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SPECIAL REVIEW SERIES

Circulating microRNAs as biomarkers and mediators of platelet activation

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Abstract

Platelets are essential mediators of physiological hemostasis and pathological thrombosis. Currently available tests and markers of platelet activation did not prove successful in guiding treatment decisions for patients with cardiovascular disease, justifying further research into novel markers of platelet reactivity. Platelets contain a variety of microRNAs (miRNAs) and are a major contributor to the extracellular circulating miRNA pool. Levels of platelet-derived miRNAs in the circulation have been associated with different measures of platelet activation as well as antiplatelet therapy and have therefore been implied as potential new markers of platelet reactivity. In contrast to the *ex vivo* assessment of platelet reactivity by current platelet function tests, miRNA measurements may enable assessment of platelet reactivity *in vivo*. It remains to be seen however, whether miRNAs may aid clinical diagnostics. Major limitations in the platelet miRNA research field remain the susceptibility to preanalytical variation, non-standardized sample preparation and data normalization that hampers inter-study comparisons. In this review, we provide an overview of the literature on circulating miRNAs as biomarkers of platelet activation, highlighting the underlying biology, the application in patients with cardiovascular disease and antiplatelet therapy and elaborating on technical limitations regarding their quantification in the circulation.

Keywords

Biomarker, cardiovascular disease, microRNA, noncoding RNA, platelets, thrombosis

History

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Introduction

Over a century ago, platelets were identified as the main mediators of physiologic hemostasis and pathological thrombosis [1]. Platelets are generated through membrane budding from megakaryocytes in the bone marrow and lungs [2,3] and hence enter the blood stream without a nucleus. Despite the absence of relevant amounts of genomic DNA, platelets inherit a vast amount of RNAs from their megakaryocyte precursors, including coding messenger RNAs (mRNAs) as well as noncoding microRNAs (miRNAs), long noncoding RNAs (lncRNAs), circular RNAs (circRNAs) and YRNAs. Among noncoding RNAs, most studies have investigated miRNAs in platelets. Given that platelets are enriched with miRNAs compared to mRNAs and that platelets are the second most abundant blood cell type, we and others demonstrated that platelets are the major source of miRNAs in plasma and serum [4–5]. Interestingly, both intraplatelet levels of certain miRNAs [9] and levels of platelet-derived miRNAs in the cell-free circulation [7] were found to correlate with platelet reactivity. Thus, miRNAs have been investigated as novel biomarkers of platelet reactivity, i.e. for monitoring the efficacy of antiplatelet therapy monitoring and assessing the risk of atherothrombosis, as highlighted in our previous reviews [6,10]. This review will provide an overview of miRNAs as biomarkers of platelet reactivity and highlight novel mechanisms that may have implications for the function of circulating miRNAs.

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MicroRNA biology

MiRNAs are evolutionary highly conserved small RNAs (~ 22 nucleotides), whose main function is to repress the synthesis of specific target proteins. This effect is typically mediated in complex with an Argonaute (Ago) protein, which guides one of the two complementary miRNA strands to the target mRNA. Through sequence complementarity of the miRNA seed region (~ 8 nucleotides) to the 3'-untranslated region of the target mRNA, protein synthesis is repressed. Until recently, this canonical RNA interference (RNAi; Figure 1) has been assumed to be the only regulatory mechanism of miRNAs. First evidence of regulatory functions that extend beyond RNAi came from studies implying ligand-like roles of miRNAs [11–15] (Figure 1): i) extracellular miRNA let-7 was shown to activate Toll-like receptors (TLRs) on microglia, macrophages and neurons [12,13]; ii) miR-21 and miR-129a secreted by tumor cells were shown to activate TLRs of immune cells [14]; and iii) extracellular miR-711 was shown to activate the transient receptor potential cation channel subfamily A member 1 (TRPA1) on neurons [15]. The fact that intracellular miRNA levels are around 13 times higher than Ago protein levels [16] and that only a fraction of miRNAs are bound (intra- or extracellularly) to Ago [16,17] provide support to this concept of a noncanonical miRNA pathway. However, concerns about artifacts remain due to the high miRNA concentrations used in these early studies. The physiological relevance of noncanonical miRNA roles has gained traction through a recent study that used sub-pmol/L miRNA concentrations (close to endogenous intracellular miRNA levels) showing that cardiomyocyte miR-1 physically

