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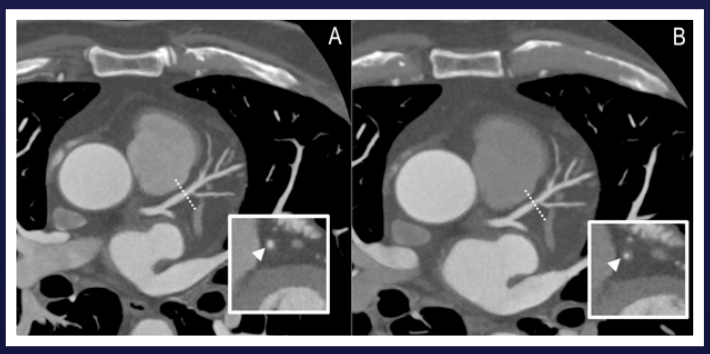

Figure 1 – *Coronary artery disease (CAD) progression on coronary computed tomography angiography (CCTA) in a 58-year-old male presenting a very mild CAD in the proximal left anterior descending coronary artery at baseline (A). Evident disease progression is seen at 13 months at the same site, with moderate luminal stenosis (B) best appreciated in the vessel's transverse plane (arrowhead). Page 397*

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Editorial

Challenges of Translational Science

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The introduction of standardized therapies for the treatment of major cardiovascular diseases, such as heart failure and myocardial infarction, significantly reduced mortality. However, cardiovascular diseases are still one of the main causes of morbidity and mortality worldwide. Consequently, a large number of experimental studies are published regarding this subject. These studies investigate, in addition to the mechanisms involved in the genesis of cardiovascular disease, potential therapeutic targets, as well as interventions that are beneficial in reducing the size of the ischemic lesion and the progression of cardiac dysfunction and, consequently, decrease mortality.

In some situations, the results of preclinical research are reproducible in clinical studies. As an example, we could cite the influence of obesity on the process of cardiac remodeling. It is accepted that the remodeling process plays a critical role in the onset and progression of cardiac dysfunction secondary to different stimuli.[1] Experimental studies have shown that obesity induces ventricular remodeling,[2] since it has been confirmed in clinical studies.[3,4]

However, not infrequently, the success of the experimental treatments studied does not replicate when applied to clinical studies. In this sense, the analysis of some recently published papers in the *Arquivos Brasileiros de Cardiologia*, in the field of basic / experimental research exemplifies this phenomenon.

One of the most interesting topics in cardiology today are the strategies to attenuate ischemia / reperfusion injury (RI). Thus, in the rat model, hypothyroidism, associated with decreased levels of nitric oxide, protected the heart from IR injury. Similarly, physical exercise,[6] administration of tramadol[7] and consumption of nitrate[8] were effective in decreasing IR-induced injury in the rat model. These and other positive results from experimental studies are obfuscated by the fact that to date, cardioprotection strategies in clinical studies have shown negative results.[9]

The reasons for this frustrating inconsistency between experimental and clinical studies are many and reflect the full complexity of translational research. The first difficulty that can be pointed out is in relation to the animals used in the experimental studies. We can observe that much of the research uses small animals, usually rodents, as the target of the intervention. It is well known that the physiology of the cardiovascular system of small rodents is not necessarily the same as that of humans. High heart rate and differences in cellular ion fluxes, including calcium flux, do not allow the extrapolation of the results of these studies to humans. In addition, small rodents used in laboratories are genetically very homogeneous and, in some situations, are virtually the same.[10] Although the experimental model with large animals is more similar to human and large animals are genetically more heterogeneous, research involving models with larger animals is much more difficult to conduct.

Another point to highlight is that most of the experimental studies use young and healthy animals, which differs significantly from the reality of the patients included in the clinical studies. It is not uncommon for patients with cardiovascular disease to have more than one comorbidity. Even when comorbidities are inserted in the experimental models, they are not treated, as is the case with patients.[11] Treatment of these comorbidities involves the use of several medications, such as angiotensin converting enzyme inhibitors and beta-blockers, which also exert a cardioprotective effect. Accordingly, some pathological modulating pathways of pathological processes may already be blocked, even partially, by such medications. Thus, the insertion of one more cardioprotective factor in clinical studies may lead to very subtle improvements in outcomes, which are not statistically significant. In addition, cardioprotection involves the activation of multifactorial mechanisms and the presence of comorbidities and medications can modify the individual panel of gene expression of patients.[12]

We must also consider that the most common experimental model to study the pathophysiological consequences of myocardial infarction is the external ligation of the anterior descending artery, whereas in humans, coronary occlusion is the result of a long inflammatory process. In this way, the activated signaling pathways can be completely different. Even when models of ApoE knockout mice are used in the induction of atherosclerosis, this happens in an artificial way and does not reproduce the reality of what happens in humans.[13] Additionally, lipid metabolism in mice is different, since in mice there is a predominance of lipoprotein HDL, whereas in humans there is a predominance of LDL and VLDL.[13]

In addition, regarding the difficulties of reproducing experimental results in clinical studies in myocardial infarction, in humans one of the pillars of the treatment is the institution of reperfusion as soon as possible. This measure alone is already successful in decreasing infarct size and mortality, and the beneficial effect of any additional intervention can be minimized in clinical studies.[14]

Another limitation of translational research is the transposition of the doses used in animals to humans, as well as the time of

Keywords

Animal Models; Ischemia-reperfusion injury; Myocardial Infarction; Signaling Pathways.

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onset and duration of treatment. The drug or substance should achieve adequate concentration in the target tissue, while at the same time it can not be excessive, because of the risks of side effects. As a consequence, subtherapeutic doses may sometimes be used in clinical studies. An example of this is the PREMIER study, which evaluated the effect of selective inhibitor of matrix metalloproteinases PG 116800 in patients after myocardial infarction. In this study, due to the risk of the onset of musculoskeletal syndrome, one of the side effects of the administration of inhibitors of matrix metalloproteinases, a dose lower than that shown to be effective in preclinical studies in pigs was used. Thus, despite promising therapy, this study did not show any effect of PG 116800 on clinical outcomes.[15]

Therefore, although the contributions of experimental research in the area of cardiology are unquestionable, the challenge that remains is getting a greater transposition, in the minimum of possible time, of the results obtained on the workbench to the clinical practice.

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Data Sharing: A New Editorial Initiative of the International Committee of Medical Journal Editors. Implications for the Editors´ Network

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**On behalf of the Editors' Network European Society of Cardiology Task Force.*

Abstract

The International Committee of Medical Journal Editors (ICMJE) provides recommendations to improve the editorial standards and scientific quality of biomedical journals. These recommendations range from uniform technical requirements to more complex and elusive editorial issues including ethical aspects of the scientific process. Recently, registration of clinical trials, conflicts of interest disclosure, and new criteria for authorship - emphasizing the importance of responsibility and accountability-, have been proposed. Last year, a new editorial initiative to foster sharing of clinical trial data was launched. This review discusses this novel initiative with the aim of increasing awareness among readers, investigators, authors and editors belonging to the Editors´ Network of the European Society of Cardiology.

Keywords

Editorial Ethics; Scientific Process; Data Sharing; Clinical Trial; Trial Registration; Authorship; Conflict of Interest; Big-data; Scientific Journals.

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The Editors´ Network of the European Society of Cardiology (ESC) is committed to promoting the implementation of high-quality editorial standards among ESC National Societies Cardiovascular Journals (NSCJ).[1-4] NSCJ play a major role in disseminating high-quality scientific research. However, they also play a relevant role in education and harmonization of clinical practice.[3] Most NSCJ are published in local languages, but many have English editions and have gained international scientific recognition.[1-4] NSCJ well complements official ESC journals and, altogether, provide an effective means to disseminate European cardiovascular research. In a globalized and highly competitive editorial environment, promoting high quality editorial standards remains of paramount importance to increase the scientific prestige of NSCJ.[1-4] From its conception, the Editors´ Network strongly advocated for the adherence to the uniform recommendations of the International Committee of Medical Journal Editors (ICMJE).[1] In its mission statement document the Editors´ Network committed to adapt NSCJ to follow these general editorial recommendations.[1] However, NSCJ are highly heterogeneous in scope and contents and these new recommendations should be embraced progressively, considering currently existing editorial policies and the editorial freedom of the NSCJ.[1-4]

Ethical issues play a growing role in ensuring the credibility of the scientific process.[5-13] Biomedical research relies on trust. However, transparency also represents a major tenet in the scientific process.[5-8] This review will discuss the new editorial recommendations on data sharing

issued by the ICMJE.[14] Novel ICMJE recommendations always appear as provocative, and often as too ambitious, when initially presented. Moreover, implementation of editorial changes is rather demanding from a technical and logistical viewpoint. Adherence to novel editorial initiatives is challenging not only for editors, but also for the entire scientific community. Therefore, many Editors have a natural tendency to avoid stepping ahead as early adopters of new editorial experiments and usually prefer to keep moving within their comfort zone until the sea change has matured.[1-4] However, experience has taught us that all editorial initiatives developed by the ICMJE eventually prevailed and played a critical role in maintaining the credibility of the scientific process.[9-13] Highly successful recent examples include trial registration, a conflicts of interest initiative and the new requirements for authorship.[9-13]

The novel ICMJE recommendations on data sharing[14] are discussed herein from a didactic perspective with the aim to provide new editorial insights and, hopefully, to be progressively adopted and implemented by the NSCJ.

Sharing clinical trial data: the new ICMJE proposal

The ICMJE considers that there is a moral obligation to responsibly share the data generated by clinical trials.[14] The rationale underlying this global endeavor is that patients have assumed a risk by accepting to participate in a trial. Accordingly, making the obtained data publicly available represents a responsible initiative to facilitate the advancement of science. Sharing the data would increase trust in the conclusions reached by trials. Indeed, data sharing allows confirmation of the results by independent research.[14] Furthermore, new hypotheses may be pursued by different groups of investigators. This initiative may foster the leveraging of data to answer different research questions not contemplated in the original study. If science becomes an open process, then many researchers would benefit by taking advantage of reliable data generated somewhere else. Therefore, data sharing emerges as the best way to ensure that all the information gathered by trials is made freely and widely available, so that it can be readily used to advance scientific knowledge.[14] The use of previously collected data to further advance science is difficult to criticize. As discussed, this honours the volunteerism of the patients who signed up and consented to participate in a trial.

Governments, funding agencies, scientific societies, the industry and even the lay society growingly demand sharing clinical trial data. Therefore, the ICMJE suggests that editors should help to meet this ethical obligation by devising new editorial policies specifically addressing this issue.[14] Proponents of open science should be pleased by this new editorial requirement of sharing clinical trial data.[14]

The first consideration is to clarify what a clinical trial is exactly. According to the ICMJE definition, a clinical trial is a study that prospectively assigns people to an intervention in order to assess the cause-and-effect relationship between that intervention and the ensuing health outcome.[5]

The ICMJE considers that sharing *de-identified* individual patient data should become part of the publication process of clinical trials.[14] This strategy protects patient´s confidentiality rights. The requirement, however, is restricted to the individual-patient data underpinning the results presented in the published article. Importantly, a clear plan for data sharing should be disclosed at the time of initial trial registration and should be also presented at the time of manuscript submission. The proposal requires clinical trialists to declare that they will share their data publically as a prerequisite for publishing the trial.[14] They should promise to freely release individual patient raw data at the time they submit the manuscript for consideration.

It is important to keep in mind that clinical trial registration was a previous ICMJE editorial initiative aimed to address problems related to publication bias (selective publication of positive trials), endpoints inconsistency and redundant research.[9,10] Potentially, public repositories provide an optimal tool not only for initial trial registration but also for individual-patient data sharing. From now on the plan for data-sharing would be an important step of the clinical trial registration initiative.[9,10,14] Details on whether the data would be freely available upon request, or only after a formal application that eventually will be approved after an agreement is reached on data use conditions, should be presented. Finally, it has been proposed that the data should be made public no more than 6 months after publication of the original study in the journal.[9,10,14] *Clinicaltrials.com*, a widely used non-for profit scientific repository,[9,10] has already adapted its registration platform to specifically clarify data-sharing plans at the time of clinical trial registration.

Obviously, this editorial initiative may have profound consequences on the planning, conduction and reporting of clinical trials and, in fact, may deeply influence research and publication strategies.[14] As a result, the idea is to implement this requirement for any clinical trial that begins to enroll patients 1 year after the official adoption of this editorial policy by the corresponding journal.[14] The initiative will also have major implications for the editorial process. Indeed, Editors are supposed to monitor the data sharing process and, eventually, address potential irregularities. These might include requests of clarification to the authors, notification to academic institutions, publication of expressions of concern or even retractions.

Finally, the ICJME acknowledges that the rights of the investigators and sponsors should be protected.[14] Moreover, credit to the original report should be granted by including a unique identifier of the data set. It is emphasized that credit should be always given to the original investigators that posted the data after publication of their research. Furthermore, additional investigators using these databases should request collaboration of the investigators that originally collected the data to ensure adequate data interpretation, management and analysis.

Challenges of data sharing

Although it appears clear that this initiative will further improve transparency and the overall integrity of the scientific literature, some remaining issues need to be addressed.

There is inherent resistance to embrace open science initiatives from some academic institutions or investigators that defend the idea of exploiting their own data.[15,16] Until now clinical researchers were discouraged from working with clinical trial data they did not generate themselves.[15,16] Likewise, trialists tended to see trial data as their personal property and would routinely refuse requests for data sharing. In fact, until very recently most researchers and pharmaceutical industry groups were opposed to making raw data available after trial publication. This practice, however, differs from other disciplines (as genomics or economics) where data sharing has been common place for a long time.[15,16]

Obtaining reliable, high-quality original data requires a major research effort. Allowing a sufficient period of time from the time of article publication to the need to share the raw data would give original investigators the possibility of publishing additional subgroup analyses from their own data.[14] This new proposal will further increase the pressure on academic investigators that frequently do not have the required resources to publish their subsequent analyses and require time to prepare the new manuscripts.[14] Notably, most researchers have no experience with the process of releasing or dealing with public data. Furthermore, the effort and resources required to organize the raw data in a way that would be comprehensible to other investigators remain a cause of major concern.[14] This would require technical support and adequate funding.

Data-access to non-trial researchers may disclose problems not recognized by the initial investigators. Although this will increase transparency and, therefore, trust in trial results, it might also generate confusion and undue scientific controversies. It is difficult to envision how the new researchers will gain the required detailed knowledge of the complicated datasets enjoyed by the original trial investigators.[14] A reliable assessment of the data requires a deep knowledge on the study background and to be able to properly address many nuances and practical considerations. These include precise information on the way variables were defined, how data was collected and how results were finally coded and entered into the database. The initiative might be fraught with problems related to incorrect analysis resulting in inaccurate results and erroneous interpretations, potentially damaging science.[14]

Finally, Editors, already deluged with work, will need to check that all of the raw data of the published articles eventually has been released as promised. Different results may emerge from misconceptions regarding what data should be analysed to answer specific questions.[14] If there are differences in results, it will be difficult to decide which analysis provides the most accurate reflection of the data. This could generate undue *scientific noise*, with contradictory results and rectifications, which may generate confusion and frustration in the scientific community. Finally, this may also promote the simultaneous publication in several journals of conflicting results from the same database by different groups.[14]

As many issues still should be clarified, the ICMJE asked for feedback on its preliminary editorial proposal on clinical trial data sharing.[14] Obviously, the initiative will only gain the required maturity from the experience gained during its adoption and implementation.

Previous initiatives on data sharing

Several leading academic entities previously have worked in this field. The *British Medical Journal* pioneered an editorial initiative of data sharing.[17] In 2012 this policy took effect only for trials on drugs and devices but, in 2015, the requirement of data sharing on request was extended to all submitted clinical trials.[17] It has been proposed that individual patient data may also be of major value during the *peer review* process by permitting independent verification of the results before final publication.[18] Although this initiative might be of potential value most reviewers are already deluged with work and this extra task could generate fatigue and burn out phenomena. In addition, many good clinical reviewers do not have the expertise required to manage data and to perform confirmatory statistical analyses.[18] Some journals, as JAMA, previously developed some related editorial initiatives including the request for independent statistical analyses by an academic statistician of industry-sponsored trials.[19]

The World Health Organization (WHO) and the Institute of Medicine (IOM) previously made important declarations on clinical trial transparency. In this regard, the IOM issued specific guidelines for trial data sharing.[20] WHO initially presented a statement on public disclosure of clinical trial results and, subsequently, encouraged sharing of research datasets whenever appropriate.[21-23] More recently, the WHO developed global norms for sharing data and results during public health emergencies, with special focus on clinical, epidemiologic, and genetic features of new infectious diseases and experimental therapeutics and vaccines. In emergency situations, data needs to be shared quickly before the information is formally published.[23]

Finally, the National Heart, Lung and Blood Institute (NHLBI) presented detailed data-sharing practices allowing public access to trial raw data and developed a data repository currently including over half a million patients from over 100 trials and observational studies.[24] In 2015 the NHLBI discussed its intent to make public the digital data from its funded trials.[24]

Platforms and repositories

Up to 30,000 clinical trials are conducted annually worldwide generating a huge volume of patient-level raw data.[25] Currently, however, available portals for data sharing are still not adequate. Most of them require a time consuming request, including a detailed research proposal with the study design, main endpoints and a statistical plan.[25] The submitted proposal is then reviewed by an independent research panel that decides whether to approve the request for data.[21,25,26] Currently, this process takes too long and when eventually the data is obtained oftentimes it is not readily usable.[25] However, the means to facilitate data sharing from the data holder to the researcher may be cumbersome and challenging to implement. Some systems provide an electronic form or template.[21] Nevertheless, when these are not available, a *de novo* proposal should be generated outlining the purpose, the statistical analysis plan, the research team, and potential conflicts of interest. The review process may come from an internal or external review panel selected by the data

holder or by a third party.[25-27] Finally, data can be shared through a public website or by direct communication between the data holder and the researcher. In most cases, however, controlled access is required. Before any analysis is started, reviewing all the accompanying documentation to assist the researcher in the understanding of the original clinical trial and the methodology used remains critical. Furthermore, the data holder may require a legally binding data sharing agreement and should be available to provide the required support should questions arise.[27]

Major care should be taken to prevent the perils that may undermine the value of data sharing.[14] Data from trials should be responsibly used.[28] A recent survey from UK Clinical Trial Units disclosed some potential risks associated with data sharing.[29] These basically included a) misuse of data, b) incorrect secondary analyses, c) resource requirements and d) identification of patients.[29,30] Researchers are responsible for presenting the data in a format amenable for external secondary use. Repositories should be prepared to make raw data available in standardized platforms in a fully comprehensive manner. Data sharing from trials with anonymized patient-level data with associated metadata and supporting information should be made available to other researchers following an independent analysis of the research proposals. Developing and adopting standard approaches to protecting patient privacy are urgently required.[14] Finally, an adequate infrastructure should be organized to support effective data sharing. In this regard, the role of the industry is significantly growing as demonstrated by some joint initiatives, such as the Yale University Open Data (YODA) project.[16,31]

Some academic research organization consortiums particularly focussed on the study of cardiovascular diseases,[32] have developed interesting tools for data sharing. This cardiovascular initiative requires presentation of a standardized request in a Web portal. Proposals are to be analyzed by a scientific committee, including members designated by the consortium and a statistician along with the trial's principal investigator. The idea is to ensure an adequate use of the data base and correct statistical analyses, while averting the problem of multiple investigators proposing the same analyses.[32]

Statistical issues

Statisticians play a key role in developing data sharing strategies.[19] They should be involved from the very beginning to organize the research strategy and the required analytical techniques.[19] In this scenario statisticians should move from their classical role as data gate-keepers to that of data facilitators.[19] A data sharing working group of medical research statisticians has been recently created from the pharmaceutical and biotechnological industry and from academia. The idea was to address the technical and statistical challenges of accessing research data for re-analyses. Specific techniques are required to ensure adequate data manipulation to convert the data initially collected and entered in the data base into data that is analytically usable. Converting raw data into standardized formats may be challenging. Moreover, familiarity with the required statistical programing language is necessary. Independent statisticians should play a major role in guiding the principles of re-analysis based on the researchers´ request while, at the same time, guarding against misleading conclusions. They should be fully aware that additional analysis may yield different results compared with the original analyses. Accordingly, they should be prepared to face criticism but, at the same time, they should be able to openly challenge previous statistical methods.[19]

Statistical guidance may be required for appropriate interpretation of results from re-analyses where different methods have been utilized. In particular, it is important to keep in mind the inherent risk of over-interpretation of the results from multiple subgroup analyses.[33] Likewise, documents for best practices in data anonymization have been developed.[34] Statisticians should be also familiar with this methodology. Risk to patient privacy can be mitigated by data reduction techniques. Data holders are responsible for generating de-identified datasets to offer protection for patient privacy through masking or generalization of main identifiers. In addition, legally binding data sharing agreements should include a compromise not to attempt to identify patients.[34] In particular, it is recommended that data use agreements are signed by the data holder and researchers. Only appropriately qualified named researchers should be granted access to the data. Finally, high security levels should be implemented for data transferring. Resources, costs and effort required to make patient-level data available for third party research may be considerable and, therefore, adequate funding should be organized.[34]

Credit to the original authors

A clear motivation for researchers to conduct randomized clinical trials is the opportunity to publish different studies in addition to the main manuscript with the primary endpoint. These secondary analyses may be of major value to unravel new findings from the original dataset.[35,36] Many have proposed that the time to open the process of data sharing should be extended to 2 years, or even to 5 years in selected complex or large studies. This will allow a precious time for original investigators to further scrutinize and analyze in depth their own data. As blinding is necessary during trial execution, once the study is completed the research teams concentrate on publishing the primary findings as soon as possible. Following this, usually there is a series of pre-planned additional analyses. These studies are organized by collaborative research teams from different institutions, but usually with relatively poor support. Secondary analyses are also very important for co-investigators and junior scientists. To respect this legitimate interest an extension from the 6 month-period after the primary data has been published has been advocated.[35,36]

Academia rewards scientists with recognition for making their discoveries public. Credit should be granted to the original researchers that create data sets that other investigators find useful.[14,15] Otherwise, original investigators may be tempted to consider research parasites those performing secondary analyses of their data. Furthermore, mechanisms are required to ensure that the external analyses are conducted adequately and not merely to undermine the original findings. Direct collaboration between primary and secondary researchers is, therefore, necessary to ensure proper data

analysis and interpretation.[14,15] The original investigators that designed and conducted the trial and obtained sources of founding deserve to receive the adequate scientific credit.[28]

Conclusions

The data transparency revolution is here to stay. This is just another step ahead into a culture of open science and it is clear that we are at the dawn of a new age.[37,38] Several European National Societies have already developed registry programs in which the registries databases are public for the use of their members.[39] Major challenges and hurdles in the adoption and implementation of the new ICMJE recommendation should still be overcome.[40] Experience gained by leading journals will eventually allow a balanced compromise between the interests of the original researchers and that of the scientific community as a whole. NSCJ should progressively adapt their policies to increase awareness of the importance of data sharing and promote policies designed to enhance transparency in biomedical research.

