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ORIGINAL RESEARCH

Low miR-19b-1-5p Expression Is Related to Aspirin Resistance and Major Adverse Cardio- Cerebrovascular Events in Patients With Acute Coronary Syndrome

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BACKGROUND: Because of a nonresponse to aspirin (aspirin resistance), patients with acute coronary syndrome (ACS) are at increased risk of developing recurrent event. The in vitro platelet function tests have potential limitations, making them unsuitable for the detection of aspirin resistance. We investigated whether miR-19b-1-5p could be utilized as a biomarker for aspirin resistance and future major adverse cardio-cerebrovascular (MACCE) events in patients with ACS.

METHODS AND RESULTS: In this cohort study, patients with ACS were enrolled from multiple tertiary hospitals in Christchurch, Hong Kong, Sarawak, and Singapore between 2011 and 2015. MiR-19b-1-5p expression was measured from buffy coat of patients with ACS (n=945) by reverse transcription quantitative polymerase chain reaction. Platelet function was determined by Multiplate aggregometry testing. MACCE was collected over a mean follow-up time of 1.01±0.43 years. Low miR-19b-1-5p expression was found to be related to aspirin resistance as could be observed from sustained platelet aggregation in the presence of aspirin (-Log-miR-19b-1-5p, [unstandardized beta, 44.50; 95% CI, 2.20–86.80; P<0.05]), even after adjusting for age, sex, ethnicity, and prior history of stroke. Lower miR-19b-1-5p expression was independently associated with a higher risk of MACCE (-Log-miR-19b-1-5p, [hazard ratio, 1.85; 95% CI, 1.23–2.80; P<0.05]). Furthermore, a significant interaction was noted between the inverse miR-19b-1-5p expression and family history of premature coronary artery disease (P=0.01) on the risk of MACCE.

CONCLUSIONS: Lower miR-19b-1-5p expression was found to be associated with sustained platelet aggregation on aspirin, and a higher risk of MACCE in patients with ACS. Therefore, miR-19b-1-5p could be a suitable marker for aspirin resistance and might predict recurrence of MACCE in patients with ACS.

Key Words: acute coronary syndrome ■ aspirin resistance ■ biomarkers ■ coronary artery disease ■ microRNAs

Cardiovascular diseases (CVD) remain a leading cause of mortality worldwide.[1,2] Coronary artery disease (CAD) is one of the most common presentations of CVD. Despite the progress in interventional and therapeutic treatment options, patients with CAD remain at high risk for recurrent events.[3,4] Aspirin for many years has been the cornerstone of treatment in patients with CAD with regard to secondary prevention.[5] However, despite aspirin therapy 10% to 20% of these patients develop a re-event.[6] Although the occurrence of recurrent events is multifactorial, failure of aspirin to prevent platelet aggregation seems to be one of them and

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CLINICAL PERSPECTIVE

What Is New?

- Lower platelet miR-19b-1-5p expression is related to aspirin insensitivity among individuals with acute coronary syndrome.

What Are the Clinical Implications?

- Present data support further exploration of miR-19b-1-5p as a suitable marker for aspirin resistance in clinical practice.

Nonstandard Abbreviations and Acronyms

MACCE	major adverse cardio-cerebrovascular events
MiRNA	microRNA
UTR	untranslated region

is often indicated by "aspirin resistance."[7] The reported prevalence of aspirin resistance varies dramatically, from 0.4% to 35%, depending on the specific in vitro test used.[8] The in vitro platelet function tests have potential limitations, ranging from interlaboratory variability to poor reproducibility and limited accuracy, making them unsuitable for the detection of aspirin resistance.[7]

We recently reported on interindividual differences in platelet microRNA (miRNA) profiles after aspirin use among healthy individuals.[9] These data suggested that a sustained platelet aggregation (eg, aspirin resistance) was associated with the downregulation of miR-19b-1-5p.[9] Since these data seemed quite promising, we questioned whether this lower expression of miR-19b-1-5p would translate into a higher risk of recurrent CVD. Since research in small study populations always harbors the potential bias of chance findings, we aimed to replicate our findings as well as to investigate the risk of recurrent CVD, in a second much larger longitudinal cohort. We therefore hypothesize that low platelet miR-19b-1-5p expression is related to sustained platelet aggregation and predicts CVD in patients receiving aspirin treatment for secondary prevention.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

