3D Cross Sectional View to Investigate the Morphology of Internal Carotid Artery Plaques. Is 3D Ultrasound Superior to 2D Ultrasound?

3D-Schnittbilder zur Darstellung der Plaquemorphologie der Arteria carotis interna

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Zusammenfassung

Ziel: Die Kenntnis der Plaquemorphologie der Arteria carotis interna spielt zunehmend eine wesentliche Rolle zur Evaluation der Patienten vor Interventionen, da in früheren Studien gezeigt werden konnte, dass Plaques mit einem niedrigen Grauwert eine höhere Komplikationsrate mit sich bringen. Eine immer wieder angegebene Limitation der Ultraschalldiagnostik ist eine lediglich zweidimensionale Darstellung in einer Schallebene, welche zur Berechnung des medianen Grauwertes herangezogen wird.

Material und Methoden: An 24 Thromboendarterektomie-Präparaten von 19 Patienten wurden anhand von 3D-Datensätzen insgesamt 71 Schnitte untersucht. Die Ultraschalldatensätze wurden mit einem 10-MHz-Schallkopf im 3D-Modus unter Zuhilfenahme einer speziellen Messkammer gewonnen und von zwei Untersuchern ausgewertet. Zusätzlich wurde von den Präparaten im B-Bild-Modus ein Longitudinal-Schnitt nach einem standardisierten Protokoll angefertigt.

Ergebnisse: Es zeigte sich eine hochsignifikante Korrelation der medianen Grauwertverteilung zwischen zwei Untersuchern ($p < 0,001$, Intraclass Correlation [ICC] $> 0,895$) und zwischen Untersuchungen zu unterschiedlichen Zeitpunkten bei der Auswertung der 2D- und 3D-Datensätze ($p < 0,001$, ICC $> 0,935$). Im Vergleich der Auswertung der Querschnitte aus dem 3D-Datensatz und dem B-Bild der Längsschnitte zeigte sich ebenfalls eine hochsignifikante Assoziation zwischen den zwei Methoden ($p < 0,001$, $R = 0,800$). 92,7% der Messungen lagen innerhalb den „Limits of agreement“ als Zeichen einer hohen Übereinstimmung zwischen den Methoden.

Schlussfolgerung: Durch diese standardisierte „In-vitro“-Untersuchung konnte bei der Evaluation der Echogenität keine Überlegenheit der

Abstract

Purpose: Previous studies have demonstrated that plaques from the internal carotid artery with lower median grayscale values are associated with higher complication rates in the perintraoperative course. A repeatedly cited limitation of ultrasound is that the single plane used to calculate the median grayscale value is only two dimensional. The goal of this study was to compare the median grayscale value as determined using two dimensional cuts versus three dimensional data sets.

Materials and Methods: Seventy-one cuts of 24 thromboendarterectomy samples from 19 patients were analyzed using 3D data sets. The ultrasound data sets were obtained using a 10 MHz probe in 3D mode in a special chamber and were evaluated by two investigators. Additionally, a longitudinal view of the samples was made using B mode according to a standardized protocol.

Results: There was a significant correlation between the 2D and 3D data as assessed by two observers ($p < 0,001$, intraclass correlation [ICC] $> 0,895$) and at different times ($p < 0,001$, ICC $> 0,935$). Comparison of the 3D transverse cuts with the longitudinal B mode cuts also showed a highly significant association between the two methods ($p < 0,001$, $R = 0,800$). 92.2% of the measured values were within the limits of agreement, reflecting the concordance of the both methods.

Conclusion: The superiority of three dimensional ultrasound with respect to two dimensional ultrasound was not able to be demonstrated using this standardized in vitro procedure to examine the echolucency in extracranial internal carotid artery plaques.

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dreidimensionalen gegenüber der zweidimensionalen Sonografie von Plaques der extrakraniellen Arteria carotis interna nachgewiesen werden.

International randomized studies have proven the benefits of revascularization of the extracranial internal carotid artery for the prevention of stroke [1–3]. In addition to the assessment of the degree of stenosis, imaging of plaque structure is becoming increasingly important, particularly because it has been shown that fatty plaques are associated with a higher risk of embolization than calcium-rich plaques.

A diagnostic procedure that allows for visualization of plaque morphology has not yet succeeded in clinics [4] because none of the available technology is able to give a completely accurate picture of the entire morphology of plaque. Ultrasound, in combination with the evaluation of median grayscale values as an indicator of the echogenicity of the plaque, is used to examine plaque morphology. Computer tomography (CT) and magnetic resonance imaging (MRI) are also used. Such studies have shown that the stroke and death rate is considerably higher for hypoechoic plaques than it is for hyperechoic plaques [5].

