AWARD NUMBER: W81XWH-20-1-0615

TITLE: Clinical Study of Vascular Plaque Determination for Stroke Risk Assessment

PRINCIPAL INVESTIGATOR: D. Geoffrey Vince, PhD.

CONTRACTING ORGANIZATION: The Cleveland Clinic Foundation, Cleveland, OH

REPORT DATE: October 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2023	Annual	15 Sep 2022 - 14 Sep 2023

4. TITLE AND SUBTITLE

Clinical Study of Vascular Plaque Determination for Stroke Risk Assessment

5a. CONTRACT NUMBER
W81XWH-20-1-0615

5b. GRANT NUMBER
PR191955

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
D. Geoffrey Vince, Russell J. Fedewa, and Sheronica L. James

E-Mail: vinceg@ccf.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Cleveland Clinic Foundation, The
9500 Euclid Avenue
Cleveland, OH 44195-0001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The high rate of diabetes for the veteran population (~20%) tracks with high rates of obesity and these both have increased complications that include carotid stenosis and risk of stroke. Composition information is not available for carotid stenosis even though it is a known predictor of stroke risk. Our hypothesis is that there exist correlations between the information provided by the **C**ompositional **A**nalysis **S**ystem by **M**achine learning (CASM) algorithm and future outcomes for patients with carotid stenosis. This research effort involves enrolling up to 1500 subjects with significant carotid stenosis. The asymptomatic patients are followed for up to 3 years to determine if there are correlations between risk of stroke and the output from CASM. Also, the output of the CASM algorithm will be analyzed for key populations: e.g., diabetic vs non-diabetic and asymptomatic vs symptomatic. A total of **513 subjects have been enrolled** at the two sites with a total of **672 carotid arteries**. In addtion,154 follow-up research ultrasound exams have been collected on 206 arteries.

15. SUBJECT TERMS

Diabetes, atherosclerosis, stroke, carotid stenosis, cerebrovascular accident, ultrasound, spectral analysis, tissue characterization, machine learning

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	22	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:**

Higher rates of diabetes in the veteran population is a contributing factor in the higher rates of carotid stenosis and subsequent stroke as compared to the general population. Currently, the degree of stenosis is a key determinant in the recommended course of treatment for carotid plaque, but this measure is blind to the risk arising from composition which is not clinically available. To address this need for composition information, the Compositional Analysis System by Machine learning (CASM) algorithm was developed based on spectral analysis of backscattered ultrasound to provide a noninvasive measure of carotid plaque composition. This research effort is designed to determine correlations between output from the CASM algorithm and future outcomes regarding stroke for patients with carotid stenosis. To accomplish this, a prospective longitudinal clinical study is currently being run to enroll up to1500 subjects at two sites. Each enrolled patient receives a research ultrasound which provides input data for the CASM algorithm and if they are asymptomatic with no interventions involving their carotid, then they are followed for up to 3 years. The measurements from the CASM algorithm will be obtained and compared between diabetic and non-diabetic, symptomatic and non-symptomatic, and over time for asymptomatic subjects. Success is defined as finding significant correlations between clinical status of the subjects and the output from the CASM algorithm. These findings will be used to obtain the interest of an ultrasound imaging system manufacturer. With the goal of adding the CASM algorithm to a clinical imaging system via a 510k application. In parallel, the results will be used to design a future clinical trial to test the findings from this clinical study.

2. **KEYWORDS:**

Diabetes, atherosclerosis, stroke, carotid stenosis, cerebrovascular accident, ultrasound, spectral analysis, tissue characterization, machine learning

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

This research project, *Clinical Study of Vascular Plaque Determination for Stroke Risk Assessment*, contains six major tasks to achieve the three specific aims.

The clinical study at the heart of this research effort has two sites: The Cleveland Clinic Foundation (CCF) and the Cleveland VA Medical Research and Education Foundation (VA).

AIM 1: Determine the Correlation between Ultrasonically Obtained Plaque Composition and Future Cerebrovascular Accident (CVA)

Major Task 1: Obtain major equipment and regulatory approval

- Update legal documents with Siemens (***goal: month 1***):
  Completed prior to the start of the contract.
- Obtain two modified research ultrasound machines from Siemens (***goal month 1*****)**
  - *Milestone*: Ultrasound system hardware delivery:
    - CCF: **Completed** prior to the start of the contract.
    - VA**:** **Completed** Month 8
  - Final Software Installation for Research Ultrasound Exam from Siemens:
    - CCF: **Completed** Month 7
    - VA: **Completed** Month 8
- *Milestone*: Local IRB Approval (***goal month 3*****)**
  - CCF: **Completed** Month 9**:** 11 Jun 2021
  - VA: **Completed** Month 9**:** 10 Jun 2021

- *Milestone*: HRPO Approval (***goal month 6***)
  - CCF: **Completed** Month 12**:** 19 Aug 2021
  - VA: **Completed** Month 11**:** 30 Jul 2021
- *Milestone*: Complete 510k Application (***goal month 48***):
  - TBD: Need to have an agreement in place with a medical device manufacturer to install the CASM algorithm prior to completing a 510k application to the FDA. (See prior Annual Report from 15 Oct 2021 for a discussion on this limitation).