The study population comprised patients undergoing invasive management for acute myocardial infarction at tertiary hospitals in Christchurch, Hong Kong, Sarawak, and Singapore between 2011 and 2015. Acute myocardial infarction was diagnosed following the criteria from the third universal definition of myocardial infarction,[10] as ascertained by the managing physician. All patients undergoing invasive management for AMI were screened. The patients who met eligibility criteria were approached to ask for their consent for participation. After the patients signed the informed consent, electronic medical records were qurated and also patients were contacted to gather data on demographic, medical history, and blood results. All patients underwent coronary angiography within 7 days of symptom onset and within 3 days of hospitalization. All of the patients were on dual antiplatelet therapy before the first blood drawn. Blood samples were collected within 72 hours after acute myocardial infarction, centrifuged at 3500*g*, and buffy coats were stored at – 80°C until analysis. Exclusion criteria included low hemoglobin concentration (<8 g/L for men and <7 g/L for women), unwillingness to give consent, or absence of obstructive CAD, defined as any stenosis ≥ 70% or left main stenosis ≥ 50%.[11] All patients were contacted by phone at 24 months after the index hospitalization to ascertain the incidence of major adverse cardio-cerebrovascular events (MACCE). Composite end point MACCE comprised myocardial infarction, stroke, or death. The study was conducted according to the Helsinki Declaration and all institutions' human ethics review boards approved the study protocol.

Selection of Candidate miRNAs

In a previous study, we performed a miRNA microarray analysis of all available miRNAs in isolated platelets before and after aspirin to identify miRNAs related to aspirin resistance. Next, we validated the candidate miRNAs in a platelet aggregation study.[9] We found 6 candidate miRNAs, of which only 1 (miR-19b-1-5p) was associated with a sustained platelet aggregation. From these previous findings, we hypothesized that individuals with a low miR-19b-1-5p while on aspirin, might be aspirin resistant and therefore prone to recurrent MACCE. This miRNA could therefore be a promising marker for aspirin resistance in clinical practice.

Platelet Reactivity Tests

Whole blood for platelet reactivity tests was drawn within 72 hours of acute myocardial infarction in plastic tubes containing anticoagulant hirudin (Cat# 06675751001; Roche Diagnostics, Basel, Switzerland). Platelet reactivity was measured using whole blood impedance aggregometry on a multiple analyzer (Roche Diagnostics, Basel, Switzerland) as per manufacturer's instruction. Briefly, whole blood

was diluted with 0.9% NaCl and platelet aggregation was determined after stimulation with a final concentration of 0.5 mM arachidonic acid (#06675816190). The arachidonic-acid-induced platelet aggregation value on the Multiplate Analyzer (ASPI test) results were expressed as the area under the aggregation curve (aggregation units × minute).

RNA Isolation

RNA was extracted from 200 µL buffy coat using 600 µl TRIzol LS reagent (Invitrogen Corp., Carlsbad, CA) and incubated for 10 minutes at room temperature. Then the sample was spiked with 10 µL of Cel-miR-39 (work dilution) to be able to monitor and correct for efficiencies in RNA isolation. Next, 160 µL of chloroform was added to each sample and the mixture was centrifuged at 12 000 g for 15 minutes. The aqueous layer was transferred to a new tube and RNA was precipitated by 400 µL isopropanol, centrifuged at 12 000*g* for 10 minutes, and washed with 800 µL 75% ETOH. RNA pellet was collected in 30 µL RNAse-free water. Nucleic acid quantification could not be performed because of the low concentration of RNA in buffy coat. DNAse and RNAse treatments were omitted since previous experiments showed no difference of miRNA expression in buffy coat with or without these treatments (data not shown).

