HHS Public Access

Author manuscript

Int J Cardiol. Author manuscript; available in PMC 2019 April 25.

Published in final edited form as:

Int J Cardiol. 2019 March 15; 279: 155–161. doi:10.1016/j.ijcard.2018.12.061.

Elevated Renalase Levels In Patients with Acute Coronary Microvascular Dysfunction – *A Possible Biomarker For Ischemia*

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Abstract

Aims—We explored the relationship between inflammation, renalase an anti-inflammatory protein, and acute chest pain with coronary microvascular dysfunction (CMD).

Methods and Results—We used cardiac Rb-82 PET/CT imaging to diagnose coronary artery disease (CAD/CALC) (defect or coronary calcification) and CMD (depressed coronary flow reserve without CAD) in patients with chest pain in an emergency department (ED). Blood samples were collected pre-imaging within 24 hours of ED presentation and were analyzed for renalase and inflammatory markers including C-reactive protein, interleukins, interferon gamma, tumor necrosis factor, vascular endothelial growth factor, and metalloproteinases. Exclusions were age ≤30 years, myocardial infarction, hemodynamic instability, hypertensive crisis, heart failure or dialysis.

Between 6/2014-11/2015, 80 patients undergoing PET/CT provided blood and were categorized as normal (18%), CAD/CALC (27%) and CMD (55%). Median renalase values were highest in patients with CMD (5503 ng/ml; IQR 3070) compared to patients with normal flows (4266 ng/ml; IQR 1503; p = 0.02) or CAD/CALC (4069 ng/ml IQR 1850; p =0.004). CMD patients had similar

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Conflicts of Interest:

G Desir is a named inventor on several issued patents related to the discovery and therapeutic use of renalase. Renalase is licensed to Bessor Pharma, and G Desir holds an equity position in Bessor and its subsidiary Personal Therapeutics.

median values for inflammatory markers as normal patients ($p > 0.05$). Renalase remained an independent predictor of CMD (OR 1.34; 95% CI= 1.1-1.7, per 1,000 ng/ml) after adjustment for smoking, family history, obesity and Framingham risk score. In a model for CMD diagnosis with Framingham risk score, typical angina history and CRP, renalase improved discrimination from C-statistic=0.60 (95% CI 0.47, 0.73) to 0.70 (95% CI, 0.59-0.82).

Conclusion—We found elevated renalase in response to ischemia from acute CMD. Its role as a biomarker needs validation in larger trials.

Keywords

coronary microvascular dysfunction; PET; chest pain; renalase; biomarker; inflammation

Introduction

Over six million patients present with chest pain each year to the emergency department (ED) in the United States, only one-fifth of which are diagnosed with obstructive coronary artery disease (CAD).[1, 2] Coronary microvascular dysfunction (CMD) is the most frequent cause of non-CAD angina and accounts for 21-63% of chest pain in patients without obstructive CAD by angiography.[3] It refers to disease of the small arterioles in the myocardium that is not detected by routine cardiac evaluation and is associated with persistent chest pain, poor functional outcomes and adverse cardiac events.[4–8] Patients with CMD have an accelerated course towards development of CAD,[7] highlighting the need for early diagnosis and treatment. However, research on CMD is lacking and effective screening approaches are sparse.

The current gold standard for diagnosis is reactivity testing using adenosine or acetylcholine during angiography, which many low-risk patients with CMD do not quality for.[9] Non-invasive alternatives include measurement of coronary flow reserve (CFR), a surrogate for microvascular health. However, non-invasive tests like cardiac positron emission tomography (PET), transthoracic Doppler echocardiography and magnetic resonance imaging (MRI) are sophisticated, costly, or only available in select centers based on local expertise and resources[6]. For these reasons, standard ED evaluations often omit routine assessment of CMD and many patients with non-CAD related chest pain are discharged home without a diagnosis or treatment plan.[10]

Thus, identifying a blood biomarker that is precise, accurate, reliable, easily obtainable and reflects the pathophysiology of CMD would be groundbreaking for CMD screening and diagnosis. Biomarkers offer the potential advantage of adding prognostic value to standard of care and could help identify patients with CMD, facilitating early primary prevention. However, the blood markers linked with the pathophysiology of CMD to date are not available for clinical use in symptomatic patients – and thus the quest continues.

Renalase is a recently discovered flavoprotein released primarily by the kidneys and heart in response to catecholamines and ischemia, and holds promise related to CMD.[11] Renalase is linked with factors associated with endothelial dysfunction, such as hypertension, insulin resistance and diabetes. More recently, renalase has also been found to have an anti-

inflammatory and anti-apoptotic effect released in response to ischemia.[12] Given the proposed mechanism of inflammation in CMD pathogenesis,[13] we conducted an exploratory analysis to assess the relationship between renalase, inflammatory markers and acute chest pain.

