Medtronic **Statistical Analysis Plan**	
Clinical Investigation Plan Title	FlowMet Blood Flow Measurement for the Diagnosis of Peripheral Artery Disease (PAD) and Critical Limb Ischemia (CLI)
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1. Version History

Version	Summary of Changes	Author(s)/Title
1.0	• 'Not Applicable, New Document'	[redacted] Principal Statistician

2. List of Abbreviations and Definitions of Terms

Abbreviation	Definition
ABI	Ankle Brachial Index
AE	Adverse Event
AUC	Area Under the Curve
CIP	Clinical Investigation Protocol
CLI	Critical Limb Ischemia
ICF	Informed Consent Form
PAD	Peripheral Artery Disease
ROC	Receiver Operating Characteristic
TBI	Toe Brachial Index

3. Introduction

It is well established in the literature and in clinical practice that diminished blood flow to the extremities, as a result of peripheral artery disease (PAD) or otherwise, may impair wound healing and overall tissue viability [1-5]. PAD is labeled a "large personal, social, and economic burden in the United States" by the American Heart Association and American College of Cardiology. PAD is estimated to affect 8-10 million in the United States alone, while having a significant mortality rate (30% mortality in 5 years) [6, 7]. Over $5B is spent annually treating peripheral vascular patients, the majority of which results from late diagnoses, ineffective interventions, and resulting lower limb amputations [8].

One end-stage manifestation of PAD is critical limb ischemia (CLI) [9, 10]. Generally, CLI is defined as: "any patient with chronic ischemic rest pain, ulcers, or gangrene attributable to objectively proven arterial occlusive disease" [9]. Early detection and treatment has the potential to keep PAD from reaching the point of CLI. Angiography is the gold standard for anatomical visualization of blood vessel structure and stenosis. However, angiographic information does not provide simple and functional metrics of blood flow, especially in the case of multiple stenoses. As such, the relationship between extremity blood flow and PAD/CLI cannot be elucidated without a more appropriate methodology for quantifying peripheral perfusion.

The ankle brachial index (ABI) is perhaps the most widely used screening technique to diagnose PAD. While the ABI is relatively simple to perform, it is a ratio of systolic blood pressures rather than a direct measurement of blood flow, and, despite its widespread usage, is only a proxy of direct perfusion measurements. Generally, a cutoff of ABI less than 0.91 is used to predict the presence of PAD, and a cutoff of ABI less than 0.4 is used to predict the presence of severe PAD indicating CLI [11].

While the noninvasive technologies mentioned above are considered standard of care in vascular medicine, there remains a lack of simple and continuous methods to assess PAD severity. A portable technology capable of quantifying disease severity simply and in real time could significantly ameliorate diagnosis, surgical treatment, and follow up care.

This study has many clinical implications. FlowMet-R is the first noninvasive technology to provide accurate and continuous quantification of microvascular perfusion. Hence, this technology may provide improved diagnostic and/or prognostic capabilities during procedures and in outpatient settings. The current study will set the foundation for future indications of FlowMet-R technology in vascular care.

The purpose of the investigation is to assess the efficacy of the FlowMet-R device in diagnosing PAD and CLI in patients scheduled for peripheral vascular examination. The results of the assessment of the FlowMet-R device will be compared to gold standard diagnostics including ABI, Toe Brachial Index (TBI), and Doppler Ultrasound.

4. Study Objectives

4.1 Objectives

4.1.1 Primary Objectives

The primary objective of this study is to determine the efficacy of FlowMet-R measurements in diagnosing PAD and CLI. To accomplish this objective, FlowMet-R data (comprised of blood flow measurements and/or feature analysis of the blood flow waveform) will be used to create a predictive model of PAD severity. This model will then be used to generate diagnostic receiver operating characteristic (ROC) curves for PAD and, independently, CLI, at multiple time points. The following endpoints will be used to assess fulfillment of this objective.

4.1.1.1 Primary Endpoints

Employ a predictive model based upon FlowMet-R data to generate ROC curves for the diagnosis of PAD and, independently, CLI. ROC curves will be generated at the initial, three month, and six month time points, and will be used to compute the peak sensitivity, peak specificity, and area under the curve (AUC).

