CARRS Cohort:
Proposed Measures
[Precision-CARRS]

MANUAL OF OPERATIONS:
CT IMAGING FOR CORONARY CALCIUM AND HEPATIC FAT

INTRODUCTION

CT imaging for Coronary Artery Calcium (CAC):

CAC is a highly specific marker of coronary atherosclerosis that is one of the most predictive CVD risk markers in asymptomatic individuals, independent of traditional risk factors.[1-33] Calcium in the coronary arteries is a result of ectopic deposition in atherosclerosis and correlates well with plaque burden.[24, 34-41] CAC demonstrates excellent accuracy for the detection of obstructive CAD[42-44] and coronary plaque as compared to intravascular ultrasound with sensitivity and specificity measures ≥90%.[45-48] A negative test has close to 100% negative predictive accuracy for obstructive CAD.[35, 49-53]

Expected prevalence: The prevalence of CAC varies by sex, age, race, and ethnicity.[54-56] A few small studies in asymptomatic South Asians have reported CAC in ~40% of the population.[57-59] The inter-scan variability of CAC scoring is <10% and CAC scores can increase by ~20-25% per year.[60-64]

Equipment: Multi-detector CT (MDCT) scanners (Siemens Somatom Force [384 Slices] in Delhi and Philips Ingenuity [128 Slice] in Chennai) will be used.

Duration of testing: 20 minutes.

Technique: CAC acquisition will be performed using prospectively ECG-triggered MDCT scanners with sequential acquisition performed at 40% (for those with heart rates [HR]≥ 80bpm) or 70% (HR <80bpm) of the cardiac cycle using the following parameters: tube voltage 120 kVp, automated tube current modulation, reference tube current-time product of 80 mAs, collimation 44×1.2 mm, gantry rotation time 0.25 s, and matrix size 512×512 pixels. Dedicated post-processing evaluation (syngo via VB10 Calcium Scoring, Siemens Healthcare, Forchheim, Germany) will be used for image analyses.

Scoring: CAC quantification will be performed independently by Dr. N Pandey at AIIMS. Plaque calcium density (measured in Hounsfield units [Hu]) must be 130 Hu or higher to qualify as a calcified plaque. The Agatston scoring method for CAC measures each discrete plaque area in mm^2 and each discrete plaque area is multiplied by 1, 2, 3, or 4, depending on the highest plaque density measurement in Hu.[4 65] The coronary segment model of the Society of Cardiovascular Computed Tomography will be used for evaluating CAC distribution (i.e. number of coronary segments and arteries affected).[66] Because recent studies have suggested improved prediction by inversely weighting for calcium density[67, 68] and the regional distribution of CAC (total number of coronary arteries with CAC),[69-71] we will also measure these parameters.

Reproducibility: Intra-observer and inter-scan correlations of plaque volume are >0.92 (p<0.001).

Manual of Operations: Detailed MOPs will be adapted from the protocol enclosed pages 3-9.

CT imaging for hepatic steatosis:

Hepatic steatosis manifests as reduced attenuation during unenhanced CT scanning of the liver and reflects liver triglyceride content.[72, 73] The normal attenuation value of the unenhanced liver parenchyma is 50-65 Hu, which is typically 8-10 Hu higher than the spleen.[74] The sensitivity and specificity of unenhanced CT to assess ≥30% macrovesicular steatosis are 100% and 95%, respectively.[75]

Expected prevalence: Using the L/S ratio <1.0 cut-point, the prevalence of hepatic steatosis was 17.2% among MESA participants; Whites and Blacks had a higher prevalence (37.7% and 32.4%, respectively).[76] In small studies in South Asians, the prevalence of steatosis, measured using different technologies, ranged between 17% and 32%.[77-80]

Technique: Liver and spleen segments will be scanned in the lower image slices of the abdomen obtained during cardiac CT imaging being performed for CAC assessments as described above using protocols similar to MESA and CARDIA.

Scoring: CT scans will be evaluated by an independent, experienced reader. The scan with the greatest coverage of the liver and spleen will be selected for liver fat measurement. Hepatic and splenic attenuation will be measured using regions of interest (ROI) >100 mm^2 in area. Two ROI will be placed in the right liver lobe and in the spleen. We will use an L/S ratio of <1 as the primary measure. Secondary endpoints will be the difference between liver and spleen of <40 Hu. Liver fat severity will be graded as mild (L/S ratio ≥0.7 to <1.0), moderate (L/S ratio ≥0.5 to <0.7) and severe (L/S ratio <0.5), corresponding to <30.1%, 30.1% to 41%, and >41% steatosis.[5, 81-84]

Reproducibility: Inter- and intra-reader correlation (0.96 and 0.99) was excellent.[76]

Coronary Calcium Scoring and Hepatic Fat Measurement

1. **Objective:**

   To conduct CT scanning for assessment of coronary calcium score (CAC), other calcium scoring and hepatic fat measurements in the CARRS cohort.

