Paper

Identification of common MicroRNAs expression signatures in antiphospholipid syndrome and thromboembolic disease: A scoping review

Lupus
2024, Vol. 33(13) 1455–1465

DOI: 10.1177/09612033241286601
journals.sagepub.com/home/lup
Sage

Camila de Oliveira Vaz[1], Bruna Cardoso Jacintho[1], Gabrielle de Mello Santos[2], José Diogo de Oliveira[1], Bruna Moraes Mazetto[1], Murilo Vieira Geraldo[3] and Fernanda A Orsi[1]

Abstract

Background: Antiphospholipid syndrome (APS) is an acquired autoimmune disorder characterized by distinct pathophysiological mechanisms leading to heterogeneous manifestations, including venous and arterial thrombosis. Despite the lack of specific markers of thrombosis risk in APS, some of the mechanisms responsible for thrombosis in APS may overlap with those of other thromboembolic diseases. Understanding these similarities is important for improving the assessment of thrombosis risk in APS. MicroRNAs (MiRNAs) are RNA molecules that regulate gene expression and may influence the autoimmune response and coagulation.
Purpose: In this scoping review we aimed to investigate shared miRNAs profiles associated with APS and other thromboembolic diseases as a means of identifying markers indicative of a pro-thrombotic profile among patients with APS.
Data collection and Results: Through a comprehensive search of scientific databases, 45 relevant studies were identified out of 1020 references. miRs-124-3p, 125b-5p, 125a-5p, and 17-5p, were associated with APS and arterial thrombosis, while miRs-106a-5p, 146b-5p, 15a-5p, 222-3p, and 451a were associated with APS and venous thrombosis. Additionally, miR-126a-3p was associated with APS and both arterial and venous thrombosis.
Conclusion: We observed that APS shares a common miRNAs signature with non-APS related thrombosis, suggesting that miRNA expression profiles may serve as markers of thrombotic risk in APS. Further validation of a pro-thrombotic miRNA signature in APS is warranted to improve risk assessment, diagnosis, and management of APS.

Keywords

Antiphospholipid syndrome, microRNAs, thrombosis, biomarkers, prediction

Date received: 13 May 2024; accepted: 9 September 2024

Background

Antiphospholipid syndrome (APS)

Antiphospholipid syndrome (APS) is a rare autoimmune prothrombotic condition characterized by the persistence of antiphospholipid antibodies (aPL). The estimated incidence ranges between 1 and 2 cases per 100,000 individuals per year, with a prevalence of 40 to 50 cases per 100,000 individuals.[1,2] It is more prevalent in females and does not exhibit racial predominance.[1,2]

Thrombotic events and obstetric complications are the primary causes of morbidity and mortality in APS.[3] APS is thought to be responsible for up to 10% of the cases of obstetric morbidity, arterial events, and venous thromboembolism in individuals under 45 years old.[1] Additionally, the mortality rate is around 50–80% higher in patients with APS compared to the general population,

[1]School of Medical Sciences, University of Campinas, Campinas, Brazil
[2]Hospital das Clínicas of University of São Paulo Medical School, University of São Paulo, Sao Paulo, Brazil
[3]Biology Institute, University of Campinas, Campinas, Brazil

Corresponding author:
Fernanda A Orsi, School of Medical Sciences, University of Campinas, R. Tessália Vieira de Camargo, 126, Campinas 13083-872, Brazil.
Email: ferorsi@unicamp.br

mainly due to consequences of thromboembolic complication.[1]

APS is categorized as secondary (SAPS) when it coexists with other systemic autoimmune diseases such as systemic lupus erythematous (SLE), while cases without any other underlying condition are classified as primary APS (PAPS).[4,5] Having multiple cardiovascular risk factors or other autoimmune disorder increases the risk of experiencing thrombotic events, that can occur in any vascular site.[6,7]

The updated Sapporo classification criteria has been used to guide the diagnosis of APS,[4] and includes the concomitance of a previous thrombotic event or obstetric morbidity with the detection of persistently positive aPL; specifically, lupus anticoagulant (LA), anti-β2-glycoprotein I (anti-β2GPI), or anti-cardiolipin (aCL) antibodies.[4] However, this classification has several limitations in clinical practice, particularly because APS manifestations are highly heterogeneous, especially in those with non-criteria antibodies or non-criteria clinical presentations.[8] In addition, the aPL profile is not sufficient to determine APS prognosis, as patient comorbidities and the inflammatory response may also play a role in the thrombotic risk.[9,10]

Moreover, while the high-risk aPL profile for example the persistence of higher titers of aPL and triple aPL-positivity are considered risk factors for thrombosis recurrence in APS,[11–14] it has been shown that patients can experience fluctuations and transient negativation in the titers or type of aPL autoantibodies during disease progression, especially after the first thrombotic event. This variability is not necessarily correlated with a lower chance of thrombosis recurrence and could be a potential confounder in assessing patients thrombotic risk.[15]

Due to these limitations in APS-related risk stratification, several efforts have been expend for the establishment of new biomarkers to accomplish the individual prognosis.[9] Recently, the American College of Rheumatology (ACR) and the European Alliance of Associations for Rheumatology (EULAR) proposed an update to the APS classification criteria, in which the most significant change was the expansion of the clinical and laboratory classification criteria to better conduct risk-stratification clinical research in APS and potentially improve patient care.[16]

A risk assessment toll to evaluate the thrombotic risk in APS is also needed. In this context, genetic and epigenetic biomarkers have been recognized as contributors to the inflammatory and hemostatic changes leading to the thrombotic clinical presentation of APS, as well as to the severity, subtype of the disease, and aPL activity.[17,18] In line with this, a study using genome-wide transcriptomic analysis of whole blood samples demonstrated that gene signatures have significant potential to distinguish patients with thrombotic PAPS and identify high-risk subgroups, such as those with recurrent thrombotic events.[19] While this provides insight into the use of genetic markers for thrombotic risk stratification in APS, there is still lack of information on epigenetic markers such as microRNAs (miRNA). Therefore, in this review, we aimed to investigate common miRNA profiles associated with APS and other venous or arterial thromboembolic diseases to identify markers indicative of thrombosis risk in APS.

MicroRNAs (miRNAs)

MiRNAs are single-stranded, non-coding RNA molecules composed of approximately 22 nucleotides, playing a significant role in post-transcriptional regulation of gene expression.[20,21] Since their discovery in *C. elegans*[22] and the recognition of their conservation in many species,[23] studies focusing on miRNAs have exponentially increased, revealing the regulatory role of miRNAs in cellular functions and the organism.[24]

Depending on the complementarity of the miRNA seed sequence (effector region) to the mRNA's 3′-UTR, miRNA can either inhibit messenger RNA transcription (imperfect complementarity) or induce its degradation (perfect complementarity). This process regulates the expression of encoded proteins,[24] constituting the canonical pathway of miRNA action.

