Supplementary Material

Novel Tech throws knock-out punch to ECG improving GP referral decisions to cardiology

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Calcagno S, *et al. Open Heart* 2022; 9:e001852. doi: 10.1136/openhrt-2021-001852

1 Clinical Investigation Plan

1.1 Abbreviations

CHART = Cardio-HART™ (Cardio-Heart Analysis Risk Test)

CTT = Cardio-TriTest™

CC = CardioClient™ (client software for CTT device)

CI = Clinical Investigation

CIP = CI Plan

Clinical Study = CS

CRF = Case Report Form

EC = Ethics Committee

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ECG or EKG = Electrocardiograph (comparator)

ECHO = Echocardiograph

GP = General Practitioner / General Physician / Family Doctor

FP = False Positives

FN = False Negatives

IAW = In Accordance With…

IB = Investigators Brochure

IMD = Investigational Medical Device (CTT and CPA)

MD = Medical Device

MT = Medical Test

RA = Risk Assessment/Analysis

UtS = Utility Study

1.2 Declaration of Helsinki

The CI shall be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki.[1] These principles protect the rights, safety and well-being of human subjects, which are the most important considerations and shall prevail over interests of science and society. These principles shall be understood, observed, and applied at every step in the CI.

1.3 Scope

The Scope of this CI is to conduct a multi-site clinical investigation (CI) study to validate and confirm the functional and operational aspects of two medical devices as each is intended to be used in clinical practice.

1.3.1 Device 1. Cardio-TriTest™ (CTT).

The CTT device is an FDA cleared medical device that captures 3 types of heart generated Bio-signals, including ECG, PCG and MCG signals or signals of an electrical, acoustic and mechanical nature emanating from the heart and non-invasively captured on the thoracic wall. Captured bio-signals are outputted as either a printable report of the measured signals, conformant with ISO-60601-2-25 for ECG or as electronic signals for analysis processing.

The CTT device is for use in patient clinical practice.

1.3.2 Device 2. Cardio-Phoenix Analyser (CPA).

CPA is an automated AI based system for the analysis of heart bio-signals, including ECG, PCG and MCG. It analyses those bio-signals and outputs four types of diagnostic findings, including ECG, PCG and MCG findings. It also analyses the combined ECG, PCG and MCG signals and outputs them as HART™ findings.

[1] See https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

The CPA outputs a report that is indicated for use by clinicians to assist them in diagnosing a patients current cardiac status.

The CPA is not for use in patient clinical practice.

Each device is independent of the other having their own functional and operational characteristics. However, the devices complement each other in that CPA can process the Bio-signals captured by the CTT, and the CTT can provide the captured bio-signals to the CPA. For references purposes, when both are discussed, they will be referred to as the CHART system. The CPA report will also be referred to as the patients CHART report.

A single Study will be used that makes use of both devices as the both the clinical population and the operational environment required for the study of each is the same.

However, each device will have its own study parameters, including endpoints, Risk Assessment, and statistical analysis. Where required, combined endpoints and risk assessments will be completed where they overlap in clinical practice.

However, each device will be studied independently in the context of one Study, based on an "at risk" target population attending a primary care facility.

Scope 1. The CTT will be studied for its Usability and Utility in clinical practice.

Scope 2. The CPA will be studied for its automated analysis and subsequently confirmed by ECHO.

Furthermore, the scope of the CI will extend to confirming the deployability, operational usefulness and cost efficiencies within the healthcare system.

1.4 Inclusion Exclusion criteria

Clinical Utility and Usability Study (CUUS) is a multi-centre, randomised, blinded, pivotal study. The study was conducted in Serbia, in three centers: Sombor, Vrsac, and Senta.

The clinical study is approved by the regulatory agency- Medicines and Medical Devices Agency of Serbia (ALIMS) in accordance with the Ministry of Health in Serbia. Ethical Committee approvals from both, hospital and the ambulances are provided.

Inclusion criteria:

a) Adults, 20≤ years of age

b) Age grouped:
i. 20-40
ii. 41-55
iii. 56-65
iv. 66-75
v. 76+

c) Gender, males, and females, approximately evenly distributed (~50/50)

d) BMI, categorized (each category should include at least 12 patients):

i. Underweight (below 18.5)
ii. Normal weight (18.51 – 24.99)
iii. Overweight (25 – 29.99)
iv. Obese (30 & higher)

e) Not currently suffering from severe medical condition

f) Race any
g) Able to provide consent
h) Risk assessment results- at least 3 identified hearth related risks factors (as per the SoC)

Other requirement criteria:

a) Patient population size: ~ 500
b) Healthy/Unhealthy split: 15%/85% based on @risk
c) Cardiology Clinics: Min 2
d) Primary Care Clinics: Min 2
e) Number of cardiologists: Min 4
f) Number of family physicians (General Practitioner- GP): Min 4

Exclusion criteria:

a) Age, Under 20
b) Persons already diagnosed for heart disease and undergoing treatment in a cardiology ward
c) Patients who have suffered and are currently undergoing pharmalogical treatments for any heart disease in cardiology ward
d) Patients who are suffering from any major illness or undergoing treatment for any disease that could influence their heart condition
e) Lactating and pregnant women
f) History of heart attack within last 120 days
g) History of Stroke within last 120 days
h) Presence of active, uncontrolled infection
i) Any psychiatric disease/disorder, irreversible cognitive disfunction or psychological issues likely to impair compliance with study protocol
j) History of organ transplant
k) Participation in any other study that can confound the study results or affect the study
l) Refusal to participate
m) Any condition that could limit survival to less than 1 week.

1.5 Bias Assessment

1.5.1 Blinding/Masking.

Blinding/Masking is not possible nor practical. The IMD and the comparator will both be used to measure their respective bio-signals, and it is impossible to hide which device is being used on the patient – nor does it matter as it is the analysed signals and the results that matter. As the CTT is also a 12 lead Standard ECG those signals and their interpretation are by default part of and included in the CHART. Masking is therefore not only redundant but counters the purpose of CI. Rather each device will be used in sequence on the same patient so that the results of each can be uniformly and consistently measured on the same patient. This will also eliminate any measurement bias resulting from different patient body types and make direct comparison of results possible.

The design of the CI protocol will ensure that the cardiologist will first review the comparator ECG results, for which they are accustomed and acquainted with, and then after making their initial diagnosis, be allowed access to the IMD for comparative diagnosis. That will allow them to understand the differences between the comparator and the IMD and identify whether the IMD contributes to broaden the range of possible heart diseases they can diagnose and better understand the patient's cardiac status.

1.5.2 Randomization.

To avoid any measurement bias, the MA will do all the MT on the patients, whereas the GP and the Cardiologist will only compare the results. MA's will be rotated through the process of conducting MT to ensure randomization of measurement techniques by different MA's, as it is expected that there will always be subtle differences that need to be accounted for in the statistical results.

Patient randomization will be determined on first-come first-served basis as would be the norm in primary clinical practice. Patients will then be interviewed for acceptance into the CI based on their risk assessment for heart disease as per the existing Standards of Care (SoC).

The random selection criteria for patient participation is based on the initial risk assessment. If three (3) risks are identified and or the patient is considered for preventative pharmalogical treatment, then they can be accepted into the CI.

A small sample of patients, that are determined to not be at risk as a result of the diagnostic assessment, will be randomly selected for next level cardiologist assessment and "ground-truth" evaluation as a control group. This will ensure that a statistically significant number of healthy patients are included in the results.

1.5.3 CRF

Diagnostic notes will be entered by the GP's and Cardiologists into an electronic system, qmsWrapper, a quality management software, that can be used to control the sequence and flow of information. Custom eCRF forms were designed to ensure data is clearly and properly captured for later processing and for quality control purposes. The forms will be approved through qmsWrapper software, which is validated and verified according to ISO/TR 80002-2:2017 standard.

The GP and Cardiologist cannot "dupe" or fool the system, except intentionally and maliciously, which is not expected as even then it can be detected. This will prevent any bias from entering into the evaluation process. Also, the eCRF forms are made in that way that every question is obligatory to answer, so the missing data is reduced to an absolute minimum.

A purpose built and verified software used in a Consensus Study, will be used during the Ground-Truth (GT) phase of the CI.

1.5.4 Number of IMDs

One IMD per participating clinic will be used. Spares will be kept by the Sponsor at the ready.

IMD's will be rotated every month, to ensure no single device bias, and verification of calibration and monitoring device condition in general.

1.6 Informed Consent

This Informed Consent is for participants in the CHART Usability and Utility Study (CUUS) in support of the development of a non-invasive heart medical device.

Principal Investigator: Dr. _____

Name of Organization: _____

Address of Organization: _____

Name of Sponsor: Cardio-Phoenix Inc.

Name of Project: CUUS (CHART Usability and Utility Study)

The Clinic is participating in a clinical study related the development of a non-invasive medical devices that could help people, much like you. Because you are visiting our clinic today, we have determined that with your risk factors for heart disease, we would like you to participate in this clinical study. You will undergo and ECG/CTT examination, and then speak to the doctor about the results. This examination will be very beneficial for you as will receive a complete cardiac status.

After the CTT examination, if the results show that you have some form of heart disease, you will be given the opportunity to undergo an Echocardiograph examination at a local Cardiology Clinic. This second examination, will confirm the CTT results and prove very beneficial to you.

