265 **PLASMA MICRORNAS AS BIOMARKERS FOR PLATELET INHIBITION**

P Willeit,[1] A Zampetaki,[2] D Kaudewitz,[2] R Lee,[3] K Dudek,[2] A King,[4] N S Kirkby,[5] H S Markus,[4] T D Warner,[6] S Kiechl,[7] A C Morton,[8] K Channon,[3] R F Storey,[8] M Mayr[2]
[1]University of Cambridge; [2]King's College London; [3]University of Oxford; [4]St George's University of London; [5]Imperial College; [6]University of London; [7]Innsbruck Medical University; [8]University of Sheffield

doi:10.1136/heartjnl-2013-304019.265

Introduction MicroRNAs (miRNAs) are small non-coding RNAs that are also detectable in the blood. Circulating levels of miRNAs have been measured in various epidemiological studies, but the effects of medication are unclear. To address these uncertainties, we aimed to **(1)** compare the levels of miRNAs in different sample types, **(2)** assess how miRNAs change in response to platelet inhibition and **(3)** correlate their plasma levels with existing markers of platelet activation.

Methods MiRNAs were assessed by real-time polymerase chain reaction in the following cohorts:

Result **(1)** Profiling for 377 miRNAs was performed in platelets, platelet microparticles, platelet-rich plasma, platelet-poor plasma, and serum. Platelet-rich plasma showed markedly higher levels of

Study population	Mean age, y	Male, %
9 volunteers participating in a dose-escalation study	<40	100%
33 patients with symptomatic carotid stenosis	72.1 ± 1.9	82%
129 patients post myocardial infarction	59.7 ± 10.4	81%

miRNAs than serum and platelet-poor plasma. Few abundant platelet miRNAs, such as miR-24, miR-197, miR-191, and miR-223, were also increased in serum compared with platelet-poor plasma.

(2) Antiplatelet therapy significantly reduced miRNA levels. Using custom-made quantitative real-time polymerase chain reaction plates, 92 miRNAs were assessed in a dose-escalation study in healthy volunteers at 4 different time points: at baseline without therapy, at 1 week with 10 mg prasugrel, at 2 weeks with 10 mg prasugrel plus 75 mg aspirin, and at 3 weeks with 10 mg prasugrel plus 300 mg aspirin. Findings in healthy volunteers were confirmed by individual TaqMan quantitative real-time polymerase chain reaction assays. Validation was performed in an independent cohort of patients with symptomatic atherosclerosis, who received low-dose aspirin at baseline. Plasma levels of platelet miRNAs, such as miR-223, miR-191, and others, that is, miR-126 and miR-150, decreased on further platelet inhibition.

(3) In a cohort of patients on dual anti-platelet therapy 30 days post myocardial infarction, plasma levels of most platelet miRNAs were positively correlated to the vasodilator-stimulated phosphoprotein phosphorylation (VASP) assay. Levels of miR-126 were also significantly associated with results from the VerifyNow $P2Y_{12}$aggregation assay. No association was observed with optical aggregometry either with arachidonic acid or with ADP.

Conclusions Our study provides evidence for a correlation of plasma miRNAs with commonly used methods in platelet testing. It highlights that miRNA levels are sensitive to medication, in particular antiplatelet therapy, and preparation of blood samples. This has to be taken into account when designing a study to investigate the relation of circulating miRNAs with cardiovascular disease.