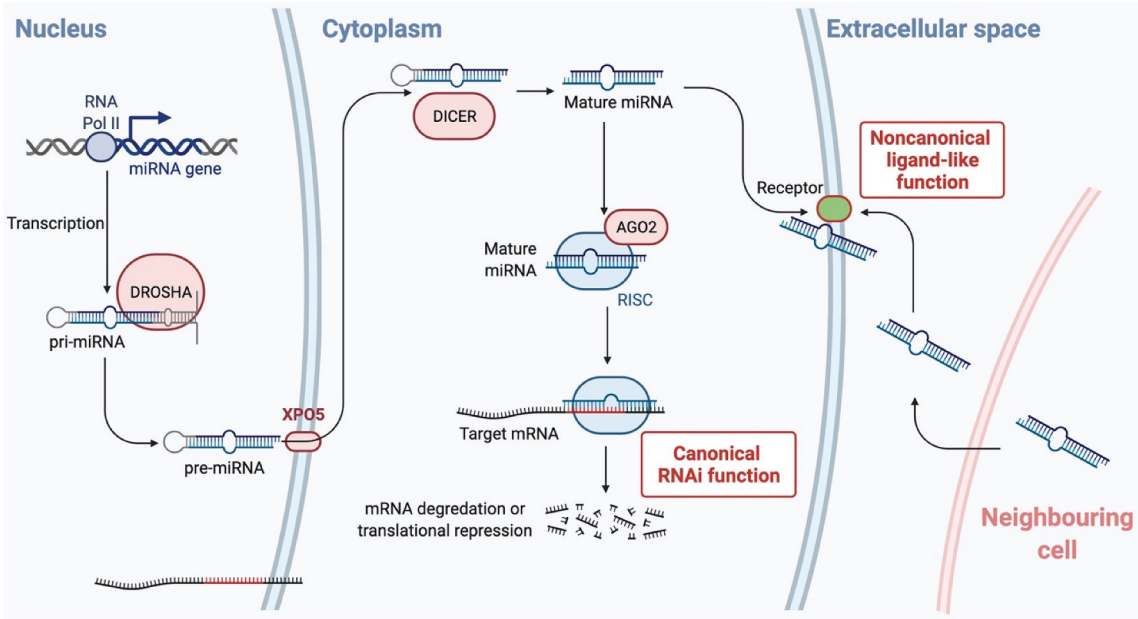

Figure 1. Canonical and noncanonical miRNA functions. In the nucleus, primary miRNA transcripts (pri-miRNA) are processed into precursor miRNAs (pre-miRNA) by a complex containing the endoribonuclease Drosha. Pre-miRNAs are then transferred to the cytoplasm through exportin-5 (XPO5), where the endoribonuclease Dicer removes the hairpin structure of pre-miRNAs and generates mature miRNA duplexes. For canonical RNA interference (RNAi), miRNA duplexes associate with Argonaute-2 (AGO2) proteins, which incorporate one of the two strands and form the RNA-induced silencing complex (RISC), guiding the miRNA seed sequence to the target mRNA. Noncanonical ligand-like roles of miRNAs have only been documented in few studies but involve a ligand-like receptor interaction of miRNAs. Noncanonical ligand-like roles may potentially be mediated by both intra- and extracellular miRNAs. Extracellular miRNAs may be shuttled within vesicles or proteins.

binds to plasma membrane Kir2.1 ion channels and modulates cardiac action potential [18]. Given that this noncanonical miR-1-Kir2.1 mechanism appeared to be evolutionary conserved between (mouse, guinea pig, canine and human) species [18], it is possible that this ligand-like interaction is part of a wider regulatory mechanism that has been overlooked; because neither screening methods, nor standard procedures used to study regulatory mechanisms would routinely identify biophysical activation of receptors by small RNAs. If confirmed, the implications could be substantial, especially for the field of circulating miRNAs given the range of different cell-free miRNAs in blood. Most studies on horizontal miRNA transfer from the circulation to the cells could not convincingly explain the mechanism how circulating miRNAs enter specific target cells, evade degradation upon cellular entry, associate with Ago and compete for biological effects with the more abundant intracellular miRNA pool of the target cell [10]. The concept that circulating miRNAs regulate cell surface receptors via physical interactions is appealing because signals induced by miRNA-receptor ligation could be amplified by downstream signaling, allowing small amounts of circulating miRNAs to mediate biological effects. One argument against this concept of miRNA-cell surface receptor ligation, however, is that miRNAs in circulation are contained in vesicles or protein complexes that protect miRNAs from degradation by RNases, which are abundant in the circulation. MiRNAs would therefore need to either be released from vesicles or disassociate from their protein carriers for binding to cell surface receptors. Alternatively, the miRNA-protein complex may be able to bind receptors. The latter hypothesis could then involve a more active role for the miRNA carrier, potentially guiding the miRNA to its receptor target, similar to the role of Ago in RNAi. Experimental evidence for a noncanonical regulatory mechanism by miRNA ligands has yet to be shown but recent ground-breaking developments in RNA biology; such as the finding that small RNAs can be glycosylated and presented on the cell surface, where they interact with receptors of immune cells [19]; are expected to prompt further studies in this evolving area of research.

Intraplatelet microRNAs and platelet function

The first comprehensive assessment of miRNAs in platelets was performed in 2009, identifying 219 miRNAs using microarray profiling [20]. This number later increased to approximately 750 miRNAs with next-generation sequencing (NGS), equivalent to approximately 30% of all known miRNA species [21]. The three most extensively studied platelet miRNAs are miR-223, miR-21 and miR-126, which we will describe in the following section.

miR-223

Based on data from multiple studies, miR-223 appears to be the most abundant platelet miRNA [6]. Interestingly, intraplatelet levels of specific miRNAs have been associated with hyporeactive and hyperreactive platelet responses to agonist stimulation [9] and conversely, several intraplatelet miRNAs are differentially expressed upon platelet activation [22], an effect that seems independent of the activation stimulus [23]. Several miRNAs were also shown to have functional roles in platelet activation (Figure 2). For instance, Landry et al. [20] have used a reporter gene assay to show that miR-223 pairs to the 3′UTR of the platelet $P2Y_{12}$ receptor and found $P2Y_{12}$ mRNA in Ago immunoprecipitates. The findings by Landry et al. [20] suggest that miR-223 regulates the platelet $P2Y_{12}$ receptor, but whether this mechanism significantly regulates