There is no risk to you. Both tests, ECG/CTT and ECHO are routine medical procedures.

What is different is that with the CTT exanimation, 4 additional non-invasive sensors are used to help detect many more types of heart diseases than is currently possible using ECG only examination. The follow-up Echo examination will be used to confirm those results.

The ECG/CTT examination test will take about 20 minutes. (this is normal)

This research will not inflict any pain or cause any harm to you.

There is no risk to you.

No drugs or medicine will be given to you related to this research. No Drugs are involved.

All test data and patient identification will be kept strictly confidential and anonymous.

Please feel free to ask us any questions about this, either now or even later. You can ask any question at any time of any of us.

If you wish to ask questions later, please contact: Valentina Milanovic +38160/0541-246

valentina.milanovic@uvaresearch.com

This research project has been approved by the Ethics Committee of the Clinic to make sure that research participants are protected from any harm and conducted in accordance with the Helsinki Protocol.

CERTIFICATE of CONSENT

I confirm that I have received the information about the CUUS study, or it has been explained to me to my satisfaction and I have no further questions at this time.

I consent voluntarily to participate as a participant in this project.

Print Name of Participant _____

Signature of Participant _____

Date: _____/_____/_____
Day/month/year

Statement by the person taking consent

I have informed the participant about the CUUS Clinical Study and to the best of my ability made sure that the participant understands that the following will be done:

1. **A normal CTT/ECG examination followed by**
2. **A doctor interview.**

I confirm that the participant was given an opportunity to ask questions and that I have answered them to the best of my ability.

I confirm that the participant has given their consent freely and voluntarily.

A copy of this Consent Form part 1 and 2 have been provided to the participant.

Print Name of person taking the consent: _____

Signature of person taking the consent: _____
Date: ___/___/_____
day/month/year

1.7 Variables

4.1.1 Medical findings

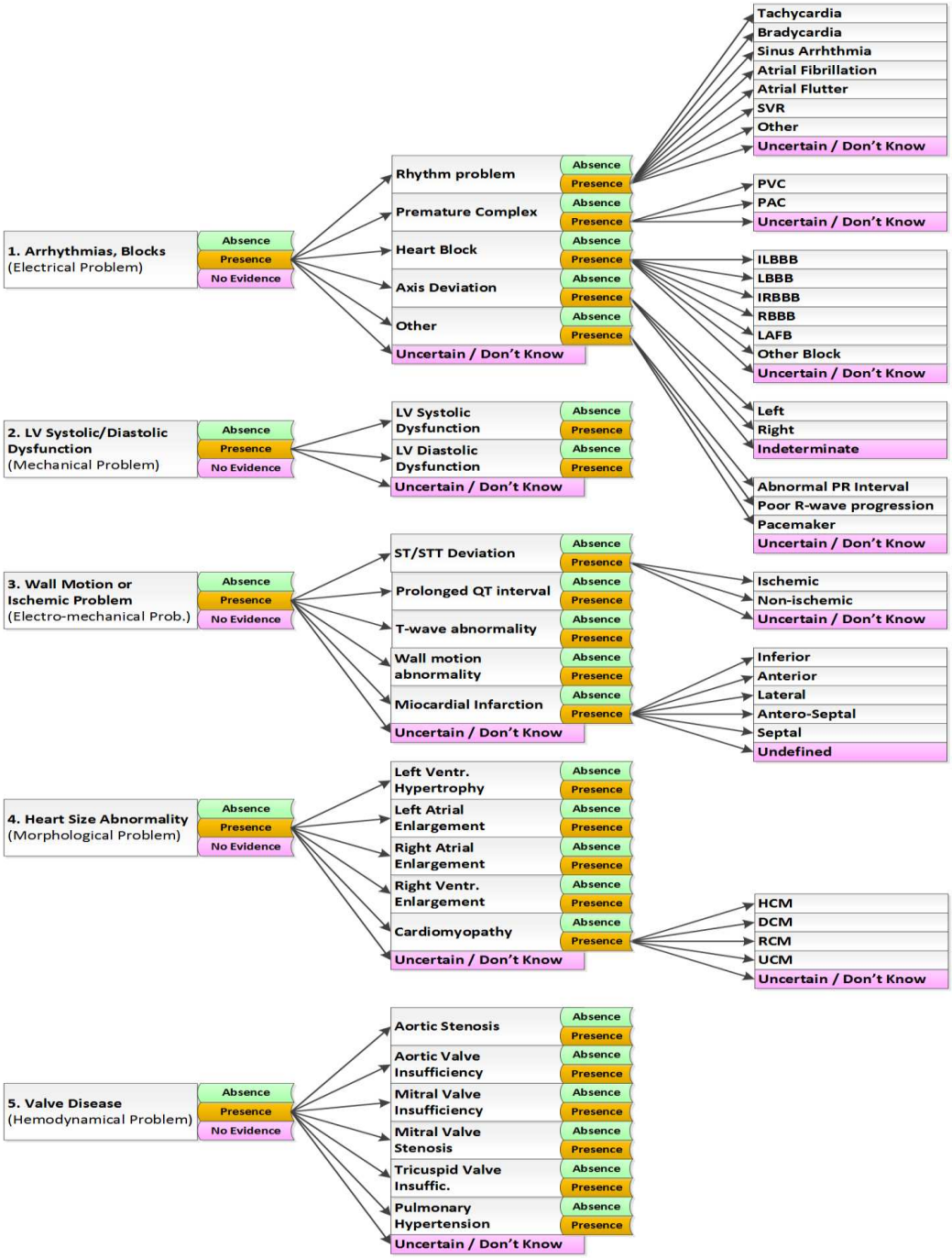

Figure 1 - ***ECG Interpretation Conditions***

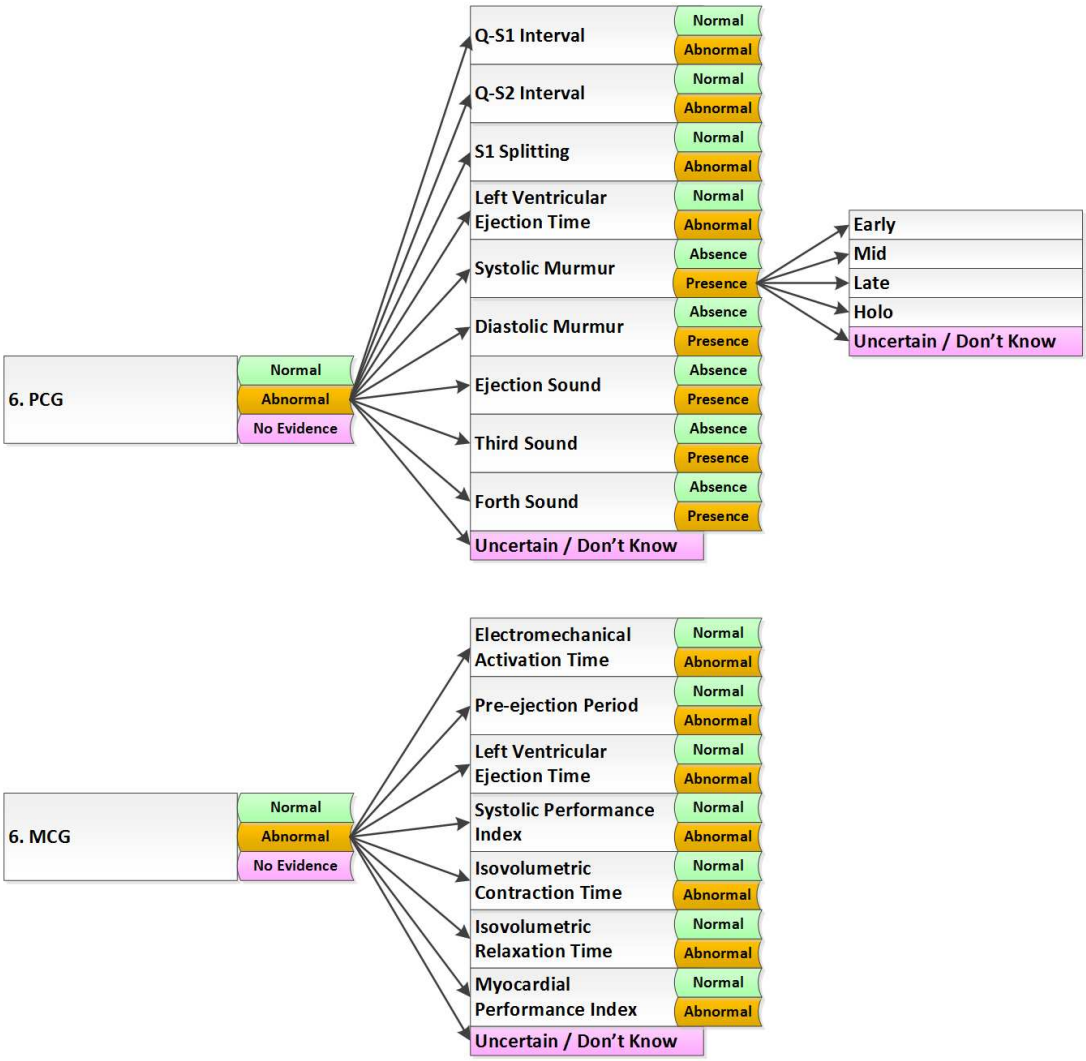

Figure 2 - *PCG/MCG Interpretation Conditions*

Based on the intended use of the CUUS clinical study, and the medical conditions shown above, the Endpoint established in this clinical study are:

1.7.1 ECG findings

CHART ECG Findings	Value domain	AHA/ACC/HRS Statement	Comment
1. Rhythm	SR	20 Sinus rhythm	
	ST	21 Sinus tachycardia	
	SB	22 Sinus bradycardia	
	SARR	23 Sinus arrhythmia	
	AFib	50 Atrial fibrillation	
	AFlut	51 Atrial flutter	
	SVR	40 Supraventricular rhythm 55 Supraventricular tachycardia	
	Other Arrhythmias	34 Ectopic atrial rhythm 37 Junctional escape complex(es) 38 Junctional rhythm 39 Accelerated junctional rhythm	Rare rhythm types in CHART intended population

		52 Ectopic atrial tachycardia 54 Junctional tachycardia 61 Fusion complex(es) 62 Ventricular escape complex(es) 63 Idioventricular rhythm 64 Accelerated idioventricular rhythm 70 Ventricular tachycardia 74 Ventricular fibrillation 76 Wide-QRS Tachycardia	
2. Pacemaker	No		
	Yes	180 Atrial-paced complex(es) or rhythm 181 Ventricular-paced complex(es) or rhythm 184 AV dual-paced complex(es) or rhythm	
3. Premature Ventricular Complex	No		
	Yes	60 Ventricular premature complex(es)	
4. Premature Atrial Complex	No		
	Yes	30 Atrial premature complex(es)	
5. Heart Axis Deviation	Normal		
	LAX	121 Left-axis Deviation	
	RAX	120 Right-axis Deviation	
	IAX	123 Indeterminate Axis	
6. Poor R-wave progression	No		
	Yes	128 Poor R-wave progression	
7. PR interval	Normal		
	LongPR	82 Prolonged PR interval	
	ShortPR	80 Short PR interval	
8. B. Branch Block	No		
	LBBB	104 Left bundle-branch block	
	ILBBB	103 Incomplete Left bundle-branch block	
	RBBB	106 Right bundle-branch block	
	IRBBB	105 Incomplete right bundle-branch block	
	IVCD	107 Intraventricular conduction delay	
9. Other Block	No		
	LAFB	101 Left anterior fascicular block	
	Other	102 Left posterior fascicular block 108 Ventricular preexcitation 24 Sinoatrial block, type I 25 Sinoatrial block, type II 81 AV Conduction Ratio N:D 83 Second-degree AV block, Mobitz I 84 Second-degree AV block, Mobitz II 85 2:1 AV block 86 AV block, varying conduction 87 AV block, advanced (high-grade) 88 AV block, complete (third-degree) 89 AV dissociation	Rare block types in CHART intended population
10. Myocardial Infarction (ECG crit.)	No		
	IMI	161 Inferior MI 162 Posterior MI	
	AMI	160 Anterior MI 166 Extensive Anterior MI	
	LMI	163 Lateral MI Anterolateral MI	
	ASMI	165 Anteroseptal MI	
	SMI	Septal MI	
	UMI	Undefined MI	
11. Ischemia	No		
	Yes	220 Acute ischemia 226 Ischemia (205 Digitalis effect, 208 Hyperkalemia)	

12. ST deviation	No		
	STdev	145 ST deviation 501 ST Elevation 502 ST Depression	
	STTdev	146 ST deviation with T-wave change	
13. T-wave Abnormality	No		
	Yes	147 T-wave Abnormality	
14. QT interval	Normal		
	Long	148 Prolonged QT interval	
	Short	149 Short QT interval	
15. Ventricular Hypertrophy	No		
	LVH	142 Left ventricular hypertrophy	
	RVH	143 Right ventricular hypertrophy	
	BVH	144 Biventricular hypertrophy	
16. Atrial Enlargement	No		
	LAE	140 Left Atrial Enlargement	
	RAE	141 Right Atrial Enlargement	
	BAE	Biatrial Enlargement (140 and 141)	
17. ECG Quality	Good		
	Poor	12 Missing lead(s) 14 Artifact 15 Poor-quality data	
	Error	10 Extremity electrode reversal 11 Misplaced precordial electrode(s) 4 Uninterpretable ECG	
18. ECG Summary	Normal ECG	1 Normal ECG 2 Otherwise normal ECG 228 Normal Variant	
	Borderline ECG	3 Abnormal ECG 301 Borderline	
	Abnormal ECG	3 Abnormal ECG	
	Uninterpretable	4 Uninterpretable ECG	

1.7.2 PCG findings

Group	***Derived/ classified from***	***Findings***	***Value domain***
Sound findings	Measurements by time-frequency representation, threshold is adjusted automatically by help of machine learning	1. S1 Intensity (S1int)	Normal/ Increased/ Decreased
		2. S2 Intensity (S2int)	
		3. Ejection Sound (ES)	Absence/ Presence
		4. Midsystolic Click (MC)	
		5. Opening Snap (OS)	
		6. Third Sound (S3)	
		7. Forth Sound (S4)	
		8. Diastolic Murmur (DM)	
		9. Wheeze (WHEE)	
		10. Artifacts (ARTF)	
		11. Systolic Murmur (SM)	Early/Mid/ Late/Holo
Systolic time interval (STI) findings	*STI measurements, what derived from PCG segmentation, threshold adjusted by literature and appropriate database*	12. S1 Splitting (S1sp) 13. S2 Splitting (S2sp) 14. Electro-Mech. Activation Time (EMAT) or Q-S1 Interval 15. Systolic Performance Index (SPI) 16. Pre-Ejection Period (PEP), 17. Left Ventricular Ejection Time (LVET)	Normal/ Abnormal
Summary	*PCG quality check algorithm*	18. PCG Signal Quality (PCGq)	Good/ Poor/ Error

	Knowledge based rules applied on findings	*19. PCG Summary*	Normal/ Abnormal/ Uninterpretable

1.7.3 MCG findings

MCG-finding	*Value Domain*
1. Electromechanical Activation Time (EMAT)	Normal/ Abnormal
2. Myocardial Performance Index (MPI)	
3. Systolic Performance Index (SPI)	
4. Pre-ejection Period (PEP)	
5. Left Ventricular Ejection Time (LVET)	
6. Isovolumetric Contraction Time (IVCT)	
7. Left Ventricular Filling Time (LVFT)	
8. Isovolumetric Relaxation Time (IVRT)	
9. Rapid Ventricular Filling Time (RVFT)	
10. MCG Signal Quality (MCGq)	Good/Poor/Error
11. MCG Summary	Normal/ Abnormal/Uninterpretable

1.7.4 ECHO and HART findings

Abbreviation	HART Findings	Validated by ECHO-finding(s)	Value Domain
1. LVH	Left Ventricular Hypertrophy	Left Ventricular Hypertrophy	Normal/Mild/Moderate/Severe
2. DCM	Dilated Cardiomyopathy	Dilated Cardiomyopathy	Normal/Mild/Moderate/Severe
3. LAE	Left Atrial Enlargement	Left Atrial Enlargement	Normal/Mild/Moderate/Severe
4. RAE	Right Atrial Enlargement	Right Atrial Enlargement	Normal/Mild/Moderate/Severe
5. RVE	Right Ventricular Enlargement	Right Ventricular Enlargement	Normal/Mild/Moderate/Severe
6. LVSD	LV Systolic Dysfunction	LV Systolic Dysfunction	Normal/Mild/Moderate/Severe
7. LVDD	LV Diastolic Dysfunction	LV Diastolic Dysfunction	Normal/Impaired Relax./Pseudonorm./ Restrictive Filling
8. WMA	LV Wall Motion Abnormality	LV Wall Motion Abnormality Ischemic Cardiomyopathy	Absent/Mild Hypok./Hypok./Akines./ Dyskin./Aneurism
9. AR	Aortic Valve Insufficiency	Aortic Valve Insufficiency	Normal/Mild/Moderate/Severe
10. AS	Aortic Stenosis	Aortic Stenosis	Normal/Mild/Moderate/Severe
11. MR	Mitral Valve Insufficiency	Mitral Valve Insufficiency	Normal/Mild/Moderate/Severe
12. MS	Mitral Valve Stenosis	Mitral Valve Stenosis Combined Mitral Defect (Mitral Vitium)	Normal/Mild/Moderate/Severe
13. TR	Tricuspid Valve Insufficiency	Tricuspid Valve Insufficiency	Normal/Mild/Moderate/Severe
14. PH	Pulmonary Hypertension	Pulmonary Hypertension	Normal/Mild/Moderate/Severe

1.7.5 RisQ report

Risk Factor

a)	Age	N/Y		
b)	Gender	N/Y		
c)	BMI	N/Y		
d)	Race	N/Y		
e)	Hypertension	N/Y	Medication	N/Y
f)	Blood Sugar	N/Y	Medication	N/Y
g)	Cholesterol	N/Y	Medication	N/Y
h)	Smoking	N/Y		
i)	Family History	N/Y		

j) Alcohol N/Y
k) Physical Activity N/Y
l) Physical Ailments N/Y (2 data points)
m) Stress N/Y (2 data points)
n) Other: _____
o) Other medications N/Y