4.1.1.1.1 Definitions of PAD and CLI

- Two types of positive diagnosis of PAD will be explored according to published literature: (1) ABI≤0.9 or ABI>1.4 and secondary verification of TBI≤0.7 with exhibition of at least mild claudication (Rutherford Category > 0), or (2) ABI≤0.9 or ABI>1.4 and secondary verification of TBI≤0.7 without exhibition of symptoms. In either case, if the patient displays normal ABI but PAD is suspected, then a postexercise ABI will be performed using a 1-minute treadmill test or heel-raises. A postexercise drop of ABI ≥ 20% or pressure drop ≥ 30mmHg will be considered a positive diagnosis [12].
- A positive diagnosis of CLI will be defined by exhibition of chronic rest pain and active wounds, verified by TBI < 0.3.
- Patients that are enrolled in the PAD positive cohort but are not positively diagnosed for PAD using the above definition (either because their disease is improving or they were screened incorrectly) will be considered PAD negative (see Section 7.11 for change to planned analysis).

4.1.2 Secondary Objectives

The secondary objectives seek to discover the relationship between FlowMet-R measurements and several additional metrics important in vascular medicine. First, the capability of FlowMet-R in the prognosis of requiring a vascular intervention or amputation within three and six months following initial visit will be determined. Additionally, the correlation between FlowMet-R measurements and peripheral artery stenosis will be investigated. Finally, the correlation between changes in ABI/TBI/Rutherford Classification and changes in FlowMet-R data between any two time points will be assessed.

4.1.2.1 Secondary Endpoints

- Utilize FlowMet-R data to create a predictive model for the prognosis of vascular intervention within a three and six month timeframe from initial visit.
- Employ the predictive model of PAD severity to generate an ROC curve for diagnosis of significant stenosis (>50%) in the iliac, femoral, popliteal, peroneal, or tibial arteries.
- Conduct a correlation test between the predictive model of PAD severity and greatest percent stenosis in the iliac, femoral, popliteal, peroneal, or tibial arteries.
- Conduct a correlation test between changes in the predictive model of PAD severity and corresponding changes in ABI, TBI, and Rutherford Classification between any two study time points.

5. Investigation Plan

This study is a non-randomized, multi-center, longitudinal study of healthy subjects and subjects with PAD who are scheduled for ABI, TBI, and either Duplex ultrasound or Angiographic assessments in a vascular clinic. Subjects will first be evaluated for study inclusion/exclusion. If the subject meets the inclusion criteria and wishes to enroll in the study, they will be asked to sign an informed consent form (ICF) and considered for enrollment. If the subject meets inclusion criteria and is enrolled, the subject will undergo approximately 3 minutes of FlowMet-R blood flow measurements in addition to their routine standard of care examination.

Subjects enrolled in the PAD cohort will be assessed according to Event Schedule A in the clinical investigation protocol (CIP) at approximately 0, 3 months, and 6 months. Subject follow ups must meet the scheduled time window within +/- 30 days. Subjects that require a vascular (re)intervention for PAD-related treatment before the final measurement (6 month follow up) will have pertinent data collected and saved in the study registry. The vascular procedure may change the timing with respect to the amount of months after baseline that the follow up visits occur. In that case, the standard of care follow up visits post intervention will be adhered to, and data will be collected from the follow up visits that correspond most closely to timepoints at 3 and/or 6 months post baseline visit.

Subjects enrolled in the Healthy cohort will be assessed according to Event Schedule C in the CIP at a single time point. Imaging outside standard of care will not be performed on the healthy subjects, and healthy cohort subjects with normal ABI and TBI values will be assumed not to have significant peripheral arterial stenosis.

5.1 Duration

The expected study duration is approximately 24-36 months. The duration of individual subject participation will vary based on timing of their enrollment and completion of the final follow-up visit; however, at a minimum, participation of an individual subject will be at least 6 months for subjects suspected of having PAD and 0 months for healthy subjects.

