Differential intensity projection (DIP) for visualisation and quantification of plaque neovascularisation in CEUS images of carotid arteries

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Abstract

Studies have shown that intra-plaque neovascularisation (IPN) is closely correlated with plaque vulnerability. In this study, a new image processing approach, differential intensity projection (DIP), was developed to visualise and quantify IPN in contrast enhanced ultrasound (CEUS) image sequences of carotid arteries. DIP used the difference between the local temporal maximum and the local temporal average signals to identify bubbles against tissue background and noise. The total absolute and relative areas occupied by bubbles within each plaque were calculated to quantify IPN. In vitro measurements on a laboratory phantom were made, followed by in vivo measurements where twenty-four CEUS image sequences of carotid arteries from 48 patients were acquired. The results using DIP were compared with those obtained by maximum intensity projection (MIP) and visual assessment. The results show that DIP can significantly reduce nonlinear propagation tissue artefacts and is much more specific in detecting bubble signals than MIP, being able to reveal microbubble signals which are buried in tissue artefacts in the corresponding MIP image. A good correlation was found between microvascular area (MVA) ($r = 0.83$, $p < 0.001$) / microvascular density (MVD) ($r = 0.77$, $p < 0.001$) obtained using DIP and the corresponding expert visual grades, comparing favourably to r = 0.26 and 0.23 obtained using MIP on the same data. In conclusion, the proposed method shows great potential in quantification of IPN in contrast enhanced ultrasound of carotid arteries.

Key words: differential intensity projection, contrast enhanced ultrasound, carotid artery, intraplaque neovascularisation, perfusion quantification

Introduction

Stroke is a leading cause of death in the world-wide (Fuster and Voûte 2005). The formation of vulnerable atherosclerotic plaque in the carotid artery increases the risk of stroke (U-King-Im et al. 2009; Mughal et al. 2011). Several studies have reported that intraplaque neovascularisation (IPN) is a precursor of intraplaque haemorrhage (IPH) and IPN could thus be a surrogate biomarker of unstable plaque (Feinstein 2006; Virmani et al. 2006; Hellings et al. 2010). Therefore, quantification of IPN can be used for the early detection and clinical management of unstable atherosclerotic plaques and hence minimise the risk of stroke.

Recently, contrast-enhanced ultrasound (CEUS) imaging with microbubble contrast agents has provided a unique tool for visualizing and quantifying IPN. It has shown promise for imaging plaque vasculature. Several groups (Feinstein 2004; Coli et al. 2008; Giannoni et al. 2009; Lee et al. 2010) have established correlations between CEUS imaging results and histological plaque neovascularisation and the risk of plaque rupture. However, in these studies, only subjective visual assessment was used to quantify the findings. Furthermore, although several computer algorithms (Hoogi et al. 2012; Akkus et al. 2013) are available to assist in the quantitative analysis of the images, they have some limitations. Hoogi et al. proposed a method for segmenting the contrast spots within atherosclerotic plaques in individual images by tracking individual microbubbles. The main advantage of this approach is that it utilises the temporal behaviour of bubble flow can be demonstrated. This makes it robust to noise and allows differentiation between blood vessels and artefacts. However, several parameters of the algorithm were determined empirically from a few sequences, which may be a variable to quantitative results. Akkus et al. developed a statistical segmentation of carotid plaque neovascularisation. An iterative expectation-maximisation algorithm was employed to solve a

mixture estimation problem to identify contrast microbubble signals. But, this technique has difficulties quantifying IPN reliably for plaques located on the far wall of the carotid artery due to nonlinear propagation artefacts (also called pseudo-enhancement artefact) (Tang and Eckersley 2006; Tang et al. 2010). Non-linear propagation of ultrasound creates artefacts in CEUS images that could significantly affect both qualitative and quantitative IPN assessments (ten Kate et al. 2012). Although there are correction methods (Renaud et al. 2012; Yildiz et al. 2015) to remove non-linear artefact, they are not available on current commercial scanners.

Moreover, the maximum intensity projection (MIP) is a common intensity-based bubble imaging method. It can visualise bubble paths (i.e. vessel trajectories) by displaying the maximum intensity over time for each pixel in CEUS images (Suri et al. 2002; van Ooijen et al. 2003; Hoogi et al. 2011). While this approach is sensitive, simple and fast, the disadvantage is that this method has low specificity to bubbles. In particular, it is difficult to distinguish between tissue artefact due to nonlinear propagation and blood vessels, and therefore it could generate over-estimated vessel paths and affect quantification results.

