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REVIEW ARTICLE

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Role of Circulating MicroRNA in Diagnosis of Acute Coronary Syndrome

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ABSTRACT

Acute coronary syndrome is the leading cause of mortality worldwide. For prompt diagnosis and management of this disease, several biomarkers have been utilized. Recently microRna which are suggestive of tissue damage and some are cardiospecific are paid special attention and might help in dearly diagnosis of this disease and furthermore help in preventing the resultant mortality. In this review cardio-specific microRnas and their importance are discussed

Key words: MicroRNA, Plasma, Serum, Biomarker, Acute Coronary Syndromes

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Introduction:

Acute Coronary Syndrome (ACS) remains one of the leading causes of morbidity and mortality in the western world. Early diagnosis of ACS is essential because of improvement in prognosis following timely interventions (1). Exploring novel approaches, which can complement and improve current strategies for ACS, is continuous. MicroRNAs (miRNAs) are a novel class of small, short non-coding RNA that post-transcriptionally regulate genes. The tissue- or cell-specific distribution features of miRNAs and its merit of stably existing in serum and plasma make them attractive biomarkers for ACS. An early and accurate diagnosis is the pre-requisite to facilitate rapid decision making and treatment and therefore improve outcome in ACS patients. This review feautures and condenses recent studies using circulating miRNAs as novel biomarkers for ACS including its role in diagnosis and prediction. Large multicenter studies are highly needed to surface the path for using circulating miRNAs as biomarkers for ACS from the bench to the bedside.

Acute coronary syndrome (ACS) is multifactorial, which includes any group of symptoms caused by coronary arteries' obstruction, ranging from unstable angina (UA), non-ST-segment elevated myocardial infarction (NSTEMI) to ST-segment elevated myocardial infarction (STEMI) (2,3,4) ACS is among the most serious cardiovascular diseases, making it a leading cause of morbidity and mortality worldwide (2). An accurate and early diagnosis of ACS can definitely help decrease the mortality rate (2) Thus, exploring novel approaches that can complement and improve current strategies for ACS diagnosis and management is important (2).

Currently, the diagnosis of ACS is based on elevation of (high-sensitive) cardiac troponin I or T (cTnI or cTnT), in the context of clinical and electrocardiographic (electrocardiogram, ECG) findings (5,6) Unfortunately, these biomarkers are not consistently elevated within the first hours after symptom onset, demanding tiresome measurements and delaying early diagnosis (7). In daily clinical practice, however, this scenario only pertains to a minority of patients presenting to the emergency department (ED) with symptoms suggestive of an ACS, making the early diagnosis of ACS a challenge and new diagnostic markers to improve early recognition of ACS are required (8). The prevalence of ACS greatly increases by ageing, while the accurate recognition of AMI, especially NSTEMI in the elderly, is still challenging (9).

Methodology

In this review, Pubmed, Google Scholar and Science direct were searched for the relevant articles during the last 10 years. Only human studies, clinical trials and reviews were included. The key words used for the search were " biomarkers, microRNA, acute coronary syndrome, and Acute myocardial infarction" all irrelevant studies including non-english articles, animal studies, cross-sectional and cohort studies, only abstracts and studies older than 10 years were excluded.

Micro-RNA in Diagnosis of ACS:

MicroRNAs (miRNAs) are endogenous, 19–25 nucleotide long, short non-coding RNAs that regulate genes post-transcriptionally (10,11) by binding to and repressing specific mRNA targets (12). To date, about 1000 miRNAs have been found in humans. Many miRNAs have been shown to contribute to various physiological and pathological conditions, including some cardiovascular diseases (13). MiRNAs regulate target genes by repressing their translation or inducing their degradation (14).

Growing evidence has indicated that miRNAs exist in the serum and plasma in a consistent, reproducible and stable manner, opening the possibility of using them as diagnostic surrogate markers for various diseases including cardiovascular disorders. MiRs are generally considered to act as intracellular mediators involved in many pathophysiological processes, including cardiovascular diseases (12, 15).

Recent studies have demonstrated that miRs are present in the human circulation in a cell-free form, can be detected in circulating blood, and thus may serve as a new class of blood-based biomarkers (16) Importantly, because specific miRs are differentially enriched in the various cell types of the heart, it was suggested that altered circulating levels of selected miRs might reflect different cardiovascular pathologies, and thus could be exploited as biomarkers for cardiovascular disease (17).

However, except for miR-208a, which is expressed exclusively in cardiac myocytes, the other muscle-enriched miRs that were proposed as potential biomarkers for acute myocardial infarction,

eg, miR-1, miR-133a/b, and miR-499, are not expressed exclusively in cardiac myocytes, but also are detected in skeletal muscle cells (10).