In general, it is estimated that approximately 60% of human messenger RNAs (mRNA) can be modulated by miRNAs, suggesting that each miRNA is capable of pairing with hundreds of different mRNAs.[25]

Following their transcription, miRNAs can either exert their action intracellularly or be excreted into the extracellular environment. Intracellularly, miRNAs concentrate in RNA processing bodies (P-bodies) and on the endoplasmic reticulum membrane.[24] When excreted, miRNAs can be detected in various biological fluids, such as plasma, urine, saliva, cerebrospinal fluid, among others.[26] Despite the high concentration of RNA-degrading enzymes in extracellular fluids, miRNAs are highly stable, primarily because these molecules exist within vesicles, such as exosomes,[27] or are associated with protein complexes like high-density lipoproteins and argonaute.[28,29]

Given the literature's demonstration of specific miRNA profiles correlating with different diseases, it is believed that circulating miRNAs can be taken up by recipient cells, thereby regulating gene transcription through intercellular communication mechanisms. Also, due to their high stability in extracellular fluids and their specific expression in different tissues or pathological states, miRNAs have come to be recognized as potential biomarkers for diagnosis, therapeutic response, and prognosis.[24,30]

In APS context, miRNAs can act as modulators of the autoimmune response and hemostatic disturbances that lead to procoagulant state and clinical thrombosis, specially by

the regulation in the tissue factor (TF) expression.[31,32] However, evidence correlating the prevalence of dysregulated miRNAs and the thrombotic risk in APS are still scarce.

Therefore, the primary aim of this scoping review was to investigate shared miRNAs profiles associated with APS and other thromboembolic diseases as a means of identifying markers indicative of a pro-thrombotic profile among patients with APS. Initially, we examined clinical studies evaluating miRNAs as potential biomarkers for APS. Subsequently, we conducted a comprehensive analysis of the role of miRNAs in predicting venous or arterial thrombosis in non-APS patients, comparing the dysregulated miRNAs within each group.

Methods

Search strategy

We conducted a comprehensive search across multiple databases, including PubMed, Scopus, BVS/Bireme, Web of Science, Embase, Cochrane Library, Ebscohost, and ProQuest. Using the search terms "MicroRNAs AND Biomarkers AND ("Antiphopholipid syndrome" OR "Thrombosis" OR "Lupus erythematous systemic")," we identified a total of 1020 unique references, which encompassed original research papers, conference abstracts, editorials, chapter of books and thesis until 2022.

Inclusion criteria

We employed the rayyan.ai tool to initially obtain the abstracts of each of the 1020 references previously founded, and two independent reviewers blinded to the study made inclusion or exclusion decisions. These reviewers are a pharmacist and a biomedical professional, both specialists in translational research and clinical analyses focused on biomarkers, and with background in studies on both APS and miRNAs. During this initial screening, we excluded systematic reviews, methodology papers, practical guides, meta-analyses, and references that described miRNAs as biomarkers for clinical conditions not related to thrombotic events or with focus in other non-coding RNAs. Any discrepancies in inclusion decisions were resolved by a third reviewer, who is a hematologist specialist in thrombosis and hemostasis and with experience in APS clinical studies. At the conclusion of this step, we were left with 217 references for the second step.

Then, in the second step we evaluated the complete version of the 217 left references using the same approach applied to the first step. At this point we excluded the remaining studies that did not analyze miRNA expression in clinical samples, had lacked correlation with thrombotic manifestations, focused on thrombotic events provoked by cancer, did not specify which miRNAs were deregulated, or were reviews and duplicates not yet excluded. We used a similar approach as in step 1, but we double-checked the full text to ensure that the analyzed references did not meet any of the exclusion criteria. The remaining references are discussed in this review and are primarily composed of original studies that assessed the circulating expression of miRNAs in biological samples from patients with thrombotic APS and compared them with patients who had thrombosis unrelated to APS or with healthy controls.

Results

Out of the 1020 unique references identified at the outset of the literary search, 45 met all the inclusion criteria and were retained in this review following the completion of the second step of the selection process. Figure 1 illustrates the study's flowchart, outlining the reasons for exclusion.

Among the remaining references, nine studies revealed 39 distinct miRNAs with differential expression in patients with APS compared to non-APS patients with thrombosis. Additionally, 16 studies identified 45 dysregulated miRNAs in patients with non-APS-related arterial thrombosis, and 20 studies described 48 differentially expressed miRNAs in non-APS-related venous thrombosis. We also observed that in most of these studies the expression of circulating miRNAs was internally validated in an independent and larger cohort of patients, ranging from a minimum of 20 to more than 100 individuals per group, after the initial identification step carried out on a small population sample. Details on all specific miRNAs and their validation status in larger cohorts are provided in the Supplementary Table. In Table 1, we present data on the miRNAs associated with APS or thrombosis in at least two different studies.

We also compared the list of dysregulated miRNAs previously described in APS with those described in non-APS arterial and venous thrombosis. Our results demonstrated the APS and non-APS arterial thrombosis share miRs- 124-3p, 125b-5p, 125a-5p, 17-5p, while APS and non-APS venous thrombosis share miRs-106a-5p, 146b-5p, 15a-5p, 222-3p, 451a. Finally, we observed that miR-126-3p was differentially expressed in both APS and non-APS thrombotic conditions compared to controls. In patients with venous thrombosis, miR-126-3p expression was increased, while in those with arterial thrombosis, it was decreased compared to controls without previous thrombosis. In thrombotic APS, miR-126-3p expression was either upregulated or downregulated, depending on the population. These results are illustrated in Figure 2.

Discussion

miRNAs in APS

Among all the references analyzed in this review, only nine clinical studies investigating the significance of miRNAs in