2. **Imaging Overview**

   CT images of the thoracic area (chest) will be collected and reviewed by Dr. Niraj Pandey at the All India Institute of Medical Sciences (AIIMS), New Delhi, and Dr. Harish Mardavada at Westminster Healthcare (WHC), Chennai, to determine the CAC/Agatston score, CAC volume score, thoracic aorta calcification score (CAC Volume and Agatston score) and the aortic valve calcification score (CAC Volume and Agatston score) and Hepatic Fat measurements.

   To ensure consistency and reproducibility, imaging equipment, method of assessment and scanning techniques at both imaging centers are to remain consistent throughout the study.

3. **Equipment**

   CT imaging will be performed using a multi-detector helical row 64 slice scanner or greater.

4. **Image Acquisition Procedure CT (Computed Tomography)**

   A non-contrast CT scan of the chest (from the top of the aortic arch to the diaphragm) will be performed at AIIMS and WHC. All images and reconstructions must be sent to AIIMS and WHC in uncompressed DICOM format.

   Scan parameters will be as follows:

   - Helical
   - 120 kVp
   - Reconstructed at 3mm slice thickness
   - Reconstructed at 0mm gap
   - Pitch must remain the same for each individual patient at baseline and at the follow up scan
   - The filter must be set as Kernal B35 heartmed medastinum (Siemens) or equivalent and remain the same for each individual patient at baseline and at the follow up scan

   Subject positioning will be as follows:

   - Head first, supine, arms above head
   - ECG gated
   - mid-late diastolic reconstructions of 70%
   - Breath hold
   - Ensure imaging extends to below rib cage to include top of liver

5. **Image Transfer Procedure**

   Imaging centers must provide CT images in raw or standard DICOM format, meaning that

they are not manipulated in any way after the scanner converts the images to DICOM from raw signal.

It is often the case that once an imaging exam is complete, the DICOM images are compressed and sent to storage or a system such as a PACS. Imaging centers **must not** send any compressed imaging data to Intrinsic Imaging.

If images are burned to CD directly from the scanner or are retrieved from PACS, they must be in standard DICOM format and cannot be compressed or in some other format, such as JPEG, TIF, etc. (as these types of image files do not provide any DICOM tag information).

Some scanners will burn DICOM header data onto an actual image so that the information is viewable as part of the images. Imaging centers **must not** submit any images that include any personal health information in the text that is placed on the image, such as Radiation Dose Reports or Subject Protocol images.

6. Image Acquisition Standards

An Image Acquisition Guide will be developed to describe how the imaging sites will collect images for this study and send them to the Core Lab.

The Image Acquisition Guide will require the imaging sites to ensure images are acquired according to the following guidelines:

- Image acquisition
- Imaging parameters consistent throughout the study
- Imaging anonymization, marking and annotations
- Images transferred to Intrinsic Imaging must be clear of any marks or annotations made at the site
- Image Quality

7. Image Processing Manual

An Image Processing Manual will be developed to describe how to process images for this study and prepare them for review.

The Image Processing Manual will include information related to:

- Data traceability
- Quality control procedures
- Query procedures
- Blinding procedures
- Documentation and storage

8. Radiological Review

A minimum of two certified radiologists (radiologist reviewers) will be trained and assigned to review the CT images for this study.

9. Reviewer Training

All reviewers will undergo training prior to completing reads on this study.

A reviewer training manual will be developed, and each reviewer will be trained on the following items:

- Imaging Charter and analysis criteria
- Study radiographic workflow and review procedure
- Systems involved in the review procedure

10. Image Review

CT Images will be reviewed by the designated reviewers as follows:

- During the processing of the images, all images will be blinded so that reviewers will not have access to any patient confidential information, local site assessments, and any treatment information.
- Images will only reach the review stage once they have been quality controlled and all queries have been resolved and/or closed.
- The CT images will be loaded into software for review by one independent radiologist.
- All CT scans will first be scored for CAC.
- Images will be analyzed as described in section 5.6.