Methods

Study population and patient flow

This was a cross-sectional study conducted in a chest pain observation unit that admitted ED patients with low-moderate risk for acute coronary syndrome (ACS) (i.e. patients with TIMI score <4, in the presence of negative troponin and non-ischemic ECG). Eligible patients included those undergoing cardiac Rubidium-82 (Rb-82) hybrid positron emission tomography (PET) and X-ray computed tomography (CT) imaging, in accordance with 2013 guidelines by American College of Nuclear Cardiology.[14] Patients were clinically indicated for PET/CT if they were obese or if they were unable to exercise adequately. A full description of patient flow in the chest pain center has been described previously.[15] Briefly, all patients presented with chest pain and underwent a standard protocol to rule out ACS with serial cardiac enzymes and electrocardiograms at 0, 3 and 6 hours before imaging (see Appendix Figure). Consenting patients provided blood samples between 7 and 11am prior to PET/CT, after an overnight rest and within 24 of hours of the chest pain symptoms. We excluded patients with myocardial infarction, hypertensive crisis (BP >180/110 mm Hg), active substance abuse, renal insufficiency, pregnancy, and inability to consent or communicate in English. The institutional review board approved this study.

Data Collection

Baseline measures—Baseline socio-demographic and clinical characteristics were documented through electronic health records and in-person interviews. Lab values for renal function, fasting lipid profile and troponins were recorded.

Assessment of CAD and calcification—Relative and absolute myocardial blood flow (MBF) were determined at rest and during regadenoson stress using Rb-82 PET/CT imaging. Full protocol is described elsewhere.[15] Attenuation corrected PET images were first evaluated visually for regional perfusion defects. Regional and global quantitative flow was determined using a commercial software package, Corridor 4DM v2013 (INVIA Medical Imaging Solutions, Inc.) that employs a previously validated generalized factor analysis of dynamic sequences (GFADS).[16, 17] CAD was defined as old or new regional perfusion defect on attenuation corrected PET/CT scans.

The CT attenuation scans were evaluated both visually and semi-quantitatively for the presence of coronary artery calcifications, using Hounsfield units (HU) threshold (>130 HU) and standard semi-quantitative criteria. PET/CT has high temporal and spatial resolution offering significant advantage for diagnostic accuracy for obstructive CAD over conventional testing such as SPECT perfusion imaging. This has been validated in several prior studies. A recent systematic review indicated the sensitivity and specificity of Rb^{82} PET/CT to be 90% and 88% respectively against luminal obstruction identified on

angiography.[18] We coupled a calcium score of 0 with absence to regional perfusion defect in low-moderate risk patients to rule out CAD.[19]

Assessment of CMD—Global and regional rest and stress flow and CFR were assessed to diagnose CMD. The rest and stress flows were corrected with the respective heart rate and blood pressure product. For the stress flow, we averaged heart rate and blood pressure values obtained at 1.5 and 2.5 minutes after initiation of the regadenoson, which represented the peak hemodynamic changes. This approach corrects for differences in resting hemodynamics and metabolic conditions between subjects, and the significant changes in the hemodynamics associated with regadenoson stress.[20] CMD was defined if patients had CFR <2.5 for uncorrected values, *and* CFR <2 for rate-pressure product corrected CFR in the absence of coronary calcification or regional defect.[15, 21]

Processing of Blood Samples—Peripheral blood was collected after overnight fasting in non-EDTA tubes, centrifuged within 30-min of collection, and then stored at −80°C.

Serum RNLS measurement by Sandwich ELISA—Human recombinant RNLS (hrRNLS) was used as the calibrator and synthesized as previously described (detailed protocol in Appendix B).[22] We generated the capture monoclonal antibody (m42-RNLS) using a previously described method.[23] The detection antibody is a commercially available goat polyclonal renalase antibody (Abcam, ab31291), generated using an unrelated peptide to that used for m42-RNLS. The secondary detection antibody is a SULFO-TAG labeled anti-goat polyclonal antibody generated in donkey (MesoScale diagnostics, Gaithersburg, Maryland, USA)