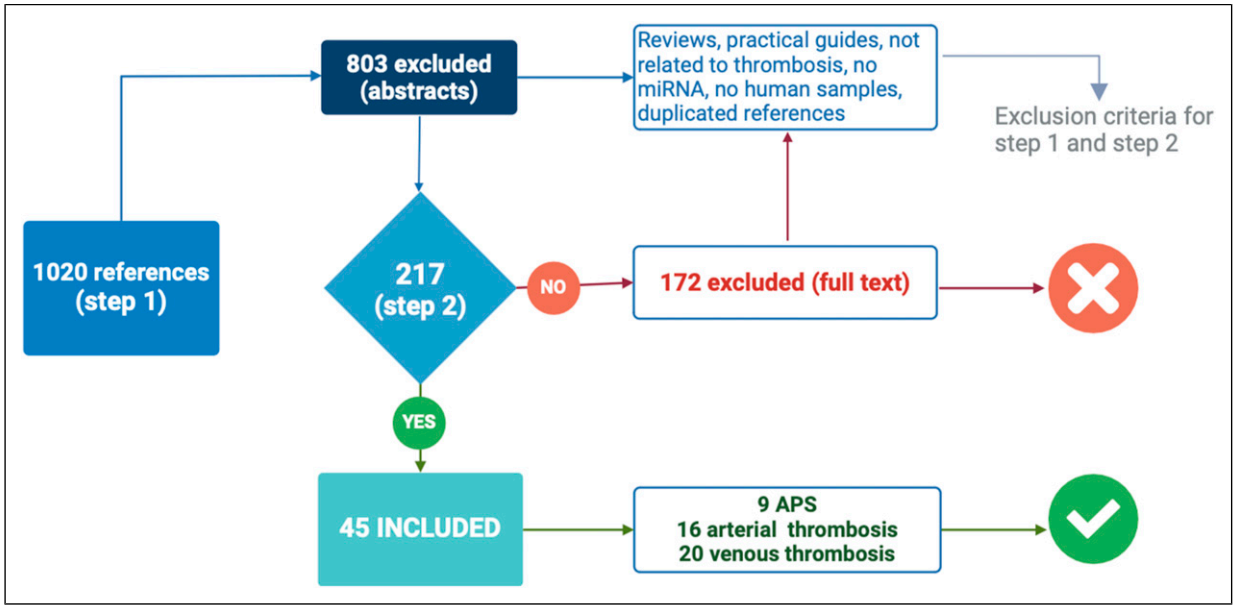

Figure 1. Flowchart of the review. In step 1, we identified a total of 1020 references using the search terms, and we evaluated the abstracts of each. Out of these, 803 were excluded for reasons summarized in the upper right corner. In step 2, we carefully analyzed 217 full texts and we excluded an additional 172 references for the same reasons considered in step 1. These exclusions could only be identified after a thorough analysis of the full text. After these exclusions, we arrived at 45 studies that met all inclusion criteria, of which 9 focused on antiphospholipid syndrome (APS), 16 on arterial thrombosis, and 20 on venous thrombosis.

categorizing thrombotic risk related to APS were found. This indicates that the literature concerning the profile of miRNA expression in APS remains limited, despite previous descriptions of miRNA activity in the pathophysiology of the disease.[31,32]

Previously, in 2016, Shazwan et al.[65] published the first systematic review identifying research papers focusing on demonstrating the differential miRNA expression profile in patients with APS. The inclusion criteria of the studies in that review ensured that the differential expression of miRNAs was investigated by the authors using biological samples from a known number of patients with APS and compared with the levels of miRNAs in biological samples from healthy individuals. However, despite analyzing a total of 357 references, only one research paper, by Teruel et al.,[33] also included in this present review, met all the requirements for analysis.[65]

In that work, Teruel et al.[33] initially conducted an in silico search to identify which miRNAs could potentially target mRNA and, consequently, change the protein levels of TF, a pro-coagulant protein that plays a crucial role in thrombosis development. Subsequently, a series of in vitro assays in monocytes from patients with APS, healthy controls and patients with SLE demonstrated an interaction between the candidate miRNAs and TF mRNA.[33] The results showed that miR-19b-3p and miR-20a-5p were decreased sevenfold in APS monocytes compared to controls. This decrease was identified as one of the factors contributing to the higher levels of TF observed in APS, as overexpression of miR-19b-3p and miR-20a-5p in vitro resulted in a 60% decrease in TF protein levels. This suggests that the down-regulation of miR-19b-3p and miR-20a-5p in monocytes could distinguish patients with APS from controls and SLE patients.[33] In this scoping review, none of these miRNAs were found in non-APS thrombosis.

A few years later, Pérez-Sánchez et al.[37] published an abstract of a study analyzing plasma samples from patients with APS and compared them with samples from healthy controls. They found that miR-19b, miR-20a, and miR-296 were differentially expressed in patients with APS. However, in contrast to the previous study, miR-19b, miR-20a, and miR-296 were upregulated at least two-fold in APS. Furthermore, they demonstrated that the combination of these three miRNAs could distinguish patients with APS from healthy donors with 70% sensitivity and specificity, and that high levels of miR-20a and miR-296 were correlated, respectively, with thrombosis recurrence and arterial thrombosis. However, complete data is not yet available.

In 2016, the same research group[38] investigated the expression profile of miRNAs associated with cardiovascular (CVD) and atherothrombotic risk in patients with PAPS compared to SLE and healthy individuals. Initially, they performed an in silico approach to identify miRNAs predicted to regulate genes involved in processes such as atherothrombosis, immune response, oxidative stress, and intracellular signaling. The most relevant miRNAs

Table 1. Differentially expressed miRNAs in more than one study for each condition.

	miRNA	References	Expanded cohort internal validation	Human biological sample type
APS	miR-20a-5p	[33–37]	Yes, yes; yes; yes; yes	Monocytes; monocytes; plasma; serum; plasma
	miR-19b-3p	[33,35–37]	Yes; yes; yes; yes	Monocytes; plasma; serum; plasma
	miR-145-5p	[32,34,35]	Yes; yes; yes	Monocytes; plasma; monocytes
	miR-124-3p	[35,38]	Yes; yes	Plasma, leukocytes
	miR-126-3p	[34,39]	Yes; yes	Monocytes; plasma
	miR-146b-5p	[32,34]	Yes; yes	Monocytes; monocytes
	miR-150-5p	[32,34]	Yes; yes	Monocytes; monocytes
	miR-17-5p	[32,34]	Yes; yes	Monocytes; monocytes
	miR-199a-5p	[32,34]	Yes; yes	Monocytes; monocytes
	miR-26a-5p	[32,34]	Yes; yes	Monocytes; monocytes
	miR-296-5p	[35,37]	Yes; yes	Plasma; plasma
	miR-30b-5p	[32,34]	Yes; yes	Monocytes; monocytes
	miR-376c-3p	[32,34]	Yes; yes	Monocytes; monocytes
	miR-4516	[34,40]	Yes; no	Monocytes; plasma
	miR-494-3p	[32,34]	Yes; yes	Monocytes; monocytes
Arterial thrombosis	miR-1-3p	[41,42]	Yes; yes	Plasma; serum
	miR-122-5p	[41,42]	Yes; yes	Plasma; serum
	miR-124-3p	[43,44]	Yes; yes	Serum; plasma
	miR-125b-5p	[44,45]	Yes; yes	Plasma; plasma
	miR-145-3p	[46,47]	Yes, yes	Serum; plasma
	miR-19-b	[46,48]	Yes, yes	Serum; plasma
	miR-223-3p	[48,49]	Yes; yes	Plasma; whole blood
Venous thrombosis	miR-126-3p	[50–52]	Yes; yes,yes	Plasma; plasma; plasma
	miR-103a-3p	[53,54]	Yes; no	Plasma; plasma
	miR-195-5p	[55,56]	Yes; yes	Whole blood; serum
	miR-197-3p	[53,57]	Yes; yes	Plasma; plasma
	miR-199a-3p	[58,59]	No; yes	Plasma; plasma
	miR-20b-5p	[58,59]	No; yes	Plasma; plasma
	miR-21-5p	[53,60]	Yes; yes	Plasma; serum
	miR-22-3p	[53,61]	Yes; yes	Plasma; whole blood
	miR-320a	[54,62]	No; yes	Plasma; plasma
	miR-320b	[54,62]	No; yes	Plasma; plasma
	miR-423-5p	[54,63]	No; yes	Plasma; plasma
	miR-424-5p	[54,64]	No; yes	Plasma; plasma
	miR-532-5p	[53,56]	Yes; yes	Plasma; serum

