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Plasma miRNAs improve prediction of coronary atherosclerosis in patients with rheumatoid arthritis

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Abstract

Objective: MicroRNAs (miRNAs) regulate gene expression and are disease biomarkers. Rheumatoid arthritis (RA) patients have accelerated atherosclerosis leading to excess cardiovascular morbidity and mortality, but traditional risk factors for cardiovascular risk stratification are inadequate. In the general population miRNAs improve cardiovascular risk estimation beyond traditional risk factors. Our objective was to develop a miRNA panel that predicts coronary atherosclerosis in RA patients.

Methods: Plasma small RNA Next Generation Sequencing (NGS) was performed on 161 RA patients whose Agatston scores for coronary artery calcium were previously measured. Random forest analysis of plasma NGS miRNA expression was used to determine which miRNAs best differentiated between those patients with and without coronary artery calcium. Top predictive miRNAs were assayed by quantitative PCR (qPCR). Elastic net regression was used to develop the most parsimonious models with qPCR-measured miRNA concentrations and clinical variables (age, sex, ACC/AHA 10-year risk score, DAS28 score, and diabetes) separately to predict presence of coronary artery calcium and high coronary artery calcium. C-statistics were used to assess performance model performance.

Results: The top miRNAs which differentiated those with and without coronary atherosclerosis based on random forest analysis included let-7c-5p, miR-30e-5p, miR-30c-5p, miR-4446–3p, miR-126–5p, miR-3168, miR-425–5p, miR-126–3p, miR-30a-5p, and miR-125a-5p. For coronary artery calcium prediction, addition of all miRNAs except miR-126–3p to clinical factors improved the c-statistic modestly from 0.86 to 0.87. For high coronary artery calcium prediction, addition of all miRNAs except miR-30c-5p to clinical factors improved the c-statistic from 0.75 to 0.80.

Conclusion: A plasma miRNA panel improved prediction of high coronary artery calcium beyond traditional risk factors and RA disease activity. Further evaluation of the miRNA panel for prediction of coronary events in RA is necessary.

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Introduction

MicroRNAs (miRNAs) are short RNA sequences approximately 22 nucleotides in length. They fine-tune gene expression, typically by binding to the 3’ untranslated region of an mRNA at a complementary region located approximately at bases 2–8 from the 5’ end, termed the seed region. Such binding of a miRNA to its target mRNA can lead to cleavage of the mRNA or translational repression [1]. miRNAs are under specific transcriptional pressures with differing patterns of expression depending on cell type and stressors [1, 2]. Some miRNAs are selectively exported out of cells [3] and transported via carriers such as extracellular vesicles, lipoproteins, and protein complexes to other cells to modify their gene expression [4–6]. Plasma miRNAs are remarkably stable, even after freeze-thaw cycles [7], making them excellent potential markers of disease.

Currently, markers of cardiovascular disease are lacking for patients with rheumatoid arthritis (RA). Traditional risk factors do not adequately assess risk of coronary atherosclerosis or coronary events among patients with RA [8, 9]. Even inclusion of RA-specific factors such as disease activity and inflammatory markers do not provide estimates that predict cardiovascular risk adequately [9]. Plasma miRNAs have been helpful biomarkers in many diseases, including RA [10, 11] and cardiovascular disease [12, 13]. Currently, there are several approved and available miRNA-based diagnostic tests including miRNA panel tests for thyroid and pancreatic cancer, identifying tumor of unknown origin, osteoporosis, and also for platelet function assessment which may be helpful for cardiovascular disease prediction and monitoring [14]. However, there has been little work identifying miRNAs which may be predictors of cardiovascular disease in RA.

Most studies assessing the utility of circulating miRNAs as biomarkers of disease have used a candidate approach based on known miRNA functions. Indeed, we previously used such a candidate approach to try to identify miRNAs which might be associated with coronary atherosclerosis in RA but were unsuccessful [15]. Because the mechanism of accelerated atherosclerosis in RA is unclear, we postulated that an unbiased approach to select candidate miRNA biomarkers might be more helpful than a candidate approach. Moreover, a panel of multiple miRNAs may be more helpful than a single miRNA. Thus, the goal of the study was to use an unbiased approach to develop a model which incorporated plasma miRNAs and clinical factors to predict the presence of 1) coronary artery calcium and 2) high coronary artery calcium, markers of coronary artery atherosclerosis.

Methods

Study population

This study included 161 patients with RA from a prior cross-sectional study examining atherosclerosis in patients with RA [8] who had coronary artery calcium measurements and plasma small RNA sequencing performed. Patients were at least 18 years of age and met classification criteria for RA [16]. Recruitment and study procedures were described previously [8]. The study was approved by the Vanderbilt Institutional Review Board and all patients gave written informed consent.