Major Task 2: Fabricate plaque volumetric determination system

- Procurement of hardware and fabrication of device (***goal month 1***)
  - Procurement of hardware: **Completed** Month 1: 2 Oct 2021
  - Fabrication of Device: **Completed** Month 6: 17 Feb 2021

- *Milestone*: Creation of control software and integration with ultrasound system (***goal month 2***)
  - **Completed** Month 10

Major Task 3: Enroll patients for Aim1, 2, and 3

- Start Enrollment **(goal month 6):**
  - CCF: **Completed** 15 Sep 2021
  - VA: **Completed** 10 Sep 2021
- *Milestone*: Reach 1500 Enrollment for Study
  - **Original goal of 1500:** original end to enrollment at month 42
    - CCF: 331 of 900 36.8% completed (original goal was 83.3%)
    - VA: 182 of 600 30.3% completed (original goal was 83.3%)
  - **Revised goal** as stated in 2022 Annual Report new target month 48 (***discussed further below***)
    - CCF: 331 of 450 73.6% completed
    - VA: 182 of 300 60.7% completed
    - **Total: 513 of 750 68.4% completed (revised goal is targeting 66%)**

Table 1: Targeted Enrollment from proposed SOW

	Year 1				Year 2				Year 3				Year 4	
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**
Site 1: Cleveland Clinic	0	0	75	75	75	75	75	75	75	75	75	75	75	75
Site 2: LS VAMC	0	0	50	50	50	50	50	50	50	50	50	50	50	50
Target Enrollment (cumulative)	**0**	**0**	**125**	**250**	**375**	**500**	**625**	**750**	**875**	**1000**	**1125**	**1250**	**1375**	**1500**

Table 2: Actual Enrollment by Quarter

	Year 1				Year 2				Year 3			
Actual Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Site 1: Cleveland Clinic	0	0	0	0	62	40	32	30	33	45	54	35
Site 2: LS VAMC	0	0	0	1	14	43	36	26	10	9	21	22
Actual Enrollment (cumulative)	**0**	**0**	**0**	**1**	**77**	**160**	**228**	**284**	**327**	**381**	**456**	**513**

AIM 2: Does the Presence of Diabetes Affect Estimate of Carotid Plaque Composition?

Major Task 1: Determination of vascular geometry and composition

- Develop and test automated border detection algorithms
  goal: start month 5 and complete month 30 : started but progress has been slow (discussed below)
  - Subtask - Manual borders production for initial data collection: **282/750* 37.6%**
    **note: have only enrolled 513 and thus the current completion is 55% of collected initial visits.*
  - Subtask - Automated algorithm development: manual border creation is just now completing sufficient output to enable this effort. We expect to be working on this until month 54 taking into account the expected no-cost extension. (discussed further below)
- Create plaque compositional images using the CASM software
  (task is repeated in SOW under AIM 2 Task 2 and AIM 3 Task 1)
  goal: start month 5 and complete month 48)
  actual: started in month 12: 130 arteries out of 672 arteries collected with an expected total 982** arteries enrolled thus **13%** complete.
  *** note: 31% of enrolled subjects have had both of their arteries enrolled.*
- *Milestone*: Geometry and composition of all vascular structures quantified
  composition is computed for 13% of the data, the best approach for computing geometry is being developed (see below for further discussion).

Major Task 2: Determine the effect of diabetes on carotid plaque composition

- Identify diabetic patients and find matched controls
  (goal: start month 36 and complete month 48)
  - Original start in month 36: **expect to start in month 49** after data collection is complete (see below for planned no-cost extension)
- Create plaque compositional images using the CASM software
  (see above: same task as AIM 2 Task 1)
- *Milestone*: Statistical correlation between plaque composition and geometry between diabetic and non-diabetic patients produced. Initial plant to start in month 37. This is revised to month 49 with completion by month 60.

AIM 3: Does Estimate of Plaque Composition Correlate with Future Changes in Plaque Composition

Major Task 1: Determine the rate of plaque progression and correlate with risk factors and medication.

- Identify all patients with baseline and follow-up data
  (goal: start month 36 and complete month 48) **expect to start in month 49**
  (see below for planned no-cost extension)
- Use segmentation software to identify vascular borders
  (goal: start month 30 and complete month 48) **expect to start in month 49**
  (see below for discussion of delay in segmentation and plan for no-cost extension)
- Create plaque compositional images using the CASM software
  (see above: same task as AIM 2 Task 1)

Milestone: Statistical correlation between plaque composition, geometry, and risk factors.
Plan to start in month 37 with completion by moth 60 (taking into account planned no cost extension request).

- **What was accomplished under these goals?**

  - **Enrollment**

    The primary focus of the research team remains enrollment. Over the past year we were able to enroll a total of 229 subjects with 167 from CCF and 62 from the VA. This is less than the 283 that we

enrolled in the prior year primarily with falloff at the VA site arising from personnel turnover and a subsequent disruption in the enrollment process. At CCF we have continued to struggle to increase enrollment. Specifically, the availability of sonographers has had a significant negative impact for enrollment which is reflected in the quarterly enrollment numbers where the best availability was in Quarter 3 which was our best performing quarter in the last year. We were able to get approval from the IRB to recruit patients who had been recently scheduled for their clinical carotid ultrasound exam, thus expanding the patient population (this ability was always available at the VA site).

Beyond the initial enrollment, the follow-up data collection has progressed with totals depicted in Table 3. Most of these follow-up cases were collected in the past year. There were 22 follow-up data acquisitions in the 2022 fiscal year with not second or third follow-up visits. In contrast, there was data collected for 109 follow-up visits, 22 second follow-up visits and one third follow-up visit in the past year.

Table 3: Number of Subjects with initial and follow-up research ultrasound data collections with the number of carotid arteries in parentheses.

	Initial	Follow-up	2nd Follow-up	3rd Follow-up
CCF site	331 (437)	77 (100)	7 (11)	1 (1)
VA site	182 (235)	54 (74)	15 (20)	0 (0)
Total	**513 (672)**	**131 (174)**	**22 (31)**	**1 (1)**

We continue to collect clinical information on each enrolled subject. At this point, we have enrolled **69 symptomatic patients (13.4%),** where symptomatic is defined as the patient having had a stroke or trans-ischemic attack (TIA) prior to enrolling. Of the enrolled asymptomatic patients, 31 have left the study after having a TIA (N=4), stroke (N=14), or died (N=13).

- **Manual Segmentation / 3D volume Estimation / Repeatability Study / Auto-Segmentation**

The second most important activity and the one taking the most amount of time is manual **image segmentation**. In the past year, the group has completed manual borders on 282 subjects for 378 arteries. This 37.6% of our goal of 750 and is 55% of the enrolled subjects. Thus we completed segmentation on an additional 144 subjects from last year. This would have been significantly higher, but we assigned our top person performing segmentation to assist in the 3D volume estimation (see next paragraph) and we are performing a reproducibility study (described below).