Complementary DNA Synthesis and Quantitative Polymerase Chain Reaction

Complementary DNA was synthesized using the qScript microRNA cDNA synthesis kit (Quanta Biosciences, Radnor, PA). First a poly (A) tail was added. Each reaction contained 3 µL of RNA, 0.5 µL nuclease-free water, 1 µL poly (A) polymerase, 2 µL poly (A) polymerase buffers, and 3.5 µL Cel-miR-54 spike-in in a concentration of 1.6×10^6 copies/µL. The reaction mixture was incubated for 60 minutes at 37°C followed by 5 minutes at 70°C. Next, cDNA was synthesized, in a reaction using 9 µL of the poly (A) tailed RNA and 1 µL of qScript Reverse transcriptase. The reaction mixture was incubated for 20 minutes at 42°C followed by 5 minutes at 85°C. cDNA was diluted 25×.

Reverse Transcription Quantitative Polymerase Chain Reaction Data Handling and Normalization

Circulating miRNA experiments are sensitive to false or inaccurate signals, which is largely explained by the often low concentrations of miRNAs in the circulation.[12] Therefore, a strict quality assessment pipeline was used to ensure the validity of each measurement and increase accuracy of the results. This pipeline is described elsewhere.[13] In brief, we distinguished 3 groups of measurements: "valid," "invalid," and "undetectable." In case of undetectable, the sample was set to a low value, which was based on the quantitative polymerase chain reaction experiment parameters. If the measurement did not pass the quality controls of the pipeline, it was marked as "invalid." Invalid measurements were taken into the analysis as missing at random and imputed using multiple imputations. If the measurement passed all the quality checks, it was marked as "valid" and the mean of the replicates was used in the analysis. Cel-miR-39 and Cel-miR-54 were used for the quality control. MiRNA expression was normalized to the geometric mean of an established miRNA normalization panel consisting of miR-130b-3p, miR-342-3p, and miR-148b-3p, as previously described in Kok et al.[14] Additionally, to correct for unavoidable interplate differences, we used a factor correction software program as recently described by Ruijter et al.[15]

Statistical Analysis

Data were analyzed using the statistical packages SPSS version 25.0 (SPSS Inc., Chicago, IL). Baseline characteristics are expressed as mean±SD for continuous variables and number (%) for dichotomous variables, except when indicated otherwise. ANOVA with post hoc Student *t* tests, Mann-Whitney *U* tests, and Fisher exact test were used to calculate differences in baseline characteristics as appropriate.

MiRNAs were expressed as normalized log-transformed starting concentrations (N0) as calculated by LinRegPCR and analyzed as such.[16] *P* values of quantitative polymerase chain reaction–based array were Benjamini-Hochberg corrected for multiple testing. Linear regression was used to define the association between the $\log_{10}$-transformed miRNA expression and platelet function multiplate data. Cox proportional hazard regression models were fitted to assess the association between log10-transformed miRNA levels and incident MACCE. The proportionality assumption was measured using the test for proportionality implemented in the survival-package in R. The *P* value of the proportionality test was found to be 0.923, so proportionality is not rejected. Cox proportional hazard regression models was designed as follows: Model I: Univariate; model II: adjusted for age and sex; model III: model II+adjusted for history of stroke/transient ischemic attack and Race or region of origin; Model IV: model III+interaction. A *P*-value<0.05 was considered statistically significant. The multivariate linear and Cox regression models were corrected for confounders that had both a relationship with platelet aggregation and MACCE. We therefore adjusted for age, sex, transient ischemic attack/stroke,

Race or region of origin, and their interaction. We also adjusted for single factors, such as body mass index, hemoglobin, creatinine, hypertension, diabetes mellitus, dyslipidemia, history of heart failure, family history of premature CAD, treatment, and their interaction, which did not influence the results.

RESULTS

Handling Missing Data: ("Valid," "Invalid," and "Undetectable" Data)

A validated algorithm was used to discover the missing data during the miRNA analysis.[17] In as few as 5 (0.5%) individuals, data on MACCE outcome were not available, so only 945 cases were included in the final analysis. In the remaining population of 945 individuals, miR-19b-1-5p was found to be "invalid" and "undetectable" in 46 (4.86%) and 41 (4.3%) individuals, respectively. Out of invalid and undetectable results, 8 (17.4%) and 7 (17.1%) had MACCE, respectively.