Clinical assessments and general laboratory measures

We collected demographic and clinical information by patient interviews and medical record review and calculated RA disease activity (DAS28 score) based on 28 joints and erythrocyte sedimentation rate (ESR) [17]. High-sensitivity C-reactive protein (hsCRP) concentrations, ESR, and fasting total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein (LDL), and triglyceride concentrations were measured by the Vanderbilt University Medical Center Clinical laboratory. Larsen score was measured as previously described [18] to assess damage to the hands, wrists, and feet was performed on 94 of the RA patients for whom radiographs of hands and feet were available.

The 2013 American College of Cardiology/American Heart Association (ACC/AHA) 10-year risk score, which predicts 10-year risk of nonfatal myocardial infarction or coronary heart disease death or stroke, was calculated for each subject based on age, race, total cholesterol, HDL cholesterol, blood pressure, treatment for hypertension, diabetes, and smoking status.

Coronary artery calcium measurement

Coronary artery calcium was measured by electron beam computed tomography and quantified by Agatston score, as previously described [19,20]. Briefly, scans were performed with an Imatron C-150 (GE/Imatron, South San Francisco, CA) with a protocol using a 100-msec scanning time and a single-slice thickness of 3 mm. Calcification within a coronary artery with a minimal attenuation of 130 Hounsfield units (HU) were identified and calcified plaque was defined present if 3 or more contiguous pixels were detected. All scans were read by one blinded investigator (PR), who is an expert at coronary artery calcium scoring. High coronary artery calcium was defined as a score ≥300 Agatston units or ≥75th percentile for age, sex, and ethnicity [21].

Small RNA Sequencing and Bioinformatics

NGS of small RNAs was performed and miRNAs quantified as previously described [10]. In brief, total RNA was extracted from stored plasma (EDTA) using Norgen Biotek Total RNA Purification kits (Thorold, Ontario, CA). Complementary DNA libraries were constructed using TruSeq Small RNA Library Preparation Kits (San Diego, California, USA). Quality assessment and size selection was performed at the Vanderbilt Technologies for Advanced Genomics (VANTAGE) core facility. NGS was performed using Illumina NextSeq500 in the VANTAGE core facility. TIGER (“Tools for Integrative Genome analysis of Extracellular sRNAs”), an in-house small RNA sequencing analysis pipeline, was used to quantify miRNAs [10, 22, 23]. Random forest analysis was used on to analyze the sequencing results and select those miRNAs that best differentiated RA patients with and without coronary artery calcium. To select the top-performing miRNAs for validation by qPCR, miRNAs which were low in abundance on NGS (baseMean <30) were excluded.

Quantitative PCR

Top-performing miRNAs selected by random forest analysis from the NGS results were validated by qPCR (Figure 1). Total RNA was extracted from the plasma as described above. After the initial lysis step, a spike-in control cocktail was added as previously described [10] to normalize for variability in RNA extraction efficiency. qScript microRNA cDNA synthesis kits (Quantabio, Beverly, MA, USA) were used to make cDNA and individual miRNA qPCR primers and PerfeCTa SYBR green supermix (Quantabio, Beverly, MA, USA) were used for qPCR. The qPCR was performed in a 384-well format with samples measured in triplicate on a CFX384 Real-Time PCR detection system (Bio-Rad, Hercules, CA, USA). The miRNA concentrations were determined from standard dilution curves of a DNA mimic of known concentration and normalized to the spike-in cocktail. A threshold of Ct ≥ 40 for ≥ half of samples was used to exclude miRNAs from further analysis; however, the lowest abundance miRNA used in model development (miR-4446–3p) had raw Ct median = 35 [interquartile range 34, 38].

Model development

Penalized logistic regression via elastic net regularization was used to develop a parsimonious model to predict 1) the presence of coronary artery calcium and 2) high coronary artery calcium. Clinical variables including age, sex, ACC/AHA 10-year risk score, DAS28 score, and diabetes, and plasma qPCR concentrations of the top 10 candidate miRNAs were included in the full model for variable selection. Clinical variables were forced into the final model and variable selection was permitted only among the miRNAs to develop the smallest miRNA panel. The elastic net regularization parameters were optimized to minimize the cross-validation error. Internal validation was performed using the .632 bootstrap method with 500 replications. The c-statistics were reported to measure model performance. We then compared models with clinical variables and miRNAs to models containing clinical variables only.

Pathway analysis

We performed pathway analysis on panel miRNAs using the Ingenuity Pathway Analysis (IPA) program (version 49932394, Qiagen). Upstream regulators and downstream targets of the panel miRNAs were filtered to include only experimentally observed or highly predicted targets in humans. Figures built using IPA were edited for clarity.