As described in the 2022 Annual Report, we have implemented the **3D volume estimation** that depends on matching images from our transverse research scans to clinical longitudinal scans to obtain a measure of the length of the plaque and the distance between collected ultrasound frames to reconstruct a 3D volume. The key task resides in matching the transverse frames to the longitudinal image. At the end of year 3, 191 arteries had been completed for 145 patients. In Quarter 2 of Year 3, we added the collection of a longitudinal image as part of the research ultrasound exam rather than relying on the clinical exam images. In Quarter 4 of Year 3, we obtained approval from the VA IRB to get a de-identified longitudinal image from the subject's clinical exam. Access to the clinical longitudinal ultrasound images was already available to CCF researchers.

To test the **reproducibility** of the 3D volume estimation and the reproducibility of borders and CASM output values, we performed a mini-study within the standard research ultrasound data collection. Specifically, for 20 consecutive research ultrasound exams we collected three consecutive video loops where the transducer is held in a transverse orientation and moved from the proximal end of the carotid to the distal end. These 60 scans will be analyzed by three members of the research team providing the ability to compare the volume computation and CASM output for repeat scans and also across three independently produced manual segmentations. All data has been collected and is currently being processed.

An intern hired for the next two years, has begun laying the foundation for **automated image segmentation**. No results to report. The collaboration with IBM to develop an auto-segmentation

approach are still in process with a draft contract completed but the contract has not been signed at this point. There continues to be a disagreement regarding how intellectual property will be handled.

- **Interim Analysis**

Our long-term goal is to produce a statistical classifier capable of providing clinically useful information. This includes producing a three-dimensional measure of plaque composition and plaque burden to include all portions of the plaque that may create the greatest risk for the subject. Histologic studies have identified a large lipid rich necrotic core, intraplaque hemorrhage, thin cap fibroatheroma [1] and amount of calcium as important structural components that define the vulnerable atherosclerotic plaque phenotype. However, whether patients with symptomatic carotid stenosis exhibit plaque characteristics distinct from those with asymptomatic carotid stenosis *in vivo* is of ongoing investigation. Further question is whether the CASM algorithm is sensitive to clinical important measure of risk for stroke.

As a test of whether our approach shows promise for distinguishing symptomatic from asymptomatic plaques, we performed the following test:

1. Radiofrequency data were collected in a transverse swipe through the plaque regions of carotid arteries from subjects enrolled in this study.
2. 130 arteries from 98 subjects were analyzed: 41 symptomatic arteries and 89 asymptomatic arteries.
3. Borders were drawn on each frame to segment the plaque from the surrounding tissue and blood.
4. The CASM classifier was used to estimate the plaque composition of the segmented frames as a combination of three tissue types: fibrous and/fibro-lipidic (F), hemorrhagic and/or necrotic core (HNC), and calcium (Ca).
5. 3D volume data for the overall plaque burden and each of the classifications was computed as described in 2022 Annual Report.

We found no significant differences between symptomatic and asymptomatic subjects for the overall volume and percent volume of F, C, or HNC as shown in Tables 4 and 5.

Table 4 Absolute volume of plaque components for symptomatic and asymptomatic subjects
Mean with the standard deviation in ***mm^3***
P-value computed using the two-sample t-test.

Tissue Type	Symptomatic (n = 41)	Asymptomatic (n = 89)	p-value
Fibrous/Fibro-lipidic	1000.20 ± 684.11	908.80 ± 650.81	.45
Calcium	187.86 ± 62.19	76.60 ± 55.94	0.73
Hemorrhagic and/or necrotic core	259.47 ± 163.07	248.02 ± 167.61	0.71

Table 5 Percent volume of plaque components for symptomatic and asymptomatic subjects
Mean with the standard deviation
P-value computed using the two-sample t-test.

Tissue Type	Symptomatic	Asymptomatic	p-value
Fibrous/Fibro-lipidic	69.16% ± 9.06%	67.82% ± 10.53%	0.54
Calcium	12.94% ± 6.82%	14.83% ± 7.86%	0.25
Hemorrhagic and/or necrotic core	17.91% ± 4.10%	17.34% ± 4.13%	0.55

In addition to the composition of the overall plaque, the frame corresponding to the point of greatest stenosis was analyzed and results are shown in Table 6. The stenosis for each frame was determined based on the manually drawn borders and computed by the entire difference of the vessel area and the

lumen area divided by the vessel area. These initial results demonstrate that the symptomatic patients have a larger Fibrous/Fibro-lipidic area as compared to the asymptomatic group.

Table 6 Mean area of plaque components at the most stenotic frame for symptomatic and asymptomatic subjects.
Mean with the standard deviation in ***mm²***
P-value computed using the two-sample t-test.

Tissue Type	Symptomatic (n = 41)	Asymptomatic (n = 89)	p-value
Fibrous/Fibro-lipidic	38.51 ± 17.36	30.02 ± 15.69	**0.009**
Calcium	7.27 ± 6.22	7.66 ± 5.59	0.73
Hemorrhagic and/or Necrotic Core	9.31 ± 5.12	8.80 ± 4.21	0.58

The results at this point are inconclusive with only the difference in fibrous at the most stenosed frame showing significance. Our prediction was that the 'hemorrhagic and/or necrotic core' (HNC) would be larger in the symptomatic population specifically at the point of greatest stenosis. The mean is nominally larger, and it is possible that when we can include all the data that there will be statistical significance. In the meantime, we are evaluating other measures as described below under our discussion of our plan for the coming year and will revisit these measures when we can include all data.

- **What opportunities for training and professional development has the project provided?**

Sheronica James attended the 2022 International Ultrasonics Symposium, 10-13 October 2022, Venice, Italy.