Description of the Study Population

The mean follow-up period was 1.01±0.43 years and the population had a mean age of 58.8±11.01 years and consisted of 804 (85.1%) male individuals. The baseline characteristics of the study population with highest ASPI tertile, meaning a sustained platelet aggregation on aspirin, are shown in (Table 1). In patients with acute coronary syndrome in the highest ASPI tertile, most sustained platelet aggregation, were more often White individuals (18.3% versus 5.1%; $P<0.05$), and had highest burden of previous stroke/transient ischemic attack (2.9% versus 2.2%; $P<0.05$) or family history of premature CAD (20.2% versus 12.3%; $P<0.05$) than lowest tertile patients. Additionally, patients with the most sustained platelet aggregation had the highest body mass index (26.8±5.9 versus 25.6±4.3 kg/m^2; $P<0.05$), but less often had dyslipidemia (152 [48.7%] versus 188 [59.5%]; $P<0.05$) compared with the most aspirin-sensitive, lowest tertile patients. As expected, patients in the highest ASPI tertile had the lowest miR-19b-1-5p expression level (0.60±1.8 versus 0.66±1.6,

Table 1. Baseline Characteristics According to Platelet Aggregation (ASPI; AUC/Min) by Tertile

	Lower (≤115)	Middle (116–204)	Upper (≥205)
Patients, n	316	317	312
Age, y, mean±SD	58.81±11.41	59.20±10.62	58.28±11.03
Male sex, n (%)	262 (82.9)	260 (82.0)	282 (90.4)
BMI, kg/m^2	25.55±4.34	26.39±4.64	26.79±5.89†
LDL, mg/dL	3.13±1.184	3.12±1.538	3.23±1.765
Hemoglobin, g/dL	14.12±1.78	14.20±1.74	14.40±1.76
Creatinine, mg/dL	108.94±118.7	110.16±145.8	95.68±53.5
Leukocyte count,	9.50±3.3	9.51±3.5†	10.2±3.2†‡
miR-19b-1-5p	0.66±1.62	0.63±2.09	0.60±1.77
Race or region of origin, n (%)			
Indian	43 (13.6)	25 (7.9)	20 (6.4)
White	16 (5.1)	46 (14.5)	57 (18.0)§
Chinese	195 (61.7)	162 (51.1)	136 (43.6)//
Malayan/others¶	62 (19.6)	84 (26.5)	99 (31.7)§//#
Comorbidities, n (%)			
Hypertension	197 (62.3)	208 (65.6)	188 (60.3)
Diabetes mellitus	109 (34.5)	117 (36.9)	93 (29.8)
Dyslipidemia	188 (59.5)	185 (58.4)	152 (48.7)*
Smokers	180 (57)	181 (57.1)	189 (60.6)
History of CAD	92 (29.1)	97 (30.6)	90 (28.8)
History of stroke/TIA	7 (2.2)	18 (5.7)	9 (2.9)*
History of peripheral arterial disease	1 (0.3)	6 (1.9)	6 (1.9)
History of heart failure	13 (4.1)	12 (3.8)	6 (1.9)
Family history of premature CAD	39 (12.3)	59 (18.6)	63 (20.2)*

Values are mean±SD or n (%).

ASPI indicates arachidonic acid–induced platelet aggregation value on the Multiplate Analyzer (ASPItest); BMI, body mass index; CAD, coronary artery disease; LDL, low-density lipoprotein; and TIA, transient ischemic attack. *$P<0.05$ for categorical variables; †$P<0.05$ compared with lower tertile; ‡$P<0.05$ compared with middle tertile; §$P<0.05$ compared with Indian; // Compared with White; #Compared with Chinese; ¶Others races: Thai, Filipino, Bangladesh, Bidayuh and Iban.

P>0.05) compared with the most-aspirin sensitive, lowest tertile patients (Table 1).

Association of miRNA With Platelet Aggregation

After adjustment, linear regression analysis revealed an inverse association between the level of miR-19b-1-5p expression and the degree of a sustained platelet aggregation (-Log-miR-19b-1-5p; B[95% CI]; 44.50 [2.20–86.80]; *P*<0.05) (Table 2).

