Circulating micro ribonucleic acids in cardiovascular disease: a look beyond myocardial injury

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Laboratory markers are of significant clinical importance in the evaluation of patients with suspected cardiac diseases. They have evolved as essential tools in cardiology over the last 50 years, i.e., lipid testing for primary and secondary prevention, creatine kinase isoenzyme MB and subsequently the more sensitive and specific cardiac troponin (cTn) testing for the diagnosis and management of acute myocardial infarction (AMI), and more recently natriuretic peptide (NP) testing for the diagnosis (in particular exclusion), risk stratification, and monitoring of heart failure (HF) (1-3). We are beginning an era when it may be possible for biomarkers to direct treatment to optimize patient management. This is already the case with cTn (1,4) but should be the final goal with all cardiac biomarkers. However, there are still some open major clinical issues, e.g., the diagnosis of myocardial ischemia. Despite huge research efforts in recent years, which were triggered by the great clinical significance and economic impact of cardiac diseases, biomarkers for the prediction of coronary artery disease (CAD) and for risk stratification in stable CAD or the general population have not yet fulfilled their manifest promise so far (5). The most established marker in this respect is high-sensitivity C-reactive protein (hs-CRP) which still remains controversial (3,6).

The 1990s were the golden era of cardiac biomarkers with the implementation of cTn and NP routine testing. Numerous additional biomarkers were discovered and immunoassays were developed which were also suitable for routine measurement. The main focus was on markers of coronary plaque formation, plaque destabilization (e.g., myeloperoxidase), intracoronary thrombus formation (coagulation and platelet activation, reduced endogenous fibrinolytic activity), and markers of myocardial ischemia (e.g., ischemia modified albumin). However, the vast majority of these markers did not make the way from research to routine application due to either preanalytical, analytical issues, or because the clinical impact for risk stratification was limited as these markers did not add much to traditional risk factors and even in multimarker approach improved risk stratification and patient reclassification only very modestly above established routine biomarkers (5). Importantly, they did not lead to direct information about how to improve patient management. More recently, copeptin, a very unspecific marker of endogenous stress, was suggested for rapidly ruling out AMI in the emergency department. However, no significant benefit compared to high-sensitivity cTn (hs-cTn) testing could be convincingly demonstrated (7). During this period also genomic biomarkers entered the field and have been particularly popular in the last two decades. Almost all of the candidate-gene era genetic biomarkers of cardiovascular disease failed to be validated after an initial period of enthusiasm (8). Rare variants may be potent but because they are rare, they do not identify large numbers of additional patients at risk. Common variants such as single genetic variants confer extremely small risks such that the usual way of calculating risk by assessment of traditional cardiovascular risk factors is better than analyses for these commonly occurring variations in deoxy ribonucleic acid (DNA) sequences. Consequently, the current consensus is not to test for commonly occurring genetic variants with weak effects (9).

Another currently very popular research topic is circulating plasma micro ribonucleic acid (miRNA) testing (10). miRNA are small (typically less than 25 nucleotides), single-stranded,

endogenous, non-coding RNAs that post-transcriptionally regulate gene expression by destabilizing messenger RNA (mRNA) or translation repression and thereby preventing proteins synthesis (11,12). Interestingly, each miRNA can target several mRNA while each mRNA can be targeted by multiple miRNAs (12). Eventually miRNAs are secreted from cells into blood being packaged in microparticles, but they are also found bound with proteins or high-density lipoproteins. The biological function of circulating miRNAs remains to be established. It is unclear whether circulating miRNAs are messengers in the cell-to-cell communication with active secretion or merely degradation products without any biological function with passive release as necrosis associated biomarkers.