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Factors Associated With Coronary Artery Disease Progression Assessed By Serial Coronary Computed Tomography Angiography

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Abstract

Background: **Coronary computed tomography angiography (CCTA) allows for noninvasive coronary artery disease (CAD) phenotyping. Factors related to CAD progression are epidemiologically valuable.**

Objective: **To identify factors associated with CAD progression in patients undergoing sequential CCTA testing.**

Methods: **We retrospectively analyzed 384 consecutive patients who had at least two CCTA studies between December 2005 and March 2013. Due to limitations in the quantification of CAD progression, we excluded patients who had undergone surgical revascularization previously or percutaneous coronary intervention (PCI) between studies. CAD progression was defined as any increase in the adapted segment stenosis score (calculated using the number of diseased segments and stenosis severity) in all coronary segments without stent (in-stent restenosis was excluded from the analysis). Stepwise logistic regression was used to assess variables associated with CAD progression.**

Results: **From a final population of 234 patients, a total of 117 (50%) had CAD progression. In a model accounting for major CAD risk factors and other baseline characteristics, only age (odds ratio [OR] 1.04, 95% confidence interval [95%CI] 1.01–1.07), interstudy interval (OR 1.03, 95%CI 1.01–1.04), and past PCI (OR 3.66, 95%CI 1.77–7.55) showed an independent relationship with CAD progression.**

Conclusions: **A history of PCI with stent placement was independently associated with a 3.7-fold increase in the odds of CAD progression, excluding in-stent restenosis. Age and interstudy interval were also independent predictors of progression. (Arq Bras Cardiol. 2017; 108(5):396-404)**

Keywords: **Coronary Artery Disease/physiopathology; Coronary Amgiography; Tomography, X-Ray Computed; Percutaneous Coronary Intervention.**

Introduction

Coronary artery disease (CAD) is the worldwide leading cause of death.[1] Clinical and revascularization approaches have been shown to decrease the morbidity and mortality from chronic CAD. Despite treatment, the clinical course of chronic CAD usually consists of progression of atherosclerosis punctuated by flares of unpredictable clinical events.[2,3] In a meta-analysis, Cannon et al. have shown that patients with previous documented CAD on secondary prophylaxis with high-dose statins in addition to contemporary clinical management still have a 7% incidence of composite events and 2% mortality per year.[4] Although CAD is a progressive inflammatory and degenerative disorder,[5,6] some studies have demonstrated the feasibility of interruption or even regression of atherosclerosis progression, as measured by invasive techniques such as intravascular ultrasound[7,8] and optical coherence tomography.[9] Previous studies have identified markers of anatomical atherosclerosis progression, but these studies were restricted to patients submitted to percutaneous coronary intervention (PCI) undergoing repeat invasive coronary angiography (ICA), as part of the study protocol.[10-12]

Coronary computed tomography angiography (CCTA) is able of noninvasively phenotyping CAD in a broader range of clinical scenarios and provides good diagnostic performance for obstructive CAD detection, as well as strong prognostic information.[13-15] A recent meta-analysis has shown a high correlation between CCTA and measures of plaque burden and stenosis severity derived by intracoronary ultrasound.[16] Able of depicting disease even with minimal luminal narrowing, CCTA offers an opportunity to track incipient CAD and obstructive coronary stenosis.

In the present study, we sought to identify the variables associated with CAD progression on sequential CCTA testing in patients with and without previous PCI.

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Methods

Subjects

Of 5055 clinically indicated CCTAs performed in 4607 patients in our institution between December 2005 and March 2013, we identified 382 individuals who underwent sequential testing at least 90 days apart. A total of 72 patients who had undergone surgical revascularization were excluded, since CAD progression in these cases may have been associated with diversion of the flow from the bypasses and not necessarily with the usual pathophysiology of atherosclerosis.[17] Additionally, 76 patients who had undergone PCI between CCTA studies were also excluded, since the quantification of the progression of native vessel disease would be biased by the artificial improvement of the treated segment. The remaining 234 patients comprised the study sample. Before each test, information on medication use, CAD risk factors, and previous coronary events and stress testing results were obtained during an interview with a physician. Baseline characteristics were established for each subject at the time of the first CCTA exam.

Each patient gave a written informed consent for inclusion of their information into our database, including clinical data and test results that were personally recorded by the physician responsible for the pretest interview and by another one in charge of the study reporting, respectively. For this study, as in every other involving this data source, access to the database by research personnel could only be made by a query, which returns a renumbered spreadsheet filled with the requested data, excluding identifying information such as patient's name and record number. Since no personal information was disclosed, institutional review board approval was not requested for this study. None of the authors of this paper was responsible for treating the patients included in this analysis or in the database in general.

CCTA imaging technique

CCTA studies were performed on a 256-slice scanner (Brilliance iCT, Philips Healthcare, Cleveland, Ohio) or one of two 64-slice computed tomography scanners (Brilliance 64, Philips Healthcare, Cleveland, Ohio, USA and Somatom Sensation 64, Siemens Healthcare, Erlangen, Germany) during contrast injection, using a bolus tracking technique aiming at acquiring images at peak coronary opacification. Prospective electrocardiogram (ECG) triggering was strongly encouraged in examinations performed on scanners with this feature. When unavailable or not recommended (*i.e.*, irregular heart rate [HR]), retrospective ECG gating was used instead.

All patients with a baseline HR above 60 bpm were given oral (100 mg) and/or intravenous (5-20 mg) metoprolol to achieve a prescanning HR of 60 bpm or less. Sublingual isosorbide dinitrate 0.4 mg was administered 3-5 minutes prior to the contrast image acquisition, unless contraindicated.

CCTA analysis

All exams were blindly reviewed by a single cardiac imaging expert (I.G.). The coronary artery tree was divided into 15 segments,[18] and coronary atherosclerosis was defined as at least 1 mm^2 of tissue structure that could be individualized within or adjacent to the lumen and differentiated from pericardial and epicardial tissue, as previously described.[18] The extent and severity of the CAD were assessed using an adapted version of the segment stenosis score (SSS), which has been previously described and validated as a strong prognostic marker.[15] Briefly, each of the 15 coronary segments was assigned a score from 0 to 4 based on the presence of atherosclerosis and degree of luminal narrowing: 0 (no atherosclerosis), 1 (1-29%), 2 (30-49%), 3 (50-69%), and 4 (70-100%). Scored segments were then added together to provide a final score ranging from 0 to 60. A progressing lesion, as seen on CCTA, is shown in Figure 1.

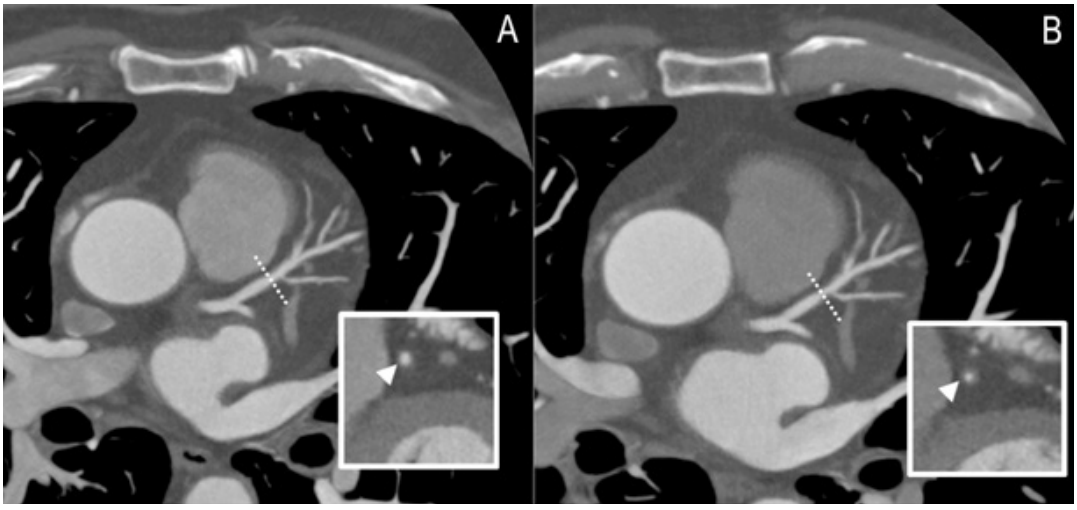

Figure 1 – *Coronary artery disease (CAD) progression on coronary computed tomography angiography (CCTA) in a 58-year-old male presenting a very mild CAD in the proximal left anterior descending coronary artery at baseline (A). Evident disease progression is seen at 13 months at the same site, with moderate luminal stenosis (B) best appreciated in the vessel's transverse plane (arrowhead).*

CAD progression definition and treatment of stented segments

The SSS from the first and second CCTA studies were calculated, and disease progression was defined as any increase in SSS from baseline to follow-up CCTA. Conversely, regression was defined as any decrease in SSS from baseline to follow-up. Stented segments were excluded from disease progression or regression calculations. For multivariable adjustments of CAD severity at baseline, each stented segment was graded as a 70-100% stenosis aiming to overestimate baseline CAD severity in patients with stents. This baseline overestimation in stented patients was done in order to increase their disease severity and, since baseline SSS was included in the multivariable model, minimize the impact of the stent acting as a marker of more "aggressive" CAD presentation.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) or median (interquartile range [IQR]), as appropriate. Categorical variables are presented as frequencies and percentages. Intergroup comparisons were analyzed using unpaired Student's *t* test or Mann-Whitney U test for continuous variables, as appropriate, and chi-square test for categorical variables. Univariable and stepwise backward multivariable logistic regression were used to assess individual predictors of CAD progression. A secondary multivariable analysis was performed in patients with evidence of atherosclerosis at baseline to identify independent predictors of CAD regression. Statistical significance was defined as a two-tailed p value below 0.05. All analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA).

Results

The study included 234 patients with a mean age of 60 ± 11 years, 79% of whom were males. The flowchart in Figure 2 shows the selection of the population. A total of 8% of the patients had a history of myocardial infarction, and 11% of them had a recent (less than 30 days before the index study) positive stress test result. A previous PCI had been conducted in 50 (21%) subjects, who had a total of 83 stented segments (mean of 1.7 per subject). Other baseline characteristics are summarized in Table 1.

During CCTA acquisition, the subjects' mean HR was 54 ± 7 bpm. The median radiation exposure was 4.7 mSv (4–6.4 mSv), and prospective ECG triggering was used in 79% of all studies. Of all exams, 35 (0.01%) segments were deemed unevaluable and were excluded from the analysis in both studies.

At baseline CCTA, 41 (17%) patients had no evidence of coronary atherosclerosis, while the CAD severity was deemed very mild (1–29%) in 60 (26%), mild (30–49%) in 65 (28%), moderate (50–69%) in 37 (16%), and severe (≥ 70%) in 31 (13%). The baseline SSS was 0 in 41 (17%) subjects, between 1 and 5 in 76 (32%) subjects, between 6 and 10 in 55 (24%) subjects, between 11 and 15 in 25 (11%) subjects, and 16 or above in 37 (16%) subjects.

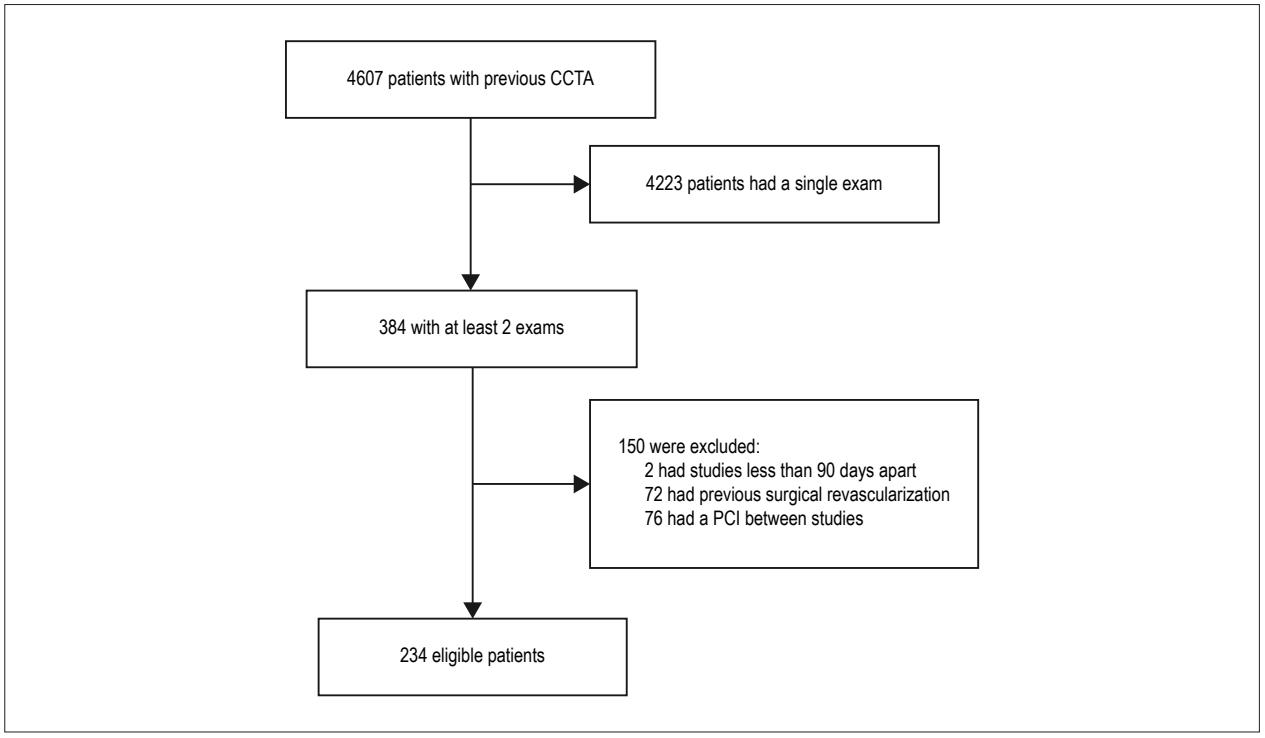

Figure 2 – *Flowchart of patient selection. The final study population comprised individuals with sequential coronary computed tomography angiography (CCTA) testing conducted at least 90 days apart and free of percutaneous coronary intervention (PCI) between studies or previous surgical coronary revascularization.*

Table 1 – Patients' baseline characteristics

Patients, n	234
Age (years), mean ± SD	59.8 ± 10.7
Male sex, n (%)	186 (79)
BMI (kg/m²), mean ± SD	27.7 ± 3.9
Exam interval (months), median (IQR)	32.4 (19.2 – 49.7)
Baseline SSS, median (IQR)	6 (2 – 11)
Clinical risk factors	
Hypertension, n (%)	117 (50)
Diabetes, n (%)	30 (13)
Dyslipidemia, n (%)	125 (53)
Family history CAD, n (%)	99 (42)
Glucose intolerance, n (%)	10 (4)
Current smoker, n (%)	25 (11)
Past smoker, n (%)	55 (24)
Positive stress test, n (%)	26 (11)
Previous MI, n (%)	18 (8)
Previous PCI, n (%)	50 (21)
Medication use	
Beta-blockers, n (%)	35 (15)
ACEI/ARB, n (%)	45 (19)
Antiplatelet, n (%)	46 (20)
Statin, n (%)	59 (25)

SD: standard deviation; BMI: body mass index; SSS: segment stenosis score; IQR: interquartile range; CAD: coronary artery disease; MI: myocardial infarction; PCI: percutaneous coronary intervention; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker.

The follow-up study was conducted at a median of 32 months (19–50 months) when 117 (50%) patients presented CAD progression.

Univariable logistic regression including all baseline characteristics revealed that age, interstudy interval, baseline SSS, and previous PCI were predictors of CAD progression. Table 2 lists the patients' characteristics according to CAD progression status. After multivariable adjustment, age, interstudy interval, and previous PCI emerged as independent predictors of progression. An independent 3.7-fold increased odds of progression was associated with a history of coronary stenting, as shown in Table 3.

Overall, 70% of the patients with previous PCI presented CAD progression, compared with 47% of those with baseline CAD but no stents (p = 0.003) and 38% without any CAD at baseline (p = 0.002). This higher rate of progression among PCI patients remained across a wide range of SSS increases, as shown in Figure 3. Differences in baseline characteristics among patients with and without stents are shown in Table 4.

On secondary analysis considering only subjects with evidence of CAD at the baseline CCTA (n = 193), disease regression was independently related only with a history of PCI with stent (OR 0.28, 95% confidence interval [95%CI] 0.10–0.77, p = 0.01), baseline SSS (OR 1.10, 95%CI 1.04–1.16, p = 0.01), and interstudy interval (OR 0.98, 95%CI 0.96–0.99, p = 0.02) on multivariable logistic regression.

Discussion

In spite of medical and invasive treatments, CAD remains a progressive disease. Several studies reveal a high incidence of events among patients submitted to guideline-based optimal therapies, underlying the limitations of currently available therapeutic approaches.[19-21] Angiographic CAD progression may identify subjects at a higher risk for cardiovascular events since plaque growth entails inflammatory activity and increased risk of rupture.[22] The identification of predictors of CAD progression is epidemiologically important and allows a better understanding of the pathophysiology of CAD.

Our cohort consisted of "real world" patients, with and without previous evidence of CAD, including those with a history of PCI. Subjects with intervening PCI procedures between CCTA studies were excluded in order to avoid the bias of decreased stenosis due to stent placement. Similarly, previously implanted stented coronary segments were excluded from the progression analysis so that restenosis would not contaminate the results. In this setting, we found a 50% rate of native vessel (non-stented) CAD progression over a median follow-up of 32 months, which is in the upper range of previous studies using ICA.[23-29] This may have been a result of the use of CCTA, which is capable of depicting three-dimensionally the coronary wall and is, therefore, not constrained by two-dimensional projections.

In multivariable analysis, age, interval between studies, and previous PCI were independent predictors of CAD progression. Specifically, previous PCI with stent placement, a potentially modifiable patient characteristic, was associated with a 3.7-fold increased odds of disease progression. Although this is the first study to our knowledge to show it using this technology, absolute causality between stent placement and progression cannot be made due to the retrospective and observational nature of this study. One potential bias could be that stents are only but a marker of faster progressing atherosclerosis biology. We vigorously tried to minimize this bias by adjusting the results to baseline CAD and other major risk factors, previous myocardial infarction and by overestimating the CAD burden for stented segments at baseline CCTA. Interestingly, a history of PCI was not only independently related to increased odds of disease progression, but also a 72% reduction in the odds of regression.

