BioSCIENCE Review Article
DOI: https://doi.org/10.55684/2024.82.e00039

Can blood and salivary troponin be early signals of acute myocardial infarction?

Podem a troponina sanguínea e salivar ser sinalisadoras precoces do infarto agudo do miocárdio?

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ABSTRACT

Introduction: Myocardial injury can be identified by biomarkers extracted from cardiac cells. Troponin is one of them and can be observed in blood and saliva. Saliva is preferred due to its ease of non-invasive and pre-hospital collection.

Objective: To review whether the expression of troponin I in salivary fluid in acute myocardial infarction in emergency screening is viable compared to its plasma levels.

Method: The literature review was carried out by collecting information published in SciELO, Bibliomed, VHL - Biblioteca Virtual em Saúde, Pubmed and Scopus in Portuguese and English. The search was based on descriptors related to the topic, identified as: "troponin, acute myocardial infarction, cardiac biomarkers, salivary fluid", with AND and OR searches.

Results: Were included 109 articles correlated to the theme.

Conclusion: Diagnosis based on saliva offers many options and could be a very interesting option for elucidating acute myocardial infarction for general practitioners, nursing homes and transport services quickly, aiding early treatment.

KEYWORDS: Troponin I. Troponin T. Acute myocardial infarction.

Central Message

Myocardial injury can be identified by biomarkers extracted from cardiac cells. Troponin is one of them and can be observed in blood and saliva. Saliva is preferred due to the ease of non-invasive and pre-hospital collection. This review verifies whether the expression of troponin I of salivary fluid in acute myocardial infarction in emergency screenings is feasible compared to its plasma levels.

Perspective

Saliva-based diagnosis offers many options and can be a very interesting option for elucidating acute myocardial infarction for general practitioners, nursing homes, and transportation services quickly, helping early treatment.

RESUMO

Introdução: A lesão miocárdica pode ser identificada por biomarcadores extraídos das células cardíacas. A troponina é uma delas e pode ser observada no sangue e saliva. A saliva é preferencial pela facilidade de coleta não invasiva e pré-hospitalar.

Objetivo: Revisar se a expressão de troponina I do fluído salivar no infarto agudo do miocárdio em triagens de emergências é viável comparado aos seus níveis plasmáticos.

Método: A revisão da literatura foi feita colhendo informações publicadas no SciELO, Bibliomed, BVS - Biblioteca Virtual em Saúde, Pubmed e Scopus em português e inglês. A busca foi baseada em descritores relacionados ao tema, identificados como: "troponina, infarto agudo do miocárdio, biomarcadores cardíacos, fluido salivar", com busca AND e OR.

Resultados: Foram incluídos 109 trabalhos.

Conclusão: O diagnóstico baseado na saliva oferece muitas opções podendo vir a ser opção muito interessante para elucidação de infarto agudo do miocárdio para o clínico geral, lares de idosos e serviços de transporte com rapidez auxiliando precocidade no tratamento.

PALAVRAS-CHAVE: Troponina I. Troponina T. Infarto agudo do miocárdio.

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Conflict of interest: None | Financial source: Partly by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) – Funding code 001 | Received: 10/10/2023 | Accepted: 21/05/2024 |
Correspondence: flaviabkubrusly@gmail.com | Associate Editor: Jurandir Marcondes Ribas Filho

How to cite:
Arsego FK, Kubrusly LF, Gewehr DM, Possiedi RD, Nassif PAN, Tabushi FI, Kubrusly FB, Ronkoski ML, Cioato PP. Podem a troponina sanguínea e salivar ser sinalisadoras precoces do infarto agudo do miocárdio? BioSCIENCE. 2024;82:e039

INTRODUCTION

Cardiovascular diseases are the most common causes of mortality in the world. Its distribution does not depend on economic, social or socio-family factors. According to data from the World Health Organization in 20211, the pandemic resulting from COVID affected more than 765 million people in the world (765,903,278) with almost 7 million deaths in these 2 years (6,927,378). These numbers caused worldwide calamity, enlisting all possible human and financial efforts from virtually the entire planet. However, in recent data (2021), the same organization estimated that 17.9 million people died from cardiovascular diseases in 2019 alone (32% of the world's mortality), that is, all the mortality presented by COVID in 2 years of the pandemic was half of the mortality of only 1 year of cardiovascular diseases, and of these, 85% were due to acute myocardial infarction.[2]