Likewise, alterations in levels of the highly endothelial cell enriched miR-126 might not reflect alterations in the coronary circulation but rather relate to systemic disturbances in endothelial cell function in patients with CAD and acute coronary syndromes (ACS) (19,20). Thygesen's study's results document that the muscle cell–enriched miR-499 and miR-133a are released from the heart into the coronary circulation on myocardial injury in patients with CAD (7) In contrast, circulating plasma levels of the endothelial cell–enriched miR-126 are reduced during the transcoronary passage in patients with ACS and evidence for myocardial injury. (21).

Circulating concentrations of the muscle-enriched miR-499 and miR-133a closely correlate with the extent of myocardial injury, as measured by hsTNT levels in the coronary circulation. In contrast, the endothelial cell–enriched miR-126 is consumed during transcoronary passage in patients with evidence of myocardial injury. Future studies will have to address whether circulating miRs may also function in intercellular communication and whether transfer of miRs between cells occurs, in addition to their potential utility as biomarkers of myocardial injury (7).

Immune cells, especially T helper (Th) cells, are critical in the development of atherosclerosis and the onset of acute coronary syndrome (ACS). To assess whether inflammation-related miRNAs (such as miR-155, 146a, 21, 125a-5p, 125b, 31) are involved in the imbalance of Th cell subsets in patients with ACS, we measured the expression of related miRNAs in patients with acute myocardial infarction (AMI), unstable angina (UA), stable angina (SA) and chest pain syndrome (CPS); analyzed the relationship between miRNA expression and the frequency of Th cell subsets; and observed the co-expression of miR-155 and IL-17A in peripheral blood mononuclear cells (PBMCs) of patients with ACS (8). Atherosclerosis is a chronic inflammatory disease that is regulated by immune cells, especially T helper (Th) cells. The imbalance of both Th1/Th2 cells and Th17/$CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells plays a critical role in the pathogenesis of atherosclerosis (10,11). According to previous studies, Th1 cells induce plaque rupture and the onset of acute coronary syndrome (ACS), and Th17 cells contribute to vascular and systemic inflammation and may modulate plaque stability, whereas Treg cells play the opposite role (12,13).

Based on the associations among miRNAs, inflammation and atherosclerosis, it is hypothesized that inflammation-related miRNAs (such as miR-155, 146a, 21, 125a-5p, 125b and 31) might show a role in the development of atherosclerosis and the onset of ACS. Furthermore, the expression of these miRNAs and their association with Th cell subgroups in patients with acute myocardial infarction (AMI), unstable angina (UA) and stable angina (SA) remain to be resolute.

Yao R et al, aimed to detect the qualified expression levels of inflammation-related miRNAs in patients with AMI, UA and SA associated to expression levels in patients with chest pain syndrome (CPS) as well as to regulate the association among the frequencies of Th cell subsets and the expression of these miRNAs (22).

Inflammation contributes to the beginning and development of atherosclerosis. In recent years, studies have indicated that a variety of miRNAs (miRNA-155, 146a, 21, 125a-5p, 125b and 31) targeted key copies of immune cells and were intricate in inflammatory diseases. To assess whether these miRNAs were convoluted in the progression of atherosclerosis, a study was conducted regarding the the relative expression of these miRNAs in both PBMCs and plasma of patients with AMI, UA, SA and CPS (22). The results demonstrated that miR-155 was downregulated, while miR-21 and miR-146a were upregulated in PBMCs of patients with UA and AMI. Additionally, the expression patterns of miRNAs in patient plasma were in line with the pattern in PBMCs, except for miR-21. miR-21 was abnormally upregulated in the plasma of patients with AMI but showed no important change in patients with UA (22). In that research the results showed that both miR-155 and miR-146a in plasma positively correlated with the expression levels in PBMCs, but miR-21 levels in the plasma did not correlate to levels found in PBMCs. miR-21 was highly expressed in injured cardiac myocytes and as well as activated immune cells, (23,24) it is guessed that the upregulation of miR-21 in patients with ACS may be due not only to over stimulation of immune cells but also to damage of cardiac myocytes (22). In this research conversely, it was observed that miR-155 was downregulated in patients with ACS, which was consistent with Fitschlerer et al (25) Th1 and Th17 cells were upregulated, whereas Treg cells were

downregulated in patients with UA and AMI, which was in harmony with Methe et al (26) Mor et al (27) and Pleister et al (28) studies results. Yao et al's research showed more studies are necessary to explain the exact relationship between miR-155 expression and Th cell distinction and the mechanisms of miR-155 variation of Th cell diversity in patients with ACS (22).

Injured heart, cardiac and skeletal muscle-specific cells would release miRNAs into circulation including miRNA-1, miRNA-133a, miRNA-133b, miRNA-499, miRNA-208a and miRNA-208b and this topic has caused a great attention. (29-42).