The objective of this study was to develop and evaluate a sensitive, specific, simple and fast microbubble detection technique for CEUS carotid artery imaging by using differential intensity projection (DIP). This technique was demonstrated in vivo, and applied to the quantification of intraplaque neovascularisation in vivo.

Methods

Differential intensity projection

The proposed algorithm worked at a pixel level to detect microbubble signals. The CEUS images contained primarily three components: tissue artefact, noise, and microbubble signals. The differential intensity projection (DIP) was defined as below to capture the microbubble signals.

$$DIP(x_i, y_i) = \text{maximun}(I(t, x_i, y_i)) - \langle I(t, x_i, y_i) \rangle \quad (1)$$

where $DIP(x_i, y_i)$ is the differential image intensity at the *ith* pixel between the temporal peak signal $I(t, x_i, y_i)$ and the temporal average intensity $\langle I(t, x_i, y_i) \rangle$. For a given bubble occasionally passing an otherwise dark image pixel, the peak intensity was expected to be much higher than the average intensity. On the other hand, the peak intensity and the average intensity were expected to be similar for tissue signal. For noise both the peak and average intensity are expected to be relatively low. As a result, the differential intensity of pixels containing microbubble signals is expected to be higher than that of tissue or noise.

Threshold selection

A threshold in differential intensity was required to separate microbubble signals from tissue and noise. It was estimated from the histogram of differential intensity projection, an example of which is shown in Figure 1. It should be noted that the threshold is automatically adjusted for each patient based on the entire image. The intensity histogram of differential intensity projection is constructed (see Figure 2). The threshold is determined at the intersection point of microbubble and tissue distributions.

Microvascular area and density

The ROI in plaque was selected manually. The number of pixels identified as containing bubble signal was defined as the microvascular area (MVA), which can then be normalized by the total number of pixels within the plaque ROI to obtain the microvascular density (MVD) measure for the ROI.

$$MVD = \frac{\text{MVA}}{area\ of\ ROI} \quad (2)$$

In-vitro study

The DIP algorithm was validated on a simple laboratory phantom constructed in-house and shown in Figure 1A. It consisted of a piece of tissue-mimicking material, above which a highly diluted microbubble suspension was gently stirred to simulate individual bubbles moving within the phantom.

Clinical application (plaque)

Forty-eight patients previously treated for head and neck cancer (HNC) with at least one risk factor for atherosclerosis were recruited from a cancer centre. These patients are asymptomatic for cardiovascular events. From this group, 24 videos with carotid plaque were selected for this study. The study was approved by the institutional research and ethics committee and each patient provided informed consent. CEUS image sequences were acquired on both sides of the neck with a clinical scanner (GE Vivid7 with a 9 MHz broadband linear array transducer). The GE scanner was used to scan the subject with the following settings: MI = 0.21, Gain = 0, DR

= 54, TGC = manually adjusted, Frequency = 3.2/6.4 MHz. The contrast mode is used to perform contrast enhanced imaging. Contrast-enhanced ultrasound video loops were taken using a commercially available ultrasound contrast agent, SonoVue^{TM} (Bracco, Milan) given as an intravenous infusion via a peripheral vein at a rate of 1.2 mL/min. The infusion was delivered over a total of 5-7 minutes. Imaging was performed in real-time prior to the arrival of and following the saturation of the carotid artery with SonoVue.

Visual assessment

IPN was graded semi-quantitatively as absent (Grade 0), limited to the adventitia/plaque base (Grade 1) or extensive and/or extending into the plaque body (Grade 2) by a clinician (Dr. Shah).

Motion compensation and DIP

The motion of carotid artery was tracked and corrected by a dedicated motion correction algorithm (Stanziola et al. 2015). The algorithm consisted of three steps: (A) Pre-processing, (B) Lumen segmentation and (C) Registration. In the first step, large rigid motions were removed by a rigid registration. Then, the algorithm used the information of the cardiac cycle and the Gabor filter responses of the corresponding frames to obtain a mixture of frames where the fragmentation of the lumen signal was largely removed. In the second step, the lumen was segmented by using thresholding and level set methods. A binary mask of the lumen region was obtained for each frame. Finally, a non-rigid registration was performed to correct the motion effect on each frame based on minimising the energy functional of non-lumen region

of two consecutive images and the energy functional of segmented lumen region of two consecutive binary masks.

Then DIP images were calculated for each CEUS image sequence using Eqn (1). Maximum Intensity Projection (MIP) images were also obtained for comparison purpose.