5.2 Rationale

The study will assess the FlowMet-R's ability to provide a simple and continuous method of assessing PAD severity. To achieve the objectives defined in Section 4.1, FlowMet-R measurements will be acquired from the limbs of enrolled patients and the relationship between the FlowMet-R data and positive diagnosis of PAD and CLI will be evaluated at multiple time points (initial measurement, 3 month follow up, and 6 month follow up). Resulting sensitivity, specificity, accuracy, and ROC curves will be generated to demonstrate diagnostic capability. Changes in FlowMet-R data for enrolled patients at each study time point will be compared to corresponding changes in ABI, TBI, or patient symptoms. Finally, the prognostic capability of FlowMet-R data will be assessed by calculating the sensitivity and specificity of predicting whether surgical (re)intervention will be necessary following initial assessment.

5.3 Study Population

Subjects shall be enrolled until the following number of limbs have been measured:

- 160 control limbs (no diagnosed PAD)
- 240 limbs with PAD
    - Of the limbs with PAD, a subset >100 limbs with CLI
    - Of the limbs with PAD, a subset >54 limbs without CLI

Any patient being seen for diagnosis of suspected PAD may be screened for enrollment in the PAD cohort. For patients undergoing intervention, supplemental data will be taken within a catheterization laboratory. Control data from a healthy cohort will also be gathered. Recruitment of healthy subjects is at the site's discretion. Third party recruitment services such as researchmatch.org may be used.

5.4 Subject Enrollment

First, the patient will be asked if he/she would like to participate in the study. If so, they will undergo the informed consent procedure.

When a subject and the principal investigator or authorized designee, as required, have personally signed and dated the IC, the subject is considered to be enrolled in the study. The date the subject signed the IC must be documented in the subject's medical records.

Subjects will be screened to ensure they meet all of the inclusion and none of the exclusion criteria prior to study enrollment.

5.5 Inclusion Criteria

Candidates for this study must meet all of the following inclusion criteria:

PAD Positive Cohort

- Subject meets PAD positive criteria set forth in Section 4.1.1.1
- Subject is willing and able to provide an informed consent.
- Subject is willing and able to comply with the study procedures.
- Subject is able to understand the study procedures.
- Subject is scheduled for vascular examination that includes noninvasive assessments as standard of care: ABI, TBI, and either a Duplex ultrasound or Angiogram.

Healthy Cohort

- Subject is willing and able to provide an informed consent.
- Subject is willing and able to comply with the study procedures.
- Subject is able to understand the study procedures.
- Subject has no history of positive PAD diagnosis and is not currently suspected of having PAD.

5.6 Exclusion Criteria

Candidates for this study who meet any of the following criteria at the time of the baseline visit are not eligible to be enrolled in this study:

PAD Positive Cohort Exclusion

- Subject is under 40 or unable to consent.
- Subject has any medical condition, which, in the judgment of the Investigator and/or designee, makes the subject a poor candidate for the investigational study.
- Subject is excluded from analysis if no stenosis is found during Doppler but Tibial disease is suspected and Tibial ultrasound is not able to be performed.
- Subject does not have a suitable finger to attach the FlowMet-R probe.
- Subject does not have a suitable 1st or 2nd digit to attach FlowMet-R probe on the limb of interest.
- Subject has undergone revascularization within the last 90 days
- Subject cannot lay safely in a supine position.

Healthy Cohort Exclusion

- Subject is under 40 or unable to consent.
- Subject has any medical condition, which, in the judgment of the Investigator and/or designee, makes the subject a poor candidate for the investigational study.
- One or more limbs has a prior or current diagnosis of PAD, or is reasonably suspected of having a diagnosis of PAD.
- Subject does not have a suitable finger to attach the FlowMet-R probe.
- Subject does not have a suitable 1st or 2nd digit to attach FlowMet-R probe.
- Subject has undergone revascularization within the last 90 days

6. Determination of Sample Size

The rate of PAD and CLI within the population seen at a typical vascular clinic is estimated from a prior investigation. In that study, 100 limbs were assessed using the ABI and graded using the Rutherford classification scheme. PAD (diagnosed via ABI score of <0.9) was present in 63% of limbs. CLI (estimated as having a Rutherford category of ≥4) occurred in 15% of limbs. FlowMet-R data was also collected from the above limbs, and the data analysis techniques used in the data analysis section below were applied to build a diagnostic model for PAD and CLI. ROC curves were then generated for the diagnosis of both Claudication and CLI. The resulting area under the curve (AUC) for these ROC curves was 0.67 and 0.80, respectively. However, the Claudicant group AUC had a tighter confidence interval due to larger sample size (84 vs. 22, respectively).