Characteristics of Individuals With and Without MACCE

When analyzing the risk of MACCE in relationship to the miR19b-1-5p expression levels, we also analyzed the baseline characteristics according to MACCE. Over the study period, 111 (11.74%) individuals developed any MACCE. This comprised 72 (7.6%) individuals developing a myocardial infarction, 12 (1.3%) individuals developing stroke, and 27 (2.9%) individuals dying of CVD. Patients in the MACCE group were older (63.66±10.67 versus 58.2±10.8; *P*<0.05), less often male (85 [76.6%] versus 719 [86.2%]; *P*<0.05), more often Chinese (65 [58.6%] versus 428 [51.3%]; *P*<0.05) or White individuals (18 [16.2%] versus 101 [12.1%]; *P*<0.05) as compared with the non-MACCE group. Furthermore, they more often had hypertension (81 (73.0%) versus 512 (61.4%); *P*<0.05), diabetes mellitus (49 [44.1%] versus 270 [32.4%]; *P*<0.05), previous stroke/transient ischemic attack (9 [8.1%] versus 25 [3.0%]; *P*<0.05), and heart failure (9 [8.1%] versus 22 [2.6%]; *P*<0.05 (Table 3).

Risk of MACCE in Relationship With the miRNA

In the multivariable Cox regression analysis, after adjustment for the confounders as mentioned in the statistical section, miR-19b-1-5p was inversely associated with MACCE risk (-Log-miR-19b- 1-5p, (HR [95% CI]; 1.85 [1.23–2.80]; *P*<0.02) (Table 4). Furthermore, a significant interaction was noted between the inverse miR-19b-1-5p expression and family history of premature CAD (*P*=0.01) on the risk of MACCE (Table 4). In the subgroup analysis, low miR-19b-1-5p levels were found to be an independent predictor of second myocardial infarction and stroke even after adjusting for confounders (Figure 1).

Table 2. Association of the Inverse Log miR-19b-1-5p With Platelet Aggregation (AUC*Min)

Model	β (95% CI)
Model I	
-Log-miR-19b-1-5p	41.09 (–1.34 to 83.43)
Model II	
-Log-miR-19b-1-5p	41.11 (–0.72 to 82.94)
Model III	
-Log-miR-19b-1-5p	44.50 (2.20–86.80)*

Model I Univariate; model II: adjusted for age and sex; model III: model II+adjusted for history of stroke/TIA and ethnicity; *P<0.05. TIA indicates transient ischemic attack.

Table 3. Baseline Characteristics According to MACCE

	No MACCE	MACCE
Patients, n	834	111
Age, y, mean±SD	58.2±10.8	63.7±10.6*
Male sex, n (%)	719 (86.2)	85 (76.6)*
BMI, kg/m^2	26.2±4.8	26.3±5.3
LDL, mg/dL	3.37±1.4	2.75±1.7
Hemoglobin, g/dL	14.34±1.7	13.49±1.9*
Creatinine, mg/dL	98.88±95.4	151.58±195.8*
Leukocyte count	9.7±3.2	10.1±3.9
miR-19b-1-5p	0.64±2.9	0.54±1.7
Race or region of origin, n (%)		
Indian	84 (10.1)	4 (3.6)
White	101 (12.1)	18 (16.2)†
Chinese	428 (51.3)	65 (58.6)†
Malayan/others¶	221 (26.5)	24 (21.6)
Comorbidities n (%)		
Hypertension	512 (61.4)	81 (73.0)*
Diabetes mellitus	270 (32.4)	49 (44.1)*
Dyslipidemia	463 (55.5)	62 (55.9)
Smokers	491 (58.9)	59 (53.2)
History of MI	200 (24.0)	34 (30.6)
History of stroke/TIA	25 (3.0)	9 (8.1)*
History of peripheral arterial disease	12 (1.4)	1 (0.9)
History of PCI	166 (19.9)	21 (18.9)
History of CABG	43 (5.2)	9 (8.1)
History of heart failure	22 (2.6)	9 (8.1)*
History of atrial fibrillation/flutter	27 (3.2)	4 (3.6)
Family history of premature CAD	141 (16.9)	20 (18.0)

Values are mean±SD or n (%).

