QUANTIFICATION OF PLAQUE IN THE CAROTID ARTERY: VALIDATION OF A NOVEL SEMI-AUTOMATED APPROACH FOR MEASUREMENT OF CAROTID ARTERY PLAQUE VOLUME

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Introduction

Approximately 10% of deaths worldwide are due to a cerebrovascular disease [1]. One of the most common cerebrovascular diseases is stroke, which occurs due to reduced blood flow to the brain. Roughly 80% of these strokes are caused by blood clots, which impede flow and thus are called ischemic strokes [2]. Atherosclerotic plaque plays a major role in ischemic strokes as it results in the narrowing of arteries and increases the chances of clotting [2]. It is important to monitor progression and regression of atherosclerotic plaque without causing harm to the patient. Imaging of the carotid artery is non-invasively acquired using B-mode Ultrasound (US) [2]. Several ultrasound measurements have been developed to measure atherosclerotic disease development.

- One-dimensional method - intima-media thickness (IMT) is the measurement of the intima-media layer thickness of the common carotid artery [4].
- Two-dimensional method - total plaque area (TPA) is a measurement of the maximum cross-sectional area of a plaque in the longitudinal view of the carotid artery [6].
- Three-dimensional methods - measurements for plaque development using 3D Ultrasound include vessel wall volume (VWV) and total plaque volume (TPV). VWV is composed of two components; a 3D cross-sectional intima-media
thickness and plaque volume [4]. TPV is a measurement that directly quantifies the size and shape of plaques by volume [3].

**Objectives**

Our focus for this study was the development and validation of TPV measurements for arterial disease progression. Currently, there is no clinical tool for measuring volume and progression of plaque in a reproducible and efficient manner [3]. Manual methods for TPV measurements previously developed rely on time consuming slice-by-slice area segmentation in the axial view [3]. Manual measurements were chosen as a reference standard for measuring TPV as this method effectively encompasses the geometries and morphologies of atherosclerotic plaque [3]. Accordingly, a new semi-automated tool was developed for measuring TPV. Our goal was to test the reproducibility of the new semi-automated tool through comparison to those obtained by the reference standard. Validation of accuracy and reproducibility was important in determining whether our semi-automated method was a viable clinical tool for measuring plaque volume.

**Hypothesis**

The null hypothesis was that there would be no significant difference in measurements obtained by the new semi-automated tool and those obtained by the manual tool.
Theory

The wall of a healthy carotid artery is made up of three distinct layers: an inner layer called the intima, a middle media layer and an outer adventitia layer [6]. The intima layer is lined with endothelial cells [6]. During endothelial cell dysfunction (usually caused by an injury or inflammation), adhesion receptors on the surface of endothelial cells are activated. These receptors bind white blood cells from the blood [6]. The cells then enter the intima layer, where they become phagocytes that oxidize and bind lipid particles [6]. This chemoattraction of white blood cells and their influence on the intima layer leads to the hardening of the artery and buildup of plaque through a process called atherosclerosis [6]. Non-laminar blood flow causes more white blood cells to bind to the adhesion receptors, promoting continuation of this process [6]. Non-laminar flow in arteries often results from a bifurcation (splitting of artery into two) or from stiffened arteries. Consequently, plaque is commonly found in these areas [6].

An important bifurcation site is where the common carotid artery (CCA) splits into the internal carotid artery (ICA) and the external carotid artery (ECA). This site is the target for monitoring atherosclerosis as it is most prone to disease development [6]. Thus we can reduce the risk of ischemic strokes by monitoring and treating atherosclerosis in this region.

Three-dimensional ultrasound (3DUS) images of the carotid artery are acquired non-invasively by translating a B-mode ultrasound (US) transducer along the neck of the patient [7]. 3D Quantify software was developed to manipulate these images and enable image analysis. Different types of imaging phenotypes are present for monitoring the development and regression of atherosclerosis. Image terminology used in 3DUS analysis
is shown in Figure 1, indicating the longitudinal (side) and axial (front) views of a 3DUS carotid artery image.

![Figure 1. 3DUS image terminology. The front facing plane is the axial view while the side facing plane is the longitudinal view.](image)

Intima media thickness (IMT) is a 1-dimensional measurement of the thickness of the intima media layer in the longitudinal view, usually in the CCA [4]. This measurement does not include plaque thus the measurements over time have a very narrow dynamic range [4].

Total plaque area (TPA) is a 2-dimensional measurement of carotid plaque [8]. The user identifies the plaque in the longitudinal view at the region where the plaque appears to be the largest [8]. Then the plaque is outlined by the user and thus the maximum area of the plaque in the longitudinal view is obtained [8].