More than 1,000 miRNAs have been identified in the human genome, but based on their tissue distribution and physiological function in the regulation of angiogenesis, apoptosis, and cell differentiation and proliferation miRNA-1, -133, -145, -208, and -499 appear to be most promising candidate markers for testing their diagnostic and prognostic potential in cardiovascular diseases (10). Regarding cardiac-specificity miRNAs-208 and -499 are promising, and in fact, particularly miRNA-499 and miRNA-208b were evaluated in patients with suspected acute coronary syndromes (ACS) with a rapid increase early after AMI with a high sensitivity within 3 hours from symptom onset (13). The hope still is to identify a miRNA profile (e.g., miRNA-1, -499, and -21) specific for myocardial ischemia (14), which would be of particular clinical interest. First studies, however, could not demonstrate an additive value of miRNA to hs-cTn testing for AMI diagnosis (13). In patients with CAD miRNA-132, miRNA-150, and miRNA-186 appear to be associated with ACS (15), and miRNAs (e.g., miRNA-145) appear to be associated with presence of CAD as well (16). However, the published data on the value of miRNAs for diagnosis and in particular for risk stratification in various cardiac diseases is still contradictory and inconclusive (10), and large clinical studies with appropriate pre-analytics and analytics remain to be done to demonstrate the additive value of miRNA measurement to conventional cardiac biomarker testing convincingly.

Currently miRNA testing is also time consuming with demanding pre-analytics and analytics (10), which precludes widespread routine use. It is very important to prepare cell free plasma to avoid in-vitro contamination from blood cells, but the methods of plasma preparation are frequently not sufficiently given in publications. Hemolysis must be avoided during blood collection and should be ruled out by oxyhemoglobin testing before miRNA testing in plasma samples. Whole blood must be processed immediately for plasma preparation as well. *In vitro* miRNA contamination from blood cells may be a particular problem if miRNAs are tested in stored frozen plasma samples which were not collected and prepared with the aim of testing miRNAs, and consequently this may lead to erroneous results and publications. Heparin plasma is not suitable for miRNA testing because heparin may inhibit complementary DNA (cDNA) synthesis and polymerase chain reaction (PCR), and quantitative reverse transcription PCR (qRT-PCR) is the most widely used method for circulating miRNA determination. Thus, it is also important to know whether patients were treated with heparin before blood collection. Another unresolved issue is the lack of harmonization of methods and of test result normalization (e.g., synthetic spike-in control miRNAs *vs.* expression or mean expression value of one or better a panel of commonly expressed miRNAs in a sample that are not associated with diseases), which makes it very difficult to compare published study results. Synthetic spike-in RNAs have the additional advantage that this can be also used to monitor the efficiency of RNA isolation, cDNA synthesis, and PCR amplification as well as to reveal potential presence of nucleases in the sample.

In conclusion, the role of biomarkers in cardiovascular diseases, such as AMI and HF, is very well established with cTn and NP testing as essential parts of patient evaluation with suspected AMI or HF (1,2,17,18). Given this powerful role of established cardiac biomarkers it is very difficult to demonstrate a significant benefit of add-on testing of new biomarkers compared with established markers (i.e., hs-cTn, NP, and hs-CRP) in cardiac diseases. Therefore, as with other heavily investigated novel markers, the coming years will show whether miRNA testing will make the way from research to routine use after an initial hype at the beginning of research, particularly as hs-cTn assays already entered routine use (17) and as even more sensitive research cTn assays ("ultra-sensitive") have been developed with significant clinical potential (17,19). In contrast to standard cardiac biomarker testing including ultra-sensitive cTn a lot of pre-analytical and analytical issues of miRNA testing also have to be solved before routine testing is feasible.

Footnote

Provenance: This is a Guest Editorial commissioned by Section Editor Zhijun Han, MD (Department of Laboratory Medicine, Wuxi Second Hospital, Nanjing Medical University, Wuxi, Jiangsu, China).

Conflicts of Interest: The author has no conflicts of interest to declare.

Comment on: Navickas R, Gal D, Laucevičius A, *et al.* Identifying circulating microRNAs as biomarkers of cardiovascular disease: a systematic review. Cardiovasc Res 2016;111:322-37.

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