General statistics

Categorical data are presented as number (percent) and continuous data as median [interquartile range]. Univariable analyses were used to measure the association between each of the miRNA plasma concentrations and cardiovascular risk factors using linear regression for continuous and logistic regression for binary data. Triglycerides and hsCRP were analyzed on a natural log scale and effect size back transformed to original units. Data were analyzed using R (version 3.6.2) and SPSS (version 26).

Results

Subject Characteristics

The 161 patients with RA had a median age of 54 years, 69% were female, median DAS28 disease activity was 3.78, and 71% were rheumatoid factor positive. Coronary artery calcium was present in 82 subjects (51%) and 62 (39%) had high coronary artery calcium. Overall study design and clinical characteristics for all patients are presented in Figure 1 and Table

1, respectively. Details for subgroups based on the presence of high coronary artery calcium are presented in Supplemental Table 1.

Initial selection of candidate miRNAs based on NGS and qPCR

Random forest analysis was performed using the relative abundance of 262 miRNAs based on NGS to predict the presence of coronary artery calcium. We excluded the miRNAs which were low in abundance from further analysis, as discussed in methods section (Figure 1), and the remaining top performing miRNAs were assayed by qPCR. These included, in order of priority based on random forest analysis, let-7c-5p, miR-320b, miR-30e-5p, miR-30c-5p, miR-4446–3p, miR-126–5p, miR-301a-3p, miR-99a-5p, miR-3168, miR-425–5p, miR-126–3p, miR-30a-5p, and miR-125a-5p. Among these, miR-320b, miR-301a-3p, and miR-99a-5p were too low in abundance on qPCR as discussed in methods section and were excluded (Figure 1).

Data reduction and model development for presence of coronary calcium

Using plasma concentrations of the ten miRNAs (by qPCR) and clinical variables, we performed elastic net regression to reduce the number of miRNA predictors for the presence of coronary artery calcium. This method selected let-7c-5p, miR-30e-5p, miR-30c-5p, miR-4446–3p, miR-126–5p, miR-3168, miR-425–5p, miR-30a-5p, and miR-125a-5p as predictors for the presence of coronary artery calcium. Thus, only miR-126–3p was removed due to lack of additional contribution to information in the model. Clinical predictors alone gave a c-statistic = 0.86 (95%CI: 0.80, 0.91) and the addition of the miRNA panel improved the prediction modestly to a c-statistic = 0.87 (95%CI: 0.82, 0.93) (Figure 2). Model characteristics are presented in Supplemental Table 2.

Data reduction and model development for presence of high-risk coronary calcium

We also used qPCR-based plasma concentrations of the ten miRNAs and clinical variables in elastic net regression to reduce the number of predictors for prediction of high coronary artery calcium. This method selected let-7c-5p, miR-30e-5p, miR-4446–3p, miR-126–5p, miR-3168, miR-425–5p, miR-126–3p, miR-30a-5p, and miR-125a-5p as predictors for high coronary artery calcium. Thus, only miR-30c-5p was removed due to lack of additional contribution to the model. Using the clinical predictors alone gave a c-statistic = 0.75 (95% CI 0.68, 0.83). The addition of the ten microRNAs improved the prediction for high coronary artery calcium to a c-statistic of 0.80 (95%CI 0.73, 0.86) (Figure 3, Supplemental Table 3).

Analysis among RA patients without diabetes

Among those with presence of coronary calcium or high coronary calcium, there were significantly more patients with type 2 diabetes mellitus. Although the analyses above adjusted for diabetes both by presence of diabetes as a separate clinical predictor and by the ACC/AHA score, which includes diabetes, we performed an additional analysis excluding those with diabetes from analysis. Among 143 RA subjects without diabetes, the clinical predictors alone had an AUC=0.85 (95%CI: 0.79, 0.91), and clinical predictors plus the miRNA panel had an AUC=0.87 (95%CI: 0.81, 0.93), only modestly improving for prediction of coronary artery calcium. For prediction of high coronary artery calcium, the clinical predictors alone had an AUC=0.74 (95%CI: 0.66, 0.82), and clinical predictors plus the miRNA panel had an AUC=0.80 (95%CI: 0.73, 0.88), which is similar to analyses with the entire cohort.

Exploratory analysis: association of individual miRNAs with cardiovascular risk factors

We performed an exploratory analysis of the relationship between the 10 individual miRNAs and cardiovascular risk factors. In univariate analyses, individual miRNAs were not significantly associated with the presence of coronary artery calcium or high coronary artery calcium (Supplemental Table 4), or traditional risk factors such as age, hypertension, or cholesterol concentrations (Supplemental Table 5). miR-126–3p was weakly associated with serum triglycerides. None of the miRNAs were associated with CRP concentrations. Several miRNAs including miR-125a-5p, miR-30e-5p, and miR-3168 were significantly positively associated serum creatinine concentration (Supplemental Table 5).