To assess the amount of total renalase in serum, samples were pre-treated as follows. 20 μl of 1 N citric acid was mixed with 40 μl of serum. Following incubation on a shaker for 15 min at RT, 40 μl of 1M Na2HPO4 was added to the mixture. Pre-treated samples were stored on ice, and diluted as needed to obtain readings in linear range. Renalase levels were then measured using a sandwich ELISA assay using Mesoscale (MSD) platform (MesoScale diagnostics, Gaithersburg, Maryland, USA). The raw data was analyzed using the Discovery Workbench software. 4-PL curve fitting was used to determine renalase concentration. Intra-assay and inter-assay coefficient of variability was calculated using calibrators run in duplicate. The calibrator intra-assay CV was <10%. The lower limit of detection (LLOD) was determined from the respective standard curve and was defined as the calculated concentration of the signal that is 2.5 standard deviations over the blank. The average lower limit of detection for renalase obtained from multiple runs was 1.28 ng/ml and upper limit of detection was 17,000 ng/ml. In our lab, the normal levels of renalase in healthy humans range ~ 4 ± 0.73 μg/mL.[24]

Analysis for inflammatory markers—Two Milliplex Map kits (EMD Millipore, Bilerica, MA) were used to analyze samples for five cytokines in one assay (IL-1β, IL-6, IFN-γ, TNF and VEGF) and 2 matrix metalloproteinases in another (MMP2, MMP9) using the manufacturer's protocol. High-sensitivity C-reactive protein (CRP) was measured by ELISA on Alfa Wassermann analyzer (ACE Alera model) using Raichem hsCRP reagents from Clinicqa Corp using ultraviolet/ Vis detection method. Biomarker values that were less

than detectable range of the kit was recorded as 0. We ran all standards and samples in duplicate and recorded averages.

Statistical methods—Baseline characteristics were summarized using frequencies for categorical variables and means (standard deviations) or medians (interquartile range) for continuous variables. The biomarkers had non-normal distributions and were therefore compared using either the Wilcoxon rank sum test or the Kruskal-Wallis test. Unadjusted associations of the presence or absence of CMD with demographics, clinical variables and renalase were analyzed using simple logistic regression. The independent association between CMD and renalase was assessed while controlling for Framingham score, obesity (defined as BMI >30), current smoking and family history of premature CAD in a multivariate logistic regression. The discriminatory ability of each biomarker for assessment of CMD was then assessed by plotting ROC curves and the C-statistic representing the area under the curve (AUC) was calculated with 95% confidence intervals. All analyses were conducted using SAS/STAT software, version 9.3 and SPSS software, version 21. P-values < 0.05 were considered statistically significant. Investigators performing the marker analysis were kept blinded to the PET results.

Results

Of the 171 ED patients undergoing clinical PET/CT scan for the evaluation of cardiac ischemia during this study period, a convenience sample of 82 patients consented to provide blood samples and 80 samples were adequate for analysis. Appendix Table 1 shows that the demographics and clinical profile of patients who consented was similar to those who did not consent, except the former group had higher rates of family history of CAD.

Table 1 shows the socio-demographic and clinical profile of patients who provided blood samples. Among study participants, 14 (18%) had normal flows, 22 (27%) had CAD or coronary calcification (CALC) and 44 (55%) had CMD. Compared to patients with new or known CAD / CALC, patients with CMD were younger, less likely to have dyslipidemia but had a lower mean CFR (mean CFR=1.61 vs. 1.98).

Table 2 summarizes the unadjusted association between CMD and typical cardiac risk factors. Female sex and renalase were predictive of CMD while history of dyslipidemia was not.

Relation of Renalase with CMD ischemia

Figure 1 shows the serum renalase values for the three groups. The median renalase values were highest in patients with CMD ischemia (median value 5503 ng/ml; IQR 3070; Kruskal-Wallis test p =0.02). For pairwise comparisons, the Wilcoxon rank sum test showed that median renalase values were significantly higher in CMD patients compared both with patients with normal flows (median: 4266 ng/ml; IQR 1503; p = 0.02) as well as CAD/ CALC (median 4069 ng/ml IQR 1850; p =0.004). Distribution of renalase did not vary significantly by patient sex (Appendix Table 3) or BMI in our cohort (Appendix Table 4).

Of note, the CAD/CALC group represented a hybrid group, including those with known CAD (with fixed known defect on PET) (n=3) and the remaining (n=19) with coronary calcifications but no regional defects. It is possible that some of these patients had concurrent CMD, although it was not confirmed by reactivity testing. This might explain the wider spread of renalase values in this subgroup (Table 3). In a subanalysis, we found a trend towards higher values for renalase in this group with CFR<2 (4239 (IQR 2293) versus patients with CFR>2 (median =3875 (IQR 2819) although the numbers were too few to reach statistical significance (Kruskal-Wallis p = 0.412).