identified were 124a-3p, miR-125a-5p, miR-125b-5p, miR-146a-5p, miR-155-5p, and miR-222-3p. Afterward, through reverse transcription polymerase chain reaction (RT-PCR) validation using leukocytes isolated from APS, SLE patients, and healthy donors, they found that all these miRNAs were significantly decreased in neutrophils from APS and SLE patients in physiological conditions. In monocytes, miR 124a-3p and miR-125a-5p were decreased, while miR-146a-5p and miR-155-5p were increased compared to controls. No significant difference in the expression of these miRNAs was observed between APS and SLE. Additionally, the authors demonstrated that the presence of aPL-IgG or anti-dsDNA-IgG autoantibodies could modulate the expression of miRNA biogenesis-related proteins (like dicer), consequently affecting the expression of mature miRNA. They also showed that these changes in the levels of mature miRNA might impact the expression of atherothrombosis-related target molecules.[38] Interestingly, we observed in this review that changes in miR-124a-3p, miR-125a-5p, miR-125b-5p expression have also been demonstrated in non-APS-related arterial thrombosis, suggesting that these miRNAs are good candidates for assessing arterial risk in APS.

A subsequent study investigated the association of miRNAs and endothelial dysfunction in APS using a NanoString approach.[34] This study identified a total of 21 miRNAs that were either decreased (miR-299-3p, -579, -494, -221-3p, -4516, -145-5p, -146b-5p, -371a-3p, -18a-5p, -26a-5p, -199a-5p, -376c, -126-3p, -let-7f-5p, -30b-5p, -106a-5p, -17-5p, -20a-5p) or increased (miR-29a-3p, -451a, -150-5p) in monocytes from patients with APS compared to healthy controls. They also demonstrated that these dysregulated miRNAs primarily targeted mRNAs of genes involved in thrombosis-linked processes, such as inflammation, lipid metabolism, mitochondrial dysfunction, and oxidative stress.[34] In our scoping review, we observed

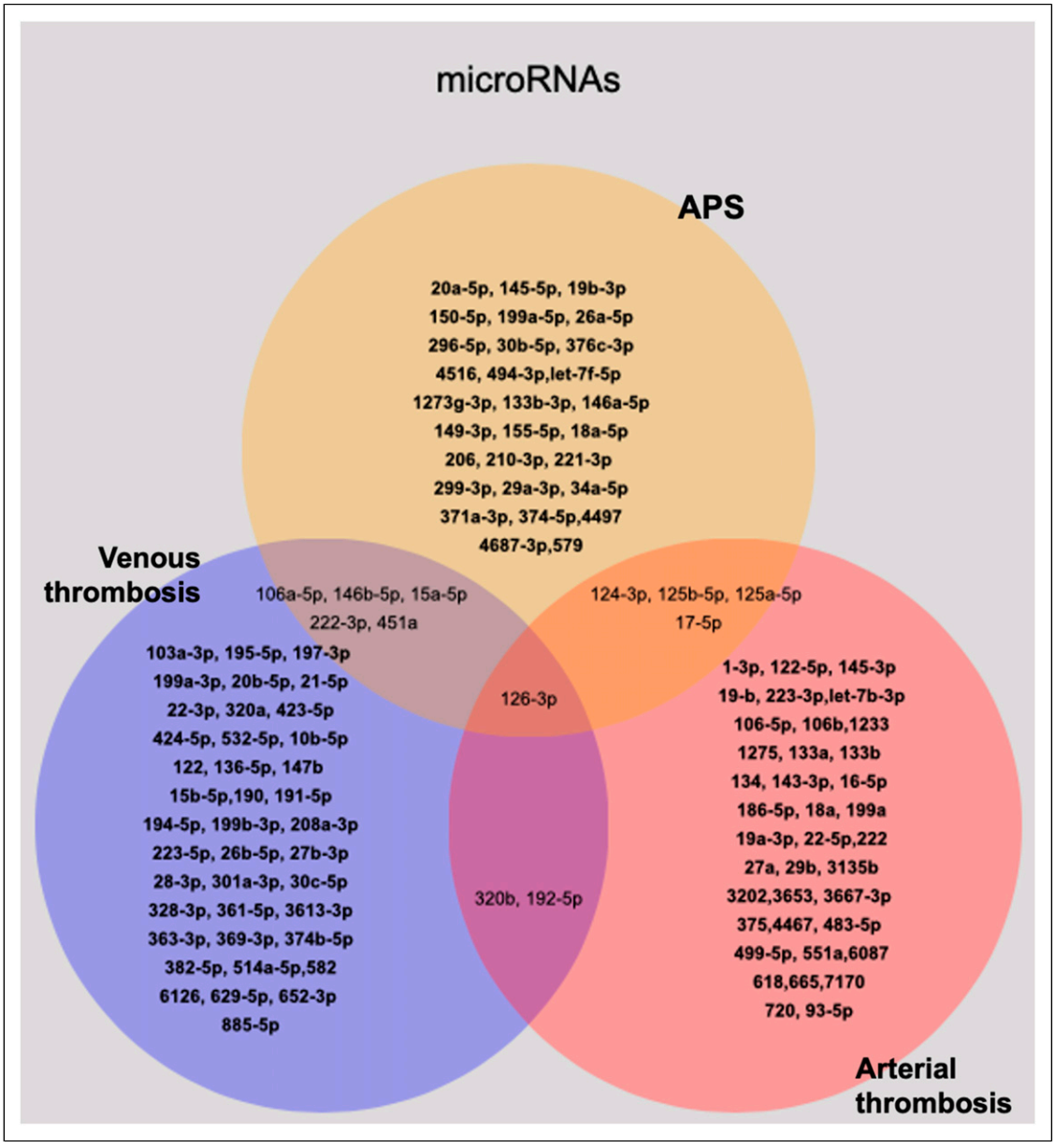

Figure 2. Differentially expressed miRNAs according to the type of thrombosis.

that miR-17-5p and miR-126-3p were also associated with endothelial dysfunction related to arterial[49,66] and venous[50–52] thrombotic processes not associated with APS. This highlights the potential of these miRNAs to participate in the establishment of a pro-thrombotic state in both APS and non-APS related thrombosis.