- Does Patient Have Allergies that could negatively affect CI? YES/NO
- Is Patient on other medication that could negatively affect CI. YES/NO
- Confirm that the identified Risk Factors conform to Standard of Care (SoC) and that patient qualifies for inclusion in the CI? YES/NO

1.7.6 Additional variables

Variable	Description	*Value Domain / Comment*
Referral decision	the GP's referral decision there are five set of such findings: • GP on ECG • GP on CHART • ORC • RC • By cardiologist consensus	DON'T: • No Action • Watch 12 months • Watch 6 months • Watch 3 months SEND: • Routine • Immediate • Urgent • Emergency Not Sure
Comparative questionnaire	ECG and CHART related questionnaire	Table 4 in article
ICD-10 diagnosis	ICD-10 diagnostic codes beside each diagnosis	
Descriptive ECHO diagnosis	Textual descriptive ECHO-interpretation and findings	
Supporting echocardiographic parameters	The measure echo parameters, images and videos supporting the ECHO-findings. These data were used in the consensus study	IVS d PW d LVID d LVID s PEEF Max LA A4C Area LA M-mode Diam s RA A4C Diam. Trans RVD M-mode d RVD M-mode s RVOTD RVOT AoV Cusp Sep M-mode AoV Peak Grad AoV Vmax Ao Root Diam Ao Reg Grade AVA MV E Vmax MV A Vmax MVA Planimetry MR Grade Vena contracta MR Jet Area TV Mean Grad TV Vmax

		TR Grade TR Jet Area TR Peak Grad RVSP (mPAP) PR grade
CTT	Cardio-TriTest records with the required basic measurements variables (body size, blood pressure)	includes ECG, PCG and MCG signals

1.8 Study size - reason study was stopped early

Without the GPs conducting the comparator protocol the original study designed for 1000 patients had to be stopped at 550.

The study was stopped early, just after the mid-point, within 2-weeks of the mid-point cross-over. The reason was that GPs previously on the CHART protocol and switched to the ECG protocol at the mid-point refused to continue the study using ECG only. Having spent months using CHART, there was open reluctance to rely on ECG having become accustomed to CHART's expansive diagnostic information. The straw that broke the camel's back came as a result of two incidents. On two separate occasions within a period of only 1 week, a female patient attended a clinic, the GP using the ECG report, failed to understand that the patient was experiencing an MI at that moment, and indicated that the patient be sent home. However, the GP using CHART, immediately called an ambulance and sent the patient directly to the hospital Emergency Department. Both patients recovered, but the GPs using ECG were so distraught afterwards, they refused to continue using the ECG only device. It was clear to them that ECG has serious limitations, and that CHART provided for more relevant and critical diagnostic support.

1.9 Study Flowchart

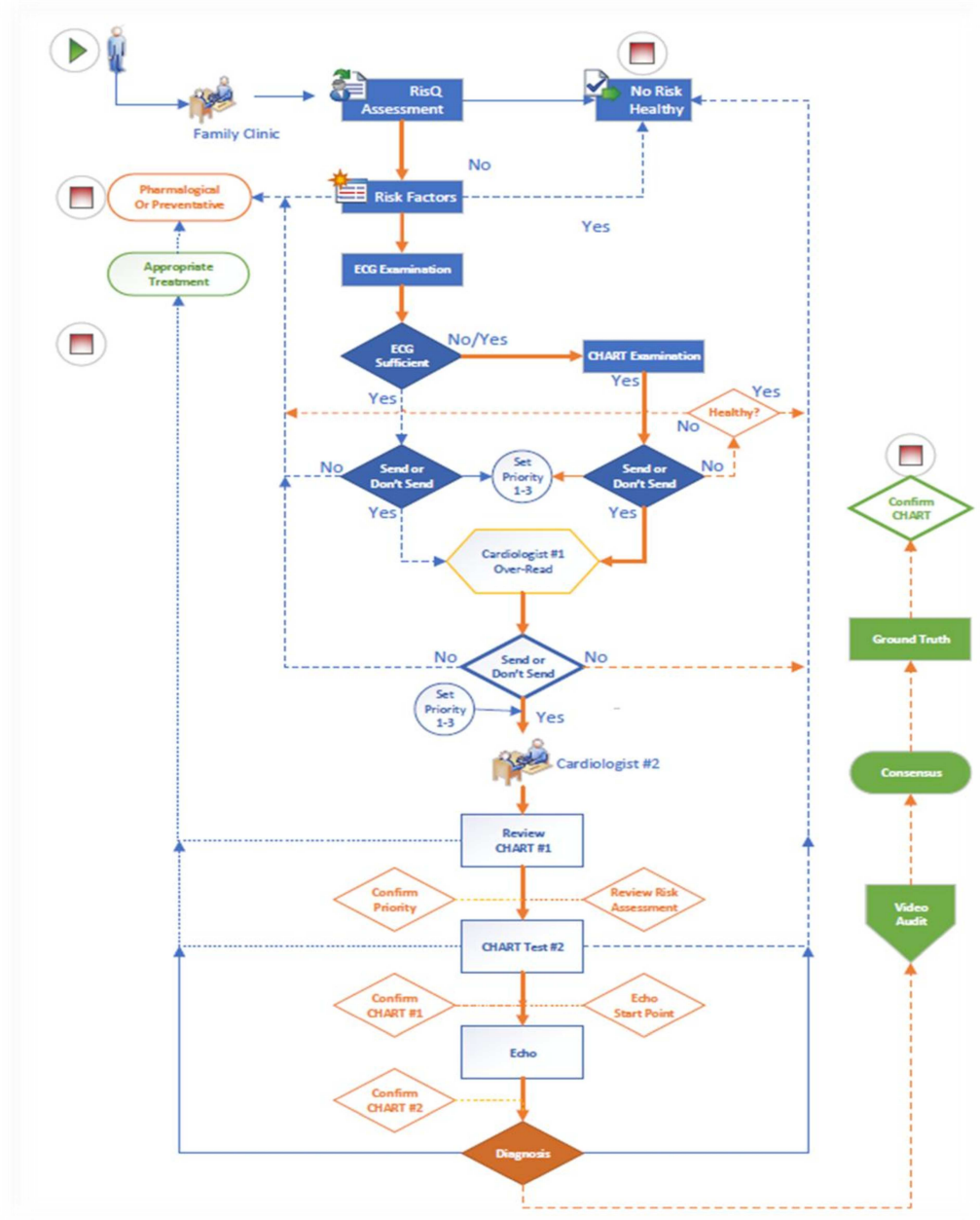

Figure 3 – CUUS flowchart

2 Statistical Plan and Performance Evaluation

All statistical methods were based on the International Conference on Harmonization (ICH) E9 document "Statistical Principles for Clinical Trials" [2] and on E9 (R1) Statistical Principles for Clinical Trials: Addendum: Estimands and Sensitivity Analysis in Clinical Trials document.[3]

All data was summarized by estimated group. For baseline characteristics and safety outputs, a total overall column was included to summarize all subjects. In summary tables of continuous variables, the minimum and maximum statistics are presented. The arithmetic mean (AM), standard deviation (SD) and standard error (SE) are extracted also, in every descriptive statistic table, if applicable and necessary.

The data was collected based on well-defined inclusion and exclusion criteria. The Gender, Age, BMI, RisQ Factors and other demographic criteria were categorically distributed, for the purpose of demonstrating the differences between the clinical study population and intended use population, that may impact the device.

2.1 Primary Objective

Characterize the effectiveness and utility of CHART analysis over ECG-only analysis in providing diagnostic assistance to Clinician's in better determining the cardiac status of the target population.

To prove that the CHART analysis is more effective than ECG only analysis in assisting General Physicians in better determining patient cardiac status in clinical practice.

2.2 Secondary Objective

1) Determine if CHART analysis is better than ECG-only in assisting the Cardiologist in better understanding the basis of the medical justification used for the referral and the prioritization of patients, from primary care to cardiology care.
2) Determine if CHART provides an effective starting point for ECHO examination.
3) Determine whether CHART examinations conducted in Primary Care are consistently reproducible and repeatable in Cardiology Care.
4) Characterize the Usability of CHART in real-world clinical care practice.

2.3 Primary Endpoints

Compare CHART analysis compared to ECG-only analysis to determine which provides Clinician's with better diagnosis decision support, measured as the reduction of false positives, false negatives and 'no-evidence/not sure" rate.

The referral tree is following.

