

Early diagnosis of doxorubicin-induced cardiotoxicity: The miRNA way

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Abstract

This short commentary focuses on the use of circulating microRNAs as biomarkers of doxorubicin-induced cardiotoxicity in cancer patients undergoing chemotherapy. Cancer is one of the leading causes of death worldwide and is usually treated following different approaches; among these, anticancer drugs have a very important role. One of the most effective molecules is Doxorubicin (DOX), a two-edged sword which combines several beneficial effects with the lack of cancer-cell specificity. DOX toxicity is known to be able to compromise the clinical effectiveness of chemotherapy, strongly impacting patients' quality of life and survival, even years after its administration. It is associated with progressive disruption of cardiac function, and can progressively develop into heart failure. Despite the availability of different cardiac biomarkers (i.e., troponins, B-type natriuretic peptide) presenting several positive features like high sensitivity, cardiac specificity and low invasivity, reliable and timely risk assessment in long-term cancer survivors is still difficult to achieve. Indeed, cardiotoxicity is usually detected late when heart impairment has already occurred and is quite evident. Evaluation of left ventricle ejection fraction (LVEF) by imaging techniques is still the only reliable and accepted diagnostic tool but does not allow early prevention. In the past years, several efforts were made to identify new biomarkers for early assessment and diagnosis of cardiovascular diseases. Lately, a new class of circulating molecules, microRNAs (miRNAs), emerged, and many groups evidenced their possible exploitation as biomarkers due to their stability in several body fluids and to the possibility to detect them even at small concentrations. Despite the clinical relevancy, only a handful of studies have focused their attention on investigating the role of microRNAs in DOX-induced cardiotoxicity, mostly relying on the use animal models. Very recently, a few groups started to examine whether circulating miRNAs could represent new and reliable early cardiotoxicity biomarkers.

Cardiotoxicity markers

Doxorubicin is widely used as anti-cancer drug to treat several kinds of tumors. It belongs to the Anthracyclines family, a group of molecules originally isolated from *Streptomyces peucetius* during the 1960s. Among DOX properties it is possible to enumerate: topoisomerase II inhibition (leading to the formation of double stranded DNA breaks), direct intercalation with double strand DNA (disrupting physiological proteins/DNA binding), and, most importantly, production of reactive oxygen species (ROS) [1,2]. DOX-based therapies result in beneficial effects, leading to an increase of cancer survival rates. Unfortunately, many patients are at risk of adverse cardiac effects (Cardiotoxicity), ranging from severe ventricular dysfunction to cardiomyopathies. Cardiotoxicity can manifest even several years after treatment, and its insurgence is very difficult to predict and diagnose, thus representing a great risk, in particular for childhood-cancer survivors [3]. Accumulating evidences indicate that DOX-induced cardiac dysfunction is caused by the perturbation of several physiological pathways, particularly the DNA-damage response pathway [4]. Unfortunately, many of the mechanisms at the base of this phenomenon are not clearly understood yet, and no reliable early toxicity biomarkers are available [5,6]. Cardiac dysfunction insurgence can be assessed by mean of different approaches: angiography, LVEF evaluation by echocardiography, and endomyocardial biopsy. These techniques, though, are characterized by important limitations: low sensitivity, high invasiveness, elevated costs and, most importantly, a late detection of cardiac impairment. At present, some circulating markers of cardiotoxicity insurgence have been identified. Among them, Brain Natriuretic Peptide (BNP) and cardiac troponins are widely used in the clinical setting during and after chemotherapy.

Increased levels of plasma BNP are indicative of the presence of heart failure, a condition presenting many similarities with late-stage DOX-cardiotoxicity. The most reliable early marker for detection of heart damage is represented by cardiac troponins, which are routinely used as circulating indicators of cardiac necrosis, a condition characterizing (among other pathologies) myocardial infarction and myocarditis [5,7]. Other possible biomarkers such as tumor necrosis factor α (TNF- α), galectin-3, IL-6, ST2 and sFlt-1 have been evaluated in order to detect subclinical cardiotoxicity after treatment with anthracyclines, but they did not show any clinical value [8].