Pathway analysis of miRNA panel- upstream regulators

Some of the top canonical pathways involved in the upstream regulation of panel of miRNAs included: the long noncoding RNA “HOX antisense intergenic (HOTAIR) regulatory pathway”, “Eukaryotic initiation factor 2 (EIF2) Signaling”, “Senescence pathway”, “Glucocorticoid receptor signaling” and “Role of macrophages, fibroblasts and endothelial cells in rheumatoid arthritis” (Supplementary Table 6, and Supplementary Figure 1). The most significant functional categories of the upstream regulators of the atherosclerosis panel miRNAs were mainly related to expression of RNA and protein, but also included fibrosis and apoptosis (Supplemental Table 7).

Pathway analysis of miRNA panel- downstream effects

The top canonical pathways which the panel miRNAs are predicted to affect include “Colorectal cancer metastasis signaling”, Molecular mechanisms of cancer”, “Systemic lupus erythematosus in B cell signaling pathway” (Supplemental Table 8). The most significant functional categories of miRNA targets were related to invasion of cells and cell movement. However, atherosclerosis was also a significant functional category (Supplemental Table 9) and the majority of miRNAs with known functions (let-7c-5p, miR-30a-5p, miR-30c-5p, miR-30e-5p, miR-125a-5p, miR126a-3p, miR-126a-5p) have direct or indirect effects on genes related to atherosclerosis (Supplemental Figure 2).

Discussion

The major finding of this study is that a panel of plasma miRNAs including let-7c-5p, miR-30e-5p, miR-4446–3p, miR-126–5p, miR-3168, miR-425–5p, miR-126–3p, miR-30a-5p, and miR-125a-5p improved prediction of high coronary artery calcium among patients with RA.

The initial random forest analysis was designed to select miRNAs and clinical variables that predicted the presence of coronary calcium. This outcome leveraged the most statistical power because of the proportion of subjects in the groups (51% with coronary calcium and

49% without) was balanced. While improvement of the model with the addition of let-7c-5p, miR-30e-5p, miR-30c-5p, miR-4446–3p, miR-126–5p, miR-3168, miR-425–5p, miR-30a-5p, and miR-125a-5p was modest, the panel could still be an important biomarker. For example, in the Framingham Heart Study, the addition of CRP to the Framingham risk score increased the c-statistic from 0.863 to 0.865 for hard coronary heart disease outcomes, and increased the c-statistic from 0.795 to 0.799 for total cardiovascular disease [24].

Although the initial analysis to prioritize miRNAs for model development was based on the prediction of coronary calcium, there was only a small improvement in the model with the addition of miRNAs. However, there was a greater improvement in the model for prediction of high coronary calcium. One possibility for this difference in predictive capacity is that age is a major determinant of the presence versus absence of coronary calcium [25, 26]; low levels of coronary artery calcium are more strongly related to aging whereas high levels are more strongly related to accelerated atherosclerosis and cardiovascular events. Also, high coronary artery calcium was more difficult to predict using clinical measures alone, as evidenced by the baseline c-statistic for clinical predictors of 0.75, thus there was more opportunity for improvement. It is possible that the model is overfit, however, this is less likely because 1) initial selection of miRNAs was based on presence or absence of coronary artery calcium rather than high coronary artery calcium, 2) initial selection of miRNAs was based on NGS data, but the final model was developed using qPCR-based measurement, and 3) we used bootstrapping with cross validation to help reduce optimism of the models.

Individually the miRNAs that contributed to the model for high coronary artery calcium were not significantly independently associated high coronary artery calcium, yet as a panel they improved the AUC for prediction. A likely explanation for this finding is that a single miRNA species by itself often has modest effects on target expression [27], but the sum of the effect of multiple miRNAs can be amplified, given that miRNAs often act in regulatory networks, with one miRNA affecting multiple targets and multiple microRNAs affecting one gene [1].

Both miR-126–3p and miR-126–5p have established relevance for cardiovascular disease and were captured in the miRNA panel for high coronary calcium. miR-126 has been cited among the top nine most frequently reported miRNAs in atherosclerosis and hypertension [28], and biologically, miR-126–3p and miR-126–5p have critical roles in atherosclerosis. miR-126–3p downregulates vascular cell adhesion molecule 1 (VCAM-1) decreasing white blood cell adhesion to the endothelium [29], and decreases plaque formation. miR-126–5p, which is increased in endothelial cells after laminal shear stress, increases endothelial cell proliferation and limits atherosclerosis by downregulating Notch1 inhibitor delta-like 1 homolog (DLK1) [30, 31]. This parallels findings that miRNA alterations in peripheral blood monocyte subsets of patients with RA and cardiovascular disease may play a role in endothelial dysfunction [32].