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[2] Statistical Principles for Clinical Trial- to establish valid Hypothesis. Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E9/Step4/E9_Guideline.pdf

[3] E9(R1) Statistical Principles for Clinical Trials: Addendum: Estimands and Sensitivity Analysis in Clinical Trials, to determine the suitable statistical analysis we are going to use in our CS. Available from: https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM582738.pdf

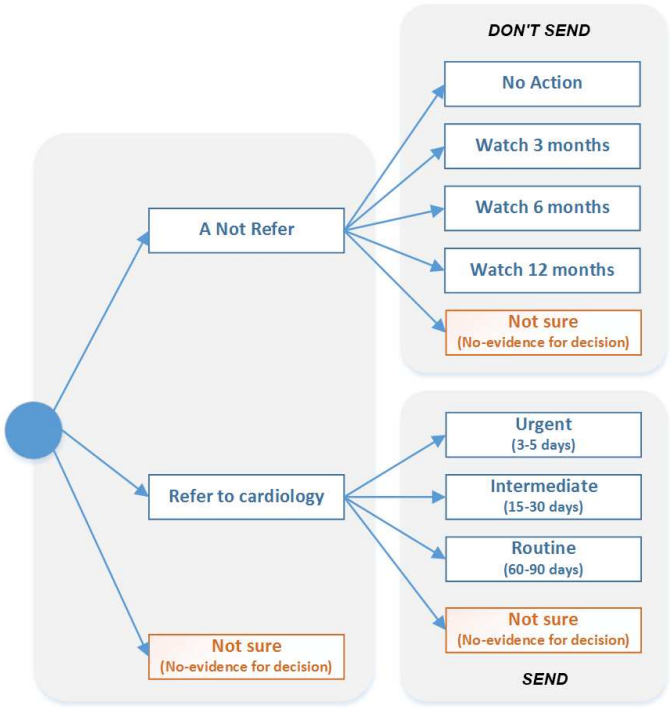


Figure 4 – Referral decision tree in CUUS

The confusion matrix "A" related to first level decision:

GP decision "A"	Consensus decision (Ground truth)	
	Not refer (negative - N)	Refer to cardiologist (positive - P)
Not refer (negative A)	True Negative (TN_A)	False Negative (FN_A)
Refer to cardiologist (positive A)	False Positive (FP_A)	True Positive (TP_A)
Not-sure / No-evidence (neutral A)	Neutral Negative (EN_A)	Neutral Positive (EP_A)

The endpoints are the change of following metrics between ECG and CHART based decision:

- False positive rate of first level - A $FPR_A = FP_A/(FP_A+TN_A)$
- False negative rate of first level - A $FNR_A = FN_A/(FN_A+TP_A)$
- No-evidence rate of first level - A $NER_A = (EN_A+EP_A)/(N+P)$

The change can be expressed as: $\mathbf{\Delta P = P_{CHART} - P_{ECG}}$

2.4 Secondary Endpoints

1) Compare the Cardiologists answers to the Clinician and the ORC (optional) about prioritization and medical justification between CHART and ECG-only, with 95% confidence interval.
2) Confirm the effectiveness of CHART in terms of indicating start point for ECHO examination by comparing the CHART report and ECHO report and confirm it with the cardiologist statement.

3) Compare the CHART#1 and CHART#2 medical tests for repeatability and reproducibility and confirm with summary of Cardiologists comments.
4) Confirm from data provided by MA that the usability of the CHART system is easy, understandable and safe, with confidence level 95% internal.

2.5 Primary Hypothesis

"CHART analysis provides better diagnostic decision support in clinical practice compared to ECG-only analysis **leading to better outcomes."**

We are going to prove this statement by

- measuring the reduction in FP and FN rates, and
- reduction in "no-evidence" and "Not Sure" answers,

between ECG-only and CHART analysis, which will be verified by consensus decision ground truth, calculated according to a 95% confidence interval.

2.6 Secondary Hypothesis

1) CHART analysis provides better prioritization and referral medical justification than ECG-only analysis. This will be accomplished by comparing the prioritization results and the diagnostic findings between the Clinician and the cardiologists, comparing CHART with ECG-only analysis. The significant increase of effectiveness measuring by answers of the referral cardiologists about prioritization and medical justification between CHART and ECG-only, with 95% confidence interval.

2) CHART can provide an effective starting point for Echo examinations. This will be accomplished by Comparing the diagnoses of CHART#2 and ECHO to show that cardiac status is correctly indicated by CHART analysis to show adequate starting point for ECHO examination. This will be confirmed by summing the cardiologists' statements about the effectiveness of CHART analysis to this end. This effectiveness will be measured by summing the cardiologists' statements about the effectiveness of CHART analysis to this end.

3) CHART medical tests are reproducible and repeatable. This will be accomplished by comparing the results between CHART#1 and CHART#2, according to 95% confidence interval. This will be confirmed by summing the cardiologists' statements related to their comparison between the two tests.

4) MA have effective clinical understanding of CHART usability. This will be accomplished by measuring the completion rate by assigning a binary value of '1' if the test participant manages to complete a Medical test and '0' if he/she does not. The equation for this measure is:

$$\text{Effectiveness} = \frac{\text{Number of MT competed succesfully}}{\text{Total number of MT undertaken}} \text{ x } 100\%$$

Concerning the post-test questionnaire with the questions about the usability and the most positive and negative aspects of the medical device, we will be confirming the Usability by summing the answers of the MA and measuring the level of confidence with the 97.5% certainty, applying I-type error.

2.7 Hypothesis calculation

The performance evaluation is necessary to accept or reject the study's hypothesis. The performance evaluation is based on some recommended performance metrics, which are recommended by FDA[4]:

- sensitivity (SE) and specificity (SP) at a clinical action point:
  - "*SE is defined as the probability that a test is positive for a population of patients with the disease/condition/abnormality*"
  - "*SP is defined as the probability that the test is negative for a population of normal patients (i.e., patients without the disease/condition/abnormality*"
- receiver operating characteristic (ROC) curve: "*ROC based endpoint allows evaluation of the device over a range of operating points*"

2.7.1 Confusion matrix

There are no different operating points (or action points) in the GP's decision about refer to cardiology, since the decisions are single:

- decided once based on one instance of ECG report
- decided once based on one instance of CHART report

The ECG and CHART are considered as "modalities" or "readers ", among which the significance of performance difference should be determined. The ground truth of decisions is also single, the ground truth will be decided based on a consensus of cardiologists. Therefore, one confusion matrix can be calculating for ECG and one for CHART:

The confusion matrix of binary decision about patient referral is the following:

		Test Decision (decision on ECG or CHART report)		
		~ Don't	**~ Send**	**~ Not Sure / No evidence**
Reference decision (Ground Truth decision)	# Don't	*TN*	*FP*	*NS⁻* – Negative Not Sure
	# Sent	*FN*	*TP*	*NS⁺* – Positive Not Sure

The number of samples in analysis N = TN + TP + FP + FN

The "Not Sure" decision answers are relatively rare, therefore in the binary decision they are not expressed separately.

In this context the ROC analysis is not applicable according to lack of a range of operating points. From these confusion matrixes the following performance metrics can be calculated separately for ECG and CHART:

- sensitivity: SE = TP / (TP+FN)
- specificity: SP = TN / (TN + FP)

[4] Clinical Performance Assessment: Considerations for Computer-Assisted Detection Devices Applied to Radiology Images and Radiology Device Data - Premarket Approval (PMA) and Premarket Notification [510(k)] Submissions, https://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm187315.pdf

- false positive rate: FPR = FP/(FP+TN)
- false negative rate: FNR = FN/(FN+TP)
- no-evidence rate: NER = (EN+EP)/(N+P)
- The Not Sure Rate is the rate of not sure answer compared to all samples

$$NSR = \frac{NS^- + NS^+}{N}$$

2.7.2 *Hypothesis calculation for Sensitivity, Specificity*

Null Hypothesis test for normal distribution is not suitable for binomial distribution, because the standard deviation σ is not available.

$$Z = \sqrt{N}\frac{\bar{x} - \mu_0}{\sigma}$$

Therefore, the significance level is calculated of using the Clopper-Pearson method[5] for exact confidence interval.

The normal approximation for binomial confidence interval is:

$$p \pm z_{1-\alpha/2}\sqrt{\frac{p(1-p)}{n}}$$

where p = proportion of interest (the performance), n = sample size, α = desired confidence, $z_{1-\alpha/2}$ = "z value" for desired level of confidence, and $z_{1-\alpha/2}$ = 1.96 for 95% confidence.

Exact Confidence Interval addressed by Clopper and Pearson[6]:

$$\sum_{k=0}^{k}\binom{n}{k}p_{UB}^{k}(1-p_{UB})^{n-k} = \frac{\alpha}{2}, \quad \sum_{k=x}^{n}\binom{n}{k}p_{LB}^{k}(1-p_{LB})^{n-k} = \frac{\alpha}{2}$$

The population proportion falls in the range p_{LB} to p_{UB} where:

p_{LB} is the confidence interval lower bound, p_{UB} is the confidence interval upper bound, n is the number of trials, k is the number of successes in n trials, α is the percent chance of making a Type I error, $1-\alpha$ is the confidence.

Solution:

$$p_{LB} = \frac{kf_{LB}}{(n-k+1)+kf_{LB}}, f_{LB} = F^{-1}\left(\frac{\alpha}{2}, 2k, 2(n-k+1)\right)$$

$$p_{UB} = \frac{kf_{UB}}{(n-k)+(k+1)f_{UB}}, f_{UB} = F^{-1}\left(1-\frac{\alpha}{2}, 2(k+1), 2(n-k)\right)$$

Where F^{-1} is the inverse of F cumulative distribution function[7].