Among these diseases, the most frequent it is coronary ischemia, followed by stroke.[3] In the U.S.' an estimated 116.4 million, or 46% of adults, have hypertension, with more than 2,300 daily deaths due to cardiovascular disease and 389.[4] daily deaths related to stroke. In Europe, an additional 4 million individuals died from cardiovascular disease in 2017.[4]

In Brazil, according to data from the System Unified Health Program (DATASUS)[5] and Brazilian Society of Cardiology, it is estimated that in 2017 alone, 395,700 deaths due to cardiovascular causes (www.cardiometro.com.br).[6]

Thus, the objectives of this review were to identify and evaluate the role of cardiac troponin in the saliva of patients to see if it can be considered a biomarker in the early diagnosis of acute myocardial infarction.

METHOD

The literature review was carried out by collecting information published in virtual access publishers and virtual platforming (SciELO – Scientific Electronic Library Online, Bibliomed, VHL - Virtual Health Library, Pubmed and Scopus). The search was based on descriptors related to the theme, identified through DeCS/MESH, namely: "troponin, acute myocardial infarction, cardiac biomarkers, salivary fluid", with AND or OR search. Initially, the title and/or abstract were considered and, later, the full text of those was read that most closely corresponded to the theme of the review.

DISCUSSION

Clinical and pathological definition of myocardial infarction

The term acute myocardial infarction (AMI) should be used when there is a lesion with clinical evidence of acute myocardial ischemia and with detection of an increase and/or decrease in cardiac troponin values with at least 1 unit above the 99th percentile of the upper limit of normal and with at least 1 of the following signs: new ischemic changes on the electrocardiogram; development of pathological Q waves in this electrode; echocardiogram and/or myocardial scintigraphy showing recent loss of viable myocardium or wall region with movement abnormality in a pattern consistent with ischemic cause. Pathological diagnosis is defined as cell necrosis due to prolonged ischemia.[7]

Pathophysiology of myocardial infarction

The first structural changes occur 10-15 min after the onset of ischemia, decreased cellular glycogen, relaxed myofibrils, and sarcomere rupture were observed.

Mitochondrial abnormalities are usually observed from 10 min after coronary occlusion and occur progressively and can be observed on electron microscopy.

It can take hours before it is possible to detect myocardial necrosis in humans, unlike what occurs in animals, where it is already possible to identify cell apoptosis within 10 min after the induction of ischemia.

Based on experimental studies, necrosis progresses from the subendocardium to the subepicardium over several hours. This time can be increased by the presence of existing collateral flow, reduced myocardial oxygen consumption, and the presence of intermittency between occlusion and reperfusion. Timely implementation of reperfusion therapies is able to reduce the extent of ischemic injury.

AMI classification

He can be classified according to its cause, transmurality and time of evolution

The case

There are 5 types of options. Type 1 is spontaneous infarction related to ischemia due to a primary coronary event such as erosion, with or without rupture, or fissure or dissection of atherosclerotic plaque. Or type 2, It is secondary to ischemia due to increased oxygen demand or decreased oxygen supply, and the causes include: coronary spasm, coronary embolism, anemia, arrhythmias, hypertension or hypotension, and illicit drug consumption. Or type 3, It is sudden death of cardiac origin, usually with symptoms suggestive of myocardial ischemia, but it occurs before blood samples are obtained, or shortly before the biomarkers are positive in the blood. Or type 4, is infarction associated with percutaneous coronary intervention (4a) or thrombosis of stent (4b) or even stenosis after coronary intervention (4c). Or Type 5, It is the one associated with myocardial revascularization surgery.[7,8]