Several members of the miRNA panel have been associated with vascular calcification. For example, decreases in miR-30c-5p cause increase in Runx2 leading increased vascular calcification in human coronary artery smooth muscle cells [33]. Additionally, miRNAs miR-30e and miR-125a are increased in atherosclerotic plaque compared to healthy vessel [34].

There are very few studies of miR-4446–3p in literature and we could find none in the context of atherosclerosis. However, most studies seeking cardiovascular biomarkers have used literature-based candidates for selection of miRNAs, so this miRNA may have been previously overlooked due to a paucity of information. Given lack of evidence associating miR-4446–3p with atherosclerosis or cardiovascular risk factors, the mechanism underlying its contribution to the predictive panels is uncertain. In cancer literature, miR-4446–3p is increased dramatically due to compressive forces in cancer-associated fibroblasts [35]. It would be interesting to know if miR-4446–3p is increased in the setting of arterial shear forces, because areas of shear stress are a major site for development of atherosclerosis [36]. Future work will be necessary to assess significance and function of miR-4446–3p.

We used a systems-based analysis to determine what potential functions the miRNAs as a group might have by assessing observed and predicted targets of the panel miRNAs. Most of the canonical pathways and functions identified were related to cancer. This might, in part, be because there is more information regarding miRNAs and cancer compared to other diseases but could also implicate mechanisms involving the immune system. Concordant with this possibility, pathways related to autoimmunity including SLE B cell and T cell exhaustion [37] were among the top targets, suggesting potential overlapping pathways in atherosclerosis and autoimmune disease. Although we found that atherosclerosis was a less significant pathway, it was interesting that even though we restricted our analysis to human data for known and highly predicted targets of the miRNAs, all but one of the miRNAs with known or highly predicted targets had a target related to atherosclerosis.

Similarly, we examined what could be driving expression of the panel miRNAs by assessing the top canonical pathways involved in their upstream regulation. The top two included the “HOTAIR regulatory pathway” and “EIF2 Signaling”. *HOTAIR* is a long non-coding RNA that increases in THP-1 macrophages treated with oxidized LDL and is instrumental in inducing oxidative stress and inflammation and apoptosis in the cells [38]. It is increased in monocytes and peripheral blood mononuclear cells from patients with coronary artery disease compared to control subjects in several small studies [39, 40]. The phosphorylation of eIF2 is induced by cellular stress, including lipid influx [41], and the eIF2 signaling pathway’s purpose is to globally block protein translation [38]. Thus, both of these identified pathways are involved in promoting or resolving cellular stresses important in the development of atherosclerosis and are potential targets for novel therapeutics [38, 41]. Lastly, components of an RA-specific pathway (“Role of macrophages, fibroblasts and endothelial cells in rheumatoid arthritis”) are upstream regulators of the miRNA panel, suggesting that RA-specific processes contribute to the composition of the miRNA atherosclerosis panel.

This study had some limitations. Basing initial selection of the miRNAs on NGS offered a broad and unbiased evaluation, but because NGS and qPCR do not have a 1:1 correlation and qPCR-based concentrations are used for the final model, it is possible that other miRNAs selected on qPCR-based concentrations could also predict coronary calcium. The study population was nearly 90% Caucasian, so it is unclear how this panel would perform in non-Caucasian populations. Although we preformed bootstrapping to improve the generalizability of the results, for development of potential biomarkers, replication in other cohorts will be necessary.

In conclusion, we determined that a panel of plasma miRNAs improves prediction of high coronary calcium in patients with RA. Replication of these findings in external cohorts and with hard cardiovascular outcomes will be helpful to fully evaluate the utility of the panel.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Point

- A plasma microRNA panel including let-7c-5p, miR-30a-5p, miR-30e-5p, miR-125a-5p, miR-126–3p, miR-126–5p, miR-425–5p, miR-3168 and miR-4446–3p improved prediction of high coronary artery calcium beyond clinical factors in patients with rheumatoid arthritis.