Using a conservative estimation that the AUC for distinguishing PAD groups from healthy controls remains within +/- 0.05 of the Claudicant group, and +/- 0.2 of the CLI group, an estimate for the necessary sampling size can be determined. A standard type I error rate (α) of 0.05 and power of 0.8 is desired, thus the following approximate sample size was calculated [13,14]: 160 control limbs and 240 limbs with PAD, of which at least 54 limbs with Claudication and at least 100 limbs with CLI. Limbs with CLI may be included as a subset of limbs with PAD. A minimum of 250 patients (400 limbs) and maximum of 400 patients will be enrolled in the study.

7. Statistical Methods

7.1 Study Subjects

7.1.1 Disposition of Subjects

Subject disposition will be summarized using a STROBE like flow diagram with follow-up compliance reported at 3 and 6 months.

7.1.2 Clinical Investigation Plan (CIP) Deviations

Protocol deviations will be reported descriptively using counts and will be reported for each type by site and for the overall study.

7.1.3 Analysis Sets

Full Analysis Set

The full analysis set will include all participants and be used for all analyses. Visits outside of visit windows will be assigned to the nearest window. If a participant is missing data for a specific analysis, they will be excluded for that analysis. Within the analysis set, the grouping participants into the healthy, PAD (definitions 1 and 2), and CLI groups are defined in section 4.1.1.1.1

7.2 General Methodology

Data analysis will be performed by Medtronic employed statisticians/programmers. All statistical analyses will be generated using SAS version 9.4 or later or R version 4.1 or later software. Demographic and baseline characteristics will be summarized descriptively and will include age, gender, ethnicity, race, body mass index and hemodynamic measures (ABI, TBI, and acceleration time from the FlowMet-R device). For continuous variables, descriptive statistics will be presented including the number of

subjects with the measurement, mean, standard deviation, median, minimum and maximum. For categorical variables, descriptive statistics will include the number with the characteristic, the number evaluated, and the percentage.

Subject enrollment and accountability, visit attendance, and end of study status will be displayed using a STROBE like diagram and descriptive statistics, as appropriate. For each study visit, the following information will be tallied: completed visits, missed visits, deaths (if any), study withdrawals, and lost to follow-up. Compliance with study visits will be summarized as the percent of subjects available at each visit.

Missing data for endpoints may result from clinical assessments that were unable to be performed or data missing from the FlowMet device. These missing values will be excluded from summaries and analyses. In general, efforts will be made to minimize missing data and imputation of missing data will not be performed.

The objectives are not hypothesis-driven and therefore their results will be presented descriptively with no adjustments for multiple comparisons.

The value from the predictive model from the FlowMet-R measurements will be derived by the Research and Development team and will consist of the acceleration time.

7.3 Center Pooling

The analysis will use the pooled data from all centers because the marginal results are of primary interest.

7.4 Handling of Missing, Unused, and Spurious Data and Dropouts

Data that is missing or incomplete will not be imputed. Complete case data will be used where all data for the respective analysis is available for a patient or visit.

7.5 Adjustments for Multiple Comparisons

No adjustments will occur for multiple comparisons. A significance level of 0.05 will be used for all analyses and 95% CI will be computed.

7.6 Demographic and Other Baseline Characteristics

Demographic and baseline characteristic variables will be summarized descriptively. The mean, standard deviation, median, minimum, 1st quartile, 3rd quartile, maximum, and the corresponding number of participants or limbs will be reported for continuous variables. The percentage and count will be reported for categorical and binary variables.