Regarding transmurality

It can be subendocardial, when ischemia involves only 1 subendocardial layer, less than 50% of the thickness of the affected area of the myocardium. It is considered transmural when there is ischemic

distress in at least 50% of the thickness of the affected myocardial area.[9]

Regarding the evolution time:

It is classified as superacute, when affected in less than 6 h; acute, between 6 h and 7 days; in healing, between 7-28 days; and healed, when equal to or greater than 29 days.[7]

Clinical and laboratory diagnosis

The identification of AMI is based on the tripod: 1) symptoms; 2) electrocardiogram; and 3) classical cardiac biomarkers of necrosis (troponin I-TnI, serum creatine kinase, myocardial fraction – CK-MB, serum concentrations of total CK and myoglobin).[10] Measurement by such markers is available in practically all hospital emergency rooms.

The time factor plays a fundamental role in decisions about medical conduct. The emergency rooms of Hospitals with quality accreditation around the world, time the times Electrocardiogram Holder (from the patient's record to the electrocardiogram) and Balloon Holder (from the recording to the moment of opening of the occluded coronary artery in the hemodynamics room) in order to reduce the period of ischemia and consequent muscle necrosis. In the popular language in emergency cardiology" Time Is Muscle". After approximately 4 h of ischemia, the loss of myocytes can impair the quality of life of this patient in relation to the low ejection fraction and consequent increase in morbidity and mortality. According to Diercks et al.[12,] every minute of delay in treatment increases the mortality rate.

Frequent reports in the literature,5,11-19 show that biomarkers have been considered for diagnosis and/or prediction of acute events in asymptomatic people. They can add prognostic information in addition to cardiovascular risk. They work as a diagnostic complement in the suspicion of ischemic lesions of the heart.[11,13]

Cardiac biomarkers must meet certain criteria: 1) availability in every emergency room; 2) diagnostic accuracy confirming the presence or absence of AMI; 3) quickly measurable; 4) Acceptable cost; and 5) prognostic evaluation capacity.[11,13-20]

Therefore with regard to management, the biomarkers should help identify other possible and even reversible causes of AMI, confirm its presence or absence, estimating its severity and the risk of disease progression.

Cardiac biomarkers are currently the most important tool in the diagnosis and treatment of coronary artery disease. They may reflect the different pathophysiological processes involved in the development and progression of these diseases, such as heart volume, pressure overload, myocyte injury, inflammation, and extracellular matrix remodeling.[20,21] They allowed for significant change in form of how patients are evaluated and managed in cardiology emergency rooms.[20,22]

Historically, the first biomarkers studied were aspartate aminotransferase,21,23 lactate dehydrogenase,21,24,25 Lactate dehydrogenase isoenzymes21,26,27 and creatine kinase.[11,20,21,24,26] However, more recently others have emerged, such as natriuretic peptides in particular B-type natriuretic peptide (BNP) and N-terminal-pro BNP,28-32 soluble ST233-36, inflammatory cytokines[37,38] GDF factor 1539-41 galectin-342-45 and sensitive troponins.[20,21,27,46] Among these, cardiac troponins are the most specific and currently most used in cardiology. They are the gold standard myocardial injury markers for the diagnosis of AMI due to their high sensitivity and specificity.[13,47,48] As reported by authors,47,49 troponin I (cTnI) and troponin T (cTnT) have practically absolute specificity for myocardial damage and also for clinical evolution, becoming fundamental for early detection of myocardial injury. They allow earlier and more accurate diagnosis of AMI, especially when the electrocardiogram is not altered, accelerating treatment and, thus, helping to reduce definitive myocardial injury.[47]

Ventricular remodeling

After the AMI with long ischemia time, it is possible that a large number of cells lose their vitality and viability in response to this aggression. The mammalian heart has a variable capacity for regeneration after suffering ischemic injury, which can range from an adequate response to the healing of the injury to a process when the lost cells are replaced by fibrotic tissue, incapable of contraction, followed by adverse cardiac remodeling that evolves with impaired systolic function and consequent heart failure.[50,51]