Most previous research on coronary atherosclerosis progression has focused on patients undergoing ICA in preparation for PCI, but they also are subject to bias.[10,20,21] Without comparing CAD progression between PCI and non-PCI patients, potential effects of the invasive treatment on disease evolution cannot be derived. Nevertheless, even in this setting, two previous studies of

Table 2 – Patients' baseline characteristics according to progression status

	All subjects		
	No Progression	Progression	p value
Patients, n	117	117	
Age (years), mean ± SD	58.3 ± 10.7	61.3 ± 10.8	0.03
Male sex, n (%)	90 (77)	96 (82)	0.42
BMI (kg/m^2), mean ± SD	27.2 ± 3.9	28.0 ± 4.0	0.11
Exam interval (months), median (IQR)	29.8 (18.8 – 42.8)	34.1 (20.4 – 55.2)	0.05
Baseline SSS, median (IQR)	5 (1 – 9)	8 (2 – 14)	0.01
Clinical risk factors			
Hypertension, n (%)	58 (50)	59 (50)	1.00
Diabetes, n (%)	15 (13)	15 (13)	1.00
Dyslipidemia, n (%)	65 (56)	60 (51)	0.60
Family history CAD, n (%)	54 (46)	45 (38)	0.29
Glucose intolerance, n (%)	3 (3)	7 (6)	0.33
Current smoker, n (%)	16 (14)	9 (8)	0.20
Past smoker, n (%)	23 (20)	32 (27)	0.22
Positive stress test, n (%)	14 (12)	12 (10)	0.84
History of MI, n (%)	6 (5)	12 (10)	0.22
Previous PCI, n (%)	15 (13)	35 (30)	0.002
Medication use			
Beta-blockers, n (%)	18 (15)	17 (15)	1.00
ACEI/ARB, n (%)	21 (18)	24 (21)	0.74
Antiplatelet, n (%)	18 (15)	28 (24)	0.14
Statin, n (%)	31 (26)	28 (24)	0.76

SD: standard deviation; BMI: body mass index; SSS: segment stenosis score; IQR: interquartile range; CAD: coronary artery disease; MI: myocardial infarction; PCI: percutaneous coronary intervention; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker.

subjects undergoing PCI have reported that a history of PCI before study entry was a significant and independent predictor of worse outcomes.[10,30]

Borges et al.[26] reported results from a study comparing subjects undergoing medical treatment alone *versus* PCI in regards to native vessel CAD progression using ICA.[26] The authors found that patients with a previous PCI had an independent 2.1-fold increased odds of CAD progression over 5 years when compared with those without prior PCI.

Limitations

Since this was a retrospective and observational study, we are unable to establish with certainty a causality between PCI and CAD progression, although we judiciously tried to adjust the model for potential confounders. Despite the biases and given the paucity of research on this subject, this study generates questions that should be answered with large prospective randomized studies.

To determine the occurrence of CAD progression, we used the results of CCTA, which has lower spatial and temporal resolution than ICA.[31] This fact may result in artifacts that hinder the CAD quantification. Although some inaccuracies may occur with this method, mostly related to stenosis overestimation, all patients were equally subjected to the same errors. Despite this limitation, the use of CCTA may offer some advantages in eccentric coronary plaque visualization and mild luminal narrowing.

Due to the limited number of subjects in our study, some questions remain to be answered by future investigations, such as the impact of gender and race on CAD progression, the relevance of the number of stented segments, differences in progression between bare metal and drug-eluting stents and, the most important of all, if this observed progression may translate into future events.

Table 3 – Predictors of coronary artery disease (CAD) progression

	Univariable analysis			Multivariable analysis		
	Odds Ratio	95%CI	p value	Odds Ratio	95%CI	p value
Age (years)	1.03	1.00 – 1.05	0.03	1.04	1.01 – 1.07	0.01
Male sex	1.37	0.72 – 2.60	0.33	1.92	0.92 – 3.98	0.08
BMI (kg/m^2)	1.06	0.99 – 1.13	0.12	1.07	0.99 – 1.15	0.08
Study interval (months)	1.01	1.00 – 1.03	0.02	1.03	1.01 – 1.04	< 0.001
Baseline SSS	1.04	1.01 – 1.09	0.02			
Clinical risk factors						
Hypertension	0.97	0.58 – 1.61	0.90			
Diabetes	1.00	0.46 – 2.15	1.00			
Dyslipidemia	1.19	0.71 – 1.99	0.51			
Family history CAD	1.37	0.82 – 2.31	0.23			
Glucose intolerance	0.41	0.10 – 1.64	0.21			
Current smoker	1.90	0.80 – 4.49	0.14			
Former smoker	0.65	0.35 – 1.20	0.17			
Positive stress test	1.19	0.53 – 2.69	0.68			
Previous MI	0.47	0.17 – 1.31	0.15			
Previous PCI	2.90	1.48 – 5.68	< 0.001	3.66	1.77 – 7.55	< 0.001
Medication use						
Beta-blockers	1.07	0.52 – 2.19	0.85			
ACEI/ARB	0.85	0.44 – 1.63	0.62			
Antiplatelet	0.58	0.30 – 1.12	0.10			
Statin	1.15	0.63 – 2.07	0.65			

95%CI: 95% confidence interval; SD: standard deviation; BMI: body mass index; SSS: segment stenosis score; IQR: interquartile range; CAD: coronary artery disease; MI: myocardial infarction; PCI: percutaneous coronary intervention; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker.

Conclusion

In a "real world" population of patients referred to sequential CCTA testing, age and history of coronary artery stenting were independent predictors of native CAD progression, while the degree of baseline CAD assessed by SSS was not independently associated with this endpoint.

Author contributions

Conception and design of the research: Camargo GC, Gottlieb I; Acquisition of data: Camargo GC, Rothstein T, Derenne ME, Sabioni L; Analysis and interpretation of the data: Camargo GC, Lima RSL, Gottlieb I; Statistical analysis: Camargo GC; Writing of the manuscript: Camargo GC, Gottlieb I; Critical revision of the manuscript for intellectual content: Rothstein T, Derenne ME, Sabioni L, Lima JAC, Lima RSL.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

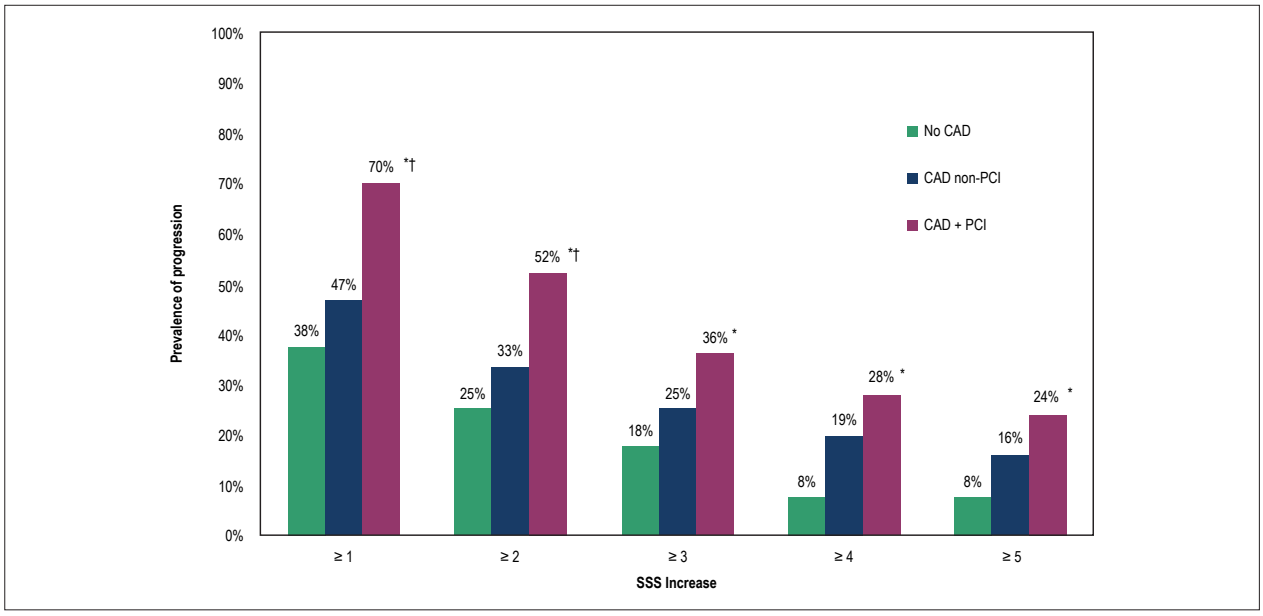

Figure 3 – *Prevalence and severity of segment stenosis score (SSS) increase according to subgroup. *p < 0.05 between coronary artery disease (CAD) + percutaneous coronary intervention (PCI) and no CAD; †p < 0.05 between CAD + PCI and CAD non-PCI.*

Table 4 – Patients' baseline characteristics according to history of percutaneous coronary intervention (PCI)

Patients, n	non-PCI	PCI	p value
	184	50	
Age (years), mean ± SD	58.9 ± 11.1	63.4 ± 9.1	0.01
Male sex, n (%)	149 (81)	42 (74)	0.35
BMI (kg/m^2), mean ± SD	27.7 ± 3.8	27.4 ± 4.4	0.56
Exam interval (months), median (IQR)	33.4 (22.0 – 53.1)	26.9 (15.0 – 37.2)	< 0.01
Baseline SSS, median (IQR)	4 (1 – 8)	16 (10 – 21)	< 0.001
Clinical risk factors			
Hypertension, n (%)	91 (49)	26 (52)	0.87
Diabetes, n (%)	20 (11)	10 (20)	0.10
Dyslipidemia, n (%)	89 (48)	36 (72)	< 0.01
Family history of CAD, n (%)	79 (43)	20 (40)	0.75
Glucose intolerance, n (%)	6 (3)	4 (8)	0.23
Current smoker, n (%)	22 (12)	3 (6)	0.31
Past smoker, n (%)	39 (21)	16 (32)	0.13
Positive stress test, n (%)	18 (10)	8 (16)	0.21
History of MI, n (%)	2 (1)	16 (32)	< 0.001
CAD progression, n (%)	82 (45)	35 (70)	< 0.001
Medication use			
Beta-blockers, n (%)	21 (11)	14 (28)	0.01
ACEI/ARB, n (%)	31 (17)	14 (28)	0.10
Antiplatelet, n (%)	18 (10)	28 (56)	<0.001
Statin, n (%)	38 (21)	21 (42)	<0.01

SD: standard deviation; BMI: body mass index; SSS: segment stenosis score; IQR: interquartile range; CAD: coronary artery disease; MI: myocardial infarction; PCI: percutaneous coronary intervention; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker.

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Relationship between Resting Heart Rate, Blood Pressure and Pulse Pressure in Adolescents

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Abstract

Background: High resting heart rate is considered an important factor for increasing mortality chance in adults. However, it remains unclear whether the observed associations would remain after adjustment for confounders in adolescents.

Objectives: To analyze the relationship between resting heart rate, blood pressure and pulse pressure in adolescents of both sexes.

Methods: A cross-sectional study with 1231 adolescents (716 girls and 515 boys) aged 14-17 years. Heart rate, blood pressure and pulse pressure were evaluated using an oscillometric blood pressure device, validated for this population. Weight and height were measured with an electronic scale and a stadiometer, respectively, and waist circumference with a non-elastic tape. Multivariate analysis using linear regression investigated the relationship between resting heart rate and blood pressure and pulse pressure in boys and girls, controlling for general and abdominal obesity.

Results: Higher resting heart rate values were observed in girls (80.1 ± 11.0 beats/min) compared to boys (75.9 ± 12.7 beats/min) (p ≤ 0.001). Resting heart rate was associated with systolic blood pressure in boys (Beta = 0.15 [0.04; 0.26]) and girls (Beta = 0.24 [0.16; 0.33]), with diastolic blood pressure in boys (Beta = 0.50 [0.37; 0.64]) and girls (Beta = 0.41 [0.30; 0.53]), and with pulse pressure in boys (Beta = -0.16 [-0.27; -0.04]).

Conclusions: This study demonstrated a relationship between elevated resting heart rate and increased systolic and diastolic blood pressure in both sexes and pulse pressure in boys even after controlling for potential confounders, such as general and abdominal obesity. (Arq Bras Cardiol. 2017; 108(5):405-410)

Keywords: Heart Rate; Arterial Pressure; Rest; Adolescents.

Introduction

High resting heart rate (RHR) has recently come to be considered an important factor for increasing the chance of mortality, and this relationship is independent of age, sex, lipid profile or blood pressure (BP) values in adults.[1] High RHR tends to be associated with myocardial infarction,[2] which could contribute to the likelihood of death from coronary heart disease in the future.

In previous studies,[3-5] a relationship has been observed between elevated RHR and high BP in pediatric populations, which suggests that elevated RHR may be a marker of cardiovascular disease in childhood. This relationship could be mediated by other important cardiovascular risk factors such as obesity,[3,4] which, through inflammatory substances, could increase RHR.[6]

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Another factor that has been associated with high RHR is pulse pressure (PP), an important marker of vascular stiffness. A recent study[7] has demonstrated a positive relationship between high RHR and PP in 227 healthy male African-American adolescents. However, as PP is affected by both BP levels and obesity status, it remains unclear whether the observed associations would remain after adjustment for these confounders.

Moreover the sex of adolescents should also be considered when analyzing these relationships. Rosa et al.[8] have found that male adolescents had significantly higher PP values than female adolescents. Higher values of heart rate variability were observed in men compared to women.[9] These findings show that hormonal and local fat deposition characteristics could influence RHR and other cardiovascular risk factors.

The purpose of this study was to analyze the association between RHR, BP and PP in adolescents of both sexes and to identify covariates of this association.

Methods

This was a cross-sectional study carried out in the city of Londrina, Paraná state, situated in southern Brazil. A meeting was held with the Secretary of Education in the city of Londrina. As a result of that meeting, information was obtained on the

six largest public schools in the downtown area, which were most likely to include students from different regions of the city (Northern, Southern, Eastern, Western and Central regions). Those schools were chosen for the study.

Londrina has approximately 17,000 enrolled students. To calculate the sample size, we used a correlation value of 0.18, an alpha error of 5%, a power of 80% and a design correction of 2.0 (as the sample was evaluated as clusters). Considering 20% losses, a minimum sample size of 624 students was required. The sample was composed of 1231 adolescents aged 14-17 years (515 boys and 716 girls). The inclusion criteria consisted in authorizing the consent form signed by parent/guardian allowing their adolescent to participate in the study, not be ingesting any medication or being in treatment related to problems of BP or heart rate. This study was approved by the ethics committee on human experimentation of the institution involved (process: 203/2010).

Measurements

Anthropometry

Body weight was measured using an electronic scale (precision, 0.1 kg), with the subjects wearing light clothing, and height was measured with a wall-mounted stadiometer (precision, 0.1 cm). Body mass index (BMI) was calculated from the values of weight divided by height squared (kg/m^2). Adolescents were classified as normal weight or overweight according to the cutoff points of the World Health Organization.[10] All anthropometric measurements were performed by the same researcher, according to standardized techniques. Waist circumference was measured using an inextensible tape-measure, according to the values recommended by Taylor et al.,[11] considering the age and sex of the adolescents.

Resting heart rate

Resting heart rate and BP were evaluated using an oscillometric device (Omron HEM-742; Omron Corporation, Kyoto, Kansai, Japan), validated for adolescents.[12] The participants sat silently in a room with their backs leaning against a chair and their arms flexed at an angle of 90 degrees. After 5 minutes of resting the first evaluation of RHR was performed, and after 2 minutes the second measurement was taken. The average of the two evaluations was used to determine RHR. These procedures were adopted according to the American Heart Society standards.[13] Adolescents located in quartile 4 were classified as having high RHR and the others as having low RHR.

Systolic BP (SBP) and diastolic BP (DBP) were measured concomitantly with RHR. The mean value was used. To indicate the presence of high levels of BP, the 95th percentile of the National High Blood Pressure Education Program cutoffs was considered, adjusted by age and height percentile.[14]

Pulse pressure was calculated (difference between SBP and DBP).

Statistical analysis

The sample characterization data were described as mean and standard deviation. As there is no specific cutoff point for RHR or PP in adolescents, we chose to classify the adolescents in the highest quartile as having a possible risk for those outcomes.

The number of adolescents classified according to the risk factors was analyzed by means of frequency and possible associations observed by using the Chi-square test. The multivariate analysis (Linear Regression) was applied and adjusted for sex and age (first model), overall fat determined by BMI (second model), and central fat (third model). The confidence interval was 95% and significance level, $p < 0.05$. All statistical analysis was performed using SPSS v.18.0.

Results

Younger age had higher RHR values (14-15 years = 80.3 ± 12.0 beats/min; 16-17 years = 76.7 ± 12.4 beats/min [p = 0.001]). Higher RHR values were observed in girls (80.1 ± 11.0 beats/min) as compared to boys (75.9 ± 12.7 beats/min) ($p \leq 0.001$). Elevated RHR was observed in 131 male adolescents (25.4%) and in 231 female adolescents (32.2%) (p = 0.011). There was no difference in BMI between boys and girls, but boys classified as having high RHR had higher BMI. The boys had higher waist circumference than girls, and the boys classified as having high RHR had higher waist circumference values. The sample characteristics are shown in Table 1.

A low correlation was observed between BMI and RHR (r = 0.06). Adolescents with higher RHR had a higher prevalence of high BP (36.4% vs. 28.4%; p = 0.050). There was no significant difference in RHR in adolescents when stratifying for nutritional status (p = 0.174) or PP values (p = 0.158). Table 2 shows the correlation between RHR and SBP, DBP and PP. Considering the relationship between heart rate and SBP and DBP, for each increased heartbeat there is a 0.090-mmHg increase in SBP of boys and a 0.063 mmHg increase in SBP of girls. Regarding DBP, the increase is 0.179 mmHg in boys and 0.161 mmHg in girls.

The linear regression models adjusted for sex, age, overall fat and central obesity indicated that RHR was associated with SBP ($p \leq 0.05$) and DBP ($p \leq 0.05$) for both sexes, and with PP (p = 0.05) only for boys (Tables 3 and 4).

Discussion

This study's results indicate that adolescents with higher RHR values have higher SBP and DBP values. High RHR was associated with higher SBP and DBP in males and females, and was inversely related to PP only in male adolescents.

This study's results are in agreement with those reported by Liu et al.,[15] who, assessing more than 8,000 individuals from different countries, aged 48-56 years, have found that high RHR associated with high BP values. Similar findings were observed by Kwok et al.,[4] who evaluated the relationship

Table 1 – Characteristics of participants by sex and presence of resting heart rate (RHR)

	Boys (n = 515)		Girls (n = 716)	
	Normal RHR	High RHR	Normal RHR	High RHR
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	15.5 (1.1)	15.2 (1.1)	15.7 (0.9)	15. 3 (1.0)
Weight (kg)	64.1 (12.7)	66.5 (16.8)[a,b]	56.9 (11.5)	56.5 (11.3)
Stature (cm)	173.2 (8.3)	171.1 (9.5)[a]	162.4 (6.7)	162.1 (6.6)
BMI (kg/m^2)	21.2 (3.4)	22.6 (5.2)[b]	21.5 (3.7)	21.5 (3.9)
WC (cm)	74.1 (8.8)	77.9 (12.2)[a,b]	70.5(8.8)	70.5 (8.4)
SBP (mm Hg)	119.7 (10.7)	121.9 (12.4)[a,b]	110.0 (3.7)	113.2 (3.9)[b]
DBP (mm Hg)	63.4 (7.5)	67.5 (8.4)[b]	64.2 (7.4)	66.8 (7.8)[b]
PP	56.2 (9.5)	54.4 (9.9)[a]	45.8 (7.0)	46.4 (7.4)

Difference between groups evaluated by two-way ANOVA; a: p < 0.05 for the difference between boys and girls; b: p < 0.05 for the difference between high RHR and normal RHR; RHR: resting heart rate; SD: standard deviation; kg: kilograms; cm: centimeters; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure.