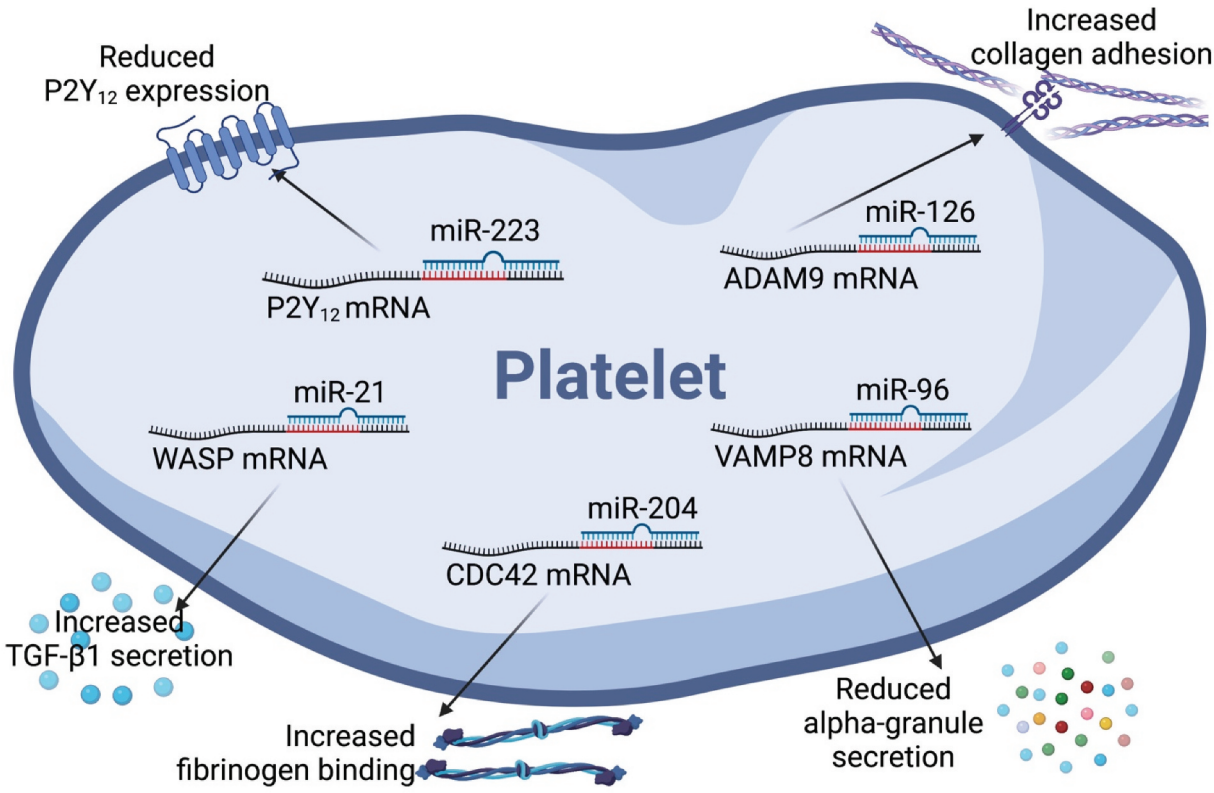

Figure 2. Intraplatelet miRNAs with known functions in platelet reactivity. MiR-126 targets ADAM9, which is an inhibitor of platelet-collagen adhesion. MiR-96 targets VAMP8, which is involved in platelet alpha-granule secretion. MiR-204 targets CDC42, leading to increased fibrinogen binding. MiR-21 targets WASP, a negative regulator of platelet TGF-β1 secretion. MiR-223 downregulates $P2Y_{12}$, a key receptor for adenosine diphosphate platelet activation pathways.

$P2Y_{12}$ mRNA and protein levels *in vivo* is unclear. MiR-223 deficiency in mice leads to increased thrombus size and prolonged clot retraction [24], providing support for a potential effect of miR-223 on platelet activation. Similarly, in patients with type II diabetes, intraplatelet levels of miR-223 are reduced, whilst $P2Y_{12}$ levels and platelet reactivity are increased [25]. The $P2Y_{12}$ receptor, being mediator of the adenosine diphosphate platelet activation pathway, is a target of antiplatelet drugs like clopidogrel, prasugrel and ticagrelor. Accordingly, non-ST elevation myocardial infarction (NSTEMI) patients with a low response to clopidogrel were found to have lower intraplatelet levels of miR-223 [26].

miR-21

In another study in patients with acute coronary syndrome (ACS), levels of miR-223, miR-221 and miR-21 were upregulated in those patients with a high response to clopidogrel [27]. Notably, miR-21 is also known for its role in tissue fibrosis and progressed to clinical trials for Alport syndrome. Our group demonstrated that miR-21 manipulation in fibroblasts induced only marginal changes in extracellular matrix protein secretion. Instead, we reported a previously unrecognized effect of miR-21 inhibition on platelets. In plasma samples from the community-based Bruneck Study, we found a marked correlation for miR-21 levels with several platelet-derived pro-fibrotic factors, including transforming growth factor beta-1 (TGF-β1). Platelet α-granule secretion of TGF-β1 is a trigger for fibrotic responses. Pharmacological miR-21 inhibition with an antagomiR reduced the platelet release of TGF-β1 in mice. Mechanistically, Wiskott-Aldrich Syndrome protein is regulated by miR-21, which is a negative regulator of TGF-β1 secretion in platelets [28].

miR-126

A functional role in platelets has also been shown for miR-126: miR-126 inhibition with antagomirs led to reduced aggregation in mice [4], whilst transfection of miR-126 into $CD34^+$-derived megakaryocytes increases reactivity of its platelet-like structures [29]. In line with these findings, a single-nucleotide polymorphism favoring miR-126 processing to a mature miRNA was found to be associated with higher levels of protein markers of platelet activation [4]. The inhibition of disintegrin and metalloproteinase domain-containing protein 9 (ADAM9; an inhibitor of platelet-collagen adhesion) and upregulation of the $P2Y_{12}$ receptor [4,30,31] might be responsible for these effects. Moreover, miR-126 appears to have a key role in platelet-supported thrombin generation, based on *in vivo* miR-126 overexpression in zebrafish, *ex vivo* miR-126 transfection of human megakaryocytes and association data from patients with cardiovascular disease ($n = 185$) [32].

Other miRNAs

A role for platelet activation despite aspirin treatment has recently been attributed to platelet miR-26b. MiR-26b targets the multidrug resistance protein-4 involved in aspirin resistance, and miR-26b was downregulated upon aspirin treatment [33]. Platelet miR-204 was reported to regulate platelet reactivity through cell division control protein 42 (CDC42) downregulation and fibrinogen receptor expression [34]. In another recent study, healthy volunteers were grouped according to very high and very low platelet reactivity as assessed by thromboelastography, identifying increased miR-150 levels in the high reactivity group [35]. These findings were then replicated in ACS patients, where patients with high on-treatment platelet reactivity had elevated miR-150 levels in platelets [35]. Another example of how platelet

Table I. Selected studies on platelet-derived miRNAs as markers of platelet reactivity.

Ref.	Year	miRNAs	Key findings
[40]	2012	miR-21, miR-24, miR-126, miR-197, miR-223	Patients with subsequent myocardial infarction showed co-expression patterns of circulating platelet-derived miRNAs.
[7]	2013	miR-20b, miR-21, miR-24, miR-126, miR-150, miR-191, miR-197, miR-223	Platelets were found to be a major source of circulating miRNAs. MiRNA levels were responsive to APT.
[41]	2013	miR-126	Platelets were found to be a major source of circulating miR-126. Aspirin use was associated with reduced miR-126 levels.
[42]	2014	miR-223	Circulating platelet miR-223 was found to be inversely associated with platelet reactivity.
[43]	2014	miR-126, miR-199a	MiRNAs in microvesicles were found to predict cardiovascular events in CAD patients.
[44]	2016	miR-96, miR-126, miR-150, miR-223	A switch from a weaker to a stronger $P2Y_{12}$ inhibitor associated with a reduction in circulating miRNAs.
[4]	2016	miR-126, miR-223	Circulating miRNA levels correlated with platelet function tests.
[45]	2017	miR-150	In ischemic stroke patients, miR-150 predicted 90-day mortality.
[46]	2019	miR-21, miR-126, miR-150, miR-223	Cessation of $P2Y_{12}$ inhibitor treatment did not lead to an expected increase of circulating platelet-derived miRNAs.
[47]	2020	miR-21, miR-24, miR-191, miR-197, miR-223	In type II diabetes patients, addition of the $P2Y_{12}$ inhibitor prasugrel to aspirin lead to a further decrease in platelet-derived miRNAs.
[48]	2020	miR-15b, miR-93, miR-126	Circulating miRNAs in ACS patients were found to correlate with platelet function tests.
[49]	2020	miR-197, miR-223, miR-223*	Rise of platelet-derived miRNAs with endotoxemia reflected platelet activation despite $P2Y_{12}$ inhibition.
[50]	2021	miR-1, miR-21, miR-23a, miR-24, miR-27b, miR-28, miR-29b, miR-33a, miR-126, miR-150, miR-191, miR-197, miR-223, miR-320a, miR-451a	In periodontitis patients, platelet-derived miRNA levels were not associated with platelet activation.
[51]	2021	miR-21, miR-126, miR-197, miR-223	Circulating platelet-derived miRNAs were found to rise with COVID-19 severity.