The standardized measurements of echogenicity made using digital data transfer for data collection with two-dimensional ultrasound led to findings that were consistently reproducible among different evaluators [6, 7]. One issue when using ultrasound to evaluate carotid plaques in this context is that the three-dimensional volume can only be seen on two-dimensional cuts.

The goal of this study was to compare the median grayscale value as determined using two-dimensional cuts versus three-dimensional data sets for in vitro imaging of internal carotid artery plaques. Another point to be investigated was whether findings from the three-dimensional method were consistently reproducible.

Materials and Method

Patients

Twenty-four consecutive internal carotid artery plaques (19 patients, 12 male, 7 female) with a level of stenosis in excess of 70% according to NASCET criteria [1] were included. The plaques were all removed by eversion endarterectomy with careful avoidance of artificial lesions. Only samples that were able to be removed en block were included in the analysis. These samples were preserved for further analysis in a 10% formalin solution. The clinical health of the patients was recorded but not analyzed since the purpose of the study was limited to investigating ultrasound as a plaque imaging technique.

Standardization of the two-dimensional B mode

The plaques were scanned to evaluate plaque morphology in a 0.9% saline solution from a distance of 1.5 cm using a 10 MHz linear probe (Logitec 500, General Electric Company, Fairfield, Massachusetts, USA). The scanner was set to a medium frame rate, time gain compensation longitudinal to blood vessel, linear post-processing curve, minimal persistence, and 60 dB dynamic range from the optimal longitudinal position.

Standardization of the three-dimensional B mode

To avoid artifacts from reflection, the sample was freely suspended in a chamber designed specifically for this purpose. The sample was able to be moved horizontally along a single plane using a crank (● Fig. 1).

The basic settings on the scanner were identical to those for the two-dimensional scans (10 MHz probe, 60 dB dynamic range, 0.9% saline solution, distance from the sample 1.5 cm). For the three-dimensional scans, the plaque was moved at a constant speed under the stationary probe. From this data set, 2 to 4 transverse cuts of the plaque were generated for the analysis, resulting in a total of 71 3D cross sectional images (● Fig. 2).

Image Analysis

The images that were obtained were saved digitally. The plaque images were analyzed with an image processing program (Adobe Photoshop D 1–6.0, Adobe, USA). Using the method de-

Fig. 1 a Chamber for suspending the plaque to allow for artifact-free scanning. b Diagram of the scanning procedure in the chamber's water bath.

Abb. 1 a Messvorrichtung mit der Aufhängung der Plaque, die somit artefaktfrei eingescannt werden kann. b Darstellung des Scan Vorgangs im Wasserbad der Messkammer.

scribed by Biasi et al., each plaque was outlined via computer mouse. The standardization was made with the help of graduation curves so that a piece of chalk was assigned a gray value of 250, and the NaCl solution a gray value of 0 to 5 [8]. In the standardized image the outline of the plaque was marked, and a histogram of the grayscale generated [9]. The median grayscale value describes the proportion of pixels and is a measure of the echogenicity of the entire plaque.

The median grayscale values of the 2D images were directly compared to the 3D data sets (● Fig. 2).

The results were read by two experienced independent investigators, A and B, at different times, 1 and 2. There was a period of three months between the first and second reading.

Statistics

Statistical analysis was performed using the SPSS for Windows software program (SPSS Inc 14.0.1, Chicago, IL, USA) and a Microsoft Excel database. The nonparametric Spearman's rank order test and intraclass correlation coefficients (ICC) with a two-way random effect for continuous variables were applied to the calculations of the median grayscale value between different investigators and between different times. An error rate of 5% was defined as the significance limit ($p=0.05$). For all agreement parameters, 95% confidence intervals (CI) were calculated.

The graphical method including the limits of agreement ($\pm 2SD$) was used to analyze the concordance between the two methods of measurement and the reproducibility of repeated measurements by each of the two methods on the same subject [10].

Results

Inter- and intra-observer correlation

Single longitudinal view

Grayscale median (GSM) analysis of the longitudinal view at the first time point resulted in a mean value of 56 (sd 13, max 81, min 34) for investigator A and 57 (sd 13, max 79, min 30) for investigator B. At the second time point, the mean GSM values for longitudinal views were 56 (sd 14, max 79, min 40) according to investigator A and 58 (sd 15, max 85, min 29) for investigator B. A highly significant correlation with $p<0.001$ and correlation coefficient $R>0.851$ was seen both between investigators and between the two time points for each investigator. The ICC ranged constantly above 0.930 (● Table 1).

