right*). The ICA at the level of the skull base and the aneurysm remnant at the left MCA, obscure on the nonsubtraction image, was clearly depicted on subtraction images

scanning parameters used for contrast-enhanced CT. When mask image acquisition is performed at lower radiation dose [9, [33\]](#page-7-0), the quality of BSCTA scans may be affected by misregistration artifacts [[29](#page-7-0)]. Although the total radiation dose increased due to mask image acquisition, this disadvantage was overcome by applying a lower tube voltage and the IR algorithm. The radiation dose of the 80-kVp protocol in this study was substantially smaller than that of previous reported BSCTA protocols [8, 9, 24, [34\]](#page-7-0), and further dose reduction might be possible. Lastly, in our 80-kVp protocol, we evaluated the image quality with only one IR strength level (S3) and additional studies are needed to evaluate the image quality at different IR strengths.

In conclusion, our comparison of an 80 kVp with SAFIRE and a 120 kVp with FBP protocol for cerebral bone subtraction CTA showed that use of the 80 kVp resulted in a substantial decrease in both the radiation and the CM dose and yielded better image quality.

Compliance with ethical standards We declare that all human studies have been approved by the institutional review board of Kumamoto City Hospital and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. We declare that all patients gave informed consent prior to inclusion in this study.

Conflict of interest We declare that we have no conflict of interest.

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