Table 2 shows the relationship between renalase and CMD as independent when tested in a logistic regression model. This relationship remained significant after adjustment for smoking, family history of premature CAD, obesity and Framingham risk score (incorporating age, sex, history of hypertension, dyslipidemia, and diabetes). With CMD as the outcome variable, we showed each 1000 ng/ml increase in renalase increased the odds of CMD by 34% (adjusted OR: 1.34, 95% CI= 1.1, 1.7; p =0.02)

Relationship between CFR and inflammatory markers

We examined select inflammatory markers that have been implicated in the atherosclerotic pathway in relation to CMD. Table 3 summarizes the median values for TNF-alpha, IFN-gamma and metalloproteinases (MMP-2 and MMP-9) as similar between the three groups. Overall, CMD patients had higher median values of CRP, and VEGF than patients with normal flows and median values for VEGF, TNF-alpha and MMP-9 were higher in CAD vs CMD patients, suggesting an underlying inflammatory state. However, none reached statistical significance to help differentiate patients with CMD-related ischemia.

Renalase in the clinical assessment of CMD

Table 4 provides the discriminatory ability of renalase and inflammatory markers for the diagnosis of CMD. Only renalase showed a fair but significant discriminatory ability for CMD ischemia (p =0.01).

Figure 2 describes the added discriminatory ability of renalase above the conventional cardiovascular risk factors that are part of the Framingham risk score and history of typical angina. The C-statistic of the base model (containing the risk factors from Framingham risk score) in prediction of CMD was 0.60. This did not change with addition of tools typically used such as history of typical angina (C-statistic=0.60, 95% CI 0.47, 0.73) or CRP (C-statistic=0.61; 95% CI 0.48, 0.73). However, the addition of renalase improved the C-statistic to 0.70 to allow better detection of ischemia (95% CI, 0.59-0.82).

Discussion

To our knowledge, this is the first study to report the relationship between renalase, inflammatory markers and acute ischemia in CMD patients presenting to an ED, providing new insight into the pathophysiology and course of this disorder. Inflammation has long thought to be the cornerstone of the structural and/or functional obstruction of the microcirculation that underlies CMD.[25] This has been demonstrated even in asymptomatic patients with CMD.[26] Renalase is released in response to a catecholamine surge from an

ischemic insult. It also has been found to have an anti-inflammatory and anti-apoptotic role.[11, 27] Our findings support a physiological role of renalase in CMD patients in response to ischemia where we found renalase levels to be significantly elevated in CMD patients compared to those with normal flows. Interestingly in the acute phase, we did not find CMD to be associated with, a) proinflammatory cytokines (IL-2, IL-6 and TNF-a), b) markers of angiogenesis (VEGF), c) markers of remodeling (MMP-2 and MMP-9), or d) other inflammatory markers (CRP). Findings were surprising, as we anticipated elevated markers of inflammation with high renalase levels. Thus, we offer two potential explanations for the seemingly suppressed inflammation observed in CMD patients.

First, it is possible that CMD has no correlation with inflammation, as has been suggested by few others.[28] In fact, the link between CMD and inflammation has been previously disputed in patients undergoing elective angiography.[28–30] We found elevated CRP levels in CMD patients suggesting an underlying inflammatory state, but it did not help differentiate them from those with normal flows.[31] It is possible that our lack of differentiation was due to the fact that we studied primarily obese patients, when an elevated CRP has been independently associated with obesity.[32] However, we believe it is more likely that the discrepancy of inflammatory markers is due to the dynamic nature of the inflammatory response to ischemia[26], rather than a lack of association.

Our hypothesis is supported by the multi-fold high levels of renalase, an anti-inflammatory marker observed in CMD patients. In animal models, renalase is released in response to ischemia and impedes oxidative stress and attenuate cardiac remodeling by suppressing inflammation.[33, 34] This is thought to be mediated through activation of hypoxia-inducible factor-1alpha gene in response to reactive oxygen species released under hypoxic conditions. The cardioprotective effects of renalase is considered in part due to its ability to metabolize excess norepinephrine that is released in response to ischemic stress. Based on results of our pilot study, we propose that high renalase levels were released in patients presenting with ischemic chest pain from CMD to counter the inflammatory cascade. A recent position paper described the adaptive processes that were found to be crucial in regulating the physiology of the microcirculation.[35] This theory is supported by the upregulation of renalase that has been observed in response to ischemia and hypoxia in animal models. In renalase-knockout mice, cardiac atrophy is seen in response to ischemia while renalase supplementation has been shown to ameliorate cardiac fibrosis and hypertrophy, restoring diastolic function.[34, 36] Similar anti-apoptotic and anti-fibrotic effects have been observed in renal and cancer ischemic animal models.[37–39]

Whether the elevated renalase observed imparts any protective role in slowing the progression to obstructive CAD or whether it acts as an innocent bystander, is unclear from a cross-sectional study. Interestingly, even within the CMD group, some patients had normal renalase levels while others had several fold higher levels. It would be important to follow the course of these patients to see whether higher renalase levels is possibly linked with improved outcomes. It is also possible that the renalase elevation is a transient phenomenon and over time, as the atherosclerotic disease advances, we might see the renalase values decline. We hope to clarify these important questions in a follow-up study with serial measurements.