Once the necrosis process begins, it follows inflammatory response. The healing process can be divided into 3 phases. First, there is a pro-inflammatory wave along with a component of degradation of the extracellular matrix, in order to reduce the amount of necrotic debris. Then, the anti-inflammatory and pro-reparative phase follows with the objective of reducing the inflammatory process and stimulating mesenchymal cells in the extracellular matrix deposits to carry out the healing process. Finally, the maturation phase occurs, where the repairing cells are removed. Several cells are involved in this coordinated response, including cardiac myocytes, neutrophils, macrophages, fibroblasts, endothelial cells, and nerve cells.[50,52]

Systolic dysfunction is related to the inhibition of contractile proteins permanently, requiring at least 20 min of ischemia so that the contractile protein can no longer regenerate spontaneously.[52]

Cardiac fibrosis is divided into 2 phases after the occurrence of infarction, restoration fibrosis and reactive fibrosis. The restoration is the one that occurs first, to prevent the rupture of the heart chamber. However, mechanical stress leads to the expansion of this fibrosis in areas remote to the infarction area, which is reactive fibrosis that leads to changes in cardiac chamber compliance and increased

ventricular stiffness, compromising contractility and systolic function. This entire area of fibrosis can also lead to abnormalities in cardiac electrical conduction, predisposing to re-entry arrhythmias, such as sustained ventricular tachycardia, bundle branch blocks, among other electrical disturbances, which contribute to the risk of sudden death in these patients.[51,52]

Troponins and other enzymes as cardiac biomarkers

Necrosis of cardiac tissue usually begins after 20 min of continuous ischemia and, when this happens, the process of temporary or permanent impairment of cardiac function begins53 by myocyte necrosis. Similarly, other injuries occur, such as myocardial trauma, neurohumoral overstimulation, inflammation, apoptosis, or a combination of these factors.[20]

Troponin levels are the gold standard for the detection of cardiac injury and evaluation of myocardial necrosis,20,48,54,55 Troponin levels in patients without myocardial injury are very low or undetectable.[47]

Altered cardiac troponin is associated with higher in-hospital mortality, regardless of other predictive variables, as observed in a clinical trial conducted by Peacock et al.[54] These authors reported the association between cardiac troponin levels and adverse events in more than 67,000 patients with acute cardiac decompensation and heart failure.[54] They concluded that patients with high troponin levels had lower ejection fraction, lower systolic blood pressure, and higher in-hospital mortality rates, which confirms the importance and usefulness of quantifying troponin levels in predicting outcomes.[54]

The study by Latini et al.[56] corroborates these conclusions, showing that most patients in a state of advanced chronic heart failure increase troponin values, thus revealing permanent myocardial changes due to volume overload in these patients.

Troponin consists of 1 complex of 3 regulatory proteins: cTnI, cTnT, and troponin C.[47,48,57] The 3-unit complex, along with tropomyosin, is located in the actin filament and is essential for calcium-mediated regulation of the skeleton and contraction of the heart muscle.[48] During muscle contractions, this complex controls the interaction mediated by calcium and myosin, i.e., it functions as a calcium ion receptor to induce structural changes in actin and myosin, thus producing contraction.[47,58,59]

There are specific isoforms of troponin I, T and C in the tissues.[48] While troponin C binds to calcium and is expressed by cardiac cells and skeletal muscle (thus unsuitable for the purpose of diagnosing AMI), troponin I, which inhibits actin-myosin interactions, and troponin T, which binds the troponin complex to actin by binding to tropomyosin, are unique to myocardial muscle.[47,48]

In particular, cTnI has in myocardial tissue the post-translational tail isoform of 32 amino acids at the N-terminus. This sequence, as well as differences of more than 40% with the sequences of other isoforms, make it possible to generate antibodies highly specific monoclonals, thus increasing the specificity of cTnI detection.[48]