Abbreviations: ACS: acute coronary syndrome, APT: antiplatelet therapy, CAD: coronary artery disease, Ref: reference.

function may be regulated by miRNAs is platelet miR-96, which targets vesicle-associated membrane protein 8 (VAMP8), involved in platelet α-granule secretion [36].

Circulating platelet-derived microRNAs and platelet function

The discovery of miRNAs in the cell-free circulation in 2008 [37] was surprising, given that RNases are abundant in the circulation and quickly degrade free RNA [38]. RNase-resistance of circulating miRNAs was later attributed to protection by proteins or vesicles, which act as miRNA carriers in the circulation [10]. A large proportion of circulating miRNAs is derived from platelets, predominantly secreted within vesicles upon platelet activation [4–8]. Differences in the miRNAome between platelet-derived vesicles and platelets have been suggested to be the result of a specific packaging mechanism rather than an unspecific bulk release [39], although experimental data for a specific release mechanism are lacking. Given that the platelet transcriptome is derived from megakaryocytes, the measurement of platelet-derived transcripts in the circulation also offers insight into the megakaryocyte transcriptome through a liquid biopsy.

In line with the concept of miRNAs being released upon platelet activation (Table I), Willeit et al. [7] analyzed changes in plasma miRNAs in response to antiplatelet therapy, showing that antiplatelet therapy was associated with significantly reduced miRNA levels [7]. Similarly, another study showed that the transfer of miR-126 from platelets to the plasma compartment upon platelet stimulation is prevented by aspirin [41]. Kaudewitz et al. [4] looked at the association of platelet-derived miRNAs with platelet reactivity in the community-based Bruneck study as well as in patients with ACS [4], showing that platelet-derived miRNAs such as miR-223 and miR-126 associate with platelet-derived proteins such as platelet factor 4, platelet basic protein and P-selectin in the general community, and associate with platelet reactivity in ACS patients as assessed by the vasodilator-stimulated phosphoprotein phosphorylation (VASP) assay [4]. MiRNAs also appear to capture rather nuanced changes in platelet reactivity, given that the switch from the weaker $P2Y_{12}$ inhibitor clopidogrel to the stronger $P2Y_{12}$ inhibitor ticagrelor was associated with a greater reduction in platelet-derived miR-126, miR-150 and miR-223 [44]. In a low dose endotoxemia model, the rise of miR-223, miR-223* and miR-197 was suggestive of platelet activation despite $P2Y_{12}$ inhibitor treatment [49]. Similarly, it was recently shown that platelet-derived miR-21, miR-197 and miR-223 rise with COVID-19 severity [51], potentially reflecting the prothrombotic state in COVID-19. Interestingly, data from the prospective Bruneck study also showed that a combination of platelet-derived miR-126, miR-223 and miR-197 levels at baseline predicted incident cardiovascular events over a 10-year observation period [40]. Similarly, another study found that the risk for arterial thrombotic events in patients with coronary heart disease is associated with circulating vesicle-bound miR-199a and miR-126 [43]. Furthermore, plasma miR-150 levels measured within 72 hours of stroke symptom onset, were found to predict 90-day mortality in a cohort of ischemic stroke patients ($n = 329$) [45]. Associations between platelet reactivity and several platelet-derived miRNAs, such as miR-126, were also found in a large clinical trial of patients with NSTEMI (TRILOGY-ACS trial, $n = 878$) [48]. Similar to many protein biomarkers, levels of miRNA biomarkers may be confounded by medication, comorbidities as well as demographic factors such as age, sex or body mass index [52]. One confounder appears to be type II diabetes, which associates with a reduction of several platelet-derived miRNAs [53], and other metabolic parameters such as obesity and lipid metabolism [54]. Data from diabetic patients on antiplatelet therapy, however, suggests that antiplatelet therapy may, at least partially, be responsible for the decrease of circulating platelet-derived miRNAs in diabetes: in a recent double-blind randomized controlled trial, treatment of diabetic patients with

the strong $P2Y_{12}$ inhibitor prasugrel led to a decrease of circulating miR-24, miR-191, miR-197 and miR-223 compared to aspirin [47]. However, not all studies found significant associations between platelet-derived miRNAs and platelet reactivity: in a small randomized controlled trial investigating the effects of periodontal treatment on platelet function, as assessed by flow cytometry and miRNA measurements, platelet activation and reactivity indices did not correlate with the levels of platelet-derived miRNAs [50]. Other conflicting data from a small study showed that circulating platelet-related miRNAs are inversely associated with platelet reactivity as suggested by Chyrchel et al. [42]. In their study, higher miR-223 levels were associated with stronger platelet inhibition [42]. Moreover, a discontinuation of long-term $P2Y_{12}$ inhibitor treatment did not lead to an expected increase of circulating platelet-derived miRNAs such as miR-21, miR-126, miR-150 and miR-223 [46]. Such conflicting data in studies of circulating miRNAs cannot only be explained by biological confounders but may also arise from preanalytical issues related to the measurement of miRNAs.

Analytical and preanalytical issues related to the measurement of microRNAs

Measurements of circulating platelet-derived miRNAs are impacted by sample preparation and data normalization (Figure 3). One major problem is the release of miRNAs from platelets that are activated after blood draw, thus overestimating *in vivo* miRNA levels. This problem can be minimized by rigorous standardization of sample collection and sample processing. The choice of anticoagulant used in blood tubes and differences in centrifugation speed or temperature may be responsible for inter-study differences [6]. The biggest discrepancies, however, are observed when platelet-derived miRNA levels are compared between plasma and serum: the preparation of serum involves activation of the coagulation cascade, which in turn results in uncontrolled platelet activation as well as potential proteolytic cleavage of protein miRNA-carriers. Thus, plasma is considered a more suitable matrix, but routine preparation of plasma often leads to substantial platelet activation or contamination with residual platelets. We have proposed the use of platelet-poor plasma for circulating miRNA measurements, generated through two consecutive centrifugation steps in the presence of the platelet inhibitor prostacyclin [6]. Residual leukocytes in plasma are also a concern, since the total RNA content of a single leukocyte is around three orders of magnitudes higher than that of a single platelet [8].