We noted low levels of renalase in the CAD/CALC patients, an unexpected finding as we anticipate CAD patients to also have endothelial dysfunction. This could be explained by the fact that these patients represented a hybrid group, those with known old infarcts, and those with coronary calcification. We hypothesize that renalase is released transiently as a physiological response to ischemia. While all our patients presented with chest pain, those with clear signs of ischemia (positive troponin or ischemic ECG changes) were treated as ACS and were selected out of the observation unit. We therefore primarily studied low-moderate risk patients without overt ischemia. It is possible that some of the CAD patients in this group had non-ischemic chest pain (suggested by normal CFR) while others had some degree of endothelial dysfunction (suggested by low CFR). Thus, it explains the wider range of renalase seen in this group, with higher levels seen in those with low CFR (<2).

While additional research is needed to confirm our hypotheses, their potential application in a clinical setting is intriguing. If renalase can be validated as a legitimate biomarker for CMD ischemia, it could be used as a screening tool for CMD in undifferentiated ED patients. Conventional methods used in clinical assessment of patients with typical angina include Framingham risk prediction scores, serial contemporary troponins, history of typical angina and CRP. Such tools guide clinicians to identify high risk individuals who exceed pre-specified risk thresholds and treat them with preventive medications. However, all of these assessments proved non-discriminatory for CMD in our study, suggesting that such thresholds may be insufficient in predicting CMD. This assumption is further supported by prior reports that similarly showed Framingham risk score to be inadequate in predicting adverse events associated with reduced CFR.[40]

In comparison, renalase demonstrated better discriminatory power for presence or absence of CMD ischemia. In the form of a peripheral blood test, renalase would be a feasible option for screening chest pain patients for CMD in emergency setting. Those who are ruled out for myocardial infarction using contemporary assays but screen positive for CMD may be referred for additional testing for CFR such as cardiac PET, Doppler echo or MRI. Renalase could have additive value to standard testing for ischemia. Prior studies have shown that strategies aimed at improving endothelial function could improve cardiovascular risk.[41–43] Early identification of such patients may provide a window of opportunity for implementing aggressive risk factor modification despite not meeting a high-risk threshold.

As with all studies, our findings should be viewed in the context of both strengths and limitations. First, our study is novel as we studied patients with acute symptoms, restricted the phenotype of CMD to those without evidence of CAD (obstructive or non-obstructive), those with normal renal function and without evidence of myocardial necrosis (normal contemporary troponins). Given this design, we were able to show that acutely symptomatic patients with ischemia from CMD had elevations in renalase compared to patients with normal flows. However, our findings should be interpreted with the limitations of a cross-sectional design, small sample size and a cohort that was obese. It is also possible that some patients with reduced CFR could have non-calcified plaques causing obstructive disease despite a calcium score of 0. However, we minimized labeling them as CMD by excluding patients with regional defects with or without calcification. Conversely, it is possible that some of the CAD/CALC patients also had concurrent CMD, which might explain the wider

IQR range of renalase in this group. Our results are hypothesis-generating and the temporal relationship between renalase, inflammation and CMD needs to be investigated in larger prospective cohorts in each subgroup with serial measurements.

Also, it is important to note that the variability of biomarker levels in our samples could lead to type II error for the inflammatory markers. However, cross-checks revealed acceptable precision with our assays and the fact that the biomarkers represented a range of mechanisms and origin reduce the chances of random error. Measurement of catecholamines would have helped further elucidate the relationship between chest pain, catecholamine surge and renalase, but this was not feasible with our sample collection methods. Peripheral blood sampling may not be specific for cardiac pathology. Future studies that analyze blood from coronary sinus during angiography might confirm our hypothesis. Many patients with CMD have chronic chest pain but in this study, we focused on acute chest pain as we believe renalase is released in response to acute ischemia. Serial measurements would allow us to study whether renalase is associated with recurrent chest pain. Also, although all our patients were ruled out for myocardial infarction, we did not use high sensitivity troponins that could have detected subclinical evidence of myocardial necrosis. Renalase could have additive value to a multimarker strategy with high sensitivity troponins in differentiating the various forms of ischemia in the ED. Finally, the majority of our patients were obese and the interpretation of these results should be limited to such patients.