Regarding cTnT, it is controlled by 3 genes, which produce 4 troponin T isoforms with variable sequences near the N-terminus and C-terminus regions. Highly specific antibodies are produced to the N-terminus of this cTnT isoform.[48] During MI or any other type of myocardial injury, the troponin complex is broken down and the individual protein with the cTnI and cTnT components is released into the bloodstream. According to Danese and Montagnana21, this leads to increased levels of troponin in peripheral blood at 3-24 h and up to peak values within 10-20 h for cTnI and 15-120 h for cTnT, returning to normal values after 10 days (cTnI) and 14 days (cTnT).[21,47,60]

For these reasons, and because of its high sensitivity and specificity for myocyte necrosis,21,47,48,61 cTnI and cTnT are used for diagnosis and follow-up of AMI. cTnI is considered a highly specific marker of coronary events, since its values are low.[47,48]

Troponin biomarkers can also help differentiate between myocardial and skeletal muscle injury, even when concentrations of other biomarkers, particularly creatine kinase, are normal or slightly increased.[61] It is important to remember that estimates of cardiac troponin in cardiomyocyte damage are nonspecific as to its cause.

Thus, differential diagnoses of elevated troponin values should be considered along with electrocardiography and clinical data. These, together, will complement the clinical evaluation with symptoms suggestive of AMI.[47]

Regarding the role of troponin as a cardiac biomarker, international consensus of the European Society of Cardiology and the American College of Cardiology recognized elevations in troponin as fundamental for the diagnosis of AMI.[48] However, in order to avoid false-positive results, they also established criteria regarding the cut-off values for the diagnosis of AMI with these cardiac biomarkers above the 99th percentile of the upper reference limit.[21,61] This percentile can be adjusted based on the patient's gender, age, or race.[48]

Biosensors for detecting troponins in saliva

The detection of cardiac troponins for clinical and therapeutic diagnosis has been performed using different technologies, such as electrochemical enzyme-linked immunosorbent assay (ELISA),[62,63] fluoroimmunoassay, chemiluminescent assay, surface plasmon resonance detection, or colorimetric protein matrix.[47,48,62] Most of these tests are based on rapid immunoassays, requiring minimal handling and sample preparation.[62] However, the need for greater sensitivity of cardiac troponin for diagnosis has led to the continuous development of other biosensors.[4,47,64-67] Due to the low concentration of troponin, these biosensors should have high sensitivity and specificity, low cost, low energy consumption,

and should preferably be miniaturizable for use outside the hospital environment.

There are already several sensitivity biosensors reported in the literature to quantify troponins in serum or whole blood in short times (<30 min), achieving low values for detection (up to a few pg/mL)[68-70] or even below 1 pg/mL71 (LOD - Limit of Detection - The lowest concentration that can be detected with statistical significance).[55,62,72-82]

To reduce the need for blood draws, efforts have been made to develop new non-invasive methods for detecting and quantifying troponins in saliva samples. Saliva is increasingly recognized as a diagnostic fluid that offers advantages in sample collection compared to blood because it is painless, less invasive, convenient, ready to be collected,[63,77,83-92] it is easier to collect for diagnostic confirmation90 and there is the possibility of collection by the patient himself in a home environment.

There are reports in the literature of a correlation between serum troponin values and unstimulated total saliva during the occurrence of AMI, compared with their respective values in control subjects.

Table 1 summarizes the studies that reported a positive correlation between serum and salivary troponin levels.

Other studies have shown lower correlations between these values, which determines the need for further studies to quantify salivary troponin compared to blood troponin for the diagnosis of AMI.[96-99] Additionally, the concentration of troponin in saliva is much lower than in serum,92 requiring devices with LOD with higher sensitivity capable of detecting these extremely low concentrations.

In addition, due to the significant differences between the properties of serum and salivary fluids, including the high viscosity of saliva, even a device and method with low serum LOD may not be able to detect the same troponin value in saliva. These challenges need to be solved before saliva can be used as a sample for point-of-care diagnostics (Table 2).[100]

Until recently, the evaluation and management of coronary syndromes depended mainly on the clinical presentation and the electrocardiogram, with biomarkers playing only a peripheral role.[11] Recently, cardiac biomarkers have played a fundamental role in the diagnosis and follow-up and prognosis of acute coronary syndromes.