Table 2 – Correlation between resting heart rate and systolic and diastolic blood pressure and pulse pressure

Independent Variable	Pearson Correlation (dependent variable: RHR)	
	r	p-value
Male		
SBP	0.10	≤ 0.001
DBP	0.23	≤ 0.001
PP	–0.10	0.016
Female		
SBP	0.19	≤ 0.001
DBP	0.24	≤ 0.001
PP	–0.01	0.658

RHR: resting heart rate, SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure.

between high values of RHR and of BP in young Chinese individuals. Corroborating our study, Fernandes et al.[3] have demonstrated similar relationships in 356 male adolescents. Even after controlling for different variables in the cited studies, high BP values were associated with high RHR. One reason is that heart rate and SBP and DBP are modulated by the central nervous system through sympathetic activity in the heart and vessels.[16]

Pulse pressure is an indicator of arterial stiffness of different arteries and vessels and has been related to arteriosclerotic processes.[17] This study's results indicated that RHR was correlated with PP only in male adolescents, which is in agreement with those reported by Song et al.[18] in a representative sample of Korean adolescents. One possible mechanism is the fact that PP is an indicator of arterial stiffness, which contributes to heart rate changes mediated by increased velocity of the pulse wave from the aorta to the peripheral vessels; in this case, the higher the speed of the anterograde wave, the higher the successive retrograde wave, causing cardiovascular overload.[19]

Another factor to be considered in this study is the relationship between obesity and increased heart rate. In a study of over 30,000 young people, Babba et al.[20] have observed that RHR was strongly associated with obesity, added to which, average values of heart rate increased according to the degree of obesity. Adipose tissue releases a variety of substances, including adiponectin, which could contribute to changes in the sympathetic nervous system and decreased parasympathetic nervous system,[21] increasing RHR values. Therefore, obesity is an important confounding factor to be considered in the analyses. The correlation between RHR and PP remained significant after adjustment for age, age + BMI, and age + BMI + waist circumference in males, suggesting an independent relationship between PP and RHR. A previous study[16] has found no correlation between total fat mass and cardiovascular variables (systolic volume, SBP and RHR) in male adolescents, unlike abdominal fat. On the other hand, in female adolescents, the relationships are absolutely inverse, the cardiovascular variables (systolic volume, SBP and RHR) are directly related to total fat mass and unrelated to waist circumference.[18]

Table 3 – Relationship between resting heart rate and systolic and diastolic blood pressure and pulse pressure in boys

	Resting Heart Rate								
	Adjusted: age			Adjusted: age and BMI			Adjusted: age, BMI and WC		
	Beta	p-value	95%CI	Beta	p-value	95%CI	Beta	p-value	95%CI
SBP	0.17	≤ 0.001	0.07;0.27	0.15	0.003	0.05;0.25	0.15	0.014	0.04;0.26
DBP	0.51	≤ 0.001	0.38;0.65	0.50	≤ 0.001	0.37;0.63	0.50	≤ 0.001	0.37;0.64
PP	–0.12	0.035	–0.02; –0.09	-0.15	0.011	–0.26; –0.03	–0.16	0.008	–0.27; –0.04

BMI: body mass index; WC: waist circumference; CI: confidence interval; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure.

Table 4 – Relationship between resting heart rate and systolic and diastolic blood pressure and pulse pressure in girls

	Resting Heart Rate								
	Adjusted: age			Adjusted: age and BMI			Adjusted: age, BMI and WC		
	Beta	p-value	95%CI	Beta	p-value	95%CI	Beta	p-value	95%CI
SBP	0.23	≤ 0.001	0.15;0.32	0.24	≤ 0.001	0.15;0.33	0.24	≤ 0.001	0.16;0.33
DBP	0.40	≤ 0.001	0.29;0.51	0.40	≤ 0.001	0.30;0.51	0.41	≤ 0.001	0.30;0.53
PP	-0.03	0.534	-0.15;0.08	-0.04	0.488	-0.17;0.08	-0.05	0.438	-0.17;-0.07

BMI: body mass index; WC: waist circumference; CI: confidence interval; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure.

Concerning sex, the present study demonstrated associations between high RHR, SBP and DBP in both sexes; however, when considering RHR and PP, this relationship was observed only in male adolescents. Pulse pressure differences in boys and girls were observed by Rosa et al.[8] after evaluating 456 adolescents, with higher PP values found in male adolescents. One possible difference to be considered is the age range of students in the current study (14-17 years), menstruation is a period of intense activity in girls and may alter the autonomic system due to intense hormonal production,[22] leading to differences compared to boys. The location of body fat could also be related to higher RHR values. This has been clearly observed by Song et al.,[18] who have found a direct relationship between cardiovascular variables (systolic volume, SBP and RHR) and waist circumference in male adolescents.

Regarding practical applications, it should be emphasized that such assessments need to be conducted from the earliest ages, aimed at preventing future cardiovascular diseases. Oscillometric devices have been used in several studies,[4,23] and in addition to their ease of use, they typically provide values of BP and heart rate, which could be assessed in the school environment, enabling the early control of those risk factors.

One limitation of this study was its cross-sectional design, which does not allow assessment of the possible cause and effect relationships. Heart rate and BP were evaluated on the same day, which is known to cause overestimation of values.[24] Another limiting aspect is the fact that PP was measured indirectly from the SBP and DBP values, and not from the pulse wave speed.

Conclusions

In conclusion, male adolescents with higher RHR had higher SBP, DBP and PP values. In female adolescents, RHR was associated with SBP and DBP, but not with PP. Thus, health promotion activities should be encouraged in young populations, because cardiovascular risk factors interact. However, inherent characteristics of sex must be considered.

Author contributions

Conception and design of the research, Acquisition of data and Statistical analysis: Christofaro DGD, Casonatto J; Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Christofaro DGD, Casonatto J, Vanderlei LCM, Cucato GG, Dias RMR.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Original Article

Self-Reported High-Cholesterol Prevalence in the Brazilian Population: Analysis of the 2013 National Health Survey

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Abstract

Background: **Data on the prevalence of dyslipidemia in Brazil are scarce, with surveys available only for some towns.**

Objective: **To evaluate the prevalence of the self-reported medical diagnosis of high cholesterol in the Brazilian adult population by use of the 2013 National Health Survey data.**

Methods: **Descriptive study assessing the 2013 National Health Survey data, a household-based epidemiological survey with a nationally representative sample and self-reported information. The sample consisted of 60,202 individuals who reported a medical diagnosis of dyslipidemia. The point prevalence and 95% confidence interval (95%CI) for the medical diagnosis of high cholesterol/triglyceride by gender, age, race/ethnicity, geographic region and educational level were calculated. Adjusted odds ratio was calculated.**

Results: **Of the 60,202 participants, 14.3% (95%CI=13.7-14.8) never had their cholesterol or triglyceride levels tested, but a higher frequency of women, white individuals, elderly and those with higher educational level had their cholesterol levels tested within the last year. The prevalence of the medical diagnosis of high cholesterol was 12.5% (9.7% in men and 15.1% in women), and women had 60% higher probability of a diagnosis of high cholesterol than men. The frequency of the medical diagnosis of high cholesterol increased up to the age of 59 years, being higher in white individuals or those of Asian heritage, in those with higher educational level and in residents of the Southern and Southeastern regions.**

Conclusion: **The importance of dyslipidemia awareness in the present Brazilian epidemiological context must be emphasized to guide actions to control and prevent coronary heart disease, the leading cause of death in Brazil and worldwide. (Arq Bras Cardiol. 2017; 108(5):411-416)**

Keywords: **Cholesterol; Dyslipidemias; Epidemiology; Coronary Artery Disease; Prevalence; Health Survey.**

Introduction

Coronary artery disease or ischemic heart disease is one of the major causes of morbidity and mortality worldwide.[1] At the beginning of the 1960s, long-term observation studies, mainly the *Framingham Heart Study*, reported that high cholesterol levels doubled the risk for myocardial infarction.[2] Detailing the cholesterol fractions has allowed identifying low-density lipoprotein cholesterol (LDL-C) as the determinant of the atherogenic process, with a strong and constant association with cardiovascular events.[3]

A meta-analysis with over 170,000 individuals randomized to receive placebo or statins or low versus high doses of those drugs has shown that, for every 40-mg/dL reduction in LDL-C, there was a relative drop of 10%, 20%, 27%, 21% and 25% in all-cause mortality, mortality due to cardiovascular disease, myocardial infarction, ischemic stroke and myocardial revascularization, respectively. There was no beneficial difference regarding the previous presence of cardiovascular disease; however, the absolute benefit was proportional to the previous risk of cardiovascular events, being twice higher in secondary prevention.[4]

Despite the importance of the relation "cholesterol – coronary disease" and the evidence that justifies the control of cholesterol levels at the population level, population surveys conducted in several countries have revealed relatively low rates of diagnosis, knowledge, treatment and control of high cholesterol levels.[5]

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The 2013 Brazilian National Health Survey (PNS) is a unique opportunity to estimate the population prevalence of self-reported dyslipidemia in adults (18 years or older), a representative sample of Brazil, its large geographic regions and federated units, urban and rural areas and self-reported educational level and race/ethnicity.

Methods

Sample

Descriptive study using 2013 PNS data. The PNS is an epidemiological survey based on households, a representative sample of Brazil, its large regions and federated units, capitals and metropolitan regions. Further information on the PNS can be found in other publications.[6-7]

The minimum sample size was 1,800 households per federated unit, with an initial total sample of 81,767 households planned. In addition, the sample was defined based on the level of accuracy desired for estimation of indicators of interest (proportions of individuals at certain categories). After data collection, registries of the interviews of 64,348 households were obtained, with 60,202 individuals interviewed. The other 4,146 residents selected were excluded because: (i) they refused to answer the specific questionnaire or (ii) had their information rejected in the automatic coherence screening performed by the Brazilian Institute of Geography and Statistics (IBGE). The rate of non-reply was 14.0%.

The PNS sample was designed in three stages. The primary units of sampling (PUS) were the census tracts or sets of sectors. The households were the secondary units, and the adult residents (≥ 18 years) were the tertiary units. Weight factors were calculated for each sample unit, considering the selection probability. The weight for the selected resident was calculated considering the household weight, non-reply adjustments by sex and calibration for the population totals by sex and age groups estimated with the weight of all residents. The PNS is part of the Integrated System of Household Surveys of IBGE, therefore, the PUS considered in this study are a subsample of the set of PUS existing in the master sample of IBGE. The households were selected based on the most recent version, available at the time, of the National Register of Addresses for Statistical Purposes. Details on the sampling process and weighing can be obtained in the previous publication on PNS results.[8]

Identification of dyslipidemia

At this stage of the survey, the information on the presence or absence of dyslipidemia was obtained via a self-report of the participants based on the results provided by their physicians about the diagnosis of "high cholesterol or triglyceride levels". The first question was *"when did you have the last blood test to measure cholesterol and triglyceride levels"*. The alternatives ranged from *"less than 6 month ago"* to *"more than 3 years ago"* and *"never"*. Only those reporting at least one cholesterol measurement were asked *"has any physician ever diagnosed you with high cholesterol?"* If the answer was positive, the following questions were asked *"how old were you when first diagnosed with high cholesterol?"* and *"has any physician or health care provider given you a recommendation on high cholesterol?"*.

Statistical analysis and ethical procedures

Using the sample base, the point prevalence estimate and 95% confidence interval (95%CI) were calculated for 'the diagnosis of high cholesterol by a physician', 'undergoing a cholesterol test once' and 'measuring cholesterol or triglyceride levels'. The frequencies were stratified by sex, age group (18 to 29 years, 30 to 59, 60 to 64, 65 to 74, and 75 years and older), educational level (none and incomplete elementary school; complete elementary school and incomplete middle school; complete middle school and incomplete high school; and complete high school) and race/ethnicity (white, black and mixed). The prevalence rates were presented for the country, its large geographic regions, federated units and urban and rural areas. The raw frequency for each specific category was presented, as was the odds ratio adjusted for the other variables, except for itself. Data were analyzed with the Stata® software, 11.0 version (Stata Corp., College Station, United States), using the set of commands for data analysis of a complex sample (survey). Statistically significant differences at the 5% level were considered in the absence of 95%CI overlapping.

The PNS was approved by the National Committee on Ethics in Research (CONEP) of the National Board of Health (CNS), of the Brazilian Ministry of Health (protocol n° 328.159, of June 26, 2013). Participation in this study was voluntary and data confidentiality was guaranteed. The adults selected to answer the interview and who agreed to participate in this study signed the written informed consent.

Results

The PNS estimates discussed in this study are based on the answers of 60,202 individuals aged 18 years and more. The proportion of participants who never had their cholesterol and/or triglyceride levels measured was relatively low, 14.3% (95%CI = 13.7-14.8). Table 1 shows that more than half of the responders had their cholesterol and/or triglyceride levels measured within the past year, with a significantly higher number of women, elderly, white individuals and individuals with complete high educational level, and a lower number of residents of the Northern and Northeastern regions.

Table 2 shows that among those who reported undergoing at least one cholesterol measurement, the prevalence of a medical diagnosis of high cholesterol in the Brazilian population was 12.5% (9.7% for men and 15.1% for women). The women's probability of having a diagnosis of high cholesterol was 60% higher than that of men. The frequency of high cholesterol according to the age group for both sexes increased up to the age of 59 years; from 60 to 74 years, the values were constant, and from the age of 75 years on, the values decreased. The educational level as a socioeconomic indicator showed similar frequencies at the extremes of the category, "no education" and "complete high school". Participants self-reporting black race/ethnicity had

Table 1 – Proportion of participants reporting cholesterol or triglyceride measurement within the past year

Variables	%	95% CI		Raw OR	95% CI		p	Adjusted OR	95% CI		p
		Lower	Upper		Lower	Upper			Lower	Upper	
Brazil	55.4	55.0	55.8								
Sex											
Male	48.2	47.3	49.0	1.00				1.00			
Female	61.8	61.3	62.4	1.74	1.69	1.80	< 0.001	1.70	1.65	1.76	< 0.001
Age											
18-29 years	41.7	39.6	43.9	1.00				1.00			
30-59 years	56.6	54.5	58.7	1.82	1.75	1.89	< 0.001	1.92	1.84	2.00	< 0.001
60-64 years	67.2	64.8	69.6	2.87	2.65	3.10	< 0.001	3.34	3.08	3.63	< 0.001
65-74 years	74.2	72.1	76.1	4.01	3.73	4.31	< 0.001	5.13	4.74	5.55	< 0.001
≥ 75 years	71.7	70.1	73.4	3.55	3.25	3.88	< 0.001	4.60	4.19	5.06	< 0.001
Schooling											
None – incomplete elementary	51.9	50.5	53.3	1.00				1.00			
Complete elementary – incomplete middle	48.7	47.0	50.3	0.88	0.84	0.92	< 0.001	1.25	1.18	1.32	< 0.001
Complete middle - incomplete high	56.0	54.6	57.4	1.18	1.14	1.23	< 0.001	1.68	1.61	1.76	< 0.001
Complete high	72.7	71.7	73.7	2.47	2.33	2.61	< 0.001	2.81	2.64	2.98	< 0.001
Race/ethnicity											
White	60.8	54.8	66.6	1.00				1.00			
Black	52.1	45.8	58.3	0.70	0.66	0.74	< 0.001	0.85	0.80	0.90	< 0.001
Mixed	48.6	41.3	56.0	0.61	0.52	0.72	< 0.001	0.53	0.45	0.63	< 0.001
Native	50.0	43.9	56.2	0.65	0.62	0.67	< 0.001	0.85	0.81	0.88	< 0.001
Region	56.9	50.7	62.9	0.85	0.66	1.09	0.201	1.21	0.94	1.57	0.146
Northern											
Northeastern	45.9	43.8	47.9	1.00				1.00			
Southeastern	48.1	46.4	49.8	1.09	1.02	1.17	0.008	1.05	0.98	1.12	0.206
Southern	60.9	59.3	62.4	1.84	1.73	1.96	< 0.001	1.50	1.40	1.61	< 0.001
West-Central	57.8	56.0	59.5	1.61	1.50	1.74	< 0.001	1.32	1.22	1.43	< 0.001
Centro-Oeste	53.6	52.2	55.1	1.36	1.26	1.48	< 0.001	1.22	1.11	1.33	< 0.001

OR: odds ratio; 95% CI: 95% confidence interval. Adjustment for the other variables.

significantly lower frequencies of medical diagnosis of high cholesterol. The lowest self-reported rates of high cholesterol were observed in the Northern and West-Central regions.

Figure 1 shows that half of the participants had their first medical diagnosis of high cholesterol in their fifth or sixth decade. One fifth of the interviewees reported having their first medical diagnosis of high cholesterol after the age of 60 years. Figure 2 shows the recommendations provided to individuals in the face of their medical diagnosis of high cholesterol, the most common being "to eat healthy", "to maintain an adequate weight" and "to practice physical activity". Drug prescription was recommended in two-thirds of the cases.

Discussion

This study shows, for the first time, the prevalence of dyslipidemia in a representative sample of the Brazilian population, in which one in every eight individuals self-reported having high cholesterol levels. The frequency of the self-reported diagnosis was higher among women, white and Asian-heritage individuals, those with higher educational level and residents of the Southern, Southeastern and West-Central regions.

According to the 2014 VIGITEL-BRASIL (Brazilian surveillance system on risk and protective factors of chronic diseases via telephone survey), performed in the 27 Brazilian capitals, the prevalence of a previous medical diagnosis of dyslipidemia was 20.0% (women, 22.2%, and men, 17.6%). In both sexes, the diagnosis of high cholesterol was associated with age increase and higher educational level. Such data are in accordance with the results of this analysis based on the PNS data.[9]

Prevalence studies of the medical diagnosis of high cholesterol have shown low sensitivity for the definitive diagnosis (55%) as shown in the *Third National Health and*

Table 2 – Proportion of individuals aged at least 18 years self-reporting a medical diagnosis of high cholesterol

Variables	%	95% CI		Raw OR	95% CI		p	Adjusted OR	95% CI		p
		Lower	Upper		Lower	Upper			Lower	Upper	
Brazil	12.5	12.1	13.0								
Sex											
Male	9.7	9.0	10.3	1.00				1.00			
Female	15.1	14.4	15.7	1.66	1.58	1.74	< 0.001	1.61	1.53	1.70	< 0.001
Age											
18-29 years	2.8	2.3	3.3	1.00				1.00			
30-59 years	13.3	12.6	13.9	5.26	4.77	5.81	< 0.001	5.04	4.55	5.57	< 0.001
60-64 years	25.9	23.2	28.6	12.02	10.65	13.57	< 0.001	11.13	9.83	12.60	< 0.001
65-74 years	25.5	23.3	27.7	11.76	10.48	13.19	< 0.001	10.79	9.57	12.17	< 0.001
≥ 75 years	20.3	17.7	23.0	8.78	7.69	10.02	< 0.001	7.89	6.87	9.05	< 0.001
Schooling											
None – incomplete elementary	15.8	15.0	16.7	1.13	1.05	1.22		0.95	0.88	1.03	
Complete elementary – incomplete middle	10.1	8.9	11.3	0.67	0.61	0.74	0.001	0.90	0.82	0.99	0.191
Complete middle - incomplete high	9.1	8.4	9.8	0.60	0.56	0.65	< 0.001	0.82	0.75	0.89	0.038
Complete high	14.3	12.9	15.6	1.00			< 0.001	1.00			< 0.001
Race/ethnicity											
White	13.4	12.6	14.1	1.00				1.00			
Black	11.2	9.7	12.8	0.82	0.75	0.90	< 0.001	0.83	0.76	0.92	< 0.001
Mixed	16.1	11.3	22.5	1.25	0.99	1.57	0.056	1.26	1.00	1.60	0.054
Native	11.8	11.1	12.5	0.87	0.83	0.91	< 0.001	0.98	0.92	1.04	0.441
Region	15.1	11.2	20.1	1.16	0.82	1.63	0.408	1.33	0.93	1.90	0.118
Northern											
Northeastern	10.2	9.2	11.1	1.00				1.00			
Southeastern	12.2	11.5	12.9	1.23	1.10	1.37	< 0.001	1.12	1.01	1.26	0.040
Southern	13.3	12.4	14.1	1.35	1.22	1.50	< 0.001	1.15	1.04	1.29	0.010
West-Central	13.0	11.8	14.2	1.32	1.18	1.48	< 0.001	1.14	1.01	1.29	0.037
Centro-Oeste	11.0	10.1	11.9	1.09	0.95	1.25	0.212	1.00	0.87	1.15	0.971

OR: odds ratio; 95% CI: 95% confidence interval. Adjustment for the other variables.

Nutrition Examination Survey carried out in the United States (1988-1994) with a representative population of that country.[10] In a survey involving 12 European countries, self-report associated with only 30% of the cases diagnosed with the specific test.[11] Despite the low sensitivity, when participants of the *Women´s Health Study* reported their cholesterol level measured, there was strong association between those self-reported values and the higher incidence of cardiovascular disease in 10 years.[12]

The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) has compared the self-report of high cholesterol medical diagnosis with the diagnosis based on an LDL-cholesterol measurement greater than 130 mg/dL or the use of lipid-lowering drugs. The following were obtained: sensitivity of 51.5% (95%CI = 50.4%-52.5%); specificity of 86.0% (65.1-86.8); and positive and negative likelihood ratios of 3.7 (3.4-3.9) and 0.56 (0.55-0.58), respectively.