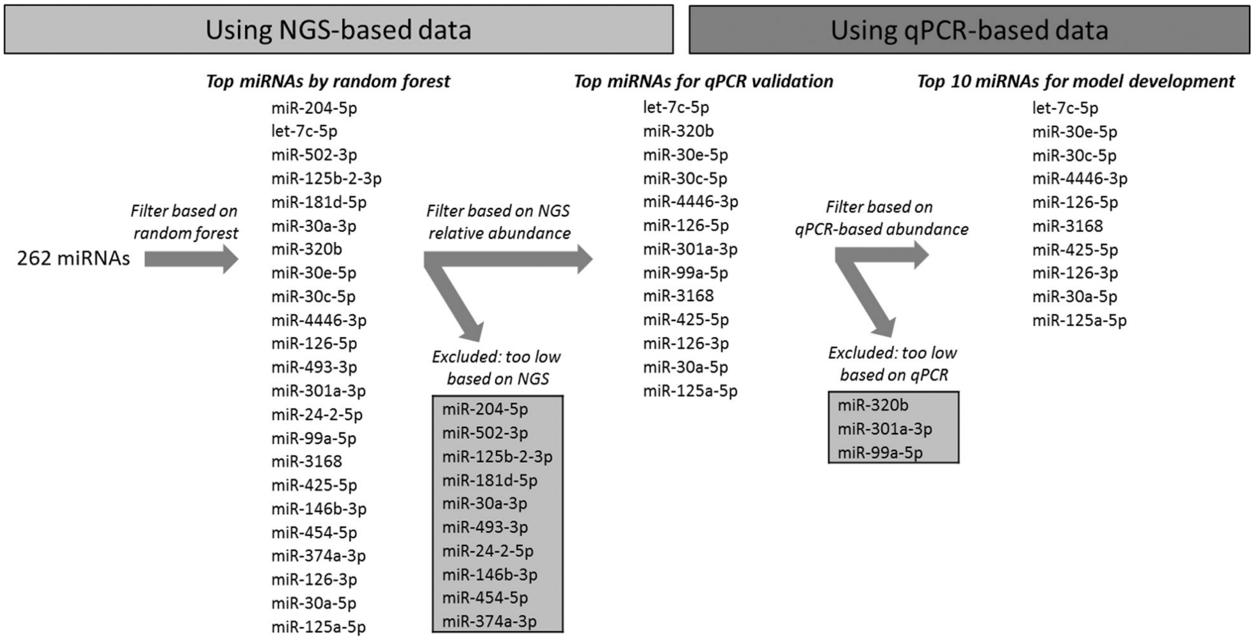

Figure 1.

Schematic of study design. The relative abundance of plasma miRNAs based on next generation sequencing (NGS) was assessed for ability to predict the presence of coronary atherosclerosis using random forest analysis and ranked based on performance. Those which were too low in abundance were excluded. Top performing miRNAs were assayed by qPCR and those which were too low in abundance were excluded until a total of ten miRNAs were available for further model development.