Diagnostic tests using biomarkers in saliva can be used within a wide range of clinical applications, from diagnosis, prognosis, pre-hospital diagnosis through home measurement systems, greatly anticipating treatment in addition to risk determination and stratification in hospital screening programs.

The studies question whether salivary troponin can be an indicator of intra- and prehospital myocardial injury, and whether salivary troponin concentration is correlated with blood concentration. Few successfully report quantification of troponins in saliva, especially raw saliva. Regardless of the extremely low concentration of biomarkers at this site, the biosensors reported in the literature for quantification of troponins in serum and blood, reached LOD in the pg/mL range, increasing the possibility of detection in saliva.

Another fact is that raw saliva samples are still challenges for the determination of troponins due to their high viscosity and the interference of raw saliva components. Microfluidics101,102 for preprocessing and passive filtration103,104 can reduce the viscosity of raw saliva making it an appropriate diagnostic fluid.

As other examples, saliva has been widely accepted for detection and quantification from cortisol to glucose88,105 including even diagnosis of some types of malignant tumors.[106,107] It, for its easy, fast and non-invasive collection, and the fact that part of its constituents are derived from local capillary blood, has the potential to become a source for the diagnosis of AMI in cardiac emergencies.[62] Its non-invasive accessibility allows it to be collected directly by patients in the prehospital phase of acute ischemic cardiac events108,110,111 making the method attractive in the diagnostic arsenal of emergency medical teams and the patient himself in his home phase or in an ambulance. Just as blood glucose measurement can be performed by patients at home

Table 1 —- Summary of studies reported in the literature that highlight the correlation between troponin values in serum and unstimulated total saliva after AMI

First author	Troponin	Detection	Troponin concentration				Correlation
			Whey		Saliva		
			MI (ng/mL)	Control (ng/m)	AMI (ng/mL	Control (ng/m)	
Mishra, V93	I	RayBio cardiac troponin-I Elisa kit	4270 ± 1790 *	158 ± 50	0.67 ± 0.10 *	0.160 ± 0.05	p < 0.001
Wirzaii-Dizgah, I94	I	Monobind Inc (USA) ELISA kit	4070 (2140-8980) *	140 (80 -230)	0.71 (0.52 - 1.07) *	0.19 (0.08 - 0.27)	p = 0.004 (total number of individuals); p=0.001 (AMI group)
Wirzaii-Dizgah, I95	T	USCG Life Science Inc (China) ELISA kit	0.2195 ± 0.235 **	0.0257 ± 0.031	0.0316 ± 0.025**	0.0089 ± 0.012	p < 0.023

* = 24 h after AMI; ** = 2nd morning after AMI

TABLE 2 — Comparison of biosensors reported in the literature for the detection of CTNI in saliva

First Author	Troponin	Detection method	Detection platform	Sample volume (µL)	Minimum Detected (ng/MI)	Linear dynamic range (ng/mL)	Analysis time
Park J103	I	Bead-based ELISA with spectrophotometric detection at 450 nm	OS beads coated with monoclonal mouse anti-cTnL antibodies	200	0.51	1.47-50.1	<20 min
Chekin F91	I	Electrochemical-differential pulse voltammetry (DPV)	Aptamer modified GC/N-prGO-COOH/PEG electrodes	Non-defined	1x10-3	1x10-3 - 100	>30 min
Rezaei Z104	I	Fluorescence spectroscopy in the 220 nm - 350 nm range	Plexcitonic hybrid system based upon GNP-QD and specific aptamer	250	7.58x10-6	9.60x10-6 - 0.06	30 s

in the face of suspicious symptoms, salivary troponin measurement could also speed up the decision-making of the patient and of a possible emergency team.[109]

CONCLUSION

Saliva-based diagnosis offers many options, with all variants of medical tests being a very interesting option for application in the exclusion of myocardial infarction for general practitioners, nursing homes54 and ambulance transport services. When a first diagnostic result is confirmed, the determination of enzyme curves can accelerate the treatment of critically ill patients with AMI.

Authors' contributions

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