Cross sectional view

The mean median grayscale values for the 3D cross sectional mode at time 1 were 56 (sd 15, max 96, min 26) and 54 (sd 16, max 92, min 25) as calculated by investigator A and B, respectively. At time 2, investigator A reported a mean of 55 (sd 16, max 99, min 27) and investigator B reported a mean of 56 (sd 16, max 93, min 28). Again, there was a highly significant correlation ($p<0.01$) between time points for each investigator as well as between investigators. The correlation coefficient R was greater than 0.85 for both comparisons (● Fig. 3). The ICC ranged constantly above 0.890.

Correlation between single longitudinal and 3D cross sectional views

Due to the strong inter- and intra-observer correlations, only the data from observer A at time 1 for the 2D and 3D data sets was

Fig. 2 a Schematic diagram of how the cross sectional view is generated from the 3D data set. b Calculation of the median grayscale value in 2D single longitudinal and 3D cross-sectional mode using image editing software.

Abb. 2a Schematische Darstellung der Generierung der Querschnitt-Ansicht aus dem 3D-Datensatz. **b** Berechnung der medianen Grauwertverteilung im 2D-Längsschnitt und im 3D-Querschnittsverfahren mithilfe eines Bildverarbeitungsprogramms.

Table 1 Demonstration of the intraclass correlation of GSM between different observers and different time points in the longitudinal and the cross sectional view

	intraclass correlation (95% CI)
<i>longitudinal view</i>	
observer A; time point 1 and 2	0.967 (0.948 – 0.979)
observer B; time point 1 and 2	0.935 (0.898 – 0.959)
observer A and B; time point 1	0.969 (0.951 – 0.981)
observer A and B; time point 2	0.952 (0.924 – 0.970)
<i>cross sectional view</i>	
observer A; time point 1 and 2	0.936 (0.899 – 0.959)
observer B; time point 1 and 2	0.935 (0.897 – 0.959)
observer A and B; time point 1	0.905 (0.852 – 0.940)
observer A and B; time point 2	0.895 (0.836 – 0.933)

analyzed. The analysis showed that there was a highly significant correlation ($p<0.001$, $R>0.800$) between the median grayscale values as determined by the two-dimensional versus three-dimensional method.

Agreement between single longitudinal and 3D cross sectional view

For the same reason mentioned above, only the data from observer A at time 1 were analyzed. The data showed good agreement between the two methods with a limit of agreement between -19.4 and 19.0 . The mean difference was -0.2 and the

Fig. 3 Inter-observer and intra-observer comparison of median grayscale values. Pictured: observer A at different time points ($p < 0.01$; $R = 0.925$; $ICC = 0.936$) **a**, the observers (A/B) at the second time point ($p < 0.01$; $R = 0.864$; $ICC = 0.895$) **b**, the two observers at time point one ($p < 0.01$; $R = 0.854$; $ICC = 0.905$) **c**, and observer B at different time points ($p < 0.01$; $R = 0.905$; $ICC = 0.935$) **d**.

Abb. 3 Dargestellt sind die GSM-Werte von Untersucher A zu verschiedenen Zeitpunkten ($p < 0.01$; $R = 0.925$; $ICC = 0.936$) **a**, die Untersucher (A/B) zum Zeitpunkt 2 ($p < 0.01$; $R = 0.864$; $ICC = 0.895$) **b**, die zwei Untersucher zu Zeitpunkt eins ($p < 0.01$; $R = 0.854$; $ICC = 0.905$) **c**, und Untersucher B zu zwei verschiedenen Zeiten ($p < 0.01$; $R = 0.905$; $ICC = 0.935$) **d**.

95% confidence interval for the lower limit of agreement ranged from -22.6 to -16.1 and for the upper limit of agreement from 15.7 to 22.8 .

Sixty-nine of 71 values (97.2%) were inside the limit of agreement and 100% were identified within the 95% confidence interval.

Measuring the repeatability of the methods, the limit of agreement ranged between -24.2 and 21.4 . Seventy values (98.6%) were inside these limits. All values were in the 95% confidence interval for the lower and the upper limits of agreement (-28.0 to -20.3 for the lower, 17.5 to 25.3 for the upper 95% confidence interval) (● Fig. 4).