With this prevalence of self-reported high cholesterol of 12.5%, the real prevalence of dyslipidemia in the Brazilian population can be estimated as 46.6%.[13]

The 2013 PNS has the limitations of a population-based survey conducted in a country of continental dimensions. However, considering the Brazilian reality, the study design and operation reached an adequate level of quality. Data generalization was relatively safe for the national and regional projections. Surveys, such as the PNS, use self-reported information on medical diagnosis, which is a limited method of assessment. Evaluation by a physician, nurse or via a previously tested standard questionnaire has better accuracy as already proved in a systematic review of population surveys using self-report.[14] However, considering Brazil's continental dimensions, that is the fastest and most inexpensive way to assess the prevalence of some conditions, such as high cholesterol.

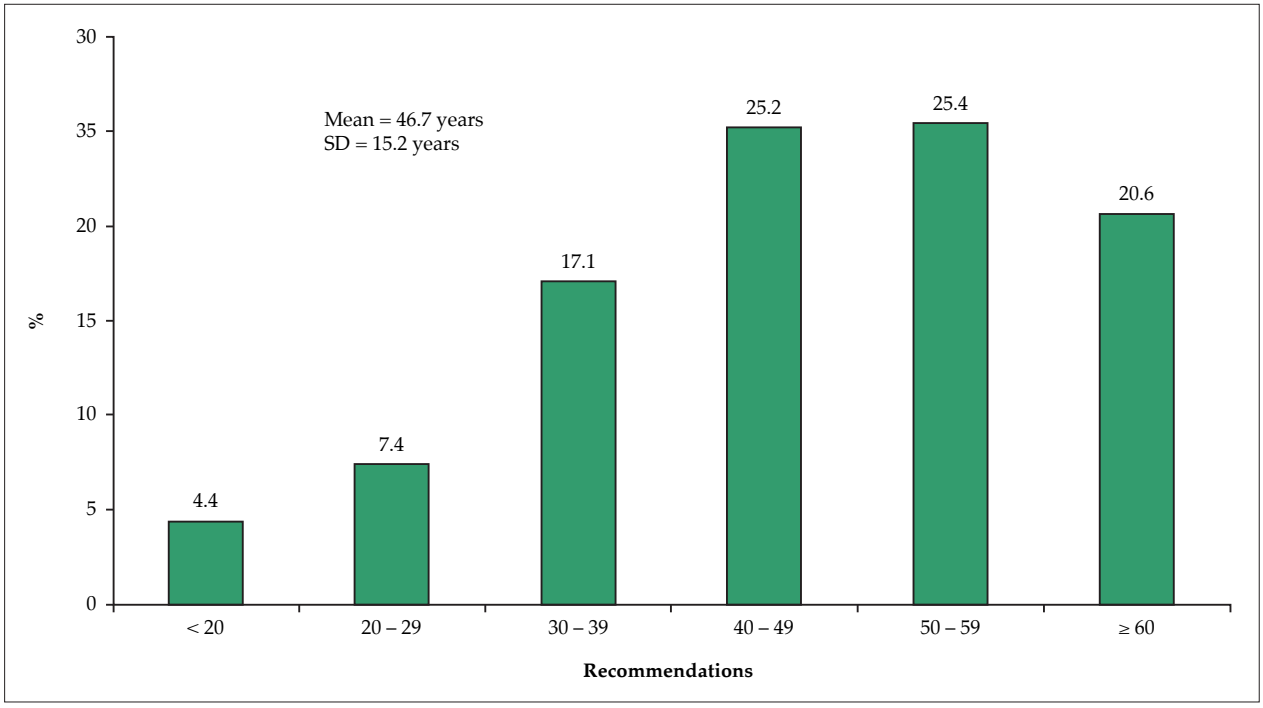

Figure 1 – *Age at the time of the first diagnosis of high cholesterol levels.*

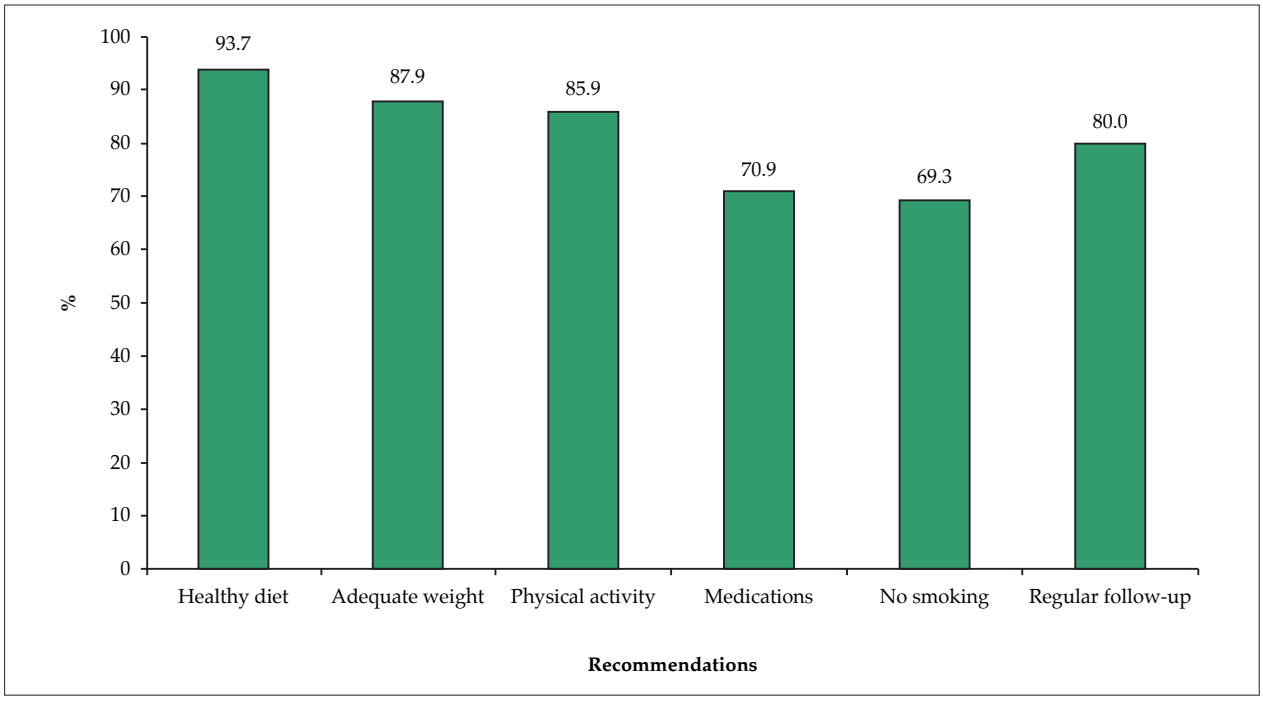

Figure 2 – *Recommendations to the participants who reported medical diagnosis of high cholesterol.*

The importance of the PNS data on the medical diagnosis of high cholesterol is justified by the data obtained from a review study of population surveys assessing the historical trend of cholesterol levels since 1980 in three million participants in Latin America, which has concluded that there were few studies on the diagnosis of dyslipidemia in those populations.[5]

The importance of dyslipidemia awareness in the current Brazilian epidemiological context should be emphasized to guide actions of control and prevention of coronary heart disease, the leading cause of death in Brazil and worldwide, and that determines higher mortality in disadvantaged social strata.[15]

Conclusion

In a representative population sample of Brazil, this study showed that 10% of the men and 15% of the women had a medical diagnosis of high cholesterol.

Author contributions

Conception and design of the research: Lotufo PA, Szwarcwald C, Stoppa SR, Malta DC. Acquisition of data: Szwarcwald C, Stoppa SR, Malta DC. Analysis and interpretation of the data: Lotufo PA, Santos RD, Prado RR, Bensenor IM. Statistical analysis: Lotufo PA, Prado RR, Stoppa SR, Malta DC. Obtaining financing: Szwarcwald C. Writing of the manuscript: Lotufo PA, Santos RD, Sposito AC, Bertolami M, Faria-Neto JR, Izar MC, Bensenor IM. Critical revision of the manuscript for intellectual content: Lotufo PA, Santos RD, Sposito AC, Bertolami M, Faria-Neto JR, Izar MC, Szwarcwald C, Prado RR, Stoppa SR, Malta DC, Bensenor IM.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Prognostic Value of Coronary Flow Reserve Obtained on Dobutamine Stress Echocardiography and its Correlation with Target Heart Rate

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Abstract

Background: Normal coronary flow velocity reserve (CFVR) (≥ 2) obtained in the left anterior descending coronary artery (LAD) from transthoracic echocardiography is associated with a good prognosis, but there is no study correlating CFVR with submaximal target heart rate (HR).

Objective: To evaluate the prognostic value of CFVR obtained in the LAD of patients with preserved (>50%) left ventricular ejection fraction (LVEF) who completed a dobutamine stress echocardiography (DSE), considering target HR.

Methods: Prospective study of patients with preserved LVEF and CFVR obtained in the LAD who completed DSE. In Group I (GI = 31), normal CFVR was obtained before achieving target HR, and, in Group II (GII = 28), after that. Group III (G III=24) reached target HR, but CFVR was abnormal. Death, acute coronary insufficiency, coronary intervention, coronary angiography without further intervention, and hospitalization were considered events.

Results: In 28 ± 4 months, there were 18 (21.6%) events: 6% (2/31) in GI, 18% (5/28) in GII, and 46% (11/24) in GIII. There were 4 (4.8%) deaths, 6 (7.2%) coronary interventions and 8 (9.6%) coronary angiographies without further intervention. In event-free survival by regression analysis, GIII had more events than GI ($p < 0.001$) and GII ($p < 0.045$), with no difference between GI and GII ($p = 0.160$). After adjustment, the only difference was between GIII and GI ($p = 0.012$).

Conclusion: In patients with preserved LVEF and who completed their DSE, normal CFVR obtained before achieving target HR was associated with better prognosis. (Arq Bras Cardiol. 2017; 108(5):417-426)

Keywords: Echocardiography, Stress; Heart Rate; Prognosis; Fractional Flow Reserve, Myocardial.

Introduction

For decades stress echocardiography has been used to assess coronary artery disease (CAD), and has been established as an important diagnostic and prognostic tool.[1-3] The most used pharmacological stressors are those that act as vasodilators (dipyridamole and adenosine) or those that increase myocardial oxygen consumption (dobutamine) by increasing cardiac work.[4] However, the literature shows that, in addition to the consistent positive inotropic effect, the action of dobutamine as a coronary vasodilator might provide important information during dobutamine stress echocardiography (DSE).[5,6]

The assessment of coronary flow velocity reserve (CFVR) in the left anterior descending coronary artery (LAD) has been often used in the clinical setting, because it adds diagnostic and prognostic value to pharmacologic stress echocardiography.[7-15] Despite their distinct mechanisms of action, the myocardial flow responses to adenosine and dobutamine in CAD have a linear correlation, dobutamine being comparable to adenosine in the same population with preserved left ventricular ejection fraction (LVEF), and both drugs provide concordant CFVR values.[5,6]

Several publications have considered a CFVR value ≥ 2 as normal and suitable to infer good prognosis or absence of significant coronary artery stenosis.[6,10-12,16-20] When CFVR values are higher at the early stages of DSE, the exam is expected to be completed with no contractile abnormality compatible with myocardial ischemia.[21] However, a low CFVR value at the early stages of DSE can anticipate the occurrence of myocardial ischemia manifest as contractile abnormality.[22]

Normal CFVR in the LAD can be obtained before (early) or after (late) submaximal target heart rate (HR) is reached.[20,23] Although the relevance of CFVR has been established, the meaning of normal CFVR obtained at the early stage of DSE has not been clarified. Thus, this study aimed at assessing the prognostic value of CFVR obtained in the LAD of patients with preserved LVEF (>50%) who completed DSE after reaching submaximal target HR.

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Methods

This is a prospective observational study performed during two years in a population selected from the previous study by Abreu et al.,[23] which has assessed CFVR during DSE.

The decision to refer patients with known or probable CAD for assessment with DSE was exclusively up to their attending physicians. After collecting the clinical history, risk factors for CAD were assessed and transthoracic echocardiography was performed. When not contraindicated, patients underwent DSE. The exclusion criteria were as follows: uncontrolled arterial hypertension; unstable angina; congestive heart failure; recent myocardial infarction (within one month from DSE); important heart valvular disease; prostate disease or glaucoma with contraindication for atropine use; and non-sinus rhythm.

The present study included patients with preserved LVEF (>50%) on transthoracic echocardiography and who completed DSE after attaining submaximal target HR. Normal CFVR (≥ 2) was classified into early or late, based on being obtained before or only after reaching submaximal HR, respectively. In all cases with abnormal CFVR values, CFVR recording was obtained at the end of DSE. The DSE protocols and CFVR recordings are described below.

Dobutamine stress echocardiography

At our service, the DSE protocol instructs patients to suspend beta-blockers 72 hours before the exam, and to resume their use after the procedure. The other drugs should be maintained. All patients were informed about the risks and objectives of the exam, which was only initiated after the patient's verbal consent. For the DSE, the Vivid 7 echocardiography device (GE Healthcare) with second harmonic image and the M4S multifrequency transducer with frequency ranging from 2 to 4 MHz were used. The left ventricle was visualized in the apical (4- and 2-chamber) and parasternal (long and short axes) views at rest and during dobutamine use at the doses of 10 (low dose), 20, 30 up to 40 μg/kg/min and 3-minute intervals. The images were obtained at rest, low-dose, peak and recovery phases, and compared on a quadruple screen. Atropine could be added after the second stage (incremental doses of 0.25 mg up to the maximal cumulative dose of 2 mg). The DSE was completed after submaximal target HR [(220 − age) x 85%] was attained and/or myocardial ischemia was found.

Ischemia was considered as the report of typical angina, new contractile abnormality or worsening of a preexisting one (except from akinesia to dyskinesia). The exam would be interrupted in the presence of intolerance to medication, hypertensive peak (blood pressure > 230/120 mm Hg), or cardiac arrhythmia. The left ventricle was divided into 16 segments, and a numerical score was given to each segment depending on contractility as follows: normal = 1; hypokinetic = 2; akinetic = 3; or dyskinetic = 4. The segmental contraction score index was calculated by dividing the points obtained by 16.[1,2,23]

Left anterior descending coronary artery assessment

Pulsed color Doppler of the LAD was recorded in the left lateral decubitus, the same position used for the DSE. The LAD was visualized in its mid-distal region with a pre-established specific preset, based on acquisition from the low parasternal long-axis, 2-chamber or modified 3-chamber view, concomitant with little adjustments of angulation or rotation of the transducer. Using a small box of color Doppler with Nyquist limit of approximately 20 cm/s, LAD appeared as a tubular image, in which the greatest possible stretching and extension were determined, as well as the smallest angulation, with the Doppler cursor, whose sample volume was 2 mm. By use of pulsed Doppler, the flow assessed was characterized by biphasic spectrum with diastolic predominance, and anterograde curves above baseline were recorded. Initially, the Doppler velocity scale was limited to 80 cm/s and could be widened during DSE, allowing the capture of subsequent velocity increases of the Doppler curves.

By using Doppler assessment of LAD synchronized with electrocardiography, peak diastolic velocities (PDV) were recorded, selecting three spectral curves at rest and during stress, not necessarily continuous, but with good quality and higher velocities. The CFVR was obtained by dividing the PDV (mean of three peaks) occurring during DSE by the baseline PDV (mean of three peaks) recorded at rest. By use of the same transducer, visualization of the two-dimensional left ventricular image and of Doppler of the LAD was alternated. Thus, the quadruple screen of the DSE was filled in the different stages, concomitantly with PDV recordings until the end of the exam. Right after completing the exam, the DSE result was defined, and CFVR, calculated.[6,18-20,22-24]

During the study, the patients' management was determined exclusively by their attending physicians. Independently of their groups, the patients were followed up to assess the occurrence of events, which were established as follows: cardiovascular death; acute coronary insufficiency; coronary intervention (hemodynamic or surgical); coronary angiography (without further intervention during follow-up); and hospitalization (due to angina pectoris, heart failure, or cardiac arrhythmia). Cardiovascular death was considered as death secondary to any of the events cited or any other condition with acute cardiac impairment. Because of the different intensities and possible gradation of events, in the absence of death, the follow-up of all patients was maintained.

Information on all patients' clinical outcome was obtained through contact with the patients, their guardians or attending physicians, and through medical record or death certificate review.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation, while categorical variables were expressed as absolute number and percentage.

Data descriptive analysis per group was performed by use of contingency tables and descriptive measures. The homogeneity of the groups in regard to the categorized variables was tested by use of Fisher exact test. The normality of the distribution of quantitative variables per group was assessed by use of Shapiro-Wilk test. The homogeneity of the groups regarding variances was assessed with Levene test. The homogeneity of the groups regarding quantitative

variables was analyzed with ANOVA (analysis of variance) for variables with normal distribution, or with nonparametric Kruskal-Wallis test of independent samples for variables with non-normal distribution. The variables whose groups differed significantly underwent sub-hypothesis tests by use of the minimum significant difference test. Overall survival for the event in the groups was analyzed by use of Kaplan-Meier regression. The groups were adjusted by use of Cox regression and Wald statistics, and underwent pairwise comparison. The analyses were performed with the SPSS software 20.0 (SPSS Inc., Chicago, IL, USA). For all analyses, $p < 0.05$ was adopted as statistically significant.

Results

Clinical characteristics

Of the 100 patients with LAD flow obtained at rest, 92 could have their LAD flow obtained during stress. However, 5 patients could not complete their DSE. Therefore, this study population consisted of an initial sample of 87 patients.

The assessment lasted 28 ± 4 months, and follow-up was performed in 83 patients of the 87 (95.4%), because 1 patient with early CFVR and 3 with abnormal CFVR were lost to follow-up. Of the 59 patients with normal CFVR, 31 had early CFVR (Group I) and 28 had late CFVR (Group II). Group III consisted of 24 patients with abnormal CFVR. The clinical data of the 83 patients studied were as follows: mean age, 63 ± 11 years; men, 48 (57.8%); hypertensive, 58 (70%); dyslipidemic, 53 (64%); diabetic, 12 (14.5%); and known CAD, 24 (29%) patients. Table 1 shows that those clinical data did not differ between the groups, and neither did the body mass index. Regarding medications, the analysis of homogeneity between the groups did not differ concerning the use of the following drugs: antiplatelet agents ($p = 0.059$); anti-hypertensive agents ($p = 0.924$); lipid-lowering drugs ($p = 0.257$); hypoglycemic agents ($p = 0.792$); and nitrates ($p = 1.000$). The time elapsed between DSE and an event occurrence did not differ between the groups. The event occurrence, however, differed.

Echocardiographic and hemodynamic assessment

Of the echocardiographic variables assessed only at baseline, LVEF was preserved, while left ventricular mass index evidenced ventricular hypertrophy and did not differ between the groups. Regarding the echocardiographic and hemodynamic variables recorded at rest and during stress, the segmental contraction score index did not differ between the groups, and the frequency of DSE compatible with myocardial ischemia was low. Heart rate and double product did not differ; however, the groups differed in the number of patients attaining maximal HR predicted for age (Table 2).

On Doppler assessment of LAD, the groups differed regarding the HR during PDV recording at rest, as well as the PDV values at rest. However, during stress, PDV did not differ, resulting in different CFVR values in the groups (Table 2). When comparing those different echocardiographic and hemodynamic variables, in Group III, PDV at rest was higher and CFVR was lower than in the other two groups; however, those variables did not differ when comparing Groups I and II. The HR during stress on PDV recording in Group I was lower than in Groups II and III, compatible with this study protocol. The LVEF differed between Groups II and III (Table 3).

It is worth noting that, on several occasions, normal early CFVR could be obtained with the infusion of dobutamine at the dose of 20 $\mu g.kg^{-1}.min^{-1}$, when HR was far below the submaximal HR calculated for the case (Figures 1 and 2).

Presence or absence of ischemia during DSE and occurrence of events

Of all DSE performed, 6 (7.2%) were positive for myocardial ischemia, 1 in Group I, 2 in Group II and 3 in Group III, and events were observed in 4 of those 6 patients, with 1 coronary angiography without further intervention and 1 stent implantation in Group II and Group III. Considering all 83 patients studied, the mean time for occurrence of events was 17 ± 8 months. During the follow-up of 28 ± 4 months, events were observed in 18 (21.6%) patients as follows: 4 deaths (4.8%); 6 coronary interventions (7.2%); and 8 coronary angiographies without

Table 1 – Clinical aspects

	Group I	Group II	Group III	p
Patients	31 (100)	28 (100)	24 (100)	
Age (years)	60 ± 10	64 ± 12	66 ± 8	0.092
Women	13 (42)	9 (32)	13 (54)	0.273
BMI (kg/m²)	27.5 ± 4.5	27.3 ± 3	28.5 ± 7	0.991
Hypertension	18 (58)	21 (75)	19 (79)	0.216
Dyslipidemia	19 (61)	19 (68)	15(62.5)	0.881
Diabetes	5 (16)	4 (14)	3 (12.5)	1.000
Known CAD	6 (19)	8 (29)	10 (42)	0.183
Time between DSE and the event (months)	28 ± 3	25 ± 8	23 ± 8	0.382
Events	2 (6.5)	5 (18)	11 (46)	0.002

BMI: body mass index; CAD: coronary artery disease; DSE: dobutamine stress echocardiography. Measures expressed as number (percentage) or mean ± standard deviation.