Vessel wall volume (VWV) is a 3-dimensional measurement of vessel wall volume similar to IMT. These measurements differ in that VWV is a measure of the intima media thickness around the vessel multiplied by the inter-slice distance [4]. This results in a volume measurement [4]. While IMT does not include plaque into its measurements, plaque is a component of VWV [4].
Total plaque volume (TPV) is the final phenotype that has the most potential for clinical use. This 3-dimensional measurement of plaque volume is different from TPA in that it quantifies plaque size based on volume rather than on area [3]. In manual methods of measuring TPV, the user must determine an axis of segmentation in the longitudinal view [9]. The user then segments the boundary of the plaque in the axial view for every slice, with an inter-slice distance of 1mm [9]. *Landry et al.* performed a 5-day variability trial to measure the intra-observer coefficient of variation (CV) for 5 repeated measurements [9]. Results as shown in Figure 2 suggest that as plaque sizes increased, the CV decreased.

![Figure 2](image-url)

*Figure 2.* Landry et al. (2004). Coefficient of variation (CV) in 5 rounds of manual measurements.

Since the manual segmentation of plaque volume is a time consuming process, we developed a new semi automated (SA) method of measuring TPV. After opening an image in 3D Quantify, plaque volume can be obtained from identifying only four contours. The user must first identify the plaque and outline it in the longitudinal view by clicking points around its edges. Following plaque identification, 3 more areas are outlined in specific locations along the z-axis in the axial view. Contours are placed at the 25th, 50th and 75th percentiles along the length of the plaque defined from the minimum and maximum
points along this z-axis axis. After all four areas are generated by the user, the algorithm computes the volume of the plaque. The process of semi-automated segmentation is summarized in Figure 3.

![Figure 3. SA-TPV measurement guide. C1 represents the first contour identified by the user in the longitudinal view. C2, C3 and C4 are the 3 contours in the axial view at the 25th, 50th and 75th percentile along the z-axis respectively.](image)

**Methods**

3DUS images were acquired using a high frequency B-mode ultrasound system with a transducer that had a central frequency of 8.5 MHz. Twenty-three echogenic plaques were selected and were measured by two observers. Each observer measured the 23 plaques five times consecutively on the same day using the SA method. Then each observer measured the same 23 plaques five more times with the same method, but with 20 hours between measurements. Finally, each user performed one round of manual measurements on each plaque for comparison as our reference standard. All measurements were performed in 3D Quantify. For SA measurements, the user outlined
areas by clicking points around the plaque. For manual measurements, segmentation boundaries were outlined using an electronic pad and pen.

**Statistics**

In order to evaluate the performance of the novel SA tool, we tested for the reproducibility of our measurements. The coefficient of variation (CV) was calculated by dividing the SD by the mean. This value was reported as a percent. SD is the standard deviation of the plaque volumes from the different rounds and the mean is the average of these volumes. We also compared the accuracy of the new SA tool to the manual tool introduced by Landry et al., using linear regression analysis and Bland-Altman plots. These plots were utilized to determine the difference between the two methods as a function of plaque volume. Linear regression and Bland Altman analyses were performed in GraphPad Prism for Windows, Version 4.01. P-values and \( r^2 \) values were also recorded.

**Results**

![Intra-observer CV plots of repeated measurements of 23 plaques at 5 min and 20 hours time intervals for two observers. The y-axis represents CV of the measurements in the 5 different rounds at both time intervals.](image)
Intra-observer analysis is shown in Figure 4 and indicates the relationship between the coefficient of variation and plaque size for the two observers. Both observers show a similar trend in measurements. As plaque size increases, the variability decreases as reported by Landry et al. This relationship was consistent among measurements 5 minutes apart as well as measurements performed 20 hours apart.

As illustrated in Figure 5, linear regression analysis shows a strong correlation between novel SA measurements and the manual segmentation method. Plots were created for each observer for both 5 minute and 20 hours time intervals between measurements. Both observers had $r^2$ values of 0.96 or greater, indicating that 96% of the
variation in semi-automated plaque volume is explained by the linear association between Mean SA PV measurements and Manual PV measurements for both time intervals.

**Figure 6.** Bland-Altman plots, graphing difference between SA and Manual measurements as a function of the mean of the two. Plots include results for measurements done 5 min and 20 hours for both observers. Dashed lines represent 95% confidence intervals.