Null Hypothesis H is rejected when the performance is higher that the upper bound or lower than the lower bound of confidence interval. In other words, this means the performance $P_{B/G}$ is significantly higher or lower that $P_{A/G}$

$$P_{B/G} > p_{UB}(k = n \cdot P_{A/G}, n) \quad \text{OR} \quad P_{B/G} < p_{LB}(k = n \cdot P_{A/G}, n)$$

[5] https://www.mathworks.com/help/stats/binofit.html

[6] http://www.sigmazone.com/binomial_confidence_interval.htm

[7] M. Abramowitz and I. A. Stegun, "Handbook of Mathematical Functions", Government Printing Office, 1964, 26.6.2

2.8 Performance metrics applied on binary referral decision

Table 1 – Performance metrics applied on binary referral decision

Metric	Description	Symbol and Formulae
Sensitivity	True positive rate compared to positive samples	$SE = \frac{TP}{TP + FN}$
Specificity	True negative rate compared to negative samples	$SP = \frac{TN}{TN + FP}$
Positive Predictive Value	provide useful insight into how to interpret positive test results	$PPV = \frac{TP}{TP + FP}$
Negative predictive Value	provide useful insight into how to interpret negative test results	$NPV = \frac{TN}{TN + FN}$
Positive Likelihood Ratio	likelihood ratio for positive results	$LR+ = \frac{SE}{1 - SP}$ [8] $LR+* = 10 \cdot LR+$
Negative Likelihood Ratio	likelihood ratio for negative results	$LR- = \frac{1 - SE}{SP}$ $LR-* = 10 \cdot LR-$
Cohens Kappa	Kappa[9] a more robust measure than simple percent agreement, since it takes into account the possibility of the agreement occurring by chance	$\mathrm{K} = \kappa = \frac{p_o - p_e}{1 - p_e}$ observed agreement $p_o = \frac{TN + TP}{N} = ACC$ hypothetical probability of chance agreement $p_e = p_- + p_+ =$ $= \frac{TN + FP}{N} * \frac{TN + FN}{N} + \frac{FN + TP}{N} * \frac{FN + TP}{N}$
Area Under Curve	Area under ROC curve. Described in next subsection	AUC is get from receiver operating characteristics (ROC) that can be calculated using the detailed referral decision, see description in supplementary material.
Positive Percent Agreement	Positive agreement is the proportion of comparative method positive results in which the test method result is positive [10]	$PPA \equiv SE$
Negative Percent Agreement	Negative agreement is the proportion of comparative method negative results in which the test method result is negative.	$NPA \equiv SP$
Positive rate	Rate of positive decision from test (GP, ORC or RC decision)	$PR = \frac{FP + TP}{N}$
Prevalence	Rate of positive decision from reference (consensus ground truth)	$PREV = \frac{FN + TP}{N}$

[8] LR+* and RL-* are used for better plottable together with percentage performance metrics

[9] Hripcsak, G., & Rothschild, A. S. (2005). Agreement, the f-measure, and reliability in information retrieval. Journal of the American medical informatics association, 12(3), 296-298.
Chmura Kraemer, Helena, Vyjeyanthi S. Periyakoil, and Art Noda. "Kappa coefficients in medical research." Statistics in medicine 21.14 (2002): 2109-2129.

[10] https://analyse-it.com/blog/2020/4/diagnostic-accuracy-sensitivity-specificity-versus-agreement-ppa-npa-statistics

2.9 Area Under Curve

Area Under Curve (AUC)[11] is calculated by moving the referral threshold on GP's decision toward ground truth binary decision. The AUC is calculated on the following ROC points, see following Table and Figure.

Table 2 – Analyis point pairs for ROC

ROC points	Simulated Positive	Simulated Negative	Reference Positive
1. Emergency	GP decision = Emergency	GP decision < Emergency	Ground truth >= Routine
2. Urgent	GP decision >= Urgent	GP decision < Urgent	Ground truth >= Routine
3. Immediate	GP decision >= Immediate	GP decision < Immediate	Ground truth >= Routine
4. Routine	GP decision >= Routine	GP decision < Routine	Ground truth >= Routine
5. Watch 3	GP decision >= Watch 3 months	GP decision < Watch 3 months	Ground truth >= Routine
6. Watch 6	GP decision >= Watch 6 months	GP decision < Watch 6 months	Ground truth >= Routine
7. Watch 12	GP decision >= Watch 12 months	GP decision < Watch 12 months	Ground truth >= Routine

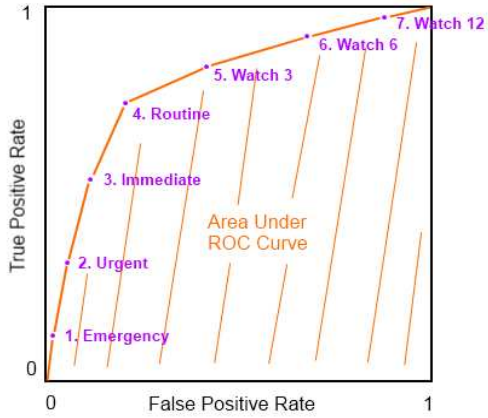


Figure 5 - Illustration of ROC analysis and AUC calculation using seven ROC points

2.10 Missing Data

There are many reasons for missing values such as:

- questions are not applicable to the respondents;
- respondents skip the questions;
- respondents do not want to reveal sensitive information etc.

Missing values can cause loss of information or skewness of the data, so because of this statement, the missing data shall be handled before starting run the analysis. In other words, they require complete data and missing values will cause errors in the analysis process.

[11] Dodd, Lori E., and Margaret S. Pepe. "Partial AUC estimation and regression." Biometrics 59.3 (2003): 614-623.

Because of the use of qmsWrapper software custom forms (CRF), each question need to have an answer (the form is designed in such way that the answers are obligatory to fill), so missing data is reduced to minimum, almost non-existing.

In some cases, only a very small percentage of the entire dataset is missing values. If after examining the missing value cases we find out that they are random and will not affect the analysis, then it could be safe to run the analysis. In other cases, in which the missing values will reduce the significant of the statistical tests, Multiple Imputation (MI) must be done to keep the cases. We are going to use this approach dealing with missing data in our study, using SPSS v23 program, and Multiple Imputation function.

The purpose of MI (Multiple Imputation) is to generate possible values for missing values, thus creating several "complete" sets of data. Analytic procedures that work with multiple imputations datasets produce output for each "complete" dataset, plus pooled output that estimates what the result would have been if the original dataset had no missing values. These pooled results are generally more accurate than hose provided by single imputation methods.

3 Results

3.1 Patient Population Analysis

3.1.1 Center distribution

The efficacy analysis was intended to be performed on the population which included all subjects who were randomised and collected during the clinical study- total 421 subjects, in three different centers- Sombor and Vrsac. The most of the subjects are enrolled in Vrsac (56.4% of the whole study population).

Center	Frequency	Percent
Sombor	111	20.2%
Vrsac	310	56.4%
Senta	129	23.4%
Total	550	100%

3.1.2 Gender distribution

The gender distribution is approximately equality (Male 51.5%, Female 48.5%).

Gender	Frequency	Percent
Male	283	51.5
Female	267	48.5
Total	550	100.0

3.1.3 Age and Obesity distribution

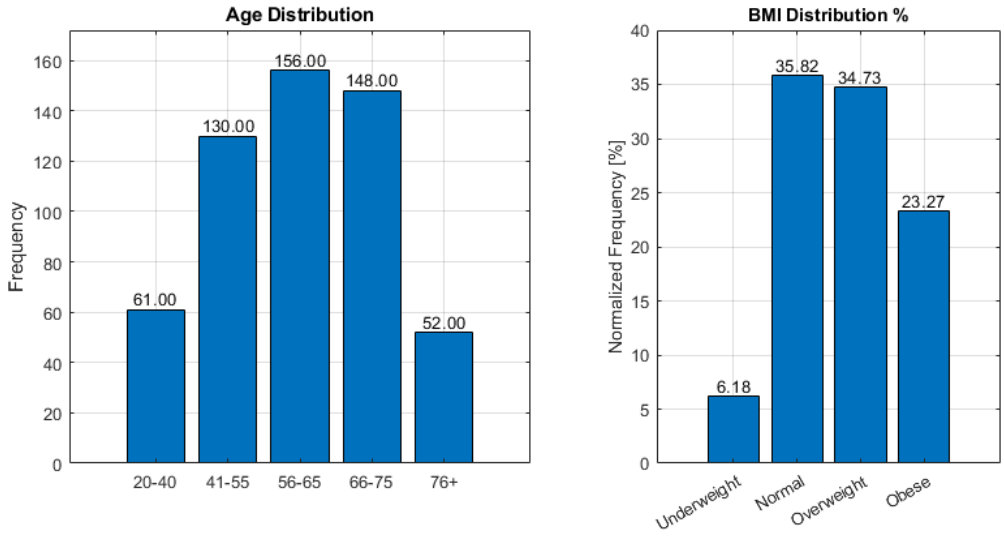


Figure 6 – Age and BMI distribution of CUUS patienst

3.1.4 Healthy/Unhealthy distribution

Healthy Status	Frequency	Percent
Healthy	71	13
Unhealthy	479	87
Total	550	100

We collect 13% of the healthy subjects, and 87% of unhealthy subjects (subjects with at least 3 risk factors established).