Table 2 – Echocardiographic and hemodynamic variables by group

Patients		Group I 31	Group II 28	Group III 24	p
Ejection fraction (%)					
	(Rest)	65 ± 7	67 ± 4	62 ± 9	0.019
LVMI (g/m²)					
	(Rest)	126 ± 29	130 ± 45	135 ± 37	0.670
SCSI					
	(Rest)	1.04 ± 0.15	1.02 ± 0.06	1.06 ± 0.21	0.086
	(Stress)	1.03 ± 0.09	1.02 ± 0.05	1.07 ± 0.24	0.949
Stress without ischemia		30 (96,8)	26 (92.9)	21 (87.5)	0.430
HR (bpm)					
	(Rest)	68 ± 12	68 ± 11	74 ± 12	0.096
	(Stress)	149 ±11	147 ± 13	147 ± 11	0.677
Maximal HR achieved		2 (6,5)	6 (21)	10 (42)	0.007
Double product (mmHg.bpm)					
	(Rest)	8548 ± 2010	8749 ± 2159	9681 ± 2020	0.107
	(Stress)	22108 ± 2896	22700 ± 3449	22215 ± 2833	0.742
PDV (cm/s)					
	(Rest)	24 ± 5	28 ± 6	38 ± 8	< 0.0001
	(Stress)	60 ± 16	68 ± 15	65 ± 17	0.143
HR at PDV					
	(Stress)	105 ± 16	135 ± 14	132 ± 17	< 0.0001
CFVR		2,53 ± 0,60	2.50 ± 0.57	1.7 ± 0.24	< 0.0001

LVMI: left ventricular mass index; SCSI: segmental contraction score index; HR: heart rate; double product: systolic blood pressure x heart rate; PDV: peak diastolic velocity; CFVR: coronary flow velocity reserve. Measures expressed as number (percentage) or mean ± standard deviation.

further intervention (9.6%). Considering all events, 6% (2/31) occurred in Group I, 18% (5/28) in Group II, and 46% (11/24) in Group III (Table 4).

Of the 8 coronary angiographies without further intervention, only 3 were performed within 1 year of follow-up (1 in Group II and 2 in Group III), while all interventions (stent implantation) were performed after 1 year of follow-up. Of the 4 deaths, 1 occurred in Group I (26.5 months after DSE) and was attributed to complications after myocardial revascularization surgery. The death in Group II (3 months after DSE) occurred during heart surgery to treat exacerbated mitral insufficiency (secondary to valve prolapse) and CAD. The other 2 deaths were observed in Group III: 1 simultaneous with pulmonary embolism; and 1 occurred 20 months after DSE during heart surgery for heart valve replacement in a patient with calcified coronary arteries. During the follow-up, we obtained no information allowing us to infer the diagnosis of the acute coronary insufficiency or of the hospitalization due to a cause other than those already cited (Table 4).

Regarding the Kaplan-Meier regression analysis of event-free survival, Groups I and II did not differ, and had a better outcome than Group III. However, after adjusting for age and LVEF, Group II did not differ from Group III, and the best event-free survival was maintained only in Group I when compared to Group III (Figure 3).

Discussion

A negative pharmacologic stress echocardiography for ischemia associates with good prognosis and less need for myocardial revascularization. However, in both micro- and macrocirculation contexts, CFVR obtained in the LAD adds value to the information provided by stress echocardiography. Patients with exams showing normal myocardial contractility and normal CFVR in the LAD have mortality lower than 1% per year, while those with impaired contractility and abnormal CFVR have mortality greater than 10% per year. Even for octogenarians, RFVC is a strong and independent predictor of death and of myocardial infarction, mainly when contractility is preserved. Those results support the measurement of CFVR during pharmacologic stress echocardiography, favoring its incorporation into routine practice.[10,12-15,25,26]

Table 3 – Comparison of the different variables by group

	Group I	Group II	Group III	p*	p**	p***
PDV (Rest)	24 ± 5	28 ± 6	38 ± 8	< 0.001	0.001	0.105
CFVR	2.53 ± 0.6	2.50 ± 0.6	1.69 ± 0.2	< 0.001	< 0.001	1.000
HR at PDV	105 ± 16	135 ± 14	132 ± 17	< 0.001	1.000	< 0.001
Ejection fraction	65 ± 7	67 ± 4	62 ± 9	0.072	0.023	1.000

PDV: peak diastolic velocity; CFVR: coronary flow velocity reserve; HR: heart rate. p (Group III vs I); p** (Group III vs II); p*** (Group II vs I).*

Figure 1 – *Male patient with target heart rate (HR) of 142 bpm. Figure 1A shows Doppler assessment in the left anterior descending coronary artery (LAD) at baseline. Figure 1B, during dobutamine stress echocardiography (DSE), dose = 20 µg.kg^{-1}.min^{-1} and HR = 68 bpm, shows normal (=2.78) and early (obtained before achieving target HR) coronary flow velocity reserve (CFVR). Normal left ventricular contractility during the entire exam.*

In several studies assessing myocardial ischemia or risk stratification by use of transthoracic echocardiography, CFVR and contractile abnormality are evaluated by use of adenosine or dipyridamole, or patients are submitted to an additional stress with dobutamine to assess the induction of contractile abnormality. However, the vasodilator effect of dobutamine in the presence of preserved LVEF is comparable to that of adenosine, which is similar to that of dipyridamole. Because dobutamine is one of the most used drugs in stress echocardiography, it is worth noting the possibility of, in the same exam, having a consistent positive inotropic effect on the cardiac muscle and a proper coronary vasodilating effect to calculate CFVR.[6,12,13,15,20,21,27]

The CFVR obtained during DSE can anticipate the probable result of the exam regarding cardiac muscle contractility, so that a better reserve associates with better contractile response, regardless of whether CFVR is obtained early or late.[21,22] However, the prognostic value of early CFVR has not been established in the literature, and this has motivated the present study.

The groups assessed in this study did not differ regarding age, sex, presence of hypertension, dyslipidemia, diabetes and known CAD, use of medications, and not even left ventricular mass index and baseline double product and HR, factors that could influence the measurement of baseline PDV, and, consequently, CFVR.[28] However, to which extent pathological conditions, such as hypertension, diabetes and dyslipidemia, affect each individual cannot be established. Thus, Group III had higher baseline PDV, which could express predominance of an abnormal microvascular component over a possible epicardial coronary artery stenosis. However, regardless of which component (micro- or macrovascular) is more important, both can be related to worse prognosis.[10,12-15,25,27]

The recording of a slightly altered segmental contraction score index at rest and during stress might have resulted from the preserved LVEF and lack of ischemic response in a greater number of exams. During the study, 4 of the 6 patients with positive DSE for ischemia had events, which might have been expected by their attending physicians or might partially represent a bias. However, although 77 of the 83 patients (92.8%) could be considered of low risk because of their negative DSE, determining the expectation of good prognosis, the CFVR measure provided better risk stratification. Regarding the patients with negative DSE for ischemia and later submitted to intervention, progression of preexisting CAD might have occurred or there might have been a false-negative DSE for ischemia. An explanation for that could be the fact that maximal HR was not achieved.[29]

Original Article

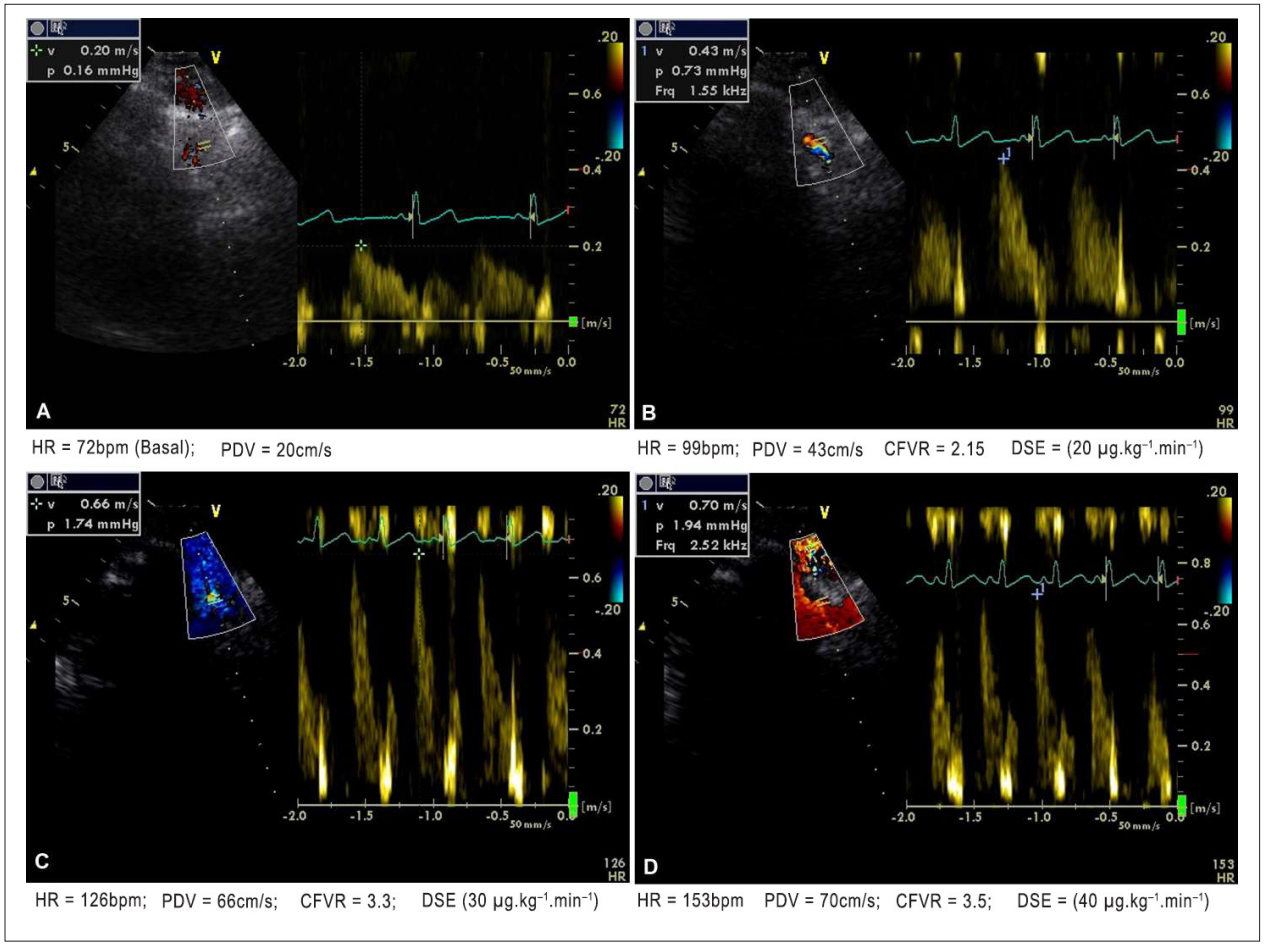

Figure 2 – *Male patient with target heart rate (HR) of 140 bpm. Figure 2A shows Doppler assessment in the left anterior descending coronary artery (LAD) at baseline. Figure 2B, during dobutamine stress echocardiography (DSE), dose = 20 µg.kg^{-1}.min^{-1} and HR = 99 bpm, shows normal (2.15) and early coronary flow velocity reserve (CFVR), which increases progressively, even after reaching target HR (Figure 2D). Normal left ventricular contractility during the entire exam.*

The hemodynamic studies were requested by the attending cardiologists, as were the further interventions, which were indicated based on the importance of the coronary artery stenosis. Most hemodynamic studies (11/14 - 79%) were performed after 1 year of follow-up. However, it is worth noting that, considering only patients with negative DSE for ischemia, in 80% (8/10) of those with hemodynamic study, that study was performed more than 1 year after the DSE, possibly expressing rather a clinical decision than a bias of the CFVR result previously informed. Those observations can suggest that the disclosure and recognition of the importance of the CFVR obtained on stress echocardiography is still limited, which could determine decisions rather related to the presence or absence of ischemia. Based on the research protocol, we do not interfere with the management of the attending cardiologists, but it is worth noting that only 1 hemodynamic study was requested for Group I patients.

The 4 deaths in this study occurred among patients with negative DSE for myocardial ischemia. In Group I, that event occurred after myocardial revascularization, which was performed 2 years after the exam. In Group II, the death occurred 3 months after the DSE, resulting from complications during surgery to repair acute mitral insufficiency in a patient with mitral valve prolapse. However, the death certificate available did not provide further information on the relevance of the CAD reported. In Group III, 1 death was attributed to pulmonary embolism, and the other death occurred in a patient who met no criteria for severe heart valve disease on DSE. The patient died 20 months after the exam, during heart surgery for heart valve replacement, when calcified coronary arteries were identified.

On the Kaplan-Meier regression analysis of event-free survival, the groups of patients with normal CFVR did not differ between themselves, and had better outcome than those with abnormal CFVR. However, after adjusting for age and LVEF, Group II did not differ from Group III, and the better event-free survival was maintained only for Group I.

The literature shows that the prognosis of patients with normal CFVR is better than that of patients with abnormal CFVR. However, in our study, the patients with preserved LVEF only had better prognosis in the presence of normal early CFVR.

Table 4 – Distribution of the cases regarding the presence or absence of ischemia during dobutamine stress echocardiography (DSE) and the occurrence of events in the groups

Groups	Ischemia during DSE	Myocardial segment affected	Events	Mean time between DSE and event (months)
Group I (CFVR ≥ 2)	No	-	Death	26.5
	No	-	Coronary angiography	13.4
Group II (CFVR ≥ 2)	Yes	Septal	Coronary angiography	1.1
	Yes	Septal	Stent	15.3
	No	-	Stent	15.4
	No	-	Coronary angiography	12.5
	No	-	Death	3.1
Group III (CFVR < 2)	No	-	Stent	28
	No	-	Coronary angiography	8.3
	No	-	Stent	21
	No	-	Stent	14.8
	No	-	Death	7.2
	No	-	Coronary angiography	14.7
	Yes	Lateral	Stent	17
	No	-	Coronary angiography	7.9
	Yes	Inferior	Coronary angiography	24.3
	No	-	Death	19.8
	No	-	Coronary angiography	22.4

Normal coronary flow velocity reserve (CFVR ≥ 2) was obtained before (Group I) and after (Group II) reaching submaximal heart rate. Coronary angiography - hemodynamic study without further coronary intervention (angioplasty, stent or surgery).

Figure 1 shows that, with a HR of 68 bpm, normal early CFVR (= 2.78) could already be obtained, demonstrating the significant vasodilating effect of dobutamine infusion (20 $\mu g.kg^{-1}.min^{-1}$). Figure 2 shows the higher baseline HR recording, but like the previous case, normal early CFVR (= 2.15) was also obtained in the second stage of DSE, with the simultaneous HR of 98 bpm. Those findings are in accordance with those by Takeuchi et al.,[21] who have reported that patients with normal CFVR recorded in the intermediate stage of DSE (20 $\mu g.kg^{-1}.min^{-1}$) belonged to the group that had no myocardial contractile abnormality next to the coronary artery assessed. In addition, Ahmari et al.[22] have reported that patients who developed no ischemia had a better CFVR with the intermediate dose of dobutamine.

In our study, all patients with normal early CFVR maintained that normal condition during all stages of DSE, and none of them showed contractile impairment of the anterior wall. This suggests that, from the time normal early CFVR is achieved, continuing its recording is no longer necessary. In a study including only patients at low risk for CAD, Forte et al.[20] have reported that, during DSE, 96% of the patients achieved normal CFVR before reaching submaximal target HR, and all of them had a negative exam for ischemia. In our study, the findings of Group I could result from a smaller impairment of the micro- and macrocirculation, which favors the attainment of normal early CFVR during DSE. However, further studies are necessary to confirm this hypothesis.

Clinical implications

Attaining normal early CFVR in the LAD identifies patients with better prognosis. In addition, in that condition, the occurrence of contractile abnormality in the anterior wall during DSE is unlikely. In the exclusive context of contractile abnormality, normal early CFVR is particularly useful when the visualization of the anterior wall is hindered during a stage with higher HR, or even when maximal HR is not achieved, because the accuracy of DSE is lower in that circumstance.[29]

Limitations

Despite the prospective nature of this study, some limitations apply. A larger sample and a longer follow-up could add more information. However, this study sample size and follow-up duration were similar to those of some studies here cited. To assess prognostic value, the CFVR was obtained through PDV recorded only in the LAD, but that condition has been validated and used in several studies cited in the present study. The most

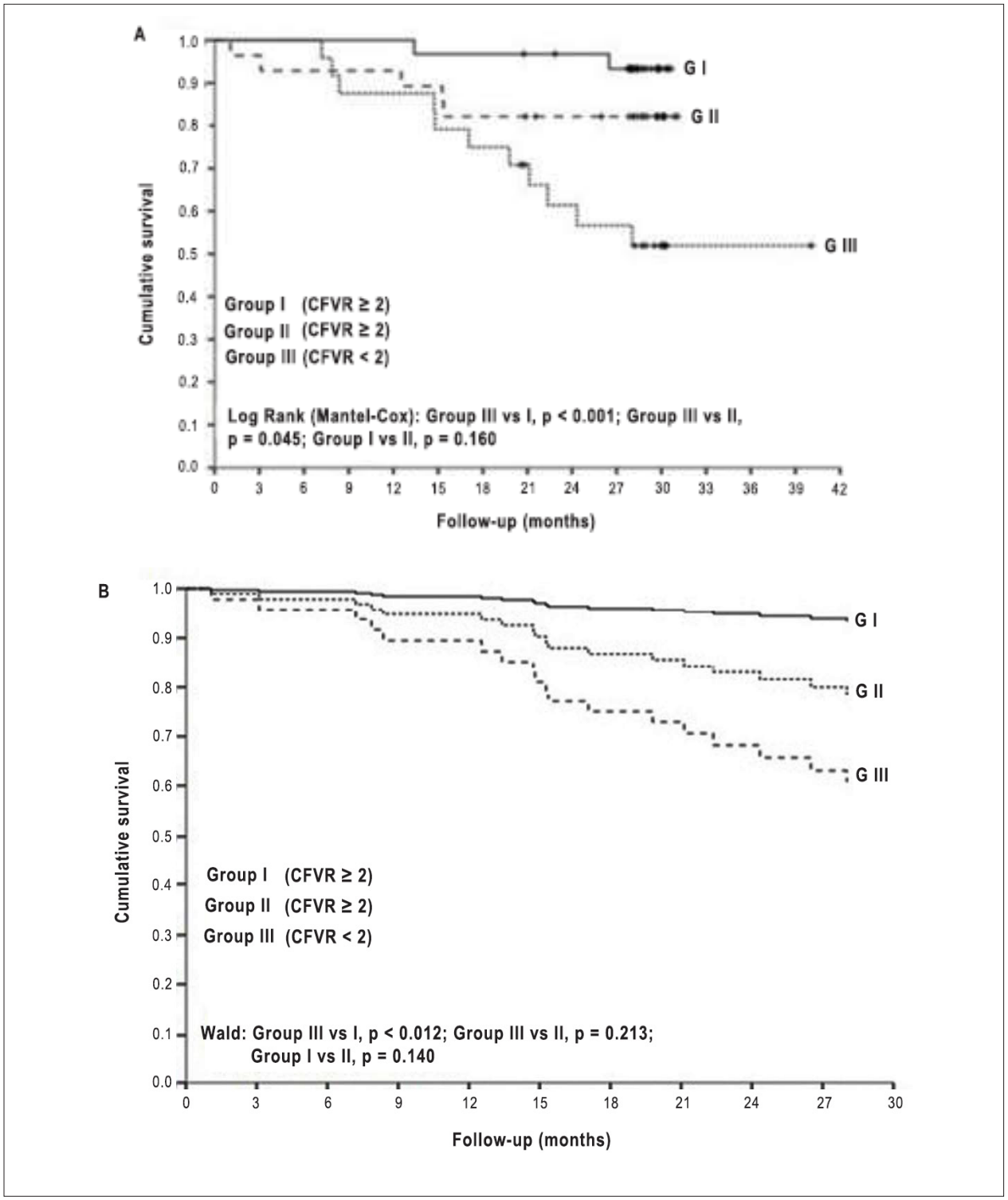

Figure 3 – *A) Kaplan-Meier regression analysis of event-free survival by group. Normal (= 2) coronary flow velocity reserve (CFVR) was obtained before (Group I) and after (Group II) reaching submaximal target heart rate. In Group III, CFVR was abnormal. Group III differed from Group I and Group II, but there was no difference between Group I and Group II. B) Survival for the event adjusted for age and ejection fraction, by use of Cox regression and Wald statistics. Group III and Group I remained different, and the better event-free survival was maintained only in Group I.*

important limitation of this study is that we neither had complete access to the therapy used by the attending cardiologists, nor knew the reasons for choosing each patient's management, mainly regarding the coronary angiographies without further intervention.