BMI indicates body mass index; CABG, coronary artery bypass graft; CAD, coronary artery disease; LDL, low-density lipoprotein; MACCE, major adverse cardio-cerebrovascular event; MI, myocardial infarction; PCI, percutaneous coronary intervention; and TIA, transient ischemic attack. *P<0.05; †P<0.05 in comparison to Indian; ¶Others races: Thai, Filipino, Bangladesh, Bidayuh and Iban.

DISCUSSION

This study shows that low miR-19b-1-5p expression is associated not only with sustained platelet aggregation while on aspirin therapy, but also with the incidence of MACCE among patients with acute coronary syndrome. With this study, we were not only able to confirm our previous findings that low miR-19b-1-5p

Table 4. Cox Regression for the Inverse Log miR-19b-1-5p and Risk of MACCE

Model	HR (95% CI)
Model I	
-Log-miR-19b-1-5p	1.86 (1.20–2.86)*
Model II	
-Log-miR-19b-1-5p	1.83 (1.20–2.78)*
Model III	
-Log-miR-19b-1-5p	1.85 (1.23–2.80)*
Model IV	
-Log-miR-19b-1-5p-Log-miR-19b-1-5p *Family history of premature CAD	1.63 (0.99–2.67) 6.69 (1.54–28.96) (*P*=0.01)

Model I Univariate; model II: adjusted for age and sex; model III: model II+adjusted for history of stroke/TIA and ethnicity; Model IV: model III+interaction; *$P<0.02$. CAD indicates coronary artery disease; HR, hazard ratio; MACCE, major adverse cardio-cerebrovascular event; and TIA, transient ischemic attack.

expression levels are related to aspirin resistance,[9] but we were also able to show that this predicted recurrent events. What mechanisms are related to the miR-19b-1-5p-related aspirin resistance were not the subject of this research, since we were interested in whether they could predict recurrent MACCE as a clinical marker.

One of the biggest challenges in miRNA research is to be able to confirm previous observations. The lack of reproducibility, which comes from a small sample error, is a common pitfall in miRNA research and therefore the value of being able to reproduce miRNA findings in an independent larger cohort often is not appreciated. In the present study we successfully validated our previous findings. This means that our previous observation of low miR-19b-1-5p expression levels related to a sustained in vitro platelet aggregation after aspirin therapy[9] was not by chance, but could infer a real association. Second, this would tell us that if this observation is real, this would mean that individuals with low miR-19b-1-5p expression levels on aspirin therapy were still at risk for recurrent events, which was indeed the case.

MiR-19b-1-5p is one of the microRNAs from the miR-17-92 cluster, which has been shown to regulate the development of the cardiovascular system and cellular proliferation,[18] and particularly miR-19 plays a critical role in CVD pathogenesis, in which it has a protective role.[19-23] Furthermore, miR-19b has been reported to have antithrombotic properties and evidence suggests that miR-19b expression inhibits endothelial tissue factor expression and its procoagulant activity, leading to thrombosis.[24,25] In addition miR-19a, the counterpart of miR-19b, plays an antithrombotic role by regulating the coagulation cascade pathway gene for tissue factor pathway inhibitor SERPINE1 and coagulation factor 3.[26] Thus, downregulation of miR-19 could increase the risk of clot formation, leading to cardiovascular events.

Mayer et al reported that a lower expression of circulating miR-19a was an independent predictor of future cardiovascular events[27] in stable patients with vascular disease (stable CAD or ischemic stroke). In addition, others also showed that individuals with low miR-19a had a higher risk of ischemic stroke.[26,28] On the other hand, other studies showed conflicting results. They could either not validate[29] the observation of low miR-19a levels with acute ischemic stroke or failed to show any differences[30] of low miR-19b levels with acute ischemic stroke, or showed an upregulation of either miR-19a or miR-19b to be related to CVD.[31] Unfortunately, most of these studies used small sample sizes, and are therefore prone to small-sample error bias.[26,29,32] Also, they do not refer to miR-19a and miR-19b as being a marker of aspirin