7.7 ABI and TBI Values

The ABI and TBI for a limb will use the value in the data. If the ABI or TBI is missing, it will be derived from the ankle and brachial pressures or the toe and brachial pressures, respectively.

7.8 Acceleration Time Values

Acceleration time will be derived by the Research and Development Team based on data from the FlowMet device. Data should be merged based on the subject identifier, visit (0, 3, or 6 months), and limb.

7.9 Interim Analyses

No interim analyses are planned.

7.10 Evaluation of Objectives

7.10.1 Primary Objectives

The primary objectives will compare the 95% CI of the AUC from the ROC curve to a value of 0.5 at 0-, 3-, and 6-months for the two definitions of PAD and one definition of CLI using the acceleration time from the FlowMet-R device. The 95% CI will not be corrected for repeated measures within a participant. If the 95% CI of the AUC excludes 0.5 at all time points for PAD or CLI, the acceleration time from the FlowMet-R device will be considered predictive of the respective endpoint. The 95% CI of the AUC will be derived using the method described in DeLong, et al (1998) [15]. Note, the definition of PAD will be defined two ways and analyzed separately. Definitions of PAD and CLI are provided in Section 4.1.1.1.1.

Three supplementary summaries will be computed. The sensitivity and specificity will be computed by selecting the point on the ROC curve closest to the upper left corner. Accuracy will be computed as the number of limbs correctly diagnosed divided by the total number of limbs.

7.10.2 Secondary Objectives

The secondary endpoints will be analyzed as follows:

1. The capability of the acceleration time from the FlowMet-R device to predict a vascular intervention or amputation will occur within three and six months following initial visit will be determined using the AUC from an ROC curve where the endpoint is vascular intervention or amputation within 7 months, which is the end of the 6 month follow-up window. The 95% CI of the AUC will be derived using the method described in DeLong, et al (1998) [15].
2. The capability of the acceleration time from the FlowMet-R device to predict a ≥50% stenosis determined using the AUC from an ROC curve where the endpoint is vascular intervention or amputation within 7 months, which is the end of the 6 month follow-up window. The 95% CI of the AUC will be derived using the method described in DeLong, et al (1998) [15].
3. The correlations between the acceleration time from the FlowMet-R device and peripheral artery stenosis, changes in ABI, and changes in TBI will be computed using Pearson correlations. The changes for ABI and TBI will be as follows: 0 to 3 months, 0 to 6 months, and 3 to 6 months.
4. The correlation between the acceleration time from the FlowMet-R device and changes in Rutherford Classification will be computed using the Spearman correlation. The changes for Rutherford Classification will be as follows: 0 to 3 months, 0 to 6 months, and 3 to 6 months.

7.11 Safety Evaluation

A listing of any site-reported Adverse Events (AEs) including Serious Adverse Events (SAEs), will be created if any are observed in the study. The listing will include the AE description, AE start and end dates, relationship to the device, whether the AE is resolved, and whether the AE was considered an SAE..

A table of all observed device deficiencies that could have led to a serious adverse device effect will be created if any are observed.

A listing of all deaths and the reason for death will be created if any are observed.

7.12 Changes to Planned Analysis

Patients will be classified based on definitions in Section 4.1.1.1.1 no matter the cohort they were originally enrolled in for the study.

8. Validation Requirements

To ensure the quality of the statistical results and datasets created for the study, the following validation requirements will be implemented. Programs that contribute directly or indirectly to the results pertaining to the primary and secondary objectives including TLG (Tables, Listings, and Graphs) development will be validated at least at level II by a statistician or a statistical programmer. Level I validation consists of a statistician or statistical programmer reviewer who independently programs output and then compares the output with that generated by the original statistician or statistical programmer according to the current work instruction. Level II: The peer reviewer reviews the code; where appropriate, performs manual calculations or simple programming checks to verify the output. Level III validation may be used for any previously validated program where only minor/administrative changes were made (e.g., change the location of the data directory). For level III validation, the original statistician or statistical programmer performs a visual inspection of the code and output to confirm functionality according to the current work instruction. Any results that are used for external publication or reports should be validated at levels I.

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