In 2018 the first study focused on the relevance of miRNAs as APS biomarkers was published.[35] In that work, initially the expression of circulating miRNAs was investigated in plasma samples from an exploratory cohort of patients with PAPS and healthy controls using PCR-Array as the screening technique. Subsequently, an in silico approach was employed to select, from all the dysregulated miRNAs identified by the screening, those which targeted the largest number of genes related to APS clinical manifestations (miR-34a-5p, 15a-5p, 145a-5p, 133b-3p, 124-3p, 206, 20a-5p, 19b-3p, 210-3p, 296-5p, and 374a-5p). These miRNAs were then validated in a subsequent cohort. The results demonstrated that these miRNAs had a strong discriminatory capacity between the APS and controls. Although different combinations of these miRNAs were able to discriminate patients with APS from individuals who had thrombotic events in the absence of associated autoimmune disease, here we observed that miRNAs 15a-5p, 124-3p are also described in non-APS arterial thrombosis, suggesting that thrombotic APS shares a similar miRNA profile with

non-aPL mediated thrombosis. It is worth noting that markers associated with atherothrombosis, such as TF, PAI-1, VEGF-A, VEGF-R1, and MCP-1, appeared to correlate with miRNA ratios and aPL titers, highlighting possible mechanisms underlying arterial thrombosis in APS.[35]

Recently, our group[40] demonstrated in a cohort of thrombotic PAPS, in which some patients also had pregnancy complications, that a plasma miRNA signature composed of five miRNAs (miR-149-3p, -1273g-3p, -4687-3p, -4497, and -4516) showed considerable potential (Area under curve 0.933; 95% CI 0.854–1.000). However, this was an exploratory study which lacks validation. Similarly, Martinez-Martinez et al.[36] explored the association between four miRNA, previously described in APS and clinical risk of thrombosis in primary and secondary APS. The results showed that miR-19b-3p and miR-20a-5p were differently expressed in APS compared to controls, with miR-20a-5p levels being associated with the presence of anticardiolipin antibodies and thrombosis recurrence. Here, although we observed that both miR-19b-3p and miR-20a-5p have been validated in multiple APS cohorts, in this review we did not confirm the association of miR-19b-3p and miR-20a-5p with non-APS related thrombosis.

Higher levels of two miRNAs previously described as regulators of the immune response, miR-126-3p and miR-146a-5p, were also demonstrated in SLE-associated APS when compared to SLE without APS and controls,[39] indicating that both miRNAs may discriminate the occurrence of APS, and possibly the thrombotic risk, in SLE. The present review also showed that miR-146a-5p has been described in non-APS venous thrombosis and miR-126-3p in non-APS venous and arterial thrombosis.

Recently, a specific mRNA-miRNA signature that discriminated primary APS from non-aPL mediated thrombosis and controls was described. The signature was composed of nine miRNAs (miR-199a-5p, -30b-5p, -494-3p, -17-5p, -26a-5p, -145-5p, -146b-5p, -150-5p, -376c-3p) associated with genes that regulate inflammatory responses, thrombosis development, CVD, and immune system response regulation.[32] Thus, this mRNA-miRNA network not only allowed the differentiation of APS from non-APS thrombosis and controls but also showed accuracy in stratifying patients with APS into three different clusters according to their clinical and laboratory profiles (cluster 1: thrombotic episodes, low obstetric morbidity, high aPL titers, high CVD risk; cluster 2: thrombotic and obstetric events, intermediate aPL titers, medium prevalence of CVD risk; cluster 3: thrombotic and obstetric complications, low titers of aPL, and low CVD risk).

Considering also that the expression levels of the miRNAs remain stable over time, we can say that the above-mentioned study demonstrated, for the first time, the relevance of using deep transcriptome analyses to identify an mRNA-miRNA network signature to characterize the clinical phenotype of patients with APS. Although, the authors pointed out the need for further studies to confirm their findings, in this review we found that the miRNAs described in the study (miR-199a-5p, -30b-5p, -494-3p, -17-5p, -26a-5p, -145-5p, -146b-5p, -150-5p, -376c-3p) have been identified in at least one other APS cohort, and miR-17-5p and -146b-5p have also been associated with non-APS arterial and venous thrombosis, respectively.

Shared miRNAs in APS and non-APS thrombosis

Using a comprehensive analysis of the published literature, we identified that some of the miRNAs differently expressed in APS was also described in non-aPL mediated thrombosis, which might corroborate with that miRNAs could influence APS-related pro-thrombotic profile.

One of the miRNAs previously described both in aPL and non-aPL mediated thrombosis was miR-126-3p, which appeared as the most frequently cited in the references analyzed in this review. Initially identified as a potential predictor of venous thrombosis, miR-126-3p was found to be elevated in plasma samples from patients with previous venous thrombotic episodes compared to healthy donors.[50,52] Also, the inclusion of families in the validation phase of the study of Rodriguez-Rius et al.[50] allow the authors to associate the miR-126-3p expression with intermediate phenotypes related to venous thrombosis, revealing that miR-126-3p expression was significatively correlated with the endogenous thrombin potential and factor XI levels.[50] Besides the association with the heritable genetic component of venous thrombosis, elevated expression of miR-126-3p in patients with a recent venous thromboembolic event was also correlated with an overall significative residual vein obstruction after 3 month of follow-up, indicating a poor prognostic correlated to the increased miR-126-3p in the baseline.[51]

However, a study quantifying miR-126-3p in whole blood from a cohort of patients who had suffered acute myocardial infarction demonstrated that decreased levels of miR-126-3p were a promising predictor of short- and long-term recurrent thrombotic events and could be used for ischemic risk stratification of patients after myocardial infarction.[49] This finding appears to contradict the previous one, as decreased, rather than increased, miR-126-3p expression was associated with worse thrombotic prognosis. However, differences in biological sample type, quantification methodology, and thrombotic etiology (arterial rather than venous) between this study and that of Rosseti et al.,[51] suggest that miR-126-3p may play an important role in both venous and arterial thrombosis, possibly by regulating different biological pathways.

Additionally, some miRNAs associated with APS were found to be dysregulated specifically in venous or arterial thrombosis, such as miR-106-5p, -146b-5p, -222-3p, -15a-

5p, and -451a. Most of these miRNAs were associated with increased cardiovascular risk prediction in APS and also with an increased risk of recurrent[53] or future venous thrombosis.[58] Conversely, miR-124-3p, 125b-5p, and 125a-5p were described as molecular predictors of acute ischemic stroke diagnosis and poor prognosis[43–45] and miR-17-5p was identified as a dysregulated marker during thromboembolic pulmonary hypertension,[66] suggesting their potential role as regulators of the atherothrombotic process.

Taken together, studies on APS and non-APS thrombosis reveal miRNA profiles that may have prognostic relevance in predicting the risk of thrombosis in APS. However, there are no data on the implementation of these miRNAs as biomarkers for thrombosis in clinical care.