11. CT Imaging Analysis for CAC

CAC acquisition will be performed using a prospectively ECG-triggered multidetector non-contrast computed tomography scanner with sequential acquisition performed at 40% (HR ≥ 80bpm) or 70% (HR < 80bpm) of the cardiac cycle using the following parameters: tube voltage 120 kVp, automated tube current modulation, reference tube current-time product of 80 mAs, collimation 44×1.2 mm, gantry rotation time 0.25 s, and matrix size 512×512 pixels. Dedicated post-processing evaluation software (syngo via VB10 Calcium Scoring, Siemens Healthcare, Forchheim, Germany) will be used for objective and subjective image analyses. Quantification of CAC on non-contrast scans will be performed by an independent observer blinded to patient characteristics and the imaging report. The plaque calcium density measured in Hounsfield units, must be 130 Hu or higher to qualify as a calcified plaque using CAC scoring. The Agatston scoring method for CAC measures each discrete plaque area in mm^2 and each discrete plaque area is multiplied by 1, 2, 3, or 4, depending on the highest density measurement in Hu in the plaque. Plaques with maximum density of 130 to 199 Hu will be multiplied by 1, those with 200 to 299 Hu by 2, those with 300 to 399 by 3, and those with 400 Hu or greater by 4. These scores are summed for all slices of the heart to give the Agatston score.[4] Finally, the Agatston score will be divided by the area score and the quotient will be the average CAC density score (range 1 to 4) for each participant. The coronary segment model of the Society of Cardiovascular Computed Tomography will be used for evaluating CAC distribution.[66] The number of coronary segments and number of coronary arteries with CAC will be recorded. Coronary dominance will be established: the system will be considered right or left dominant if both posterior descending artery and posterolateral branch originate from the right coronary artery or left circumflex coronary artery, respectively. The coronary system will be considered codominant if posterior

descending artery originated from the right coronary artery and posterolateral branch originated from the left circumflex coronary artery. The proximal coronary segments will be defined as segment 1 (proximal right coronary artery), segment 2 (mid right coronary artery), segment 5 (left main coronary artery), segment 6 (proximal left anterior descending coronary artery), and segment 11 (proximal left circumflex coronary artery). The presence of proximal CAC will be considered positive if CAC was present in segments 1 or 2 (right dominant); segments 5, 6, or 11 (left dominant); and segments 1, 2, 5, 6, or 11 (codominant).

Radiologist reviewers will use a workstation to analyze CT image series slice-by-slice. CAC/Agatston Score, CAC volume scoring system will be assessed at the following locations: coronary arteries, thoracic aorta and aortic valve.

For CAC Agastson scoring of the coronary arteries, the reviewer will identify each lesion within each of the major vessel distributions (LM, LAD, LCX, RCA). The score for each vessel will be summed to generate a single overall coronary artery CAC Agaston score. The left main coronary artery will be summarized as a single score (LM). The left anterior descending coronary artery, circumflex coronary and right coronary artery and their respective branches will be summarized as a single score (LAD-LCX-RCA).

The aortic valve calcification (Agaston and volume) scores will include the aortic valve leaflets and aortic annular tissue (AV). Calcium within the aortic sinus or on the aortic wall will be excluded from and not be measured as part of the aortic valve calcification scores. Calcium outside of the aortic valve leaflets and aortic annulus will be included in the thoracic aortic calcification scores.

Characterization of the thoracic aortic calcification (Agatston and volume) score will include the following segments and will be summarized as a single score (Thoracic Aorta):

- Aortic sinuses of valsalva
- Ascending thoracic aorta
- Transverse arch excluding the great vessels of the head and neck
- Descending thoracic aorta

At the discretion of the radiologist reviewers, additional findings considered relevant to the analysis may be documented in the comment section of the analysis.

The following situations may occur (but are not limited to) that render baseline and follow-up scans not directly comparable:

- Motion artifact: this could cause a vessel that was visible in the baseline scan to not be evaluable in the follow-up scan. In this case the vessel affected by motion on the follow-up scan will be removed from both scans
- Scan field of view different from baseline: the z-axis (superior-inferior span) is larger in the baseline scan than in the follow-up scan. In this case the baseline scan will be re-assessed including only the superior-inferior span visible in both scans.
- Anatomy previously scanned is cut off in follow-up visit: in this case the anatomy originally include in the baseline scan interpretation will be removed from the follow-up scan as well.
- If amending the baseline assessment does not allow the proper comparison of the two timepoints, the case will be deemed unevaluable.

12. Hepatic Fat Analysis:

Liver and spleen segments will be scanned in the lower image slices of the abdomen obtained during cardiac CT imaging being performed for CAC assessments as described above. The technique is similar to assessments in the MESA and CARDIA studies.

CT scans will be evaluated by an independent, experienced reader. The scan with the greatest coverage of the liver and spleen will be selected for liver fat measurement. Hepatic and splenic attenuation will be measured using regions of interest (ROI) >100 mm^2 in area. Average liver attenuation is calculated by placing the circular region of interest (ROI) multiple places in the liver, covering all the hepatic segments. The measurements should be avoid inclusion of macroscopic vessels and close to fissure. Average splenic attenuation is measured by placing ROI at its upper, mid, and lower poles.