- **How were the results disseminated to communities of interest?**

Poster presentation and proceedings paper: James S, Fedewa R, Lyden S, Vince DG, *Integrated Backscatter Versus Spectral Parameters for in Vivo Estimation of Human Carotid Plaque Composition*, 2022 IEEE International Ultrasonics Symposium, Oct 10-13, 2022.

Copy of the proceedings paper is in the Appendix.

- **What do you plan to do during the next reporting period to accomplish the goals?**

We plan to apply for a no cost extension to permit more time for both enrollment and analysis. For the upcoming year, enrollment and manual image segmentation will remain the primary activities of the laboratory and will shift to more analysis as the year progresses.

- **Enrollment**

  We are planning to continue enrollment through year 4 and complete analysis during a no-cost extension of the grant. This continuation will help to offset the lost time (roughly 6 months) for enrollment at the start of the project as we had delays in obtaining the modified ultrasound system (see 2021 Annual Report). Also, the extension provides the opportunity to reach 750 enrolled subjects. The CCF site has been consistent over the last 2 years with enrollment totals of 164 and 167. The VA site enrollment decreased significantly from 119 to 62. We anticipate closer to 80 subjects in Year 3 at the VA site since the last two quarters they were slightly over 20 subjects enrolled each quarter. Thus we predict a total enrollment of 160 from CCF and 80 from the VA site resulting in a total of 240 subjects which is sufficient to reach 750 enrolled subjects. This level of enrollment will provide roughly 980 carotid arteries based on 30% of enrolled subjects qualifying for both arteries.

  For follow-up cases, we anticipate exceeding the 132 that were collected in the past year.

- **Repeatability Study**

  The repeatability study will be completed with each of three research team members creating manual segmentation for each subject where three sets of data were collected from the artery. Analysis will permit the comparison of

  - Intra-operator reproducibility: for the same person creating borders on the same patient, differences in the CASM outcomes and overall plaque burden will be compared.
  - Inter-operator reproducibility for manual segmentation: for the same data sets the differences across the three researchers performing segmentation will compared.

- **Interim Analysis: Part 2**

  The limited results from the initial interim analysis are not surprising, although a bit disappointing. We had hoped for sharper differences between the symptomatic and asymptomatic populations that would be apparent at this early stage. Specifically, the histologic findings in carotid plaques showed no significant difference in the amount of lipid-rich necrotic core and calcification between symptomatic and asymptomatic plaques [2], [3] which we expect to play an important role in risk of stroke. These results have sparked a re-evaluation of the literature where the relationship between hemorrhage and symptoms remains unclear. Some studies have reported no significance between the two groups with respect to hemorrhage and the presence of necrotic core [3]–[5], while others have reported the opposite [6], [7]. Most studies, including our initial analysis, have quantified hemorrhage and necrosis independently of location. A few magnetic resonance studies have reported symptomatic plaques as having greater juxta-luminal hemorrhage compared with asymptomatic ones [7], [8]. A ruptured thin fibrous cap is known to be the precursor for most acute atherosclerotic events, including ischemic cerebral events (stroke, TIA) associated with carotid atherosclerosis. Intraplaque hemorrhage, defined by the accumulation of blood components within the plaque, is not necessarily associated with atherosclerotic plaque rupture [9], but is major contributor to the enlargement of the necrotic core underlying the thin fibrous cap [10]. Cap rupture and intraplaque hemorrhage has also been reported to occur more frequently upstream of the maximum stenosis [11]. Thus, the proximity of HNC to the lumen surface and location within the length of the plaque may be a more sensitive measure of stroke risk.

  In the next year we will be investigating the following:

  1. Phenotype based analysis: e.g. Computing the proximity of HNC to the lumen surface where thrombus formation occurs.
  2. Differences in HNC composition/percentages between the distal and proximal regions of the plaque
  3. Comparison of different approaches for determining which portion of the plaque to analyze for future stroke risk. (e.g. We may need to look proximal to the region of greatest stenosis.)
  4. Continued investigation of additional parameters that can be used to improve the CASM algorithm building on the proceedings paper that is included in the appendix.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

  Nothing to Report

- **What was the impact on other disciplines?**

  Nothing to Report

- **What was the impact on technology transfer?**

Nothing to Report

- **What was the impact on society beyond science and technology?**

  Nothing to Report

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

  Nothing to Report

- **Actual or anticipated problems or delays and actions or plans to resolve them**

  - **Enrollment**

    We continue to under enroll at both sites and are still working to reach half the original enrollment target of 1500 subjects. Over the last year this has been negatively impacted by the following issues:
    - A lack of sonographers at the CCF site, this was truly an issue for most of the last year except for quarter 3 which was the best enrollment quarter of the past year.
    - Personnel turnover at the VA site had a negative impact on enrollment which did not rebound until quarter 3 of the past year.

    To mitigate the slow enrollment and to reach the 750 target we plan to request a no cost extension in the next year in to keep enrolling through the next year and then use the extension to complete the analysis stage. We are still seeing that roughly 30% of enrolled subjects have both carotid arteries qualify for the study. At that rate, we anticipate that 750 subjects translate into 975 carotid arteries.

  - **Automating Image Segmentation**

    The IBM collaboration remains in the negotiation stage in month 36 we are in the final stage of contract negotiation. We still are hopeful that a collaboration with IBM as part of the Cleveland Clinic-IBM Discovery Accelerator will occur, but we are not planning on them being able to contribute prior to the end of this grant period. We have begun working on ultrasound image segmentation and will continue to move towards implementing a convolution neural network approach. We now have a data set from 282 patients for 378 arteries as a starting point for training an AI approach for automation.

  - **Interim Analysis**

    First, not finding that the symptomatic subjects have greater amounts of HNC is not definitive at this point and the true test is when we have collected and analyzed the complete data set. However, it does raise the potential that the amount of HNC may not be an important measure for clinical care. In response to current null findings, we have begun an analysis based on phenotype where we will produce measures of the location of the HNC with respect to the lumen surface among other measures (see above). This further analysis has the risk of becoming a 'fishing expedition'. To mitigate the risk of finding a 'false' correlation. We plan on continuing to use these 130 arteries to look for correlations and trends that will then be tested with the remainder of the data being acquired during this project.