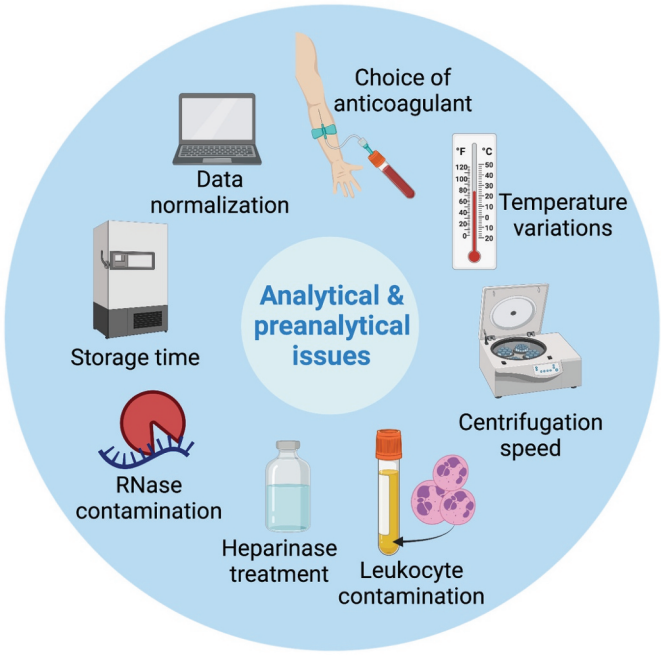

Figure 3. Analytical and preanalytical issues related to the measurement of miRNAs.

Another important confounder is heparin, which is commonly administered to hospitalized patients or to patients undergoing coronary interventions. Heparin interferes with the polymerase chain reaction [55]. This effect can be overcome by treating RNA extracts with heparinase [56]. Most studies fail to report the administration of heparin or the treatment of RNA with heparinase. In addition to its effect on polymerase chain reactions, heparin may increase the release of platelet-derived extracellular vesicles, thus artefactually increasing platelet-derived miRNAs [57,58]. Moreover, neutrophil extracellular trap formation may be increased in heparinized samples [59]. Other important confounders are RNases, which can be introduced during or after RNA isolation and substantially affect the miRNA measurements even in trace amounts through RNA degradation. Similarly, RNA degradation may occur due to prolonged storage or upon repeated freeze–thaw cycles.

Finally, different data normalization strategies may yield different results [6]. Various single miRNAs or combinations of endogenous miRNAs, such as miR-16, miR-93, miR-103, miR-423 and miR-425 as well U6 RNA, have been proposed as normalizers based on stable expression of these RNAs [6]. Universal applicability of these normalizers, however, is questionable because the effect of many diseases or drugs on miRNA normalizers has not been sufficiently investigated yet. In the absence of a universal normalizer, the most robust approach may be to normalize to the average expression of a large panel of miRNAs [60], in addition to using an exogenous normalization control (e.g. Cel-miR-39-3p), that is spiked into the samples during RNA isolation [6].

Besides measurements of circulating miRNAs being impacted by analytical and preanalytical factors, miRNAs may also serve as markers of sample quality in platelet concentrates used for transfusion purposes. Platelet concentrates stored in blood banks may undergo storage lesions, which impair platelet structure and function [61]. Several studies have looked at the differential expression of miRNAs during prolonged storage and have revealed platelet miRNAs that are up- and downregulated with prolonged storage [61]. Accordingly, the ratios of certain miRNAs, such as a low miR-127/miR-320 ratio, have been implicated as a marker of platelet storage lesions [62].

Conclusions and perspectives

Recent developments in RNA biology, such as the finding that miRNAs may have functional roles as receptor ligands independent of RNAi [18], or that small RNAs anchored on the cell surface may interact with immune cell receptors [19], have potential to advance our understanding of the biological function of circulating cell-free miRNAs. A substantial proportion of circulating cell-free miRNAs are derived from platelets, and the levels of platelet-derived miRNAs are correlated with platelet activation. The clinical utility of miRNAs as biomarkers for platelet reactivity remains to be demonstrated in large clinical trials, and preanalytical issues need to be taken into consideration. The use of "biomarker signatures", i.e. the use of a combination of measurements rather than single markers, is certainly applicable to the miRNA field as well. The need for novel biomarkers of *in vivo* platelet reactivity is highlighted by the fact that existing *ex vivo* platelet function tests might not be as reliable, in

particular in acute settings such as MI due to an *in vivo* pre-activation of platelets leading to a hypo-responsiveness *ex vivo* [63]. Accordingly, existing platelet function tests are not commonly used to inform clinical decisions with regard to choice and dosage of antiplatelet therapy [64]. It remains to be seen whether platelet reactivity signatures based on miRNAs or other molecular measurements can address this clinical need or improve risk management of thrombotic complications.

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Disclosure statement

M.M. has filed and licenced patent applications on miRNAs as platelet biomarkers.