3.1.5 Excluded patients

During the evaluation of the collected data, we excluded 13 patients from the analysis because the MA at one site did not follow the protocol, and the medical tests were not completed and valid.

Also, some of the patients withdrew in the middle of the study, after their Medical Test but they didn't go to the Cardiologist level, so we missed their CHART#2 reports and ECHO examinations. For that reason, in the RC evaluations we included only 515 valid subjects and 549 subjects for ORC evaluation.

Withdrawal of patients were mainly due to delays in attending the cardiologist level and having to travel some distances to the center.

A confirmatory analysis was performed on the per-protocol population which included all subjects that completed the tests and ECHO, and did not meet any major protocol violation during the study period. The safety population included all subjects who has at least 3 risk factors for cardiac diseases (in 85% of the population).

3.2 Time delay statistics between CHART#2 and CHART#2

The original requirement was for a minimum of 3 days delay between the first and second CTT, i.e. CHART#1 and CHART#2 reports.

Except 39 patients the minimum 3 days is fulfilled, the average delay is 9.5 days, the maximum is 80[12] days, the minimum is 4 hours. The distribution of time delay expressed in days can be seen on Figure 4, colored by centers.

[12] 80 Days represents patients that were tested at start of trial but never returned. They were later convinced to return to complete the protocol. This true for most of 30+ Days patients.

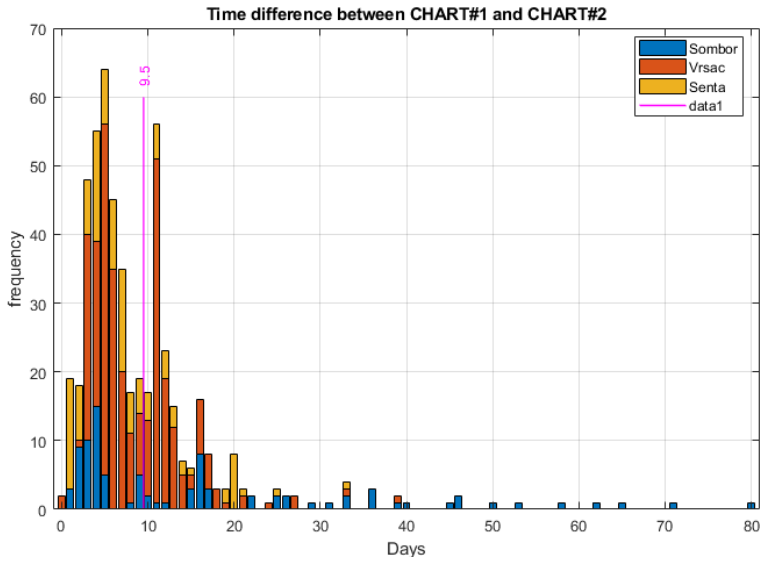


Figure 7 - ***Time delay distribution between two CTT-CHART#2 and CHART#2 reports***

3.3 Additional Performance results

3.3.1 Confusion matrix - GP referral decision on ECG and CHART

Table 3 - Confusion matrix of GP referral decision on ECG and CHART reports compared to by consensus ground truth

	Ground Truth	GP decision		*Summary*
ECG report		Don't	Send	
	Don't	246	67	*313*
	Send	122	115	*237*
CHART report		Don't	Send	
	Don't	263	50	*313*
	Send	85	152	*237*

3.3.2 Confusion matrix - GP and ORC and RC decision

Table 4 - Confusion matrixes of between GP and ORC decisions and GP and RC decisions

Reference situation	Reference decision	Test decision - GP		
ORC and GP decision on **ECG** report	**ORC decision**	Don't	Send	*Summary*
	Don't	271	78	*349*
	Send	96	104	*200*
ORC and GP decision on **CHART** report	**ORC decision**	Don't	Send	
	Don't	279	64	*343*
	Send	68	138	*206*
RC and GP decision on **ECG** report	**RC decision**	Don't	Send	*Summary*
	Don't	266	73	*326*

	Send	78	98	*189*
RC and GP decision on **CHART** report	**RC decision**	Don't	Send	
	Don't	262	64	*326*
	Send	61	128	*189*

3.3.3 Subgroups performance comparison

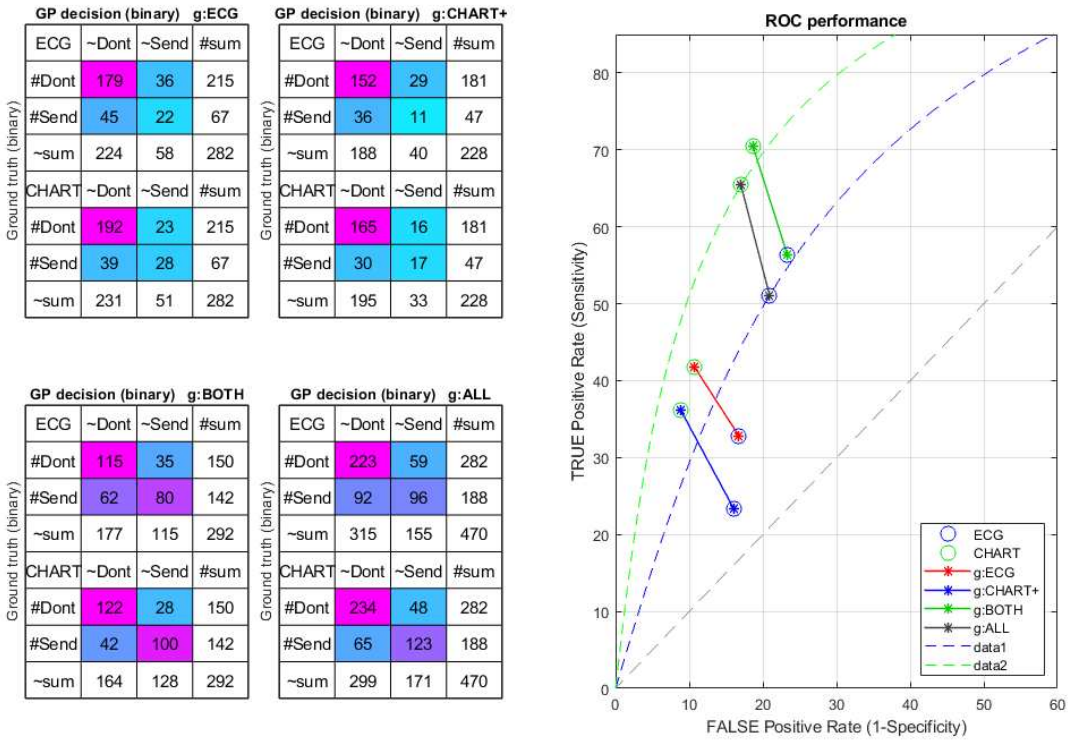
In the diagnosis we can distinguish typical ECG diagnosable (e.g. arrhythmia or ischemia) and typical CHART+[13] - diagnosable (e.g. systolic dysfunction or valve disease), see details in Table 13 in section 8.5.1.

In this analysis the GP decision performance four subgroup of patients based on the type of diagnoses diseases:

1. g:ECG – positive ECG diagnosable findings, plus the normal set (no cardiac disease)
2. g:CHART+ – positive CHART+ diagnosable findings, defined as non-ECG diagnosable plus the normal set
3. g:BOTH – patients having both ECG based and CHART+ based diagnosable findings in parallel plus the normal set. This excludes patients having only ECG or only CHART+ diagnosable findings (abnormal set from the previous two subgroups)
4. g:ALL – include all the patients, all abnormal, all normal

The confusion matrix and ROC performance analysis are plotted in the Figure 13.

[13] CHART**+** because CHART includes ECG findings as well, but shows the additional non-ECG findings, which denotes the "+" after "CHART"



GP decision (binary) g:ECG

Ground truth (binary)	ECG	~Dont	~Send	#sum
	#Dont	179	36	215
	#Send	45	22	67
	~sum	224	58	282

Ground truth (binary)	CHART	~Dont	~Send	#sum
	#Dont	192	23	215
	#Send	39	28	67
	~sum	231	51	282

GP decision (binary) g:CHART+

Ground truth (binary)	ECG	~Dont	~Send	#sum
	#Dont	152	29	181
	#Send	36	11	47
	~sum	188	40	228

Ground truth (binary)	CHART	~Dont	~Send	#sum
	#Dont	165	16	181
	#Send	30	17	47
	~sum	195	33	228

GP decision (binary) g:BOTH

Ground truth (binary)	ECG	~Dont	~Send	#sum
	#Dont	115	35	150
	#Send	62	80	142
	~sum	177	115	292

Ground truth (binary)	CHART	~Dont	~Send	#sum
	#Dont	122	28	150
	#Send	42	100	142
	~sum	164	128	292

GP decision (binary) g:ALL

Ground truth (binary)	ECG	~Dont	~Send	#sum
	#Dont	223	59	282
	#Send	92	96	188
	~sum	315	155	470

Ground truth (binary)	CHART	~Dont	~Send	#sum
	#Dont	234	48	282
	#Send	65	123	188
	~sum	299	171	470

Figure 13- Performance of GP decision compared to ground truth for subgroups

Conclusion:

- The benefit of CHART over ECG is very consistent through these subgroups. This means the GP decision is more accurate using the CHART report for each subgroup, so benefit is independent from the type of diagnosis, e.g. it is not dependent on whether it is a typical ECG abnormality or a CHART+ abnormality.
- Both the positive decision rate or prevalence are significantly different in these subgroups, in other words the rate of diseases patients is different. The ECG only and CHART+ diagnosis subgroups have smaller positive ground truth and decision compared to BOTH subgroups. This means that when both ECG and CHART+ indicate any abnormality, then it is more likely that the patient should be Sent to cardiology.