Final considerations and conclusion

Over the past years, there has been a notable increase in studies examining the association between APS manifestations and miRNA expression levels. However, these studies remain limited. Despite the growing body of evidence implicating miRNAs as biological regulators in the development and progression of APS and non-aPL mediated thrombosis, their translation into clinical practice as reliable biomarkers remains elusive.

While circulating miRNAs hold promise as disease-associated markers due to their stability in most blood fluids, non-invasive assessment, and regulatory potential, their measurement remains challenging. Factors such as sample selection, pre-analytical and analytical variables can introduce artifacts that impair accurate quantification of circulating miRNAs, thereby limiting their potential as reliable biomarkers.[49,67] In this review, significant discrepancies were observed among the clinical studies analyzed regarding the screening and quantification methodologies for miRNA identification, as well as variations in miRNA expression due to different sample types. This may, at least in part, explain the high variability in identified microRNAs between studies. Another issue is the heterogeneity of the populations in these studies, many of which lack detailed information on patient demographics and clinical characteristics. Given that miRNA expression could be altered by anticoagulant and immunosuppressive treatments, such as warfarin, glucocorticoids, and hydroxychloroquine, as well as by the co-existence of autoimmune conditions, as seen in SAPS, the lack of detailed clinical characterization of patients could be a confounding factor.[68–71] This makes it challenging to determine whether the observed differences in the expression of circulating miRNAs in APS are due to the presence of the disease or influenced by other factors.

Furthermore, as APS is a rare autoimmune disease with a heterogeneous clinical presentation, it is important that the sample size is sufficient to distinguish miRNAs differentially expressed in the disease. The study population should also comprise patients without other autoimmune disorders, such as SLE, to avoid bias in the results.[31]

Despite differences in study methodology, we observed that consistent findings emerge when a large number of studies are evaluated together. In this scoping review, we observed that APS shares common miRNA signatures with other non-APS thrombotic conditions. This suggests that miRNA expression profiles hold promise as potential markers for assessing thrombotic risk in APS. Validation of an APS-specific pro-thrombotic miRNA signature is essential to improve the accuracy of risk assessment, diagnosis, and management strategies for this condition.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the São Paulo Research Foundation – FAPESP (grant numbers: 2020/07922-4; 2020/12630-2), FAEPEX – University of Campinas (grant number: 519292/232120), and National Council for Scientific and Technological Development (CNPq) (grant numbers: 406990/2021-2; 308452/2021-6).

ORCID iD

Camila de Oliveira Vaz https://orcid.org/0000-0002-4860-1054

Supplemental Material

Supplemental material for this article is available online.

References

1. Dabit JY, Valenzuela-Almada MO, Vallejo-Ramos S, et al. Epidemiology of antiphospholipid syndrome in the general population. *Curr Rheumatol Rep* 2022; 23: 85. DOI: 10.1007/s11926-021-01038-2.
2. Gaspar P, Sciascia S and Tektonidou MG. Epidemiology of antiphospholipid syndrome: macro- and microvascular manifestations. *Rheumatology* 2024; 63: si24–si36. DOI: 10.1093/rheumatology/kead571.
3. Ruiz-Irastorza G, Cuadrado MJ, Ruiz-Arruza I, et al. Evidence-based recommendations for the prevention and long-term management of thrombosis in antiphospholipid antibody-positive patients: report of a task force at the 13th International Congress on antiphospholipid antibodies. *Lupus* 2011; 20: 206–218. DOI: 10.1177/0961203310395803.
4. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb*

Haemostasis 2006; 4: 295–306. DOI: 10.1111/j.1538-7836.2006.01753.x.
5. Hughes G. Hughes syndrome: the antiphospholipid syndrome--a clinical overview. *Clin Rev Allergy Immunol* 2007; 32: 3–12.
6. Ruiz-Irastorza G, Crowther M, Branch W, et al. Antiphospholipid syndrome. *Lancet* 2010; 376: 1498–1509. DOI: 10.1016/s0140-6736(10)60709-x.
7. Niznik S, Rapoport MJ, Avnery O, et al. Patterns of recurrent thrombosis in primary antiphospholipid syndrome-multicenter, real-life long-term follow-up. *Front Immunol* 2022; 13: 843718. DOI: 10.3389/fimmu.2022.843718.
8. Bradacova P, Slavik L, Ulehlova J, et al. Current promising biomarkers and methods in the diagnostics of antiphospholipid syndrome: a review. *Biomedicines* 2021; 9: 166.
9. Chaturvedi S and McCrae KR. Clinical risk assessment in the antiphospholipid syndrome: current landscape and emerging biomarkers. *Curr Rheumatol Rep* 2017; 19: 43. DOI: 10.1007/s11926-017-0668-2.
10. Sciascia S, Sanna G, Murru V, et al. GAPSS: the global antiphospholipid syndrome score. *Rheumatology* 2013; 52: 1397–1403. DOI: 10.1093/rheumatology/kes388.
11. Bakow BR, Yanek L, Crowther MA, et al. Low recurrent thrombosis rates in single positive antiphospholipid syndrome regardless of type of anticoagulation. *Thromb Res* 2024; 237: 88–93. DOI: 10.1016/j.thromres.2024.03.013.
12. Dufrost V, Risse J, Reshetnyak T, et al. Increased risk of thrombosis in antiphospholipid syndrome patients treated with direct oral anticoagulants. Results from an international patient-level data meta-analysis. *Autoimmun Rev* 2018; 17: 1011–1021. DOI: 10.1016/j.autrev.2018.04.009.
13. Lim W. Thrombotic risk in the antiphospholipid syndrome. *Semin Thromb Hemost* 2014; 40: 741–746. DOI: 10.1055/s-0034-1390003.
14. Tektonidou MG, Andreoli L, Limper M, et al. EULAR recommendations for the management of antiphospholipid syndrome in adults. *Ann Rheum Dis* 2019; 78: 1296–1304. DOI: 10.1136/annrheumdis-2019-215213.
15. Chighizola CB, Willis R, Maioli G, et al. Deciphering the clinical significance of longitudinal antiphospholipid antibody titers. *Autoimmun Rev* 2024; 23: 103510. DOI: 10.1016/j.autrev.2023.103510.
16. Barbhaiya M, Zuily S, Naden R, et al. The 2023 ACR/EULAR antiphospholipid syndrome classification criteria. *Arthritis Rheumatol* 2023; 75: 1687–1702. DOI: 10.1002/art.42624.
17. Rosa dos Santos AP, de Oliveira Vaz C, Hounkpe BW, et al. Association between interferon-I producing plasmacytoid dendritic cells and thrombotic antiphospholipid syndrome. *Lupus* 2022; 31: 1067–1077. DOI: 10.1177/09612033221101731.
18. Jacintho BC, Mazetto Fonseca BM, Hounkpe BW, et al. Evaluation of a gene signature related to thrombotic manifestations in antiphospholipid syndrome. *Front Med* 2023; 10: 1139906. DOI: 10.3389/fmed.2023.1139906.
19. Verrou K-M, Sfikakis PP and Tektonidou MG. Whole blood transcriptome identifies interferon-regulated genes as key drivers in thrombotic primary antiphospholipid syndrome. *J Autoimmun* 2023; 134: 102978. DOI: 10.1016/j.jaut.2022.102978.
20. Baek D, Villén J, Shin C, et al. The impact of microRNAs on protein output. *Nature* 2008; 455: 64–71. DOI: 10.1038/nature07242.
21. Selbach M, Schwanhäusser B, Thierfelder N, et al. Widespread changes in protein synthesis induced by microRNAs. *Nature* 2008; 455: 58–63. DOI: 10.1038/nature07228.
22. Lee RC, Feinbaum RL and Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993; 75: 843–854. DOI: 10.1016/0092-8674(93)90529-y.
23. Pasquinelli AE, Reinhart BJ, Slack F, et al. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* 2000; 408: 86–89. DOI: 10.1038/35040556.
24. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; 116: 281–297. DOI: 10.1016/s0092-8674(04)00045-5.
25. Friedman RC, Farh KK, Burge CB, et al. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 2009; 19: 92–105. DOI: 10.1101/gr.082701.108.
26. Weber JA, Baxter DH, Zhang S, et al. The microRNA spectrum in 12 body fluids. *Clin Chem* 2010; 56: 1733–1741. DOI: 10.1373/clinchem.2010.147405.
27. Cortez MA and Calin GA. MicroRNA identification in plasma and serum: a new tool to diagnose and monitor diseases. *Expet Opin Biol Ther* 2009; 9: 703–711. DOI: 10.1517/14712590902932889.
28. Vickers KC, Palmisano BT, Shoucri BM, et al. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat Cell Biol* 2011; 13: 423–433. DOI: 10.1038/ncb2210.
29. Arroyo JD, Chevillet JR, Kroh EM, et al. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc Natl Acad Sci U S A* 2011; 108: 5003–5008. DOI: 10.1073/pnas.1019055108.
30. Zhang Z and Zhang R. Epigenetics in autoimmune diseases: pathogenesis and prospects for therapy. *Autoimmun Rev* 2015; 14: 854–863. DOI: 10.1016/j.autrev.2015.05.008.
31. Kotyla PJ and Islam MA. MicroRNA (miRNA): a new dimension in the pathogenesis of antiphospholipid syndrome (APS). *Int J Mol Sci* 2020; 21: 21062076. DOI: 10.3390/ijms21062076.
32. Pérez-Sánchez L, Patiño-Trives AM, Aguirre-Zamorano MÁ, et al. Characterization of antiphospholipid syndrome atherothrombotic risk by unsupervised integrated transcriptomic