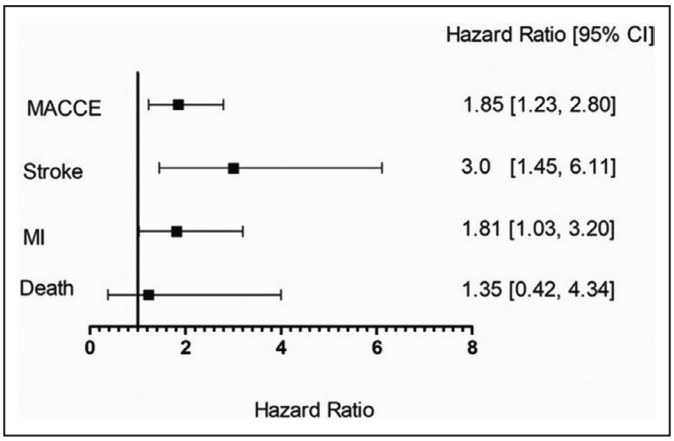

Figure. Forest plot showing multivariate Cox regression analysis of the effect of miR-19b-1-5p expression on MACCE and individual events.
MACCE indicates major adverse cardio-cerebrovascular events; and MI, myocardial infarction.

resistance[26-28,31] and do not show a relationship with recurrent cardiovascular events, which is a crucial finding in terms of secondary prevention of cardiovascular events in these patients.

On the other hand, others have found similar results on miR-19b and aspirin resistance, showing that expression of miR-19b was related to platelet reactivity.[33] However, we are the first to combine these data, showing not only that low miR-19b levels are related to aspirin resistance, but also to recurrent cardiovascular events.

Our study was designed to show the usefulness of miR-19b-1-5p as a marker in a clinical setting. We propose that it reflects aspirin resistance, since aspirin-related impaired platelet aggregation is suggested to be related to aspirin-induced modulation of the NO-cGMP signaling pathway, incorporating *GUCY1A3*, *NOS3*, and *PDE5* genes.[34-39] Therefore, if miR-19b-1-5p would be involved mechanistically in this pathway, there should be a putative binding site for miR-19b-1- 5p in the 3′ untranslated region (3′UTR) of the mRNA encoding *GUCY1A3, NOS3*, and *PDE5* genes. When consulting miRDB (http://mirdb.org/), TargetScan (http://www.targetscan.org/vert_72/), and miRDIP (http://ophid.utoronto.ca/mirDIP/index.jsp#r), it was reported that the 3′UTR of the mRNA of *GUCY1A3* had 5 putative binding sites for miR-19b-1-5p, 3′UTR mRNA of *PDE5* had 1 high-affinity binding site, and that the 3′UTR mRNA of *NOS3* also had 1 binding site. Therefore, it is reasonable to expect that miR-19b-1-5p binds to the 3′UTR of *GUCY1A3* and to a lesser extent, *NOS3* and the *PDE5* genes, which in turn regulate the NO-cGMP signaling pathway and could modulate the aspirin sensitivity. Kessler et al indeed showed that a genetic variant in *GUCY1A3* impaired platelet aggregation and inhibited the production of cGMP after exposure to an NO donor.[35] One of the possibilities of a decrease in miR-19b-1-5p after aspirin therapy could be the shedding of miRNAs into the circulation, with ongoing platelet aggregation, which has been reported to be the case in a study investigating patients with myocardial infarction as compared with healthy individuals.[40]

Our study has several strengths and limitations. The strength of this study lies in the fact that we were able to replicate the result of our previous study in a larger independent cohort. Furthermore, a robust technical approach, in the form of triplicate measurement, interplate variance correction, and utilization of 3 endogenous and 2 technical normalizers was used, which makes the data quite robust. In addition, a data-handling pipeline, which has been reported to increase both precision and accuracy of miRNA measurement, was used for further quality check and handling missing data.[17] A limitation of this study was that the patients were receiving dual antiplatelet therapy, which could have influenced platelet aggregation and/or miR-19b-1-5p expression. Furthermore, antiplatelet treatment compliance data were not available for the study cohort. Noncompliance with the medications can have an impact on the MACCE outcome and can bias its association with the miRNA expression data. On the other hand, we have no reason to believe that the compliance would have been any different from any other study, in which compliance rates of around 80% to 90% are reported.[41-43]

CONCLUSIONS

In conclusion, a low platelet miR-19b-1-5p expression level was associated with sustained platelet aggregation and the risk of future MACCE, suggesting aspirin resistance.

ARTICLE INFORMATION

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Disclosures

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