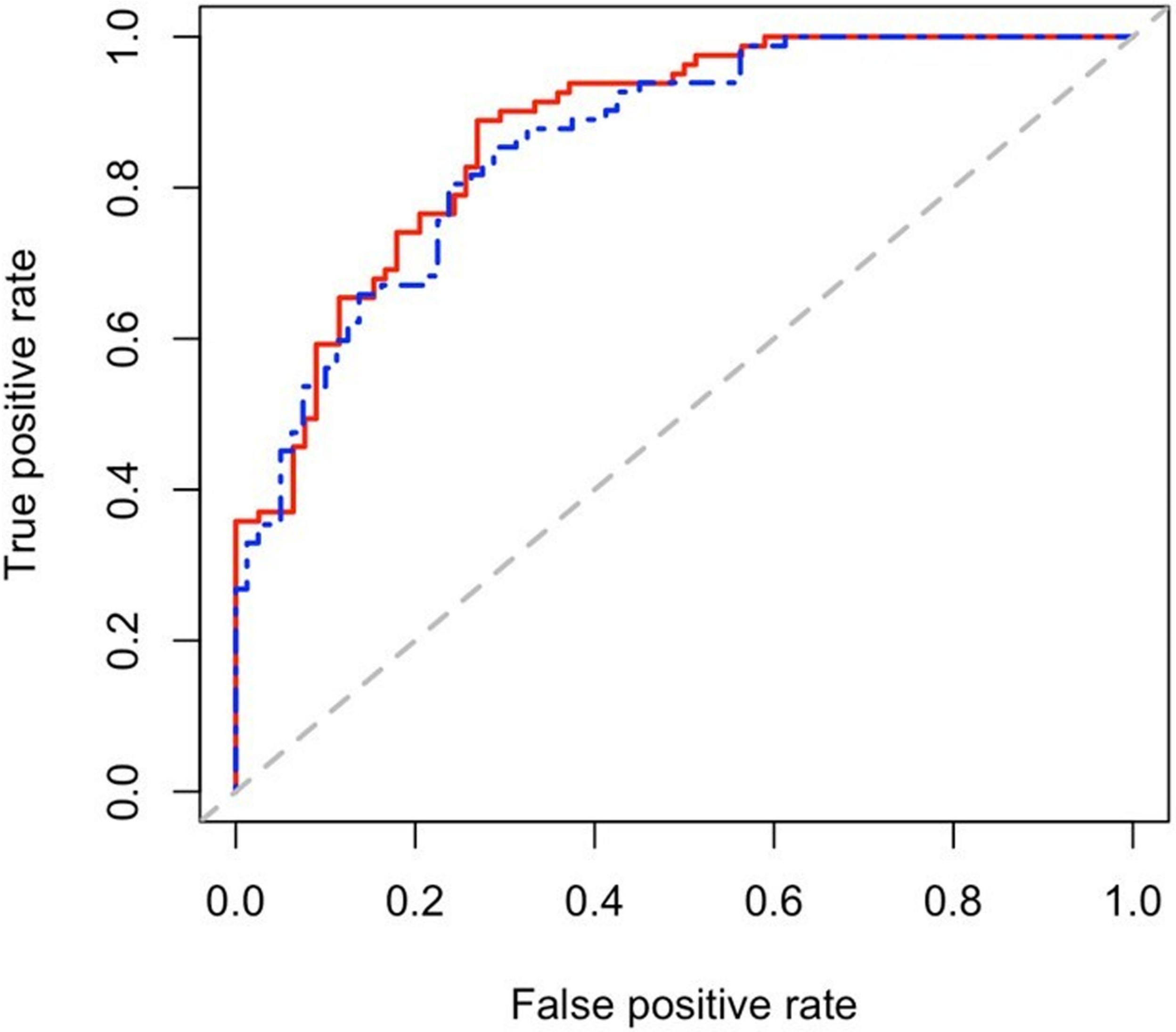

Figure 2.

Receiver operating characteristic curve for prediction of presence of coronary calcium among patients with rheumatoid arthritis. Blue line represents the curve for clinical predicators alone (c-statistic = 0.86 (95% CI: 0.80, 0.91)) and red line represents the curve for clinical predictors plus let-7c-5p, miR-30e-5p, miR-30c-5p, miR-4446–3p, miR-126–5p, miR-3168, miR-425–5p, miR-30a-5p, and miR-125a-5p (c-statistic = 0.87 (95% CI: 0.82, 0.93)).