Discussion

The importance of diagnosing plaque morphology is not limited to the evaluation of ipsilateral cerebro-vascular risk. Current discussion about carotid angioplasty as an alternative to surgical

revascularization emphasizes the importance of risk analysis [11, 12]. Well investigated is that echolucent plaques are associated with a higher rate of embolization in spontaneous process [6, 13, 14]. However, numerous studies have not been able to demonstrate a correlation between ultrasound findings and plaque histology [8, 15]. One of the reasons for this is that in vivo evaluations are not three-dimensional [16, 17]. Another consideration that strengthens the argument for understanding plaque morphology is the fact that large atherosclerotic lesions have histological characteristics that vary over the area of the plaque and therefore do not result in a GSM analysis that accurately represents the entire plaque [18]. Consequently, a three-dimensional method of assessing echogenicity was needed. It was not the aim of this evaluation to determine histology. Because of the comparable results between 2D and 3D imaging, we assume no improvement in this aspect. Nevertheless, further evaluation of pathology should follow to investigate this topic specifically. Three-dimensional ultrasound has already been evaluated as a means of measuring plaque volume and the probable volume

Fig. 4 Difference against mean for GSM data. **a** Between 2D and 3D evaluation. **b** Between 2D and 3D evaluation using repeated measurements. **Abb. 4** Bland-Altman-Analyse zur Berechnung der Differenz gegenüber dem Mittelwert bei der medianen Grauwertverteilung. **a** Zwischen 2D- und 3D-Untersuchungen. **b** Zwischen wiederholten Untersuchungen.

progression and was shown to be considerably better than two-dimensional studies [19]. It was possible to obtain good observer-independent results with the three-dimensional method [20] which led to the use of the 3D method for evaluating therapeutic effects, e.g. after treatment with statins [21]. However, there are no studies in the current literature that compare echogenicity determined by cross-sectional view versus a volume model.

In this paper, two-dimensional views were compared to views generated by three-dimensional data sets on the basis of median grayscale values.

Since the investigation was intended only to examine the relationship between 2D and 3D modes of ultrasound, no clinical patient data was analyzed. However, previous studies have investigated the connection between multi cross sectional view and the occurrence of clinically relevant events. Those studies showed a good differentiation between symptomatic and asymptomatic plaques [22].

The plaque analyses in the study presented here were conducted in vitro. Standardized conditions and the avoidance of artifacts allowed maximum validity. A special chamber was developed in which the samples were freely suspended and therefore were able to be scanned without generating mirror-image artifacts. Because of this special chamber and the ability to scan the samples with a linear velocity, the conditions were optimal for generating and analyzing the three-dimensional data sets.

The primary analysis was focused on the reproducibility of results. In this series normalized image analysis of carotid plaques achieved highly significant inter- and intra-observer reproducibility.

To allow comparison between the two- and three-dimensional data, each analyzed B-mode view was compared with several of the cuts from which the three-dimensional data set was generated. The GSM analysis of the single longitudinal view produced the same results as the multiple cross sectional analysis from the three-dimensional data set. We identified nearly complete concordance between the two methods. Repeat measurements with each of the two methods used in our study applied to the same subject resulted in a good agreement as well. Summarizing our findings, we postulate that the methods can be interchanged. Based on these findings, evaluation of GSM of internal carotid artery plaques with a three-dimensional method offers no advantages over the two-dimensional method that has been used thus far.

One limitation of the study is that we focused our evaluation on one aspect of carotid plaque imaging. Further aspects to demonstrate plaque morphology are plaque luminal morphology, and plaque volume. Therefore, the option of a 3D investigation could exceed the clinical value of a 2D measurement and might improve the visualization of carotid plaque morphology.

Using intravascular ultrasound in addition to these other methods might be another way of improving ultrasound imaging of plaque morphology. Intravascular ultrasound of coronary arteries agreed well with histological findings [23, 24]. The results of GSM analysis could also be improved using pixel distribution analysis and/or color mapping of particular regions of interest within the plaque. Evaluations utilizing these methods provided additional information about the histology of the plaque and had a strong correlation with the occurrence of neurological events and mortality [13, 25]. Additional studies will be necessary to see if these detailed analyses are possible in vivo and under reduced resonance conditions, and whether they can also achieve successful acoustic shadowing of more calcified plaques.

Conclusion

The goal of this paper was to investigate in vitro whether 3D ultrasound is superior to 2D ultrasound for the evaluation of the GSM of atherosclerotic plaques in the extracranial internal carotid artery. For this purpose, a special chamber was designed to facilitate standardized scans while minimizing the number of artifacts. The results from GSM analysis based on 2D and 3D studies were comparable and highly reproducible. Therefore, in this regard there was no advantage to using the three-dimensional rather than two-dimensional method.

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