analyses. *Arterioscler Thromb Vasc Biol* 2021; 41: 865–877. DOI: 10.1161/ATVBAHA.120.315346.
33. Teruel R, Pérez-Sánchez C, Corral J, et al. Identification of miRNAs as potential modulators of tissue factor expression in patients with systemic lupus erythematosus and antiphospholipid syndrome. *J Thromb Haemostasis* 2011; 9: 1985–1992. DOI: 10.1111/j.1538-7836.2011.04451.x.
34. Pérez-Sánchez C, Aguirre M, Ruiz-Limón P, et al. Ubiquinol effects on antiphospholipid syndrome prothrombotic profile: a randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol* 2017; 37: 1923–1932. DOI: 10.1161/atvbaha.117.309225.
35. Pérez-Sánchez C, Arias-de la Rosa I, Aguirre M, et al. Circulating microRNAs as biomarkers of disease and typification of the atherothrombotic status in antiphospholipid syndrome. *Haematologica* 2018; 103: 908–918. DOI: 10.3324/haematol.2017.184416.
36. Martinez-Martinez La S-MF, Chacon-Perez MJNN, Juarez-Vicuña Y, et al. Serum chemokines and miRNA levels and its association with cumulative organ damage in patients with antiphospholipid syndrome: a bench to bedside study. *Arthritis Rheumatol* 2018; 70(suppl 9): 172.
37. Perez-Sanchez CIM, Aguirre Zamorano M, Ruiz-Limon P, et al. Circulating mirnas as potential disease biomarkers in antiphospholipid syndrome patients. *Arthritis Rheumatol* 2015; 67(suppl 10): 2005.
38. Pérez-Sánchez C, Aguirre MA, Ruiz-Limón P, et al. Atherothrombosis-associated microRNAs in antiphospholipid syndrome and systemic lupus erythematosus patients. *Sci Rep* 2016; 6: 31375. DOI: 10.1038/srep31375.
39. Nagy D, Shaheen NH, Selim HM, et al. MicroRNA-126 and 146a as potential biomarkers in systemic lupus erythematosus patients with secondary antiphospholipid syndrome. *The Egyptian Rheumatologist* 2020; 42: 201–206. DOI: 10.1016/j.ejr.2020.05.002.
40. Oliveira Vaz CVGM, Ferreira AL, Nascimento Silva Vasconcelos PE, et al. Micrornas as potential biomarkers in thrombotic primary antiphospholipid syndrome. *Res Pract Thromb Haemost* 2020; 4: 950–951. DOI: 10.1002/rth2.12393.
41. D'Alessandra Y, Devanna P, Limana F, et al. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. *Eur Heart J* 2010; 31: 2765–2773. DOI: 10.1093/eurheartj/ehq167.
42. Badacz R, Kleczyński P, Legutko J, et al. Expression of miR-1-3p, miR-16-5p and miR-122-5p as possible risk factors of secondary cardiovascular events. *Biomedicines* 2021; 9: 1055. DOI: 10.3390/biomedicines9081055.
43. Zhou X and Qi L. miR-124 is downregulated in serum of acute cerebral infarct patients and shows diagnostic and prognostic value. *Clin Appl Thromb Hemost* 2021; 27: 10760296211035446. DOI: 10.1177/10760296211035446.
44. He X-W, Shi Y-H, Liu Y-S, et al. Increased plasma levels of miR-124-3p, miR-125b-5p and miR-192-5p are associated with outcomes in acute ischaemic stroke patients receiving thrombolysis. *Atherosclerosis* 2019; 289: 36–43. DOI: 10.1016/j.atherosclerosis.2019.08.002.
45. Tiedt S, Prestel M, Kautzky V, et al. Profiling of circulating miRNAS after human ischemic stroke with next-generation sequencing. *Int J Stroke* 2015; 10: 40.
46. Napoleão P, Cabral LBP, Selas M, et al. Stratification of ST-elevation myocardial infarction patients based on soluble CD40L longitudinal changes. *Transl Res* 2016; 176: 95–104. DOI: 10.1016/j.trsl.2016.04.005.
47. Gigante B, Papa L, Bye A, et al. MicroRNA signatures predict early major coronary events in middle-aged men and women. *Cell Death Dis* 2020; 11: 74. DOI: 10.1038/s41419-020-2291-9.
48. Li L, Li S, Wu M, et al. Early diagnostic value of circulating microRNAs in patients with suspected acute myocardial infarction. *J Cell Physiol* 2019; 234: 13649–13658. DOI: 10.1002/jcp.28045.
49. Hromadka M, Motovska Z, Hlinomaz O, et al. MiR-126-3p and MiR-223-3p as biomarkers for prediction of thrombotic risk in patients with acute myocardial infarction and primary angioplasty. *J Personalized Med* 2021; 11: 508.
50. Rodriguez-Rius A, Lopez S, Martinez-Perez A, et al. Identification of a plasma MicroRNA profile associated with venous thrombosis. *Arterioscler Thromb Vasc Biol* 2020; 40: 1392–1399. DOI: 10.1161/atvbaha.120.314092.
51. Rossetti P, Goldoni M, Pengo V, et al. MiRNA 126 as a new predictor biomarker in venous thromboembolism of persistent residual vein obstruction: a review of the literature plus a pilot study. *Semin Thromb Hemost* 2021; 47: 982–991. DOI: 10.1055/s-0041-1726341.
52. Bai XSJ, Zhao Y, Zhang W, et al. Detection of MicroRNA-126 as a plasma biomarker for diagnosis of DVT. *Res Pract Thromb Haemost* 2020; 4: 2292.
53. Wang X, Sundquist K, Svensson PJ, et al. Association of recurrent venous thromboembolism and circulating microRNAs. *Clin Epigenet* 2019; 11: 28. DOI: 10.1186/s13148-019-0627-z.
54. Starikova I, Jamaly S, Sorrentino A, et al. Differential expression of plasma miRNAs in patients with unprovoked venous thromboembolism and healthy control individuals. *Thromb Res* 2015; 136: 566–572. DOI: 10.1016/j.thromres.2015.07.005.
55. Jin J, Wang C, Ouyang Y, et al. Elevated miR-195-5p expression in deep vein thrombosis and mechanism of action in the regulation of vascular endothelial cell physiology. *Exp Ther Med* 2019; 18: 4617–4624. DOI: 10.3892/etm.2019.8166.
56. Qin J, Liang H, Shi D, et al. A panel of microRNAs as a new biomarkers for the detection of deep vein thrombosis. *J Thromb Thrombolysis* 2015; 39: 215–221. DOI: 10.1007/s11239-014-1131-0.
57. Zhou X, Wu Q, Hao T, et al. Expression and diagnostic value of circulating miRNA-190 and miRNA-197 in patients with