Interpretation:

We will use an L/S ratio of <1 as the primary measure. Secondary endpoints will be the difference between liver and spleen of <40 Hu. Liver fat severity will be graded as mild (L/S ratio ≥0.7 to <1.0), moderate (L/S ratio ≥0.5 to <0.7) and severe (L/S ratio <0.5), corresponding to < 30.1%, 30.1% to 41%, and > 41% steatosis.[5, 81-84] Inter-reader and intra-reader correlation (0.96 and 0.99) was excellent.[76] In small studies in South Asians, the prevalence of steatosis, measured using different technologies, ranged between 17% and 32%.[77-80]

13. Saving Analysis Results

CT imaging will be saved within the workspace menu in the main menu bar.

14. Reviewer Workstations

In order to maintain a consistent review, all reviewer workstations will be restricted from any automatic updates or changes to the analysis software (no software changes will be made automatically).

Review software will not be updated unless necessary and with assurance that it will not change the ability to evaluate the images, the nature or quality of the review, and is thoroughly validated and approved for use.

15. CRF Quality Control

The radiologist reviewers will enter the analysis results into storage (TBD). Upon completion, the analysis results will be provided to Niraj Pandey at AIIMS and Manoj Srinapakkathori at WHC or their designees and they will enter the results into the CRF. The CRF will use automated edit checks (required fields, data validation, etc.) to prospectively prevent errors. In addition, a separate investigator will manually verify the following on the CRF after the data has been entered in the CRF:

- The following participant key identifiers will be used:
    - Patient ID
    - Visit Date
- All required fields are completed
    - Image quality has been assessed

- CAC/Agatston Score and CAC volume
- Aortic Valve calcification Agatson Score and volume
- Thoracic Aortic calcification Agatson Score and volume

- Required fields are clearly indicated on the form
- Field responses are clear and legible
- Measurements have been correctly entered
- Intrinsic CRFs are properly signed and dated

16. QC Findings and Resolution Procedure

CRFs that pass the Quality Control verification will be considered complete and no further action will be taken.

CRFs that do not meet quality control verification will be required to be addressed by the radiologist according to the Resolution Procedures presented in section 5.10.

17. Resolution Procedure

CRFs that do not meet quality control verification will be resolved based on the nature of the issue.

18. Case Report Forms corrections

Case Report Forms that may require corrections will be reviewed by the radiologist.

- If a radiologist reviewer or Intrinsic Imaging Study Team Member determines that an amendment is required to a field on a CRF, the radiologist will securely log in using their name and password and amend the field of the original CRF as may be required. All changes will be tracked by an appropriate audit trail that indicated the reader who made the change, the date of the change, and the reason for the change.

19. Re-Reads

As the study progresses there is a possibility that scans read by the radiologist reviewer must be re-read.

Examples of images requiring a re-read include, but are not limited to;

- Reviewer Error
- Incorrect exam
- Incorrect patient
- Criteria change
- Any error/change in protocol that affects imaging
- Additional images have been received
- Scheduled timepoints that have been received out of sequence or previously believed to have not been imaged will automatically be read (a re-read may be required if additional images are received that are out of sequence and subsequent images have already been

reviewed).

- Unscheduled imaging will be received and reviewed by Intrinsic Imaging if it is pertinent to the criteria and after a case-by-case discussion. Unscheduled imaging is defined as any imaging performed outside of the scheduled visits presented in Table 1.

20. Monitoring Plan

The imaging site must acquire and send images to AIIMS. AIIMS will then quality control the images according to the Image Processing Manual. Queries are issued to the imaging site and re-queried or escalated as per the Image Processing Manual.

21. Charter Modifications

Any modifications to the Imaging Charter after imaging of the patient that impact the acquisition, analysis, interpretation or patient safety will be discussed

- may require re-training of the Radiologists
- will be documented appropriately in the revision history
- will have its document version number incremented accordingly to ensure traceability

22. Imaging and Data Transfer and Storage

Imaging sites will submit images directly to Dr. Niraj Pandey at AIIMS and Dr. Harish Mardavada at WHC.

23. CT Scan Quality Review Process

Dr. Niraj Pandey will complete an over read of the first ten subjects' Screening CT CAC scores). Dr. Harish Mardavada will then complete a 10% monthly over read of subjects cases for the duration of the study.

CAC(Agatston) Score Thresholds (Over Reads):

- A 10% or lower difference between the CAC score (Agatston) measured by the two reviewers is considered acceptable variability.
- A greater than 10% difference between the CAC score (Agatston) measured by the two reviewers will be discussed between the two and resolved. If required it will be referred to a third reviewer, the Head of Cardiac Radiology at AIIMS, Prof. Sanjeev Sharma.
- If, after the joint review of the case, the CAC score (Agatston) is revised to be +10% of the original score, the score will be updated.

If the number of over read cases requiring revision exceeds 8 (10% of the total expected over reads (80 scans)) but revisions are across study reviewers, the over read percentage will increase by 10% of monthly scans. If the overread cases requiring revision trend to one Independent Image Reviewer, that reviewer will undergo refresher training.

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