Bland-Altman analysis was performed to further compare segmentation methods. As seen in Figure 6, results were consistent for both time intervals in between measurements and also between observers. In every case, the size of large plaques was
slightly underestimated by the novel SA tool, as these data points were outside of our 95% confidence intervals.

**Discussion**

Ultrasound scanning of the carotid artery provides several phenotypes to monitor the progression of atherosclerosis. However, limitations specific to each measurement provide motivation for the development of a semi-automated measurement that directly quantifies plaque volume.

For example, IMT has a very narrow dynamic range of 0.5mm to 1mm, making it difficult for long term comparisons [4]. IMT is usually only recorded in the CCA and does not include any plaque in its measurements. We have seen before that it is important to include plaque in our measurements as changes in volume allow us to characterize the atherosclerosis disease process and to evaluate potential drug therapies with imaging.

VWV is a better representation of IMT but it also has limitations. It is a slice-by-slice segmentation of the vessel wall thickness all around the artery, accounting for plaque [4]. When the vessel wall thickness is multiplied by the inter-slice distance, it results in a measurement of vessel wall volume. This is a time consuming process, as it requires the user to outline the intima and media layer at every slice.

TPA is another useful measurement for research studies. However, this phenotype is limited in that it relies on the user identifying the largest area of a plaque in the longitudinal view. Since plaques can change their shapes and sizes in all directions, measuring a 3-dimensional change with a 2-dimensional tool is not accurate or reproducible. As a plaque develops over time, it can maintain its area in the longitudinal view but it could change its dimensions in the axial view. The user could easily fail to
observe these changes since they are not detectable by TPA. It would be difficult to assess true changes in disease progression with this method. For example, a plaque with no change in volume at a follow up visit may be interpreted as disease progression or regression as TPA was measured at a different maximum longitudinal location.

To improve the estimation of plaque TPV was introduced. Measuring volume gives us a more accurate measurement of plaque development as it encompasses 3-dimensional morphological changes. Manual segmentation was shown to have decreasing variability with increasing plaque size by Landry et al. From Figure 2 we can see that plaques larger than 300 mm³ can be reproduced with variability less than 5 percent. Plaques that are smaller in size showed higher variability as plaques with smaller than 100 mm³ were reproduced with a variability of 15-20 percent.

Our goal was to create a tool that is faster than manual measurements but equally as reproducible. This would enhance the potential of TPV for clinical use. The algorithm estimates the volume of the plaque based on 4 areas outlined by the user. Two variability trials were completed of 5 rounds. One, where 5 minutes was allowed between measuring the same image again, and one where 20 hours was allowed between measurements. The purpose of the user measuring the same image with 5 minute time interval was to reduce user variability and to see how this would compare to measurements where more time was introduced between measurements. After completion of the variability trials, intra-observer variability data, plotted in Figure 4, agreed with those found by Landry et al. Larger plaques had lower variability while smaller ones had higher variability. Increasing time from 5 minutes to 20 hours between plaque measurements did not influence this trend. We suggest that the variability present
in the measurements is due to the tool itself and not the user. To further validate the novel tool with manual segmentation, linear regression and Bland-Altman plots were constructed in Figures 5 and 6 respectively. Linear regression plots showed at least a 96% correlation between the measurements obtained using the two methods for both observers and both time intervals. Bland-Altman plots also showed a strong correlation between the two methods. All measurements fell between our 95% confidence intervals except for one of the larger plaques. This means that for this measurement, the SA tool slightly underestimated the size of the plaque. Since the user is limited to only outlining three areas in the axial view, some of the volume may have been lost in between the outlines. This may be a limitation of our tool but further improvement may help eliminate such underestimation.

Future work could focus on incorporating information such as voxel intensity into measurements. Currently, the user must select the four outlines based on their best judgment, but we could reduce variability by incorporating boundary detection into the algorithm. None-the-less, based on our variability and accuracy analyses we have shown that manual measurements could be replaced by the novel semi automated tool. If recruited for clinical use, it would significantly reduce the time required for characterizing atherosclerosis. Therefore we fail to reject our null hypothesis, which states that our two methods are going to be the same.

**Conclusion**

Several different phenotypes can be used to monitor atherosclerosis, the underlying cause of ischemic strokes. Phenotypes that provide direct and safe measurements of atherosclerosis include IMT, VWV, TPA and manual segmentation of
TPV. Currently, many of these measurements are limited to research studies. Semi-automated TPV directly estimates plaque volume and reduces measurement times while still achieving results that were highly correlated to manual TPV measurements. Future studies could involve building on this interface to incorporate image intensity information that could reduce user variability between measurements.
References