Conclusion

In patients with preserved LVEF and who completed the DSE, the normal CFVR obtained before achieving submaximal target HR associated with better prognosis. This study suggests that, after attaining normal early CFVR, continuing its recording is no longer necessary.

Author contributions

Conception and design of the research: Abreu JS, Rocha EA; Acquisition of data: Abreu JS, Machado IS, Parahyba IO, Rocha TB, Diogenes TCP; Analysis and interpretation of the data: Abreu JS, Rocha EA, Paes FJVN, Diogenes TCP, Abreu MEB, Farias AGLP, Carneiro MM; Statistical analysis and Writing of the manuscript: Abreu JS; Critical revision of the manuscript for intellectual contente: Abreu JS, Rocha EA, Farias AGLP, Carneiro MM.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any thesis or dissertation work.

Erratum

In the Original Article "Prognostic Value of Coronary Flow Reserve Obtained on Dobutamine Stress Echocardiography and its Correlation with Target Heart Rate", published in the Arquivos Brasileiros de Cardiologia [Arq Bras Cardiol. 2017; 108(5): 417-426], please be aware that the correct spelling for Isabelle O. Parahyba is Isabelle Oliveira Parahyba and Thais Brito Rocha is Thaís de Brito Rocha.

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Degree of Agreement between Cardiovascular Risk Stratification Tools

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Abstract

Background: **Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in Brazil, and primary prevention care may be guided by risk stratification tools. The Framingham (FRS) and QRISK-2 (QRS) risk scores estimate 10-year overall cardiovascular risk in asymptomatic individuals, but the instrument of choice may lead to different therapeutic strategies.**

Objective: **To evaluate the degree of agreement between FRS and QRS in 10-year overall cardiovascular risk stratification in disease-free individuals.**

Methods: **Cross-sectional, observational, descriptive and analytical study in a convenience sample of 74 individuals attending the outpatient care service of a university hospital in Brazil between January 2014 and January 2015. After application of FRS and QRS, patients were classified in low/moderate risk (< 20%) or high risk (≥ 20%).**

Results: **The proportion of individuals classified as at high risk was higher in FRS than in QRS (33.7% vs 21.6%). A synergic effect of male gender with systemic arterial hypertension was observed in both tools, and with for geriatric age group in QRS ($p < 0.05$) in high-risk stratum. The Kappa index was 0.519 (95%CI = 0.386-0.652; $p < 0.001$) between both instruments.**

Conclusion: **There was a moderate agreement between FRS and QRS in estimating 10-year overall cardiovascular risk. The risk scores used in this study can identify synergism between variables, and their behavior is influenced by the population in which it was derived. It is important to recognize the need for calibrating risk scores for the Brazilian population. (Arq Bras Cardiol. 2017; 108(5):427-435)**

Keywords: **Cardiovascular Diseases / mortality; Cardiovascular Diseases / morbidity; Risk Assessment; Cardiovascular Diseases / epidemiology; Period Analysis.**

Introduction

Cardiovascular disease (CVD) is the main cause of mortality and morbidity in Brazil. It accounts for 20% of deaths in individuals over the age of 30 years, and its high prevalence is associated with inadequate control of risk factors. Identification of asymptomatic subjects at higher risk for CVD is a crucial step in public health policies, since effective control of these factors can reduce the mortality rate by up to 44%.[1,2]

The presence of risk factors allows identifying individuals at higher cardiovascular risk (CVR). Nonetheless, intuitive estimates may either overestimate the low risk or underestimate the high risk, for not considering the interaction between them.[3]

A variety of scores have been developed for CVR stratification, such as the Framingham (FRC) and QRISK-2 (QRS) score, which estimate overall CVR in ten years. FRC was improved in 2008 m and has been widely used in Brazil and in the world along with aggravating factors. QRS was created in the UK, and has been used for cardiovascular prevention in primary care.[4,5]

Despite its practicality and widespread use, FRS may underestimate or overestimate the risk in Hispanic and European populations. This fact motivated the development of QRS. However, studies have pointed out disagreements between both scores in estimating high risk, which may lead to the use of different therapies for the same patient.[6]

Considering both the importance of identifying asymptomatic patients at high risk for CVD, and therapeutic implications of each risk stratification score, we evaluated the degree of agreement between FRS and QRS in 10-year CVR in disease-free individuals attending a teaching hospital.

Methods

Study design

This was a cross-sectional, observational, descriptive, analytical study.

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Subjects

From January 2014 to January 2015, patients who attended scheduled appointments at the Internal Medicine outpatient clinic of a university hospital in the south of Brazil were invited to participate in the study. A total of 120 patients were assessed and, using a convenience sample, our final sample consisted of 74 patients.

Data collection instruments and procedure

Standard form

Data were obtained by interview with participants or from their medical records by medicine students. A standard form (Appendix I) was filled out with these data, including identification, clinical, and laboratory data, as follows.

1. **Identification** – Age (years), considering geriatric (≥ 60 years) and non-geriatric age (< 60 years); sex (female or male); ethnicity (white or non-white); self-defined ethnicity (white or non-white); family income per dependent (self-reported income in minimum wages – MW, BRL724.00[7] – divided by the number of family members), which was classified into high income (> 1MW) or low income (≤ 1 MW); educational attainment – low educational attainment (primary education or none) and high educational attainment (from 'some high school education' to 'bachelor's degree').
2. **Clinical examination** – History of CVD (left ventricular hypertrophy, angina and/or acute myocardial infarction; coronary revascularization or stent; congestive heart failure; intermittent claudication; stroke or transient ischemic attack); type 1 or type 2 diabetes mellitus (DM) (previous diagnosis or in treatment); treated systemic arterial hypertension (SAH) (previous diagnosis and/or use of anti-hypertensive drugs); history of premature coronary artery disease (CAD) (myocardial infarction or sudden death before the age of 55 of patient's father or other male first degree relative, or before the age of 65 of patient's mother or other female first degree relative);[4] rheumatoid arthritis; atrial fibrillation; chronic kidney disease (previous diagnosis) and smoking – non-smokers or smokers (current smokers or who had quit smoking less than 2 years prior to the study).[8] Weight (kg) and height (m) were measured using an anthropometric scale. Systolic arterial pressure (SAP) (mmHg) was measured with patient in the supine position, using an automatic, oscillatory, sphygmomanometer, after five minutes of rest. The highest SAP value between the two upper limbs was considered for analysis.[9] Body mass index (BMI) was calculated considering overweight and obesity as BMI ≥ 25 kg/m² and > 30 kg/m², respectively.[10]
3. **Laboratory tests** – Lipid profile was analyzed by total cholesterol (TC) (mg/dL) and HDL cholesterol (HDL-c) (mg/dL) levels in the last 12 months. An altered lipid profile was considered as TC ≥ 240 mg/dl and/or HDL-c < 40.[3]

Risk stratification scores to estimate the 10-year overall CVR

Framingham risk score (FRS)

FRS estimates CVR based on the variables sex, age, SAP, SAH therapy, smoking, DM, HDL-c and TC. These data were used in the calculators available at Framingham Heart Study website.[11]

Individuals with a 10-year overall CVR ≥ 20% were classified as at high risk. Participants without recent data of lipid profile had their risk estimated by a calculator that used the BMI (in place of lipid data).[12]

QRISK-2 score (QRS)

QRS estimates CVR based on the variables gender, age, ethnicity, smoking, DM, family history of premature CAD, atrial fibrillation, SAH therapy, rheumatoid arthritis; chronic kidney disease; TC/HDL ratio and BMI. QRS was calculated using the risk calculator available at ClinRisk database website.[13] Patients with a risk probability ≥ 20%[14] were classified as at high risk (Table 1).

Participants without recent lipid profile data had their risk determined by multiple imputation technique for missing data.

Criteria definition

Inclusion criteria

Patients of both sex aged between 30 and 74 years (which was the widest age range common for both scores) with no evidence of CVD were included.

Exclusion criteria

Patients with CVD, out of the age range established by the online CVR calculators, or with missing or unreadable information in the medical records were not included in the study.

Statistical analysis

Continuous variables with normal distribution (determined by the Kolmogorov-Smirnov test) were expressed as mean and standard deviation, whereas categorical variables as absolute frequency and proportion. Associations between variables were assessed by the chi-squared test (χ^2), and statistically significant variables were adjusted by linear regression model, which considered the relationship between two risk strata (low/moderate *vs* high risk) as dependent variables. Models were adjusted by backward stepwise analysis and calculation of odds ratio (OR).

Kappa statistics was used to quantitatively assess the agreement between the scores. Kappa coefficients range from -1 to 1 and are classified, according to Landis & Rock (1977):[15] < 0, no agreement; 0-19, poor agreement; 0.20-0.39, fair agreement; 0.40-0.59, moderate agreement; 0.60-0.79, substantial agreement; 0.80-0.99, almost perfect

Table 1 – Characteristics of 10-year cardiovascular risk stratification tools

Score	Local/Studies on instrument derivation	Age	Gender	Variables	Outcomes	Risk
FRS, CVD in 10 years; 2 versions, FRS with lipid levels and FRS with BMI	8,491 participants, Framingham, Massachusetts, United Sates of America, 12 years of follow-up	30-74 years	Male and female	Age, gender, SAP, SAH treatment, TC, HDL-c, DM, smoking, BMI	10-year risk for AMI, coronary insufficiency, angina, ischemic stroke, hemorrhagic stroke, PAOD, heart failure	0-6% low; 6-20% moderate; ≥ 20% high
QRS, CVD in 10 years	2.3 million participants, QRESEARCH database (extracted from primary health care in the United Kingdom), 5.7 years of follow-up	25-84 years	Male and female	Age, gender, ethnics, zip code, smoking, DM, angina, family history of premature CAD in first-degree relative, CKD, AF, SAH treatment, RA, TC / HDL-c ratio, SAP, BMI	10-year risk for AMI, angina, CAD, stroke and TIS	0-10% low; 10-20% moderate; ≥ 20% high risk

FRS: Framingham risk score; QRS: QRISK-2 score; BMI: body mass index; SAP: systemic arterial pressure; SAH: systemic arterial hypertension; TC: total cholesterol; HDL-c: HDL-cholesterol; DM: Diabetes Mellitus; CAD: coronary artery disease; CKD: chronic kidney disease; AF: atrial fibrillation; RA: rheumatoid arthritis; TIS: transient ischemic stroke; PAOD: peripheral artery obstructive disease.
SOURCE: Framingham Heart Study[11] and ClinRisk®[13]

agreement; 1, perfect agreement. Statistical significance was set at $p < 0.05$, and analyses were performed using the SPSS® (Statistical Package for the Social Sciences), version 22.0.

Ethical aspects

The research was approved by the Ethics Committee (project number 1973.8713.8.0000.0121), and informed consent was obtained from all participants before entering the study.

Results

A total of 120 patients were assessed, and the final sample consisted of 74 patients (33 were excluded for CVD and 13 were out of the pre-established age range). Sociodemographic, clinical and laboratory characteristics of patients are described in Table 2. Fourteen (19%) patients did not have recent data of lipid profile.

After risk stratification, low/moderate risk was predominant in both scores. The proportion of individuals classified as 'high risk' was higher in FRS (33.7% vs 21.6%) (Figure 1).

In both instruments, percentages of male, white individuals, non-smokers, and individuals with high SAP and BMI were higher in the high-risk stratum and similar between both scores. DM patients and older hypertensive patients were predominant in QRS and FRS, respectively, in the same stratum (Table 3).

Kappa analysis revealed moderate agreement between FRS and QRS ($K = 0.519$; 95%CI 0.386-0.652; $p < 0.001$).

In both instrument, significant relationships were observed between high-risk stratum and older age (FRS and QRS [$p < 0.001$ and $p = 0.001$]), male sex (FRS and QRS [$p < 0.001$ and $p = 0.001$]), treated SAH (FRS and QRS [$p < 0.001$ and $p < 0.001$]), DM (FRS and QRS [$p < 0.001$ and $p < 0.001$]), and elevated SAP (FRS and QRS [$p = 0.002$ and $p < 0.003$]) (Table 4). OR was higher in the presence of these variables in both FRS and QRS, with statistically significant relationship (Table 5). In both scores, DM was associated with high risk, and in QRS, high risk was strongly related with treated SAH and older age (Table 5).

Multivariate logistic regression revealed that male sex had a synergistic effect with SAH treatment in the high-risk status in both scores, and with older age in QRS. The variable SAP was excluded from the final model (Table 6).

Discussion

CVR stratification scores are constructed from population-based cohort studies, via logistic regression analysis. Risk estimates may be influenced by many factors, including calendar year, geographic region, number of visits to physician assistant office, time of patients' follow-up, quality of data collection, and prevalence of CVD risk factors. Therefore, agreement between scores, and consequently the number of individuals allocated in each risk stratum may vary substantially according to the score adopted,[14] which was observed in this study. We found a moderate agreement ($p < 0.001$) between FRS and QRS in the estimate of overall 10-year CVR, which is consistent with other similar studies in the literature.[14,16] However, a comparative study of North American and European risk scores, used in Latin American population, showed a weak agreement between scores.[17] Thus, application of a score that had not been calibrated for the Brazilian population may lead to different therapeutic decisions.

CVR is estimated by the sum of the weights assigned to each risk factor, and multiplicative effect of these factors. Due to interaction complexity, intuitive risk estimates usually lead to underestimation or overestimation of data, and identification of these associations is made by risk scores.[3] In the present study, FRS showed a fourteen-time higher risk for male individuals being classified as at high risk when the variable was associated with old age (OR 14.25; 95%CI 2.65-76.74; $p = 0.002$). QRS also showed increased probability of high risk for men (OR 9.56; 95%CI 1.27-71.78; $p = 0.028$) and hypertensive subjects

Table 2 – Sociodemographic, clinical and laboratory profile of the study population by continuous and categorical variables, University Hospital, Florianopolis, Brazil, 2015

Continuous variables (n = 74)	Mean (± DP)
Age	53.2 (± 11.0)
SAP (mmHg)	141.52 (± 24.3)
BMI (kg/m^2)	28.4 (± 5.0)
Continuous variables (n = 60)	**Mean (± DP)**
CT (mg/dl) *	195.46 (± 47.7)
HDL-c*	52.0 (± 14.4)
Continuous variables (n = 64)	**Mean (± DP)**
Family income †	2073.1 (± 1267.1)
Categorical variables (n = 74)	**Total (%)**
Elderly age range	21/74 (28.3)
Female gender	48/74 (64.8)
White race	68/74 (91.8)
Low educational attainment	57/74 (77.0)
Smoking	10/74 (13.5)
Elevated SAP	38/74 (51.3)
Elevated BMI	57/74 (77.0)
Treated SAH	35/74 (47.2)
Female	23/48 (47.9)
Male	12/26 (46.1)
Geriatric	13/21 (61.9)
Non-geriatric	22/53 (41.5)
Diabetes Mellitus	18/74 (24.3)
Female	8/48 (16.7)
Male	10/26 (38.5)
Geriatric	8/21 (38.1)
Non-geriatric	10/53 (18.9)
Categorical variables (n = 64)	**Total (%)**
High family income †	37/64 (57.8)

** 14 patients did not have recent lipid profile data; † 10 patients did not declare their family income. SD: standard deviation; TC: total cholesterol; HDL-c: HDL – cholesterol; SAP: systemic arterial pressure; BMI: body mass index; SAH: systemic arterial hypertension.*

(OR 19.22; 95%CI 1.76-210.41; p = 0.015), when associated with old age, suggesting a synergistic effect between these risk factors. The moderate agreement between the two scores used in this study may be partly due to these results, which is in agreement with the literature.[5,18]

There was an agreement between FRS and QRS algorithms in grouping men with high SAP and BMI into high risk, indicated by the higher proportion of these categories in the stratum. Relatively more importance was given to older and hypertensive individuals by the QRS, since greater proportions of these subjects (68.6% and 97.5%, respectively) were found in the high-risk stratum. As compared with the FRS, the QRS adopts a wider age range (24-84 years) for risk calculation, and assigns more weight to age, which explains the greater proportion of elderly subjects considered as at high risk by the score. Similar trend was observed for SAH, due to its direct, linear relationship with age.[9]

The highest individual contribution to high risk was given by DM (FRS and QRS [OR 9.53 and 16.03, respectively]; p < 0.001), indicating the need to identify and control this condition. However, the proportion of diabetics was higher in low/moderate risk strata, in contrast to the Adult Treatment Panel III and the Brazilian Society of Cardiology recommendations, which consider DM as a coronary disease, with high CVR.[4,19] These recommendations are questioned by some authors of prospective studies conducted in the United Kingdom (UK), who suggest that DM should be assessed according to the age of onset and disease duration for proper risk stratification.[20] A systematic review indicates that CVR stratification scores currently available in the literature are able to stratify the risk in these patients, differently from other instruments specific for DM, and can be used in the making decision process. However, if the physician intends to calculate the risk, as part of preventive strategy to motivate the patients to change habits and adhere to treatment, an individualized, clinical assessment of patients, without the use of instruments dominated by fixed variables may be more adequate.[21]

Bivariate analysis showed that elderly age range and male sex are associated with high risk in both scores. Aging independently contributes to the increase of CVR in every decade of life, and is related to other conditions, including obesity, treated SAH, diabetes and dyslipidemia.[22] Male gender is an independent risk variable for coronary disease, a frequent, early manifestation of CVD, commonly reported in population studies.[12,23,24] The association of treated SAH, increased SAP and DM with high risk is in agreement with a multi-country, case-control study that identified these factors as major contributors to attributable risk for acute myocardial infarction (AMI).[25] These findings suggest that preventive and therapeutic strategies may be used in different populations, aiming at preventing new cases of early IAM. Also, they may serve as a base for the use of FRS and QRS, even if they had not been calibrated for the Brazilian population, as an auxiliary tool for decision making in primary health care.

The relatively higher number of patients classified as at high risk by the FRS compared with QRS (33.7% versus 21.6%) is consistent with a study conducted in the United Kingdom, which suggests that the FRS may overestimate the risk by 5% in that population.[5] A study analyzing the use of North American instruments in the UK population revealed that the scores overestimated the results by 25-115%. This variation may be justified by the fact that these tools derived from a cohort of predominantly white men decades ago, when the prevalence of CVD in the United States was higher than today.[6]

The QRISK-2 derives from a more recent and heterogeneous cohort in the UK, in a period when the prevalence of CVD has decreased. This may explain the lower proportion of patients in the high-risk stratum identified by this score.[5] Other factors include the use of preventive therapies (aspirin, lipid-lowering and antihypertensive drugs), which

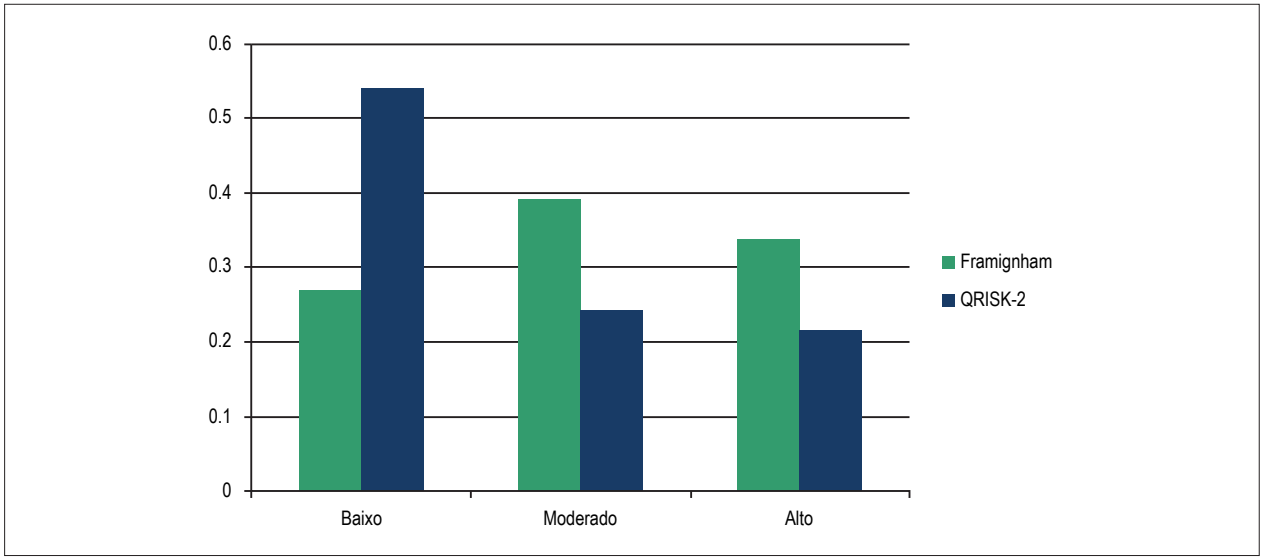

Figure 1 – *Ten-year overall cardiovascular risk stratification. University Hospital, Florianopolis, Brazil, 2015.*

reduce the incidence of CVD and modify the quantitative relationship between risk factors and cardiovascular events in an unpredictable manner, contributing to lower agreement between the instruments.[6]

The high proportion of individuals at high risk in both scores may be attributed to characteristics of the sample, which constituted patients attending a teaching hospital that treats highly complex cases, referred from the primary health care level.