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References

1. Bizzozero G. Su di un nuovo elemento morfologico del sangue dei mammiferi e della sua importanza nella trombosi e nella coagulazione. L'Osservatore 1881;17:785–787.
2. Wright JH. The origin and nature of the blood plates. Bost Med Surg J 1906;154:643–645. doi:10.1056/NEJM190606071542301.
3. Lefrançais E, Ortiz-Muñoz G, Caudrillier A, Mallavia B, Liu F, Sayah DM, Thornton EE, Headley MB, David T, Coughlin SR, et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. Nature 2017;544:105–109. doi:10.1038/nature21706.
4. Kaudewitz D, Skroblin P, Bender LH, Barwari T, Willeit P, Pechlaner R, Sunderland NP, Willeit K, Morton AC, Armstrong PC, et al. Association of MicroRNAs and YRNAs with platelet function. Circ Res 2016;118:420–432. doi:10.1161/CIRCRESAHA.114.305663.
5. Laffont B, Corduan A, Ple H, Duchez A-C, Cloutier N, Boilard E, Provost P. Activated platelets can deliver mRNA regulatory Ago2bulletmicroRNA complexes to endothelial cells via microparticles. Blood 2013;122:253–261. doi:10.1182/blood-2013-03-492801.
6. Sunderland N, Skroblin P, Barwari T, Huntley RP, Lu R, Joshi A, Lovering RC, Mayr M. MicroRNA biomarkers and platelet reactivity. Circ Res 2017;120:418–435. doi:10.1161/CIRCRESAHA.116.309303.
7. Willeit P, Zampetaki A, Dudek K, Kaudewitz D, King A, Kirkby NS, Crosby-Nwaobi R, Prokopi M, Drozdov I, Langley SR, et al. Circulating MicroRNAs as novel biomarkers for platelet activation. Circ Res 2013;112:595–600. doi:10.1161/CIRCRESAHA.111.300539.
8. Teruel-Montoya R, Kong X, Abraham S, Ma L, Kunapuli SP, Holinstat M, Shaw CA, McKenzie SE, Edelstein LC, Bray PF. MicroRNA expression differences in human hematopoietic cell lineages enable regulated transgene expression. PLoS One 2014;9:e102259. doi:10.1371/journal.pone.0102259.
9. Nagalla S, Shaw C, Kong X, Kondkar AA, Edelstein LC, Ma L, Chen J, McKnight GS, López JA, Yang L, et al. Platelet microRNA-mRNA coexpression profiles correlate with platelet reactivity. Blood 2011;117:5189–5197. doi:10.1182/blood-2010-09-299719.
10. Gutmann C, Joshi A, Zampetaki A, Mayr M. The landscape of coding and noncoding RNAs in platelets., antioxid. Redox Sig 2021;34:1200–1216. doi:10.1089/ars.2020.8139.
11. Dragomir MP, Knutsen E, Calin GA. SnapShot: unconventional miRNA functions. Cell 2018;174:1038–1038.e1. doi:10.1016/J.CELL.2018.07.040.
12. Lehmann SM, Krüger C, Park B, Derkow K, Rosenberger K, Baumgart J, Trimbuch T, Eom G, Hinz M, Kaul D, et al. An unconventional role for miRNA: let-7 activates Toll-like receptor 7 and causes neurodegeneration. Nat Neurosci 2012;15:827–835. doi:10.1038/nn.3113.
13. Park C-K, Xu -Z-Z, Berta T, Han Q, Chen G, Liu X-J, Ji -R-R. Extracellular MicroRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. Neuron 2014;82:47–54. doi:10.1016/J.NEURON.2014.02.011.
14. Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, Lovat F, Fadda P, Mao C, Nuovo GJ, et al. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. Proc Natl Acad Sci USA 2012;109:E2110–6. doi:10.1073/pnas.1209414109.
15. Han Q, Liu D, Convertino M, Wang Z, Jiang C, Kim YH, Luo X, Zhang X, Nackley A, Dokholyan NV, et al. miRNA-711 binds and activates TRPA1 extracellularly to evoke acute and chronic pruritus. Neuron 2018;99:449–463.e6. doi:10.1016/J.NEURON.2018.06.039.
16. Stalder L, Heusermann W, Sokol L, Trojer D, Wirz J, Hean J, Fritzsche A, Aeschimann F, Pfanzagl V, Basselet P, et al. The rough endoplasmatic reticulum is a central nucleation site of siRNA-mediated RNA silencing. EMBO J 2013;32:1115–1127. doi:10.1038/emboj.2013.52.
17. Flores O, Kennedy EM, Skalsky RL, Cullen BR. Differential RISC association of endogenous human microRNAs predicts their inhibitory potential. Nucleic Acids Res 2014;42:4629–4639. doi:10.1093/nar/gkt1393.
18. Yang D, Wan X, Dennis AT, Bektik E, Wang Z, Costa MGS, Fagnen C, Vénien-Bryan C, Xu X, Gratz DH, et al. MicroRNA biophysically modulates cardiac action potential by direct binding to ion channel. Circulation 2021;143:1597–1613. doi:10.1161/CIRCULATIONAHA.120.050098.
19. Flynn RA, Pedram K, Malaker SA, Batista PJ, Smith BAH, Johnson AG, George BM, Majzoub K, Villalta PW, Carette JE, et al. Small RNAs are modified with N-glycans and displayed on the surface of living cells. Cell 2021;184:3109–3124.e22. doi:10.1016/J.CELL.2021.04.023.
20. Landry P, Plante I, Ouellet DL, Perron MP, Rousseau G, Provost P. Existence of a microRNA pathway in anucleate platelets. Nat Struct Mol Biol 2009;16:961–966. doi:10.1038/nsmb.1651.
21. Bray PF, McKenzie SE, Edelstein LC, Nagalla S, Delgrosso K, Ertel A, Kupper J, Jing Y, Londin E, Loher P, et al. The complex transcriptional landscape of the anucleate human platelet. BMC Genomics 2013;14:1. doi:10.1186/1471-2164-14-1.
22. Osman A, Fälker K. Characterization of human platelet microRNA by quantitative PCR coupled with an annotation network for predicted target genes. Platelets 2011;22:433–441. doi:10.3109/09537104.2011.560305.
23. Cimmino G, Tarallo R, Nassa G, De Filippo MR, Giurato G, Ravo M, Rizzo F, Conte S, Pellegrino G, Cirillo P, et al. Activating stimuli induce platelet microRNA modulation and proteome reorganisation. Thromb Haemost 2015;114:96–108. doi:10.1160/TH14-09-0726.
24. Elgheznawy A, Shi L, Hu J, Wittig I, Laban H, Pircher J, Mann A, Provost P, Randriamboavonjy V, Fleming I. Dicer cleavage by calpain determines platelet microRNA levels and function in diabetes. Circ Res 2015;117:157–165. doi:10.1161/CIRCRESAHA.117.305784.