CHART provides important additional diagnostic power beside ECG findings, and can more clearly indicate that the patient has some measure of cardiac disease and should therefore be referred to cardiology. – This conclusion is derived from the previous conclusion point.

3.4 GP Interview

3.4.1 Dr. D. - switched from CHART to ECG protocol in the GP evaluation

In the initial phase of the CUUS, Dr. D. was initially on the CHART protocol. Midway through the CUUS, she was switched to the ECG protocol.

In the referral diagnosis (ICD10 code) we found CHART+ based diagnosis evaluated on ECG-only report from Dr. D. The Risk assessment and the ECG report does not provide any medical justification to diagnose the following heart diseases that CHART+ is engineered to do:

- I05.0 - Rheumatic mitral stenosis
- I05.2 - Rheumatic mitral stenosis with insufficiency
- I06.8 - Other rheumatic aortic valve diseases
- I07.0 - Rheumatic tricuspid stenosis
- I07.1 - Rheumatic tricuspid insufficiency
- I08.9 - Rheumatic multiple valve disease, unspecified
- I34.0 - Nonrheumatic mitral (valve) insufficiency
- I35.0 - Nonrheumatic aortic (valve) stenosis
- I35.1 - Nonrheumatic aortic (valve) insufficiency
- I35.2 - Nonrheumatic aortic (valve) stenosis with insufficiency
- I35.8 - Other nonrheumatic aortic valve disorders
- I36.1 - Nonrheumatic tricuspid (valve) insufficiency
- I42.0 - Dilated cardiomyopathy

In the initial phase of the study, the online processing system to capture clinical study notes, did not hide the OTHER report. It was expected that GPs would only access the Report based on the protocol they were assigned. They were not prevented from accessing the other report.

After an interview with the GP, the following was found:

Dr. D had initially been on the CHART protocol, she had become reliant on CHART. So, when switched to the ECG protocol, whenever she felt that the ECG report did not adequately explain what she suspected her patient was suffering from, she simply reached into the file and read the CHART report. Dr D had completed some 150 patients using the CHART report only. As such, she was well aware of the additional information the CHART report provided and when the ECG report did not provide her the diagnostic assistance, she simply reached into the patient file and opened the CHART report. She found it not only very useful but necessary to diagnose various diseases such as Valve disease, PCG and MCG related diagnostic questions. Additionally, she felt it also provided her with more confidence in her overall decisions – so she used it.

Altogether 17 patients were found to have CHART+ based diagnosis recorded by her on ECG forms, where the CHART+ based diagnoses were deleted.

From these 17 patients, 8 decisions were corrected from “Send” to “Don’t” decision category, where there was no ECG diagnosable abnormality only CHART+.

3.4.2 Interview with Dr. B

Dr. B is a GP, when working on the ECG protocol, she referred almost everybody in the first 150 patients. Her decision answers showed a large bias toward “Send” compared to other GPs in the referral decision on ECG report.

After an interview with GP, the following reason was found: In the case of a patient with normal ECG but increased risk factors, such as hypertension, she referred the patients to higher level of care to discover the real cardiac situation. She “send” most all patients with no medical justification.

All the patients affected by her decision were re-verified and the “Send/Don’t Send” decisions corrected based on her actual diagnosis. Altogether 107 patient decisions were corrected, from which 100 patients were changed from false “Send” to “Don’t” decision category, with her approval.

She was re-educated on the correct interpretation of the protocol, and her remaining patients were correctly processed. However, she remained the weakest in terms of diagnostic accuracy when compared to her peers and the ground truth.

However, when she switched to the CHART protocol, both her bias and her diagnostic weakness disappeared. She was the one GP who showed the greatest improvement in diagnostic accuracy. When re-intereviewed, she suggested that she better understood CHART which increased her confidence.

3.5 Summary

The Clinical Utility and Usability Study (CUUS) was a pivotal, multicentre (Sombor, Vrsac, and Senta), randomized, blinded study the goal of which was to determine utility of CHART, its usability for its intended use, in a clinical environment, by its intended users, in a study population representative of the target population.

This study was designed with the assistance of and approval by the FDA. The study was designed to collect the data to confirm the safety and effectiveness of the CHART system, in particular the IMD- Cardio-TriTest (CTT for short) and the CHART Processing Algorithm (CPA).

The data collected would confirm the hypothesis that CHART analysis is more effective than ECG only analysis in assisting the GP in determining their referral decision (Send/Don't Send) and the basis for it.

550 patients were recruited into the CUUS. More than 500 patients' clinical results were evaluated to measure the diagnostic and decision support capability of CHART report compared to ECG report. This study population was representative of the target population according to the intended use of CHART. Some 43,0% of the patients are classified as Sent to referral cardiology based on the consensus-based ground truth.

The results confirm that in many ways CHART analysis is more effective than ECG only analysis. False-negative rates are significantly decreased (CHART produced a 15.8% decrease in False-positive) and False-Positive rates decreased by 5% (False-positives in the patient referral decision by GP as compared to ECG-only based decisions).

Furthermore, doctors (GP, ORC and RC) were significantly more sensitive in their referral decision when based on CHART report.

Reproducibility is better than the predicate ECG, and the Usability results shows that the system itself, like their devices separately, are easy to use, user-friendly and that there were no problems or additional risks, not previously understood and mitigated, established while working with the devices.

No adverse events (AE) or effects were reported.

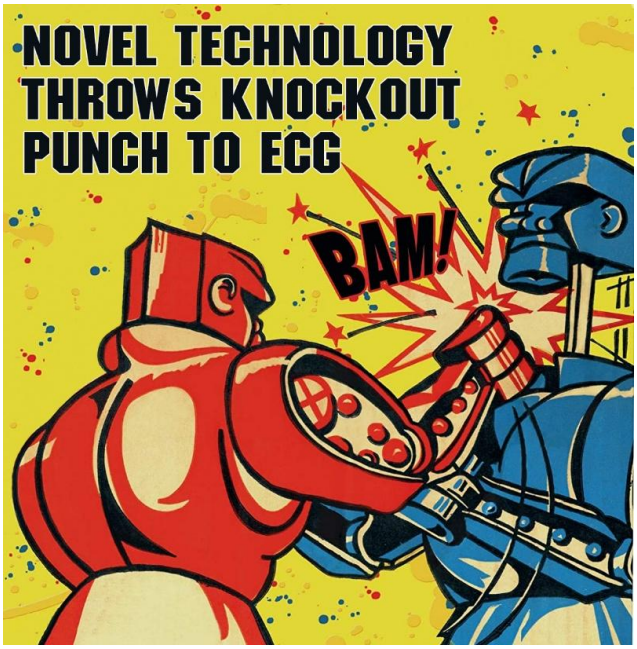
The benefits of this study and its results are:

1. Better able to detect and confirm onset of heart disease earlier (when treatment options are more effective and cost-efficient).
2. No additional risk compared to predicate ECG devices. Device is as safe to use as Predicate ECG devices.
3. Increased effectiveness for a much wider range of disease conditions.
4. Easy to use, little or no additional operator training was required for existing operators of predicate devices.
5. Fits into current workflows for normal Standards of Care (SoC).

6. Is an effective assistant to Primary Care physicians in helping them to better understand their patient's cardiac status.
7. Reduction of FN by 15.8%.
8. Reduction of FP by 5%.
9. Widespread benefit to all patients attending Primary Care, or patient care clinics.
10. Helps Cardiologists with Collaborative triage of patient appointment priorities.
11. Helps Cardiologists identify a start point for Echo examination, saving time, and costs.

Establishing the risk factors based on the CHART report, early detection and prompt treatments are likely to improve clinical outcomes. Overall, the probable benefits outweigh the probable risks given the available information concerning the benefits and risks. There is reasonable assurance of the safety and effectiveness for this system for the intended use.

4 Graphical Abstract and Bullet points



- Clinical Study comparing GP decisions using ECG versus GP using Cardio-HART, a breakthrough technology for diagnosing heart disease.
- GPs using CHART increased positive diagnostic rate from 8.5% to 26.7%, uncertainty decreased from 24% to 1.7%.
- Using CHART, GP referral decisions were same as those of Overreading Cardiologists, within 1,7%.
- CPs using CHART reduced FP rate by 5%, and FN rate by 15.8%. compared to peers using ECG.

5 Author Contributions

- Simone Calcagno, MD, MMSc, FHFA and Giuseppe Biondi Zoccai, MD, MStat, unrelated and independent of the clinical study, completed an independent review of the results of the clinical study.
- Tatjana Stankovic, MD, was the principal investigator and assisted with data and verification of ground truth.
- Erzsebet Szabo, MD, and Aniko Berta-Szabo, MD assisted with data and verification of ground truth.
- Istvan Kecskes, PhD, assisted in the statistical assembly and data preparations