In conclusion, we report an association between elevated renalase and symptomatic CMD in patients presenting with chest pain. As our quest for biomarkers continues, renalase holds promise as a potential peripheral blood biomarker for the detection of CMD and should be validated in larger studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

The publication was made possible by the Yale CTSA grant UL1TR000142 from the National Center for Advancing Translational Science (NCATS), NIH. We thank Fangyong Li for statistical support.

Disclosures:

This work was supported in part by Veterans Affairs Connecticut (G.V. Desir), National Institute of Health grants RC1DK086465, RC1DK086402, and R01DK081037, (G.V. Desir), and R01CA216846 (G.V. Desir), and H. Kluger.

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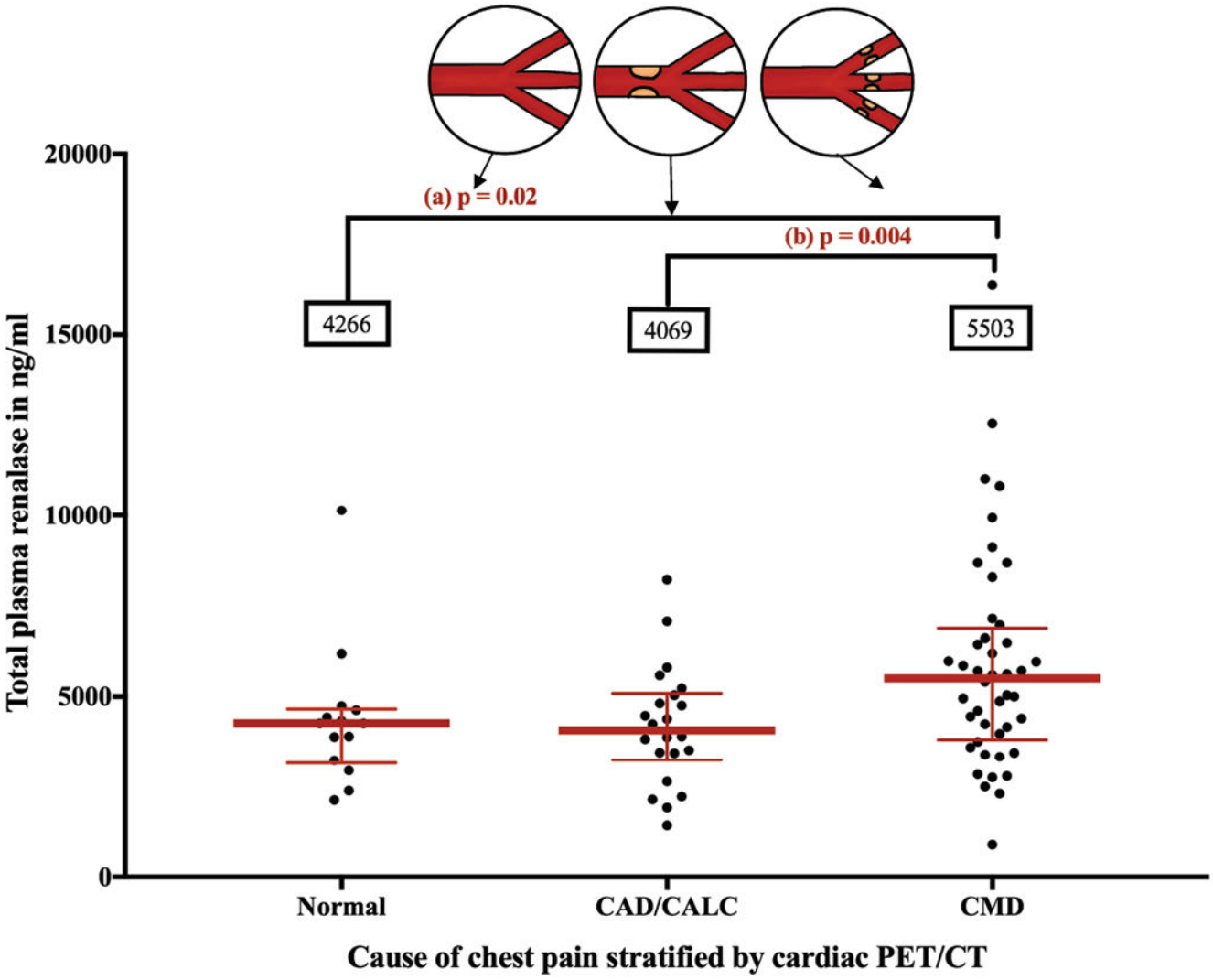