Regions of interest (ROI) analysis

Analysis of CEUS video sequences was performed off-line using software developed in-house using MATLAB (The MathWorks, Natick, MA, USA). Carotid plaques were segmented manually as the regions of interest (ROIs) by a clinical expert (Dr Chahal) using both CEUS sequence and maximum intensity projection (MIP) (Figure 2, first and second columns). Both MVA and MVD were calculated for each plaque, and results compared with visual grading.

Statistical analysis

The sample size was small and not normally distributed. Therefore, non-parametric statistical analyses were used in this study. The correlation between the visual grade and the MVA/MVD derived from our method was tested by Spearman rank correlation. The differences between the mean rank of MVA/MVD and the visual grade groups were tested by Kruskall-Wallis test with alpha set at 0.05. Statistical analyses were performed using SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, New York, USA). Further, a comparison between MIP and DIP using patient data was performed

Results

In-vitro study

The CEUS image of the phantom, MIP and DIP images were shown in Figure 1. It can be seen that while the microbubble detection is similar between the MIP and DIP, the tissue linear artefact at the lower part of the image in the CEUS image and the MIP was completely removed in DIP image.

Differential Intensity Histogram and Threshold selection

By examining the histogram of differential intensity projection (0.25) (Figure 2, dotted line), it can be seen that there are peaks in the histogram corresponding to microbubble, tissue and noise.

Visual Assessment and Differential intensity projection

Among the 24 video sequences, Grade 0 IPN was seen in 12/24 videos, while Grade 1 IPN in 8/24 videos and Grade 2 IPN in 4/24 videos. Examples of CEUS images and the MIP images with each visual grade are shown in Figure 3. The corresponding DIP images are shown in Figure 2 (third column).

By examining MIP and DIP images of the same dataset against visually confirmed bubble signals by clinician experts (solid arrows in the images), DIP showed not only much less nonlinear tissue artefact and better image contrast, but also clearly revealed IPN signals which were buried by tissue artefacts in the corresponding MIP image (Figure 2B).

Clinical evaluation (MVA and MVD)

The average and the median of MVA for each visual grade are shown in Table 1. It can be observed that both mean and median of MVA increased with the visual grade. Furthermore, Table 2 shows the average and the median of MVD for each visual grade. It can be seen that both mean and median of MVD increased with the visual grade.

The box plots of visual grade vs MVA or MVD were displayed in Figure 4.

Spearman's rank correlation coefficient

Both MVA and MVD were significantly correlated with the visual grade (R= 0.83 and 0.77 respectively, (p < 0.001 for both) for DIP. This is a significant improvement over those obtained by MIP (MVA: R = 0.26, MVD: R = -0.23).

Kruskal-Wallis Test

Table 3 shows the visual grade groups comparison. For the group grade 0 vs grade 1, there was a significant difference in MVA or MVD at α = 0.05. Similarly, there was a significant difference in MVA or MVD at α = 0.05 for the group grade 0 vs grade 2. However, for the group grade 1 vs grade 2, the difference in MVA or MVD was not significant at α = 0.05, while the difference in MVD was significant at α = 0.1.

Discussion

In this study a new image processing approach, differential intensity projection (DIP), was developed to visualise and quantify plaque IPN in CEUS image sequences of carotid arteries in vivo. Compared with existing method MIP, the proposed DIP can significantly reduce nonlinear propagation tissue artefacts and improve imaging specificity, as validated in the in vitro study where ground truth is available. Two quantitative measures, MVA which is related to the total vascular areas occupied by IPN in the plaque, and MVD which is a vascular density measure, were generated based on each DIP image. The in vivo data on human carotid artery analysed by DIP showed a strong and much higher correlation between MVA/MVD and visual IPN grade than that by MIP. There was also a significant difference in MVA/MVD between patient groups (i.e. grade 0 vs grade 1 or grade 0 vs grade 2).

Quantification of IPN as a novel surrogate marker for stroke risk can be highly valuable in clinical diagnosis. Recently, several groups have developed various methods for IPN quantification. Huang et al. (Huang et al. 2008) proposed a dynamic evaluation of the plaque enhancement by a time intensity curve analysis (TIC). TIC is commonly used in analysing large and well perfused organs, for example, the liver, prostate and heart. However, plaques in the carotid artery are often small and weakly perfused. Therefore, TIC analysis may not be appropriate to quantify microvessels in plaques. Hoogi et al. (Hoogi et al. 2012) adopted electrocardiogram (ECG) gating to correct for motion and only one CEUS image per cardiac cycle was used. Hence, the connection of microvessel paths after time integration may be lost. More importantly, these algorithms can be significantly affected by the nonlinear propagation tissue artefact. The DIP method has a unique advantage of being able to efficiently reduce such tissue artefacts.