- **Changes that had a significant impact on expenditures**
  - The limited enrollment is directly associated with the cost for ultrasound technician time (i.e. sonographer).
  - The expected replacement for Tanujit Dey (Key personnel, statistician) has been delayed. We have a specific candidate are gathering the information to submit in order to add the new statistician.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  - **Significant changes in use or care of human subjects**

    Nothing to Report

  - **Significant changes in use or care of vertebrate animals.**

    Nothing to Report

  - **Significant changes in use of biohazards and/or select agents**

    Nothing to Report

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**

  - **Journal publications.**
    Nothing to Report

  - **Books or other non-periodical, one-time publications.**
    Nothing to Report

  - **Other publications, conference papers, and presentations.**
    James S, Fedewa R, Lyden S, Vince DG, "Integrated Backscatter Versus Spectral Parameters for in Vivo Estimation of Human Carotid Plaque Composition", 2022 IEEE International Ultrasonics Symposium, Oct 10-13, 2022.
    Proceedings paper is included in the Appendix.

- **Website(s) or other Internet site(s)**
  Nothing to Report

- **Technologies or techniques**
  Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Nothing to Report

- **Other Products**

  We have collected a partial data set for 513 enrolled subjects (see section 3 above for details) that includes the following:
  - Initial research ultrasound data set comprising a 3D visualization of the carotid artery and plaque
  - Clinical data associated with each subject
  - Manual borders (partially complete)
  - CASM output (partially complete)

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

D. Geoffrey Vince – no change
Michael A. Rosenbaum – no change
Sheronica L. James – no change
Jaqueline Loftis – no change
Maya Mays – no change

Name:	Russell J. Fedewa
Project Role:	Co- Investigator at CCF site
Researcher Identifier (e.g. ORCID ID):	0000-0002-0690-9472
Nearest person month worked:	10
Contribution to Project:	Managing regulatory submissions with key sites and collaborators. Development of clinical study protocol and support documents with assistance from co-investigators. Oversight and review of lab personnel for image segmentation. Support for signal and image processing and programming with support for image segmentation and lead for automatic segmentation effort.
Funding Support:	NA

Name:	Lauren Hetzel
Project Role:	Research Technician at CCF site
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	11
Contribution to Project:	Screening, enrolling, and data acquisition are the primary tasks with secondary task of image segmentation.
Funding Support:	2 months funded from internal Cleveland Clinic funds

Name:	Allison Smollen
Project Role:	Clinical Research Coordinator at CCF site
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	12
Contribution to Project:	Screening of patients, initial contact and follow-up, capturing data from medical records, handling communications with IRB, record keeping for the research study operation.
Funding Support:	NA

Name:	Jesse Wu
Project Role:	Research Intern
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	4
Contribution to Project:	Developing auto-segmentation for ultrasound images
Funding Support:	NA

Name:	Jerad Williams
Project Role:	Research Nurse / Study Coordinator at VA site
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	2
Contribution to Project:	Mr. Williams has been responsible for the screening, recruitment, and consenting of subjects. He has also assisted with study data collection.
Funding Support:	NA

Name:	Manda Double
Project Role:	Research Regulatory and Compliance specialist at VA site
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	1
Contribution to Project:	Ms. Double has managed the IRB submission and documentation for the Northeast Ohio VA Health System IRB, tracking of human subjects' certification and Conflicts of Interest documentation, and assisted with regulatory audits.
Funding Support:	NA

Name:	Abdelrahman Eisa
Project Role:	Study Coordinator at VA site
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	9
Contribution to Project:	Mr. Eisa has been responsible for the screening, recruitment, and consenting of subjects. He has also assisted with study data collection.
Funding Support:	NA

Name:	Sarah Dzigiel
Project Role:	Study Coordinator at VA site
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Ms. Dzigiel was responsible for the screening, recruitment, and consenting of subjects. She also assisted with study data collection. She filled in as the study coordinator after Mr. Williams left the project until when Mr. Eisa took over as the primary study coordinator
Funding Support:	NA

:

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  D. Geoffrey Vince – no change
  Russell J. Fedewa – no change
  Natalia Fendrikova-Mahlay – no change

  Name/Role: Michael A. Rosenbaum / Co-Investigator
  Description of Change: Participation in the following research effort has **ended**
  Title: **Treatment of Deep Venous Thrombosis via Targeted Inhibition of the FXII-uPAR-pAkt2 Axis in Neutrophils**
  Funding Agency: Department of Veterans Affairs Biomedical Research and Development Service, 1I01BX003851
  Start and End Date: 10/1/2019 – 09/30/2023
  Level (%) of Effort in the Project: 10%
  Potential Overlap: None
  Role: Co-Investigator

Name/Role: Michael A. Rosenbaum / Co-Investigator
Description of Change: Participation in the following research effort has **ended**
Title: **Effects of Lipids on Vascular Graft Healing**
Funding Agency: National Institutes of Health/National Heart, Lung, and Blood Institute, 2R01 HL064357
Start and End Date: 12/01/2017 – 11/30/2023
Level (%) of Effort in the Project: 10%
Potential Overlap: None
Role: Co-Investigator

Name/Role: Michael A. Rosenbaum / Co-Investigator
Description of Change: Participation in the following research effort has **begun**
Title: **Src family kinase-induced PI3K p85α activation mediates TRPC6 channel activation and impaired arterial healing after injury**
Funding Agency: Cleveland Veterans Affairs Medical Research and Education Foundation
Start and End Date: 09/21/2023 – 03/31/2025
Level (%) of Effort in the Project: 25%
Potential Overlap: None
Role: PI

Name/Role: Michael A. Rosenbaum / Co-Investigator
Description of Change: Participation in the following research effort has **begun**
Title: **A Phase 3, Randomized, Double-Blind, Double-Dummy, Parallel Group, Active-Controlled Study to Evaluate the Efficacy and Safety of Milvexian, an Oral Factor XIa Inhibitor, Versus Apixaban in Participants With Atrial Fibrillation**
Funding Agency: Janssen Research & Development, LLC; NCT05757869
Start and End Date: 7/1/2023 – 06/30/2027
Level (%) of Effort in the Project: 10
Potential Overlap: None
Role: Co-Investigator

- **What other organizations were involved as partners?**

Organization Name: Siemens Medical Solutions USA, Inc.
Location of Organization: 51 Valley Stream Parkway, Malvern PA 19355, USA
Partner's contribution to the project: In-kind support
Equipment loan: 2 Siemens Sequoia Ultrasound Systems with associated probes, software, service, and engineering support.