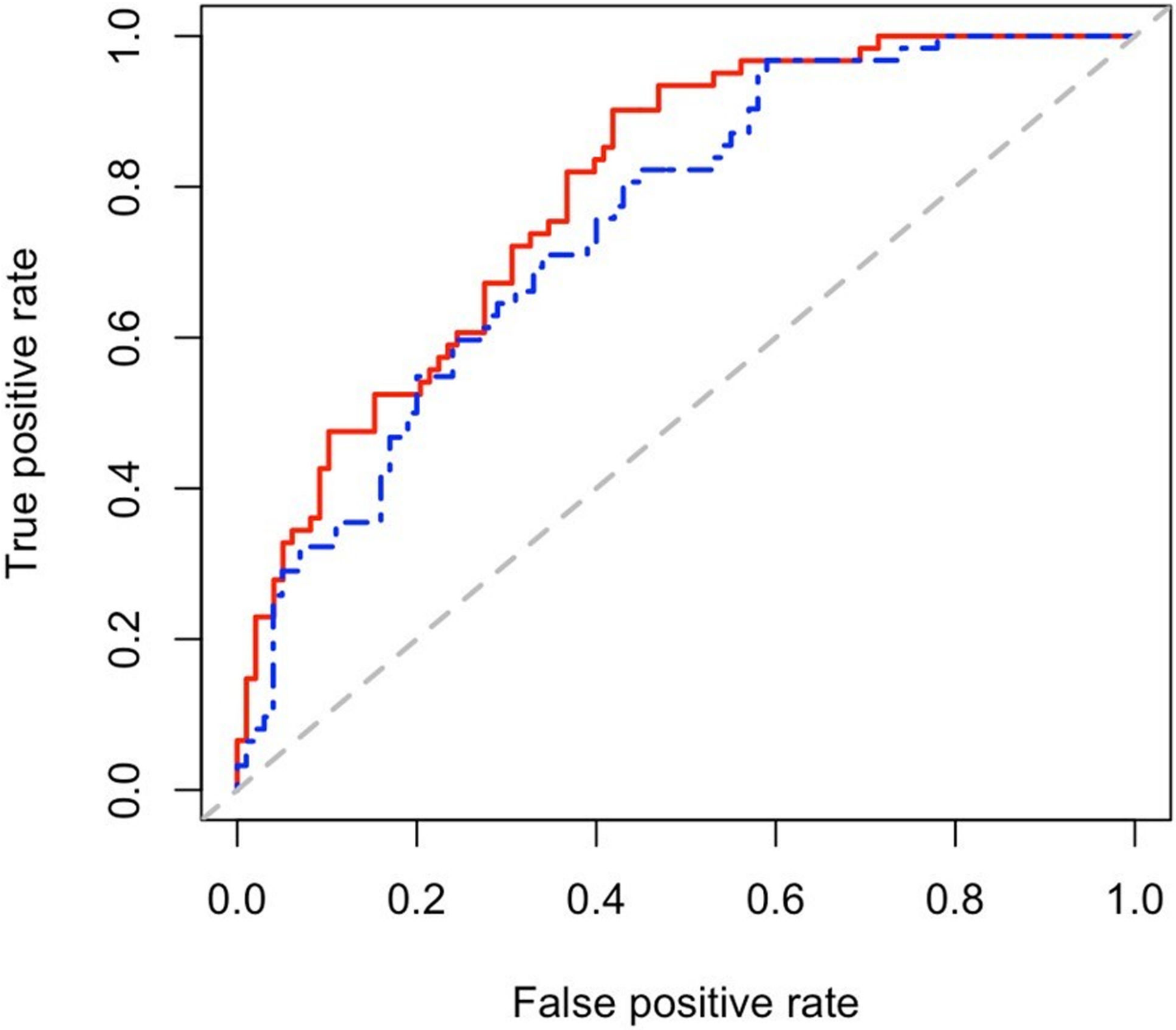

Figure 3.

Receiver operating characteristic curve for prediction of presence of high-risk coronary calcium among patients with rheumatoid arthritis. Blue line represents curve for clinical predicators alone (c-statistic = 0.75 (95% CI: 0.68, 0.83)) and red line represents curve for clinical predictors plus miRNA panel let-7c-5p, miR-30e-5p, miR-4446–3p, miR-126–5p, miR-3168, miR-425–5p, miR-126–3p, miR-30a-5p, and miR-125a-5p (c-statistic = 0.80 (95% CI: 0.73, 0.86)).

Table 1.

Clinical characteristics of patients with RA

	All RA (N=161)	Without coronary calcium (n=79)	With coronary calcium (n=82)	P value
Age, years	54 [45, 63]	48 [40, 54]	60 [54, 68]	<0.001
Sex, female	111 (69)	64 (81)	47 (57)	0.002
Race, Caucasian	143 (89)	68 (86)	75 (91)	0.40
DAS28, units	3.83 [2.62, 4.75]	3.46 [2.39, 4.40]	4.05 [2.84, 5.01]	0.04
RF, positive	110 (71)	52 (69)	58 (72)	0.80
hsCRP, mg/L	4 [1, 11]	3 [1, 10]	5 [2, 12]	0.09
Disease duration, years	4 [2, 18]	3 [2,12]	11 [2,20]	0.003
Larsen score, points	2 [0, 16]	0 [0, 13]	2 [0, 19]	0.38
Coronary artery disease, #	17 (11)	0 (0)	17 (21)	<0.001
Hypertension, presence	83 (52)	29 (37)	54 (66)	<0.001
Systolic BP, mmHg	134 [119, 146]	129 [116, 138]	137 [121, 151]	0.004
Diastolic BP, mmHg	75 [68, 82]	72 [67, 80]	76 [69, 85]	0.13
Waist-hip ratio, units	0.88 [0.81, 0.95]	0.84 [0.78, 0.92]	0.91 [0.83, 0.99]	<0.001
BMI, kg/m^2	28.3 [24.0, 33.0]	28.3 [24.0, 33.5]	28.3 [23.7, 31.5]	0.34
Total cholesterol, mg/dL	185 [155, 211]	183 [156, 204]	188 [153, 214]	0.70
HDL cholesterol, mg/dL	43 [37, 54]	44 [38, 55]	43 [36, 52]	0.31
LDL cholesterol, mg/dL	112 [89, 135]	105 [88, 134]	114 [90, 136]	0.44
Triglycerides, mg/dL	110 [80, 158]	108 [81, 149]	112 [80, 164]	0.58
DMII, presence	18 (11)	4 (5)	14 (17)	0.03
Anti-hypertensive, use	60 (37)	16 (20)	44 (54)	<0.001
Corticosteroid, use	87 (54)	42 (53)	45 (55)	0.95
Methotrexate, use	115 (71)	61 (77)	54 (66)	0.16
Anti-TNF, use	31 (19)	17 (22)	14 (17)	0.61

Data are presented as median [interquartile range] for continuous variables and number (percent) for categorical variables. Abbreviations: RA= rheumatoid arthritis, DAS28=disease activity score based on 28 joints, RF=rheumatoid factor, hsCRP=high sensitivity C-reactive protein, ESR=erythrocyte sedimentation rate, BP=blood pressure, BMI=body mass index, DMII= diabetes mellitus type 2, TNF=tumor necrosis factor alpha. Larsen score is available for 93 subjects (40 without coronary artery calcium and 53 with coronary artery calcium).