One challenge of quantifying neovascularisation in plaque is tissue motion. It is caused by the expansion and contraction of blood vessels, breathing and swallowing. Our dedicated motion compensation algorithm (Stanziola et al. 2015) was applied to improve the quantification of IPN. The software performs better than other current available methods. It should be noted that even if motion compensation is applied, some out-of-plane motion could still affect the quantification. Any non-corrected motion will potentially introduce artefacts into DIP images. Further studies to take into account of out-of-plane motion could further improve the quantification results.

Besides the nonlinear propagation tissue artefact and motion compensation, attenuation is also an important consideration that may affect quantification. Whilst it appeared in our study that quantification was not significantly affected by attenuation, it may not always be the case. Recently, Cheung et al (Cheung et al. 2015) have developed an automated attenuation correction and normalisation algorithm to improve the quantification of contrast enhancement in ultrasound images of carotid arteries. The algorithm firstly corrects for attenuation artefact and normalises intensity within the contrast agent-filled lumen and then extends the correction and normalisation to regions beyond the lumen.

The proposed method can generate more specific visualisation of vessels and more reliable IPN quantification. It could have important implications for clinical screening, diagnosis and management of this important disease. Specifically, such quantitative information on plaque vascularisation enables improved patient risk stratification and potentially improves drug treatment by providing a tool for monitoring treatment.

The DIP is simple and computationally efficient and can be implemented in real time, as it only involves simple mathematical operations. The quantification process is semi-automated, only requiring manual input for segmenting the plaques. Fully automated segmentation is possible but requires further studies.

It should be noted that there is some overlap in MVD between grade 1 and grade 2 plaques and the difference was not statistically significant. This is likely due to the the small sample size of the analysis (n=4 for grade 2). More patient data in future studies would help demonstrate any significance in quantification results between the two groups using our method.

In our clinical data only two out of the twenty four plaques are located in the near wall, while there are 22 plaques found in the far wall. Due to the low number of the near wall plaques it is not possible to draw any conclusion on how our method performs on plaques located at the different sides of the wall. However, it should be noted that the correlation of the far wall quantification by DIP improved significantly over MIP.

Conclusions

DIP is demonstrated to be a specific, simple and fast technique for visualisation and quantification of small vessels in CEUS images and has potential for clinical assessment of intraplaque neovascularisation.

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Figure 1: (A) The CEUS image of tissue mimicking phantom (B) Maximum intensity projection (C) Differential intensity projection.

Figure 2: Histogram of differential intensity projection

Figure 3: First column: CEUS image with ROI. Second column: Maximum intensity projection. Third column: Differential intensity projection. (A) plaque with grade 0 (B) plaque

379 with grade 1 (C) plaque with grade 2, tissue artefact is indicated by a dashed arrow and bubble
380 signal is indicated by a solid arrow.

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382 Figure 4: Box plot of visual grade versus (A) MVA (B) MVD, outlier is indicated by a circle
383 with number.

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385 Video: A CEUS video sequence of a carotid artery with IPN (Grade 2), where microbubbles are seen
386 passing through the plaque (red arrows).

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388 Table 1: The average and median of MVA for each visual grade

Visual Grade	*Mean ± SD*	*Median*
Grade 0	1.42 ± 2.90	0
Grade 1	95.67 ± 105.58	48
Grade 2	538.50 ± 701.27	228.5

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390 Table 2: The average and median of MVD for each visual grade

Visual Grade	*Mean ± SD*	*Median*
Grade 0	0.08 ± 0.47 (%)	0%
Grade 1	1.21 ± 1.40 (%)	0.40%
Grade 2	8.26 ± 12.88 (%)	2.18%

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392 Table 3: Visual grade groups comparison by Kruskal-Wallis test

Visual Grade Groups Comparison	*MVA*	*MVD*
Grade 0 vs Grade 1	$p = 0.001^{*}$	$p = 0.006^{*}$
Grade 1 vs Grade 2	$p = 0.126$	$p = 0.062$
Grade 0 vs Grade 2	$p = 0.001^{*}$	$p = 0.001^{*}$
* significant at $\alpha = 0.05$		

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