Organization Name: Cleveland VA Medical Research and Education Foundation
Location of Organization: 10701 East Blvd, Cleveland, OH 44106-1702, USA
Partner's contribution to the project: Collaboration and Facilities
Second site for clinical study and scientific collaboration with site personnel.

8. **SPECIAL REPORTING REQUIREMENTS**

Nothing to Report

9. **APPENDICES:**

- **Bibliography**

- **Proceedings Paper:** James S, Fedewa R, Lyden S, Vince DG, *Integrated Backscatter Versus Spectral Parameters for in Vivo Estimation of Human Carotid Plaque Composition*, 2022 IEEE International Ultrasonics Symposium, Oct 10-13, 2022.

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Integrated backscatter versus spectral parameters for in vivo estimation of human carotid plaque composition

Sheronica James
Dept. of Biomedical Engineering
Cleveland Clinic
Cleveland, Ohio, USA
jamess4@ccf.org

Russell Fedewa
Dept. of Biomedical Engineering
Cleveland Clinic
Cleveland, Ohio, USA
fedewar@ccf.org

Sean Lyden
Dept. of Vascular Surgery
Cleveland Clinic
Cleveland, Ohio, USA
lydens@ccf.org

D. Geoffrey Vince
Dept. of Biomedical Engineering
Dept. of Cardiovascular Medicine
Cleveland Clinic
Cleveland, Ohio, USA
vinceg@ccf.org

***Abstract*— Carotid plaque composition is a missing piece of information in the treatment of carotid stenosis. This study evaluates a spectral analysis-based approach versus an intensity only approach (comparable to an idealized grayscale) using both fundamental and harmonic bandwidths. The intensity approach utilizes the integrated backscatter (IB), while the spectral analysis approach uses the slope, intercept and mid-band fit from an estimate of the backscatter transfer function. Backscattered ultrasound RF data were acquired in *vivo* from 134 subjects prior to carotid endarterectomy. Serial histology slides of the surgically excised plaque were matched to grayscale images created from the RF data. 1.2 mm by 1.2 mm regions of interest (ROI) were selected in the RF data corresponding to homogenous regions within the histology, determined as calcified (Ca), fibrous or fibro-lipidic (F), and hemorrhagic and/or necrotic core (HNC). A balanced data set of 130 Ca, 120 F, and 125 HNC ROI's was randomly selected to train and test three random forest classifiers relying on 1) IB, 2) spectral linear fit parameters, or 3) both IB and spectral linear fit parameters as inputs. Color-coded maps for 30 randomly selected matched frames were produced from the classifiers and compared to the matched histology based on a blinded expert review. HNC accuracy and specificity were slightly better for the spectral linear fit based approach than IB (accuracy, 0.63 ± 0.05 vs 0.57 ± 0.06 and specificity, 0.77 ± 0.1 vs 67 ± 0.1), while sensitivity was the same for both (0.36 ± 0.07). The spectral linear fit parameter-based model provided the best representation of the plaque for 24 of 30 frames. These results support the understanding that spectral information can improve on the performance of intensity only based approaches for ultrasound-based tissue characterization of carotid plaque.**

Keywords—ultrasound, carotid, atherosclerosis, spectral analysis, tissue characterization, harmonic imaging

I. Introduction

Carotid atherosclerosis is a major cause of ischemic stroke, which accounts for roughly 87% of all strokes [1]. While a well-established combination of clinical information and diagnostic imaging features, namely degree of stenosis, have been used in cerebrovascular risk stratification [3], guidelines from the American Society of Neuroradiology [2] and the European Society of Cardiology have indicated an association between plaque morphology and risk of ischemic stroke. Plaques exhibiting ulceration, intraplaque hemorrhage, lipid-rich necrotic core and active inflammation are associated with a high risk of stroke [3]–[6]. Thus, plaque composition is likely to be a more powerful indication of patient risk for future cerebrovascular events than the current standard of carotid stenosis but is unavailable to the vast majority of patients receiving treatment for carotid atherosclerosis.

Multiple imaging modalities are attempting to address this clinical need. Ultrasound grayscale (B-mode) analysis-based approaches have initially shown potential to improve risk stratification, but are highly operator-dependent, and therefore limited in clinical usefulness [7], [8]. Computed tomography can quantify calcium burden as well as detect presence of lipid and ulceration, but expose patients to ionizing radiation [9]. While, magnetic resonance has the ability to distinguish lipids, hemorrhage, and necrotic core regions [10]–[12], it is expensive and not applicable for clinical implementation in a screening or monitoring role.

Ultrasound spectral analysis of backscattered radiofrequency (RF) data provides information about tissue microstructure that is lost during grayscale/intensity image formation. Studies analyzing plaque composition *in vivo* using integrated backscatter (IB) of the normalized power spectrum have been able to differentiate fibrous, fatty and calcified lesions of the [13]–[16] arterial wall. However, hemorrhage or necrosis have rarely been considered. Additionally, since the IB parameter is a sum of the area under the normalized power spectrum curve of the backscattered signal, it does not provide information on the variation of scattering with respect to frequency or traditionally include the nonlinearly generated harmonic portion of the backscattered signal.