pulmonary thromboembolism. *J Clin Lab Anal* 2021; 35: e23574. DOI: 10.1002/jcla.23574.

58. Starikova I, Hindberg K, Martos L, et al. Plasma levels of miRNAs are associated with future risk of incident venous thromboembolism. *Research and Practice in Thrombosis and Haemostasis* 2017; 1: 566–567. DOI: 10.1002/rth2.12012.
59. Morelli VM, Starikova I, Hindberg K, et al. Plasma levels microRNAs and future risk of incident venous thromboembolism. *Research and Practice in Thrombosis and Haemostasis* 2017; 1: 566–567. Conference Abstract. DOI: 10.1002/rth2.12012.
60. Du X, Hong L, Sun L, et al. miR-21 induces endothelial progenitor cells proliferation and angiogenesis via targeting FASLG and is a potential prognostic marker in deep venous thrombosis. *J Transl Med* 2019; 17: 270. DOI: 10.1186/s12967-019-2015-z.
61. Liang H, Chen Y, Li H, et al. miR-22-3p suppresses endothelial progenitor cell proliferation and migration via inhibiting onecut 1 (OC1)/vascular endothelial growth factor A (VEGFA) signaling pathway and its clinical significance in venous thrombosis. *Med Sci Mon Int Med J Exp Clin Res* 2020; 26: e925482. DOI: 10.12659/msm.925482.
62. Jiang Z, Ma J, Wang Q, et al. Combination of circulating miRNA-320a/b and D-dimer improves diagnostic accuracy in deep vein thrombosis patients. *Med Sci Mon Int Med J Exp Clin Res* 2018; 24: 2031–2037. DOI: 10.12659/msm.906596.
63. Yunhe CCW, Zheng Y and Wang Q. Circulating microRNA-423-5p serves as a potential diagnostic biomarker in elderly patients with deep-vein thrombosis. *Int J Clin Exp Med* 2019; 7065–7073.
64. Wang X, Sundquist K, Elf JL, et al. Diagnostic potential of plasma microRNA signatures in patients with deep-vein thrombosis. *Thromb Haemostasis* 2016; 116: 328–336. DOI: 10.1160/th16-01-0071.
65. Shazwan MS, Muhammad Aliff M, Asral Wirda AA, et al. MicroRNA expression in antiphospholipid syndrome: a systematic review and microRNA target genes analysis. *Malays J Pathol* 2016; 38: 273–283.
66. Gong J, Yang Y, Wang J, et al. Expression of miR-93-5p as a potential predictor of the severity of chronic thromboembolic pulmonary hypertension. *BioMed Res Int* 2021; 2021: 6634417. DOI: 10.1155/2021/6634417.
67. Krepelkova I, Mrackova T, Izakova J, et al. Evaluation of miRNA detection methods for the analytical characteristic necessary for clinical utilization. *Biotechniques* 2019; 66: 277–284. DOI: 10.2144/btn-2019-0021.
68. Chafin CB, Regna NL, Hammond SE, et al. Cellular and urinary microRNA alterations in NZB/W mice with hydroxychloroquine or prednisone treatment. *Int Immunopharm* 2013; 17: 894–906. DOI: 10.1016/j.intimp.2013.09.013.
69. Kabiri Rad H, Mazaheri M and Dehghani Firozabadi A. Relative expression of PBMC MicroRNA-133a analysis in patients receiving warfarin after mechanical heart valve replacement. *Avicenna J Med Biotechnol (AJMB)* 2018; 10: 29–33.
70. Long H, Wang X, Chen Y, et al. Dysregulation of microRNAs in autoimmune diseases: pathogenesis, biomarkers and potential therapeutic targets. *Cancer Lett* 2018; 428: 90–103. DOI: 10.1016/j.canlet.2018.04.016.
71. Pierouli K, Papageorgiou L, Mitsis T, et al. Role of microRNAs and long non-coding RNAs in glucocorticoid signaling (review). *Int J Mol Med* 2022; 50: 147. DOI: 10.3892/ijmm.2022.5203.