Figure 1: Quantification of total plasma renalase levels in ED chest pain patients

Median plasma renalase levels with IQR shown in red - (a) higher renalase levels seen in CMD patients compared with those with normal flows (b) higher renalase levels in CMD patients compared with those with CAD or coronary calcification

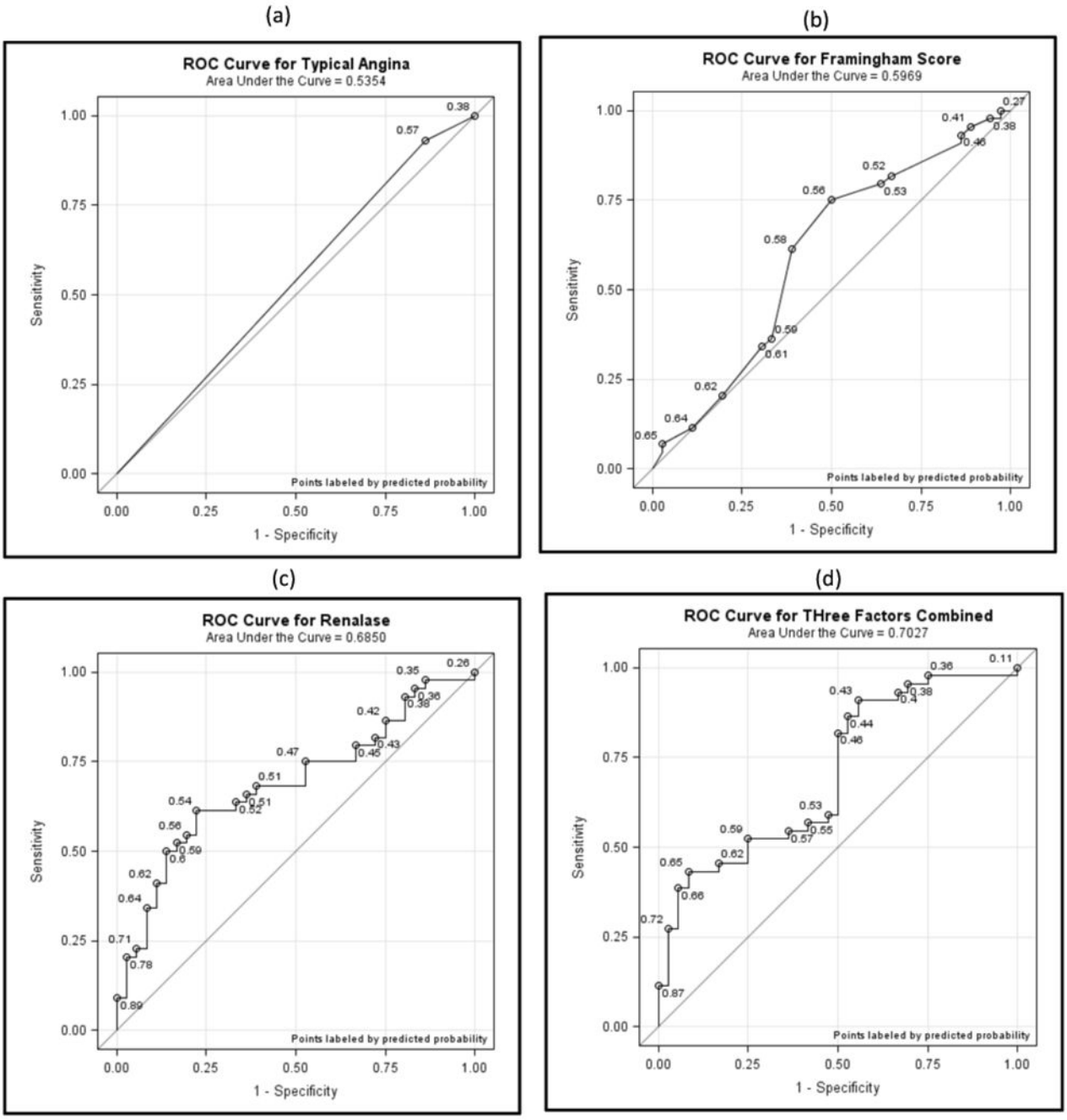

Figure 2:

ROC Curves for diagnosis of CMD using a)History of typical angina, b) Framingham risk score, c) Renalase Alone and d) All three factors combined. The direction of the curves should be interpreted with Table 3 that shows that **Framingham score and Typical angina are more predictive of CAD while Renalase is more predictive of CMD diagnosis.**

Table 1:

Baseline Sociodemographic and Clinical Profile

	Normal N=14 (18%)	CAD/Calcification N=22 (27%)	CMD N=44 (55%)
Mean age in years (SD)	50 (11.6)[f]	58 (9.9)[†]	52 (6.6)
Female Sex	8 (57%)	12 (55%)	34 (77%)
White Race	12 (86%)	18 (82%)	30 (68%)
Hispanic	0 (0%)	3(14%)	9 (21%)
Cardiac risk factors			
Hypertension	9 (64%)	18 (82%)	27 (61%)
Diabetes	5 (36%)	11 (50%)	13 (30%)
Dyslipidemia	6 (43%)	16(73%)[†]	16 (36%)
Current smoker	1(7%)	5 (23%)	4 (9%)
Family history of CAD	6 (43%)	8 (36%)	16 (36%)
Obese	5 (83%)	10 (91%)	27 (87%)
Mean BMI (SD)	35 (6)	38 (8)	40 (9)
Chest pain (%)			
Typical angina	1 (7%)	4 (18%)	3 (7%)
Atypical angina	10 (71%)	15 (68%)	28 (64%)
Non anginal	3 (20%)	3 (14%)	13 (29%)
Current medications			
Aspirin	7 (50%)	9 (41%)	12 (27%)
Beta-blockers	2 (14%)	13 (60%)[†]	10 (23%)
ACEI/ARB	7 (50%)	15 (68%)[†]	16 (36%)
Clopidogrel	0	4 (18%)[†]	0
Calcium Channel Blocker	1 (7%)	6 (27%)	12 (27%)
Statins	4 (29%)	16 (73%)[†]	10 (23%)
Mean resting HR (SD)	68 (13)	67 (11)	74 (12)
Mean resting SBP (SD)	122 (14)	137 (16)	128 (17)
Mean stress HR (SD)	94 (14)	96 (13)	104 (11)
Mean stress BP (SD)	126 (15)	138 (13)[†]	134 (23)
Mean labs (SD)			
Creatinine	0.8 (0.2)	0.8 (0.2)	0.8 (0.2)
Glomerular Filtration Rate	>60	>60	>60

	Normal N=14 (18%)	CAD/Calcification N=22 (27%)	CMD N=44 (55%)
Total Cholesterol	174 (52)	171 (33)	176 (41)
High density lipoprotein	46 (16)	43 (11)	47 (11)
Low density lipoprotein	102 (46)	90 (29)	98 (31)
Triglycerides	165 (148)	179 (90)	136 (93)
Glucose	147 (84)	121 (34)	115 (39)
Troponin-I	0.01	0.01	0.00
Mean CFR (SD)	2.52 (0.8)[f]	1.98 (0.8)[†]	1.61 (0.3)

BMI=Body mass index; SBP= Systolic blood pressure; HR=Heart rate; CFR= Coronary flow reserve; ACEI/ARB = Angiotensin converting enzyme inhibitor/ Angiotensin II Receptor Blocker

[f] *denotes p value < 0.05 when Normal compared with CMD group;*

[†] denotes p value < 0.05 when CAD/CALC compared with CMD group

Table 2:

Profile of inflammatory markers and renalase for patients with CMD

Median values (Q1, Q3)	Normal N=14	CAD/Calcification N=22	CMD N=44	P-value
CRP (mg/ml)	2.81 (1, 8)	3.50 (2, 11)	5.84 (2, 12)	0.30
TNF-alpha (pg/ml)	16.38 (5, 24)	20.84 (9, 28)	11.64 (7, 24)	0.40
VEGF (pg/ml)	119.47 (37, 258)	154.50 (55, 362)	129.50 (34, 267)	0.80
IL-1-Beta (pg/ml)	1.94 (0, 10)	0.17 (0, 2)	1.76 (1, 5)	0.12
IL-6 (pg/ml)	6.24 (0, 27)	3.68 (0, 25)	6.50 (0, 40)	0.58
IFN-gamma (pg/ml)	4.26 (0, 58)	1.19 (0, 29)	2.93 (1, 27)	0.49
MMP-9 (ng/ml)	11.30 (6, 23)	18.89 (5, 23)	10.84 (6, 20)	0.49
MMP-2 (ng/ml)	6.18 (5, 8)	7.36 (6, 9)	6.49 (6, 8)	0.24
Renalase (ng/ml)	4266 (3155, 4658)	4069 (3236, 5086)	5503 (3804, 6874)	0.02

Lowest values converted to 0 as follows: TNF-alpha < 5.45; VEGF < 12.45; IL-2 beta < 0.17; IL-6 <2.32; IFN-gamma < 0.7. MMP values of > 22605.37 in dilution were converted to 22610 for final values.