This study evaluates a spectral analysis-based approach versus an intensity only approach (comparable to an idealized grayscale) to characterize carotid plaque. The intensity approach utilizes the integrated backscatter, while the spectral analysis approach uses the slope, intercept and mid-band fit

This work was supported by the Office of the Assistant Secretary of Defense and Health Affairs, through the Peer Reviewed Medical Research Program under Award No. W81XWH-16-1-0608. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the Department of Defense or U.S. Army.

Research supported by AHA SDG Award 15SDG25700258

Ultrasound imaging system loaned by Siemens Medical Solutions USA, Inc.

from the linear fit to an estimate of the backscatter transfer function. Parameters are extracted from normalized power spectra for both fundamental and harmonic bandwidths. Plaque is classified into three types: calcified (Ca), fibrous or fibro-lipidic (F), and hemorrhagic and/or necrotic core (HNC). Quantitative parametric images of carotid plaque specimens are constructed from the analysis of ultrasonic RF measurements and compared to the matched histology based on a blinded expert review.

II. METHODS

A. Patients and specimens

We enrolled 140 patients undergoing a carotid endarterectomy (CEA) for carotid atherosclerosis. Age was restricted to greater than 40 years, since stenosis in younger patients is often due to processes other than atherosclerosis. Only patients with atherosclerotic plaque located from the distal end of the common carotid artery through the internal carotid artery, including the common carotid artery bulb, were permitted to enroll. Enrollment was limited to those without prior ipsilateral carotid intervention (e.g., CEA, stent, radiation therapy). The excised plaque was collected following surgical removal. Specimens were immersed in saline and ultrasonically imaged on the same day as the CEA.

B. In vivo data acquisition

Post-beamformed pulse inversion RF data were acquired from 134 subjects using a Siemens S2000 or S3000 ultrasound system (Siemens Medical Solutions USA, Inc., Malvern, PA), 9L4 probe and Axius Direct Ultrasound Research Interface (URI) software, which records the RF signals prior to image processing. Data collection was performed prior to CEA. First, RF data were collected at static sites while holding the transducer in the transverse orientation with respect to the carotid artery. Sites were separated by roughly 1 cm from the proximal to distal ends of the carotid plaque region. This 1 cm step size provides sampling independence between neighboring sites. Next, one or more RF data loops of 54 frames each were collected while moving the transducer from the proximal end of the plaque to the distal end. These loops served as a backup to the static sites and provided context for matching *in vivo* frames to histology. Reference RF data were acquired from a 0.5 dB/cm-MHz tissue-mimicking phantom (Ultrasound Resolution Phantom model 044, Computerized Imaging Reference Systems, Inc., Norfolk, VA, USA).

Identical system settings were used for both the *in vivo* data collection and the phantom data collection. The depth was set to 4 cm with a transmit focus of 2 cm. Each frame of RF data is composed of 456 lines by 2076 points digitized at 40 MHz.

C. Histology matching to ultrasound data

The excised plaque tissue was imaged in saline to locate regions of the plaque corresponding to the *in vivo* static sites. The tissue was then placed in 10% buffered formalin for at least 24 hours followed by two rounds of de-calcification (Cal-Rite, Thermo Fisher Scientific, Runcorn, UK). Prior to paraffin embedding, the target sites for matching were marked on the tissue with dye. Serial histology was performed with slide pairs taken at 1 mm steps before and after the marked region and 0.5 mm steps through the target region. One slide of each pair received hematoxylin and eosin stain while the other slide received Movat's pentachrome stain. The best matching slide to each *in vivo* grayscale site was found by one member of the research team and reviewed by a different member. Discrepancies were discussed and resolved. A pathology expert then read the matched slide and the nearest neighbor slides defining regions of homogenous tissue across all three slides as calcified, fibrous or fibro-lipidic, and hemorrhagic and/or necrotic core.

Matches to histology were found for 231 *in vivo* sites from 111 of the 134 CEA subjects. Inability to match slides was due to the following: plaque not being removed, plaque sent to pathology, plaque removed in pieces and thus interfering with matching, and simply low or no confidence in locating a matched histology slide to the grayscale image frame. Homogenous regions were identified for 186 slide-frame pairs from 92 subjects.

D. Signal processing

Grayscale B-mode images were formed from ultrasound backscatter RF data acquired *in vivo* prior to subjects receiving a CEA. Customized Matlab software (The Mathworks Inc., Natick MA, USA) was used to draw regions of interest (ROI's) within the grayscale *in vivo* images corresponding to homogenous tissue types. The RF data corresponding to these 1.2mm by 1.2 mm (15 scanlines by 64 points) ROI's was extracted. The fundamental signal was obtained by computing the pulse inversion difference and the nonlinearly generated second harmonic signal, by computing the pulse inversion summation. An average power spectrum for each ROI was obtained by computing the power spectrum of each scanline within the ROI using a Yule-Walker Autoregressive method of order 24, converting to dB scale, and then averaging over all 15 lines. The -20dB bandwidths were obtained from the phantom data: 2.5 MHz to 6.9 MHz for the fundamental and 4.9 MHz to 10.1 MHz for the harmonic. These signals were normalized using a reference phantom approach [17] to obtain an estimate of the backscatter transfer function (eBTF).

Four spectral parameters were extracted from the eBTF for each frequency band: integrated backscatter and linear fit parameters slope, intercept, and mid-band fit. These served as input parameters for three random forests based on the following parameter sets: integrated backscatter alone, spectral linear fit parameters, and both sets of parameters in combination. Random forest classifiers were created within Matlab using these parameter sets as inputs. Classifiers were grown to 100 trees.