Among them, miRNA-499, miRNA-208a and miRNA-208b belong to the same family named the miRNA-208 family. MiRNA-208a and miRNA-208b have identical nucleotide sequences of the seed region with only three different nucleotides in the rest of the sequences. MiRNA-208a is located in an intron of the Myh6 gene and is expressed in the heart, while miRNA-208b is in an intron of the Myh7 gene and is expressed in the heart and skeletal muscle (43-52).

In the previous reviews the use of circulating miRNAs as ACS biomarkers in detail has been summarized and also several other outstanding reviews have provided sufficient informative data (43-53). Interestingly, miRNAs were also found to be present in human serum and plasma and altered expression profiles were observed in cancer and other diseases like diabetes (54-56).

Circulating miRNAs have been proposed as potentially useful novel biomarkers for detecting cardiac injury (57, 1). Regarding to previous studies, a hypothesis developed that miRNAs might be released upon cardiac injury and that the detection of cell-free miRNAs – including cardiac-related miR-1, miR-499 and miR-208a - could be used for the diagnosis of ACS (58, 59).

The usefulness of three cardio-enriched circulating miRNAs, miR-1, -208b and -499-5p, previously shown to be elevated in plasma following myocardial infarction, both for distinguishing myocardial infarction (MI) from non-MI chest pain in the acute phase and for long-term prognosis of death and development of heart failure in a large population of patients presenting with acute coronary syndrome (ACS) was assessed in a study (60).

In this research ST-elevation myocardial infarction (STEMI) diagnosis was based on ECG criteria, non-STEMI (NSTEMI) on troponin levels together with clinical symptoms of the patients. Normalization for variations in RNA input was conducted using miR-17, which is stable and abundant in plasma and is unaffected by myocardial damage (61). In this research the samples where miR-17 was not detected were regarded as poor quality RNA and those patients were excluded from the analysis ($n = 10$). (60) In this study to confirm that miR-208b and miR-499-5p are released directly from the myocardium as a result of tissue damage, analysed miRNA levels in the coronary sinus of CABG-patients before and immediately after cardioplegia was analysed. miR- 208b and miR-499-5p were undetectable in the coronary sinus before cardioplegia but became readily detectable immediately after. The levels of miR-208b were significantly higher in the coronary sinus than in the periphery after cardioplegia ($p < 0.01$). For miR-499-5p, levels were higher in the coronary sinus than in the periphery but the difference did not reach statistical significance.(60) The best discriminatory characteristics for a miRNA were observed for miR-208b, with an area under the ROC curve (AUC) of 0.82. miR – 499 - 5p had an AUC of 0.79 whereas the AUC for miR-1 was 0.57. The current cardiac marker Troponin T (TnT) had an AUC of 0.95 (60).

Oerlemans et al performed a study combined of miR-1, miR-499 and miR-21 which were compared with the combination of three established markers of myocardial necrosis (hs-troponin, myoglobin and CK-MB) in the model with clinical risk factors and the results showed that in the total population, hs-troponin negative patients and early presenting patients, the combination of miRs performed better than the combination of these three necrosis markers (1). In Oelermans' research especially three of the investigated miRs (miR-1, miR-499 and miR-21) seem very promising, as their combined diagnostic performance is statistically better than hs-troponin. Additionally, this miR-combination also led to higher AUC (area under curve) values than the combination of three myocardial necrosis markers (hs-troponin, myoglobin and CK-MB) (1). This research also showed that in patients suspected of ACS presenting to the ED within 24 h of symptom onset, circulating microRNA levels (miR-1, miR-208a, miR-499, miR-21 and miR-146a) are higher in those with an ACS and are already increased in suspected ACS patients with initially negative troponin levels and in those presenting within 3 h of symptom onset (1). Addition of miR-1, miR-499 or miR-21, significantly increased

the diagnostic value compared to hs-troponin T and these three miRs were independent predictors of ACS. Interestingly, the combination of these three miRs resulted in a higher AUC than hs-troponin T, including the hs-troponin negative patients. These findings demonstrate that circulating miRNAs hold great potential as novel early biomarkers of cardiac injury. MiRNAs might be useful for better management of suspected ACS patients, in particular those with UA pectoris and NSTEMI in whom diagnostic uncertainty is high (1). The recent research showed that using a real-world population of suspected ACS patients presenting to the ED, this study shows that circulating miRNAs hold a great potential as novel early biomarkers of cardiac injury. These findings have important clinical implications as miRNAs might be useful for better management of suspected ACS patients than can be achieved by the current biomarker of the choice, high-sensitive troponin T. This is of particular interest in patients in patients with unstable angina pectoris and NSTEMI in whom diagnostic uncertainity is high (1).

Conclusion:

In patients suffering from NSTEMI, or suspected ACS circulating microRNA are of a great importance and many lead to precise diagnosis and thus reduce the ACS mortality.

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