25. Fejes Z, Póliska S, Czimmerer Z, Káplár M, Penyige A, Gál Szabó G, Debreceni IB, Kunapuli SP, Kappelmayer J, Nagy B. Hyperglycaemia suppresses microRNA expression in platelets to increase P2RY12 and SELP levels in type 2 diabetes mellitus. Thromb Haemost 2017;117:529–542. doi:10.1160/TH16-04-0322.
26. Shi R, Ge L, Zhou X, Ji W-J, Lu R-Y, Zhang -Y-Y, Zeng S, Liu X, Zhao J-H, Zhang W-C, et al. Decreased platelet miR-223 expression is associated with high on-clopidogrel platelet reactivity. Thromb Res 2013;131:508–513. doi:10.1016/j.thromres.2013.02.015.
27. Peng L, Liu J, Qin L, Liu J, Xi S, Lu C, Yin T. Interaction between platelet-derived microRNAs and CYP2C19*2 genotype on clopidogrel antiplatelet responsiveness in patients with ACS. Thromb Res 2017;157:97–102. doi:10.1016/j.thromres.2017.07.011.
28. Barwari T, Eminaga S, Mayr U, Lu R, Armstrong PC, Chan MV, Sahraei M, Fernández-Fuertes M, Moreau T, Barallobre-Barreiro J, et al. Inhibition of profibrotic microRNA-21 affects platelets and their releasate. JCI Insight 2018;3. doi:10.1172/jci.insight.123335.
29. Garcia A, Dunoyer-Geindre S, Zapilko V, Nolli S, Reny J-L, Fontana P. Functional validation of microRNA-126-3p as a platelet reactivity regulator using human haematopoietic stem cells. Thromb Haemost 2019;119:254–263. doi:10.1055/s-0038-1676802.
30. Wendel Garcia PD, Fumeaux T, Guerci P, Heuberger DM, Montomoli J, Roche-Campo F, Schuepbach RA, Hilty MP. Prognostic factors associated with mortality risk and disease progression in 639 critically ill patients with COVID-19 in Europe: initial report of the international RISC-19-ICU prospective observational cohort. EClinicalMedicine 2020;100449. doi:10.1016/J.ECLINM.2020.100449.
31. Schober A, Nazari-Jahantigh M, Wei Y, Bidzhekov K, Gremse F, Grommes J, Megens RTA, Heyll K, Noels H, Hristov M, et al. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. Nat Med 2014;20:368–376. doi:10.1038/nm.3487.
32. Zapilko V, Fish RJ, Garcia A, Reny J-L, Dunoyer-Geindre S, Lecompte T, Neerman-Arbez M, Fontana P. MicroRNA-126 is a regulator of platelet-supported thrombin generation. Platelets 2020;31:746–755. doi:10.1080/09537104.2020.1775804.
33. La Rosa G, Biasucci LM, Mandolini C, Massimi I, Copponi G, Pulcinelli FM, Crea F. Platelet miRNA-26b down-regulates multidrug resistance protein 4 in patients on chronic aspirin treatment. J Cardiovasc Med 2018;19:611–613. doi:10.2459/JCM.0000000000000691.
34. Garcia A, Dunoyer-Geindre S, Nolli S, Strassel C, Reny J-L, Fontana P. miR-204-5p and platelet function regulation: insight into a mechanism mediated by CDC42 and GPIIbIIIa. Thromb Haemost 2021. doi:10.1055/a-1497-9649.
35. Liu J, Qin L, Wang Z, Peng L, Liu J, Wang X, Du R, Zou Y, Wu Y, Yin T. Platelet-derived miRNAs as determinants of the antiplatelet response in clopidogrel-treated patients with ACS. Thromb Res 2020;186:71–74. doi:10.1016/j.thromres.2019.12.016.
36. Kondkar AA, Bray MS, Leal SM, Nagalla S, Liu DJ, Jin Y, Dong JF, Ren Q, Whiteheart SW, Shaw C, et al. VAMP8/endobrevin is overexpressed in hyperreactive human platelets: suggested role for platelet microRNA. J Thromb Haemost 2010;8:369–378. doi:10.1111/j.1538-7836.2009.03700.x.
37. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee M-LT, Schmittgen TD, et al. Detection of microRNA expression in human peripheral blood microvesicles. PLoS One 2008;3:e3694. doi:10.1371/journal.pone.0003694.
38. Tsui NBY, Ng EKO, Lo YMD. Stability of endogenous and added RNA in blood specimens, serum, and plasma. Clin Chem 48;2002:1647–1653. [accessed August 24, 2019]. http://www.ncbi.nlm.nih.gov/pubmed/12324479
39. Diehl P, Fricke A, Sander L, Stamm J, Bassler N, Htun N, Ziemann M, Helbing T, El-Osta A, Jowett JBM, et al. Microparticles: major transport vehicles for distinct microRNAs in circulation. Cardiovasc Res 2012;93:633–644. doi:10.1093/cvr/cvs007.
40. Zampetaki A, Willeit P, Tilling L, Drozdov I, Prokopi M, Renard J-M, Mayr A, Weger S, Schett G, Shah A, et al. Prospective study on circulating MicroRNAs and risk of myocardial infarction. J Am Coll Cardiol 2012;60:290–299. doi:10.1016/j.jacc.2012.03.056.
41. de Boer HC, van Solingen C, Prins J, Duijs JMGJ, Huisman MV, Rabelink TJ, van Zonneveld AJ. Aspirin treatment hampers the use of plasma microRNA-126 as a biomarker for the progression of vascular disease. Eur Heart J 2013;34:3451–3457. doi:10.1093/eurheartj/eht007.
42. Chyrchel B, Totoń-Żurańska J, Kruszelnicka O, Chyrchel M, Mielecki W, Kołton-Wróż M, Wołkow P, Surdacki A. Association of plasma miR-223 and platelet reactivity in patients with coronary artery disease on dual antiplatelet therapy: a preliminary report. Platelets 2015;26:593–597. doi:10.3109/09537104.2014.974527.
43. Jansen F, Yang X, Proebsting S, Hoelscher M, Przybilla D, Baumann K, Schmitz T, Dolf A, Endl E, Franklin BS, et al. MicroRNA expression in circulating microvesicles predicts cardiovascular events in patients with coronary artery disease. J Am Heart Assoc 2014;3:e001249. doi:10.1161/JAHA.114.001249.
44. Carino A, De Rosa S, Sorrentino S, Polimeni A, Sabatino J, Caiazzo G, Torella D, Spaccarotella C, Mongiardo A, Strangio A, et al. Indolfi, modulation of circulating MicroRNAs levels during the switch from clopidogrel to ticagrelor. Biomed Res Int 2016 ;2016:1–5. doi:10.1155/2016/3968206.
45. Scherrer N, Fays F, Mueller B, Luft A, Fluri F, Christ-Crain M, Devaux Y, Katan M. MicroRNA 150-5p improves risk classification for mortality within 90 days after acute ischemic stroke. J Stroke 2017;19:323–332. doi:10.5853/jos.2017.00423.
46. Jäger B, Stojkovic S, Haller PM, Piackova E, Kahl BS, Andric T, Vargas KG, Wojta J, Huber K. Course of platelet miRNAs after cessation of P2Y12 antagonists. Eur J Clin Invest 2019;49:e13149. doi:10.1111/eci.13149.
47. Parker WAE, Schulte C, Barwari T, Phoenix F, Pearson SM, Mayr M, Grant PJ, Storey RF, Ajjan RA. Aspirin, clopidogrel and prasugrel monotherapy in patients with type 2 diabetes mellitus: a double-blind randomised controlled trial of the effects on thrombotic markers and microRNA levels. Cardiovasc Diabetol 2020;19:3. doi:10.1186/s12933-019-0981-3.
48. Becker KC, Kwee LC, Neely ML, Grass E, Jakubowski JA, Fox KAA, White HD, Gregory SG, Gurbel PA, de P. Carvalho L, et al. Circulating MicroRNA profiling in non-ST elevated coronary artery syndrome highlights genomic associations with serial platelet reactivity measurements. Sci Rep 2020;10:6169. doi:10.1038/s41598-020-63263-6.
49. Braza-Boïls A, Barwari T, Gutmann C, Thomas MR, Judge HM, Joshi A, Pechlaner R, Shankar-Hari M, Ajjan RA, Sabroe I, et al. Circulating MicroRNA levels indicate platelet and leukocyte activation in endotoxemia despite platelet P2Y12 inhibition. Int J Mol Sci 2020;21:2897. doi:10.3390/ijms21082897.
50. Heber S, Laky M, Anscheringer I, Wolschner L, Mussbacher M, Krammer T, Haririan H, Schrottmaier WC, Volf I, Hackl M, et al. Platelet activation is not always associated with platelet-related plasma microRNA abundance – results from a randomized controlled trial of periodontal patients. Front Physiol 2021;12:191. doi:10.3389/fphys.2021.613515.
51. Gutmann C, Khamina K, Theofilatos K, Diendorfer AB, Burnap SA, Nabeebaccus A, Fish M, McPhail MJW, O'Gallagher K, and Schmidt LE, et al. Association of cardiometabolic microRNAs with COVID-19 severity and mortality. Cardiovasc Res 2022;118(2):461-474. doi:10.1093/cvr/cvab338.
52. Rounge TB, Umu SU, Keller A, Meese E, Ursin G, Tretli S, Lyle R, Langseth H. Circulating small non-coding RNAs associated with age, sex, smoking, body mass and physical activity. Sci Rep 2018;8:17650. doi:10.1038/s41598-018-35974-4.
53. Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, Mayr A, Weger S, Oberhollenzer F, Bonora E, et al. Plasma MicroRNA profiling reveals loss of endothelial MiR-126 and other MicroRNAs in type 2 diabetes. Circ Res 2010;107:810–817. doi:10.1161/CIRCRESAHA.110.226357.
54. Sangiao-Alvarellos S, Theofilatos K, Barwari T, Gutmann C, Takov K, Singh B, Juiz-Valiña P, Varela-Rodríguez BM, Outeiriño-Blanco E, Duregotti E, et al. Metabolic recovery after weight loss surgery is reflected in serum microRNAs. BMJ Open Diabetes Res Care 2020;8:e001441. doi:10.1136/bmjdrc-2020-001441.
55. Kaudewitz D, Lee R, Willeit P, McGregor R, Markus HS, Kiechl S, Zampetaki A, Storey RF, Channon KM, Mayr M. Impact of intravenous heparin on quantification of circulating microRNAs in patients with coronary artery disease. Thromb Haemost 2013;110:609–615. doi:10.1160/TH13-05-0368.
56. Schulte C, Barwari T, Joshi A, Theofilatos K, Zampetaki A, Barallobre-Barreiro J, Singh B, Sörensen NA, Neumann JT,