MicroRNAs

Recent studies focused their attention to miRNAs as possible circulating and tissue biomarkers of several diseases [9,10], comprising cardiac-related maladies [11,12]. Indeed, miRNAs play a key role in many biological processes, including cardiac functions like conductance of electrical signals, heart muscle contraction, growth, and pathogenesis of cardiovascular diseases [13]. Several groups employed small animal models of DOX-induced cardiotoxicity to investigate the role of miRNAs in the drug-induced toxicity, as these closely mimic the clinical setting much more than cellular models. Consequently, an extensive amount of literature on the detrimental effects of DOX

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on the hearts of small animals is present, but limited information was acquired from these miRNA-centered studies.

One of the most common issues among all investigations represented by the selection of only a few miRNAs of cardiac interest (e.g. miR-208) to be analyzed, thus limiting the novelty of acquired knowledge [14-19]. This choice obviously led to a strong limitation in novel acquired knowledge. In addition, a key issue of many studies was the less-than-optimal assessment of cardiotoxicity itself. Indeed, in many cases, heart dysfunction insurgence did not rely on direct instrumental evaluation (e.g. echography) or by dosage of circulating markers (e.g. Troponins). Thus, the correlation between miRNA expression and cardiotoxicity was not always as reliable as needed.

Circulating miRNAs

Very recently, two groups evaluated the expression of circulating miRNAs, not only in animal models [20], but also in breast cancer patients undergoing Dox-based therapy [21]. In the latter case, once more only a handful of miRNAs were selected for expression analysis basing on literature and on a previous preliminary investigation regarding the failure of one of the most promising cardiac miRNA, miR-208, as biomarker [22]. For the very first time, plasma samples of 59 female patients with breast cancer under the first round of chemotherapy with Doxorubicin were used to assess the effect of treatment on miR-1, miR-133b, miR-146a, miR-208a, miR-208b, and miR-423-5p. MiRNA expression levels were evaluated during 4 cycles of drug administration every 3 weeks. Of note, every detectable miRNA showed a trend of upregulation in all patients in comparison to baseline levels, but only miR-1 was significantly upregulated in cardiotoxicity-affected patients. This finding, together with the ROC analysis assessing the miRNA ability to discriminate Dox-affected patients from unaffected, demonstrated once again the great potential of circulating miRNAs as possible diseases markers.

A couple of interesting remarks can be made about this latter investigation. The first is that all investigated miRNAs presented a significant upregulation in comparison to baseline levels at least in one time point during treatment, regardless of cardiotoxicity insurgence. One possible explanation is that DOX could trigger an increase in expression for all considered miRNAs. Another possible explanation, though, could be represented by a normalization issue. Indeed, as commonly observed in many studies focusing on circulating miRNAs, an exogenously added miRNA, cel-miR-39, was used for qRT-PCR normalization, since plasma RNA is impossible to quantitate by standard methods. Nevertheless, the addition of a fixed amount of exogenous RNA to the plasma does not take into account possible variations in the RNA content of the samples, thus leading to possible biases in the collected data. The second remark lies once again in the choice of miRNAs selected for investigation, which was based on previous literature about cardiac disease and on the results of the first in vivo investigation about miRNAs and cardiotoxicity [14]. Since the time frame of the study was restricted to the period of drug treatment, a regulation of miRNAs known to be regulated in response to acute cardiac injury, like miR-1 and -133, could be expected. Conversely, the choice of miR-423-5p, previously associated to heart failure [23], is less clear, since anthracycline-triggered cardiac dysfunction is usually observed late after treatment [24]. An additional good candidate for evaluation could be represented by miR-34, which was indicated as regulated by Dox from several groups, even in the plasma of rats [20,25,26]. Given the high novelty and potential clinical relevance of this kind of study, probably a screening of expression of circulating

miRNAs would have been preferable to literature based selection.

Conclusion

Despite the strong clinical interest on this topic and the highly promising and encouraging results about the possible use of circulating miRNAs as cardiac biomarkers, to date there are no strong evidences regarding future exploitation of their potential “at the bedside”. The in-vivo based studies showed great limitations in the number of miRNAs chosen for investigation and on the methods of choice itself. The only investigation focusing on human subjects demonstrated that coping with the clinical setting leads to addressing a number of issues which are not replicated by animal models, like the administration of combinations of drugs and the time and money needed to adequately monitor patients during the study. However, the discovery of novel biomarkers with high and early predictive value is mandatory. Standardization of methods, long follow-up periods and screening based selection of miRNAs of interest could represent the key to open the way to new studies with a real clinical potential, hopefully leading to new therapeutic strategies to contrast cardiotoxicity and improve the quality and expectancy of cancer survivor's lives.

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