Our initial dataset contained a total of 737 ROI's (Ca = 135, F = 125, HNC = 477) from 186 *in vivo* ultrasound frames. A random subsample of 130 Ca, 120 F, and 125 HNC ROI's was selected as a balanced dataset. Stratified random sampling was used to partition this smaller dataset into a training set and test set with the same relative class

frequencies in each. The training set contained two-thirds of the data, while the remaining one-third was used for testing. The predicted outcomes of the testing sets were compared to the known plaque types from histology to obtain the predictive accuracy, sensitivity, and specificity of the classification models.

The three classifiers were used to estimate the composition of 30 randomly selected matched frames using a 75% axial and lateral overlap of ROI's. Color-coded map overlays of the plaque composition were constructed from the classifier output and ranked by two experts based on similarity to histology. Reviewers were presented with a set of images for each of the 30 matched frames that included the Movat pentachrome stained histology image, grayscale match and color-coded map overlays based on the 3 classifier outputs. The map overlays were placed in randomized order, and the reviewers were blinded as to which algorithm each image was obtained from. Reviewers were also given a set of detailed histology notes for each slide. For each set, they were instructed to assign integer ranks to the map overlays from 1 to 3, where 1 was the most similar to histology and 3 was the least similar.

III. Results and Discussion

A tally of the blinded review ranking results are shown in Table 2. The spectral linear fit based model provided a better representation of the plaque histology for 24 of 30 output images. The combination of all parameters was found to be worse than either parameter set by itself.

The accuracy assessment of each classification system was based on the confusion matrix constructed from the predicted and known values of each data set. Plaque ROI's were classified into three types: calcium; fibrous or fibro-lipidic; hemorrhagic and/or necrotic core. Results in Table I focus on HNC versus not HNC, since this tissue type is associated with vulnerable or rupture-prone plaques. Sensitivity was the same for both (0.36 ± 0.07), but HNC accuracy and specificity were better for the spectral linear fit based approach than IB (accuracy, 0.63 ± 0.05 vs 0.57 ± 0.06 and specificity, 0.77 ± 0.1 vs 0.67 ± 0.1). While not statistically significant, this difference was not surprising when seeing the example compositional color overlay in Fig. 1. Both models identified the calcified HNC region on the left, but the IB based model overestimated the extent of the HNC region. The IB based model also appears to be more susceptible to misclassifying fibrous regions when the fibers are perpendicular to the ultrasound propagation direction. This can be seen in Fig. 1, where the IB based model misidentifies fibrous tissue as HNC along the top of the vessel. Both IB and spectral linear fit parameters identify the calcified nodule on the bottom, but the spectral linear fit parameters appear to misclassify part of the nodule as HNC.

TABLE I. HNC classification model performance for IB and spectral linear fit parameters

STATISTIC	**MODEL**	
	IB	***Spectral linear fit***
Accuracy	0.57 ± 0.06	0.63 ± 0.05
Sensitivity	0.36 ± 0.07	0.36 ± 0.07
Specificity	0.67 ± 0.1	0.77 ± 0.1

TABLE II. Blinded review rankings of 30 classification output images based on similarity to histology

MODEL	**RANKING**		
	First	***Second***	***Third***
IB	4	18	8
Spectral linear fit	24	5	1
IB + Spectral linear fit	2	7	21

IV. Conclusion and Limitations

Spectral analysis of backscattered RF ultrasound provides additional discriminatory information not available in intensity-based approaches for estimating carotid plaque composition. These results demonstrate the potential for using spectral parameters to distinguish hemorrhagic and/or necrotic core from calcified and fibrous tissue within carotid plaque.

No attempt was made to remove outliers in the data or determine parameter importance in the random forest models. ROI selection is not exact. Some of these homogenous regions may include mixed tissue types. These misplacements arise from the warping of the tissue during histology processing and the inherent error of matching a single slice of tissue that is microns thick to an ultrasonic slice that is orders of magnitude greater.

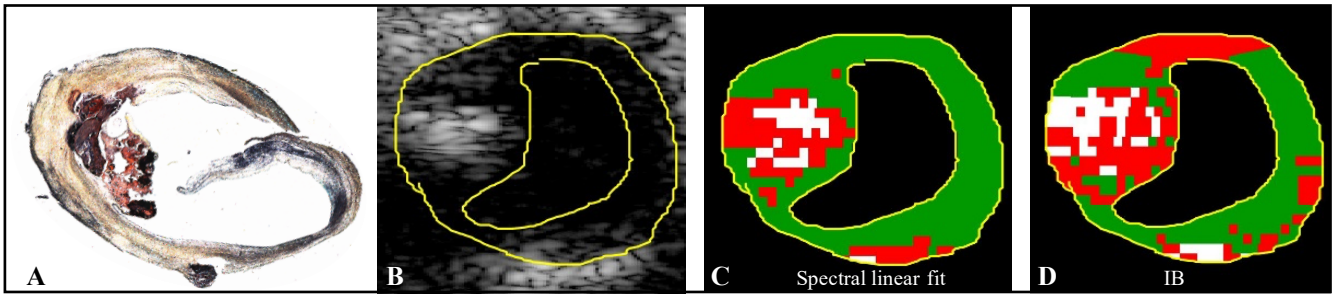

Fig. 1: Matched Data: (A) Movat pentachrome histology slide; (B) grayscale ultrasound; (C) & (D) Color maps based on classification of spectral linear fit parameters and IB parameter respectively. For the Color Maps: calcified (white); fibrous or fibro-lipidic (green). hemorrhagic and/or necrotic core (red); Histology depicts a calcified hemorrhagic and/or necrotic core on the left with a calcified nodule on the bottom and the remainder is fibrous.

Intensity based approaches have not been able to successfully translate to the clinical setting despite demonstrating correlation with increased stroke risk. The addition of spectral information to intensity measures demonstrates improved fidelity to the tissue composition. However, the definitive test for this methodology will depend on the value it brings to clinical care.

ACKNOWLEDGEMENT

The authors thank the skilled contributions provided by the sonographers within the Heart and Vascular Institute Vascular Laboratory at Cleveland Clinic. Study data were collected and managed using REDCap electronic data capture tools hosted at Cleveland Clinic[18], [19].

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