Zeller T, et al. Comparative analysis of circulating noncoding RNAs versus protein biomarkers in the detection of myocardial injury. Circ Res 2019;125:328–340. doi:10.1161/CIRCRESAHA.119.314937.

57. Lacroix R, Judicone C, Poncelet P, Robert S, Arnaud L, Sampol J, Dignat-George F. Impact of pre-analytical parameters on the measurement of circulating microparticles: towards standardization of protocol. J Thromb Haemost 2012;10:437–446. doi:10.1111/j.1538-7836.2011.04610.x.
58. György B, Pálóczi K, Kovács A, Barabás E, Bekő G, Várnai K, Pállinger É, Szabó-Taylor K, Szabó TG, Kiss AA, et al. Improved circulating microparticle analysis in acid-citrate dextrose (ACD) anticoagulant tube. Thromb Res 2014;133:285–292. doi:10.1016/j.thromres.2013.11.010.
59. Lelliott PM, Momota M, Shibahara T, Lee MSJ, Smith NI, Ishii KJ, Coban C. Heparin induces neutrophil elastase-dependent vital and lytic NET formation. Int Immunol 2020;32:359–368. doi:10.1093/intimm/dxz084.
60. Mestdagh P, Van Vlierberghe P, De Weer A, Muth D, Westermann F, Speleman F, Vandesompele J. A novel and universal method for microRNA RT-qPCR data normalization. Genome Biol 2009;10:R64. doi:10.1186/gb-2009-10-6-r64.
61. da S. Maués JH, de F. Aquino Moreira-nunes C, Rodriguez Burbano RM. MicroRNAs as a potential quality measurement tool of platelet concentrate stored in blood banks-a review. Cells 2019;8. doi:10.3390/cells8101256.
62. Pontes TB, de F.a. Moreira-nunes C, da S. Maués JH, Lamarão LM, de Lemos JAR, Montenegro RC, Burbano RMR. The miRNA profile of platelets stored in a blood bank and its relation to cellular damage from storage. PLoS One 2015;10:e0129399. doi:10.1371/journal.pone.0129399.
63. Joshi A, Schmidt LE, Burnap SA, Lu R, Chan MV, Armstrong PC, Baig F, Gutmann C, Willeit P, Santer P, et al. Neutrophil-derived protein S100A8/A9 alters the platelet proteome in acute myocardial infarction and is associated with changes in platelet reactivity. Arterioscler Thromb Vasc Biol 2021;ATVBAHA121317113. doi:10.1161/ATVBAHA.121.317113.
64. Aradi D, Storey RF, Komócsi A, Trenk D, Gulba D, Kiss RG, Husted S, Bonello L, Sibbing D, Collet J-P, et al., Working Group on Thrombosis of the European Society of Cardiology. Expert position paper on the role of platelet function testing in patients undergoing percutaneous coronary intervention. Eur Heart J 2014;35:209–215. doi:10.1093/eurheartj/eht375.