In the present study, predominance of older (71.6%), female (64.86%) patients is similar to that observed in other patient groups in this outpatient care center[26,27] and in another Brazilian hospital.[28] The predominance of white individuals (91.9%) is compatible with the 2010 census, conducted by the Brazilian Institute of Geography and Statistics, that reported a similar proportion in the city (94.47%).[29]

Low socioeconomic status (low income, low educational attainment and/or poor regions) is considered a psychosocial factor for CVD that negatively affects the adherence to a healthy life style, medical advice and treatment. The present study showed a predominance of patients with low educational attainment, and is consistent with another study performed in our center, showing an association between this variable and higher risk for hypertension. The higher proportion of high-income individuals may be associated with data bias due to the private nature of this question.

An Australian study[30] related the high risk for cardiovascular disease with low socioeconomic status, suggesting the use of such association in the early detection and correct management of individuals at high risk. This strategy was successfully used in the UK, by using the QRISK-2 associated with Townsend index (which identifies each region by its zip code), and could be adapted to Brazil, in which primary healthcare centers are also strategically distributed, as in Australia and in the UK.

The elevated proportion of individuals with high SAH, SAP, overweight / obesity, and DM found in this institution reflects its position as a referral center, and corroborates the relevance of these modifiable risk factors in the study population. This may also justify the adoption of public health strategies focused on these factors.

There is as a considerable proportion of subjects without recent lipid levels (19%), which makes difficult the use of scores based on this laboratory parameter. Gaziano et al.[31] found that the estimation of CVR by using easily obtained risk factors, such as arterial pressure, hypertension treatment, socioeconomic status, smoking, BMI and history family of DM, is able to estimate CVR as efficiently as laboratory tests, which is valuable for poorer areas. The authors also suggest the sedentary lifestyle, an important risk factor for CVD, may be included as a risk score, since it is influenced by behavior and due to the increasing prevalence of overweight/obesity worldwide. This was evidenced by a high proportion [77.0% (57/74)] of overweight/obesity detected in our study.

Limitations, contributions and future perspectives

This study used a convenience sample, which may not reflect the behavior of the general population. Also, analysis of the effect of the combination of DM and elevated SAP with the other variables in the multivariate analysis is limited by the small sample size. The study investigated characteristics of two instruments normally used in CVR stratification in patients attending the internal medicine outpatient care of a university hospital. These data may be used as a base for the development of instruments for cardiovascular prevention, specific for local reality.

Conclusion

In our study population, there was a moderate correlation between FRS and QRS in estimating 10-year overall CVR.

Table 3 – Distribution (relative and absolute frequencies) of independent variables in the high-risk stratum for cardiovascular disease, University Hospital, Florianopolis, Brazil, 2015

Variables	High risk	
	Framingham (n = 25), %[n]	QRISK-2 (n = 16), % [n]
Age		
Non-geriatric	48.0 [12]	31.3 [5]
Geriatric	52.0 [13]	68.6 [11]
Gender		
Male	64.0 [16]	62.5 [10]
Female	36.0 [9]	37.5 [6]
Race		
White	100 [25]	100.0 [16]
Non-white	0.0 [0]	0.0 [0]
Treated SAH		
No	24.0 [6]	12.5 [2]
Yes	76.0 [19]	97.5 [14]
Family history of PCAD		
No	72.0 [18]	62.5 [10]
Yes	28.0 [7]	37.5 [6]
Smoking		
No	88.0 [22]	87.5 [14]
Yes	12.0 [3]	12.5 [2]
Diabetes Mellitus		
No	52.0 [13]	68.8 [11]
Yes	48.0 [12]	31.3 [5]
SAP		
Normal	24.0 [6]	25.0 [4]
Increased	76.0 [19]	75.0 [12]
BMI		
Normal	16.0 [4]	18.8 [3]
Increased	84.0 [21]	81.3 [13]

CVD: cardiovascular disease; SAH: systemic arterial hypertension; PCAD: premature coronary artery disease in first-degree relative; SAP: systemic arterial pressure; BMI: body mass index.

The scores assign different weights to the variables, which may have a synergistic effect and be affected by local population. This finding should be recognized for clinical purposes, as well as the need for calibrating risk scores for the Brazilian population.

Author contributions

Conception and design of the research: Garcia GT, Stamm AMNF, Rosa AC, Marasciulo AC, Marasciulo RC, Battistella C; Acquisition of data: Garcia GT, Stamm AMNF, Rosa AC, Marasciulo RC, Battistella C; Analysis and interpretation of the data and Statistical analysis: Garcia GT, Stamm AMNF, Rosa AC, Marasciulo AC, Marasciulo RC; Writing of the manuscript: Garcia GT, Stamm AMNF, Rosa AC; Critical revision of the manuscript for intellectual content: Garcia GT, Stamm AMNF, Rosa AC, Marasciulo AC.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

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Study Association

This study is not associated with any thesis or dissertation work.

Table 4 – Bivariate analysis of independent variables in the high-risk stratum, University Hospital, Florianopolis, Brazil, 2015

Variables	Sample (%) [n = 74]	High risk			
		Framingham		QRISK-2	
		χ^2	P†	χ^2	p†
Age					
Non-geriatric	71.6 [53]	10.36	0.001	16.37	< 0.001
Geriatric	28.4 [21]				
Gender					
Male	35.1 [26]	13.80	< 0.001	6.70	0.01
Female	64.9 [48]				
Race					
White	91.9 [68]	††	††	1.80	0.18
Non-white	8.1 [6]				
Treated SAH					
No	52.7 [39]	12.47	< 0.001	13.23	< 0.001
Yes	47.3 [35]				
Family history of PCAD					
No	20.3 [15]	††	††	3.75	0.53
Yes	79.7 [59]				
Smoking					
No	86.5 [64]	0.74	0.78	0.18	0.89
Yes	13.5 [10]				
Diabetes Mellitus					
No	75.7 [56]	15.71	< 0.001	21.88	< 0.001
Yes	24.3 [18]				
SAP					
Normal	48.6 [36]	9.18	0.002	4.57	0.03
Increased	51.4 [38]				
BMI					
Normal	23.0 [17]	1.03	0.30	0.20	0.65
Increased	77.0 [57]				

** Bracketed values indicate the absolute number of participants. † chi-squared test; †† variables not predicted by Framingham risk score; SAH: systemic arterial hypertension; PCAD: premature coronary artery disease in first-degree relative; SAP: systemic arterial pressure; BMI: body mass index.*

Table 5 – Independent variables and high-risk chance, University Hospital, Florianopolis, Brazil, 2015

Variables	Score					
	Framingham			Q-RISK 2		
	OR	95%CI	p*	OR	95%CI	p*
Low/Moderate *vs.* high risk						
Male gender	6.93	2.37-20.25	< 0.001	4.37	1.365-14.02	0.013
Geriatric age range	5.55	1.86-16.52	0.002	10.56	3.002-37.14	< 0.001
Treated SAH	6.53	2.18-19.52	0.001	12.33	2.552-59.60	0.002
Increased SAP	5.00	1.69-14.76	0.004	3.69	1.064-12.81	0.04
Diabetes mellitus	9.53	2.83-32.06	< 0.001	16.03	4.283-59.98	< 0.001

** Logistic regression analysis; OR: odds ratio; CI: confidence interval; SAH: systemic arterial hypertension; SAP: systolic arterial pressure.*

Table 6 – Multivariate analysis between risk strata and independent variables, University Hospital, Florianopolis, Brazil, 2015

Variables	Score					
	Framingham			Q-RISK 2		
	OR	IC 95%	p*	OR	IC 95%	p*
Low/Moderate *vs.* High risk						
Male gender	14.25	2.65-76.74	0.002	9.56	1.27-71.78	0.028
Geriatric age range	4.74	1.19-18.89	0.028	19.58	2.69-142.78	0.003
Treated SAH	10.79	1.88-61.88	0.008	19.22	1.76-210.41	0.015
Increased SAP†	-	-	-	-	-	-
Diabetes mellitus	3.52	0.81-15.26	0.092	9.98	1.58-62.98	0.014

** Logistic regression analysis; †increased SAP was removed from the model; OR: odds ratio; SAH: systemic arterial hypertension; SAP: systemic arterial pressure; CI: confidence interval.*

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Effects of a Single Bout of Resistance Exercise in Different Volumes on Endothelium Adaptations in Healthy Animals

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Abstract

Background: Resistance exercise (RE) has been recommended for patients with cardiovascular diseases. Recently, a few studies have demonstrated that the intensity of a single bout of RE has an effect on endothelial adaptations to exercise. However, there is no data about the effects of different volumes of RE on endothelium function.

Objective: The aim of the study was to evaluate the effects of different volumes of RE in a single bout on endothelium-dependent vasodilatation and nitric oxide (NO) synthesis in the mesenteric artery of healthy animals.

Methods: Male Wistar rats were divided into three groups: Control (Ct); low-volume RE (LV, 5 sets x 10 repetitions) and high-volume RE (HV, 15 sets x 10 repetitions). The established intensity was 70% of the maximal repetition test. After the exercise protocol, rings of mesenteric artery were used for assessment of vascular reactivity, and other mesenteric arteries were prepared for detection of measure NO production by DAF-FM fluorescence. Insulin responsiveness on NO synthesis was evaluated by stimulating the vascular rings with insulin (10 nM).

Results: The maximal relaxation response to insulin increased in the HV group only as compared with the Ct group. Moreover, the inhibition of nitric oxide synthesis (L-NAME) completely abolished the insulin-induced vasorelaxation in exercised rats. NO production showed a volume-dependent increase in the endothelial and smooth muscle layer. In endothelial layer, only Ct and LV groups showed a significant increase in NO synthesis when compared to their respective group under basal condition. On the other hand, in smooth muscle layer, NO fluorescence increased in all groups when compared to their respective group under basal condition.

Conclusions: Our results suggest that a single bout of RE promotes vascular endothelium changes in a volume-dependent manner. The 15 sets x 10 repetitions exercise plan induced the greatest levels of NO synthesis. (Arq Bras Cardiol. 2017; 108(5):436-442)

Keywords: Exercise; Endothelium; Physical Conditioning, Animal; Muscle, Smooth, Vascular; Nitric Oxide; Vasodilatation; Rats.

Introduction

Physical activity induces physiological adaptations of the endothelium, contributing to the local control of vascular tone.[1] Besides, the beneficial effects of regular exercise on sympathetic and parasympathetic tone,[2] blood coagulation,[3] myocardial contractility[4] and release of endothelium-derived relaxing factors[5] are likely to improve cardiovascular health and reduce the risk of diseases.

Exercise training is nowadays one of the main non-pharmacological tool in the maintenance of a healthy life. Its effects involve recurrent exposure to changes in cardiovascular hemodynamics promoted by bouts of physical activity. In response to acute exercise, numerous intra- and extracellular pathways are activated to increase blood flow to active muscles.[6,7] At the onset of exercise, the mechanical action of skeletal muscle creates a 'muscle-pump', which causes an immediate increase in blood flow.[7,8] This abrupt change in blood flow towards the exercised tissues, promotes a reduction in blood supply in visceral region, phenomena known as exercise hyperemia.[7,8]

Many evidences suggest that striation in the vascular wall, associated with pulsatile flow, intermittent hypoxia and release of catecholamines are essential factors for nitric oxide (NO) production during a single bout of exercise.[9-11] Recently, our group have showed that acute resistance exercise induce an intensity-dependent effect on NO synthesis and vascular relaxation in mesenteric arteries of healthy rats.[12,13] The mesenteric artery regulates 20% of the blood flow and effectively participates in the total peripheral resistance, and thus, is directly involved in vascular changes promoted by exercise.[9]

In addition, our group has recently demonstrated a strong, positive relationship between the magnitude of

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vascular adaptations to exercise and exercise intensity.[12] Nevertheless, exercise prescription depends on two different aspects – intensity and volume of exercise. The volume of exercise directly affects the demand of oxygen and other nutrients in attempt to recover from the stress promoted by consecutive muscle contractions. Thus, it is expected that changes in training volume promote different vascular adaptations, i.e., the higher the volume of exercises the higher the metabolic demand.

In addition, there are no studies investigating acute vascular adaptations to different volumes of resistance exercise. This information could guide the prescription of long-term training in cardiovascular disease conditions. Thus, our study aimed to evaluate the influence of the volume of resistance exercise on endothelium-dependent vasodilatation and NO synthesis in mesenteric artery of healthy animals.

Methods

Animals

Twenty-four male Wistar rats (250-350 g, 8–10 weeks old) were used for all experiments. The rats were randomized into three groups: control (Ct, n = 8), low-volume (LV, n = 8) and high-volume resistance exercise (HV, n = 8). All procedures were in agreement with the Brazilian Society of Laboratory Animal Sciences and were approved by the Ethics Committee on Animal Research XXX (omitted to the review process), Brazil (protocol # 80/2010).

Resistance exercise protocol

Animals were exercised following a model described by Tamaki et al.[14] Electrical stimulation (20 V, 0.3 s duration, at 3 s intervals) was applied on the tail of the rat through a surface electrode. The animals underwent three days of familiarization; firstly, they were placed on the apparatus and left on exercise position for 5 min to reduce the stress caused by the equipment and handling of the animals. After the familiarization period, the animals performed one maximum repetition (1RM) test, which consisted of determining the maximum lifted load in one repetition. After 2 days, the animals underwent the protocol of leg extension exercise – 5 (LV) or 15 (HV) sets with 10 repetitions and a 180s resting period between each set. The animals exercised in intensity of 70% of 1RM. Animals of the Ct group were maintained under the same conditions of the LV and HV animals but at resting position.

Vascular reactivity studies

Immediately after exercise, the animals were sacrificed. The superior mesenteric artery was removed, stripped of connective and adipose tissues, and sectioned into rings (1–2 mm). Rings were suspended in organ baths containing 10 mL of Tyrode's solution by fine stainless steel hooks connected to a force transducer (Letica, Model TRI210; Barcelona, Spain) with cotton threads. This solution was continually gassed with carbogen (95% O_2 and 5% CO_2) and maintained at 37°C and the rings maintained at a resting tension of 0.75 g for 60 min (stabilization period). The functionality of the endothelium was assessed by the ability of acetylcholine (ACh, 1 μM; Sigma-Aldrich, USA) to induce more than 75% relaxation of precontracted vascular rings with phenylephrine (Phe, 1 μM; Sigma-Aldrich, USA). After that, changes in vascular reactivity were assessed by obtaining concentration-response curves for insulin (Novo Nordisk, Bagsvaerd, Denmark) (10^{-13}–10^{-6} M). The rings were then washed out and new insulin-induced relaxation was obtained after incubation with a non-specific inhibitor of nitric oxide synthase, L-N^G-Nitroarginine methyl ester (L-NAME, 100 μMol/L; Sigma-Aldrich, USA), for 30 min. This was used to evaluate the role of NO on insulin-induced vascular relaxation.

Measurement of NO Production

NO production in mesenteric artery ring was determined by using a fluorescent cell permeable dye for NO, DAF-FM (4,-amino-5 methylamino-2',7'-diaminofluorescein diacetate, Molecular Probes, USA), as previously described.[15] In order to detect NO, freshly isolated mesenteric artery was loaded with 10 μM of the probe for 40 min at 37°C. Twenty minutes after the onset of the probing, some rings were stimulated with 10 nM of regular human insulin for 20 min and then washed for 40 min with Tyrode´s solution. Mesenteric segments were frozen and cut into 20μm-thick sections. Images were recorded using a fluorescence microscope (IX2-ICB, Olympus®, USA) under identical settings. The fluorescence intensity was measured using ImageJ software (NIH, USA). A minimum of ten regions were randomly selected from the endothelial and smooth muscle layers of each mesenteric section. It is worth to note that smooth muscle exhibits an autofluorescence, therefore, in order to avoid misleading fluorescence measurements, analyses of images were carefully performed selecting the region of interest within the smooth muscle fibers.

Statistical Analysis

Initially, all data underwent the Kolmogorov-Smirnov test to determine whether the probability distributions were parametric or non-parametric. All data had normal distribution. The values were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by the Bonferroni`s test were performed using GraphPad Prism Software (San Diego, CA, USA). The NO fluorescence microscopy images were analyzed according to the intensity of the fluorescence per normalized area, represented in arbitrary unit (a.u.). The values were considered statistically significant when $p < 0.05$.

Results

Acute effect of different resistance exercise volumes on endothelium-dependent vasodilation

As shown in the Figure 1a, in all groups, insulin caused a concentration-dependent vasodilation in superior mesenteric arteries. Despite the tendency to increase the insulin-induced vasodilation in LV group, no significant difference was found

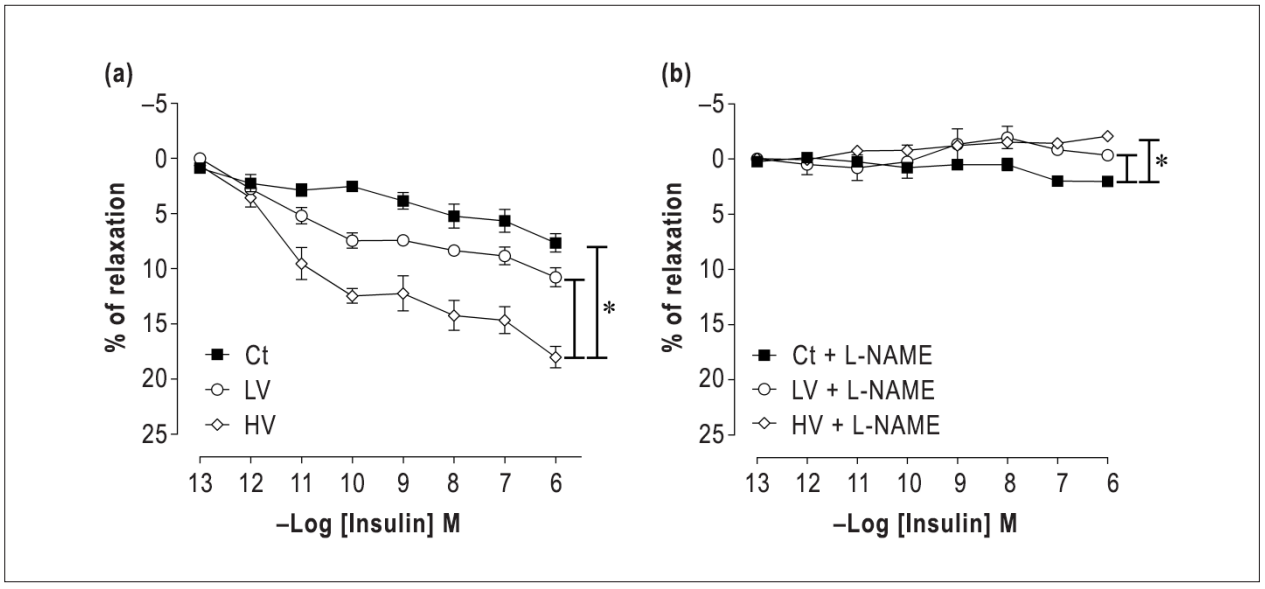

Figure 1 – *Effects of a single bout of resistance exercise in different levels of volume on endothelium-dependent relaxation. (a) Concentration-response curves for insulin (10^{-13} - 10^{-6}M) in rings isolated from the superior mesenteric artery with functional endothelium and pre-contracted with phenylephrine (Phe) (1 μM). (b) Concentration-response curves for insulin in rings pre-incubated with nitric oxide inhibitor (L-NAME: 100 μM). Ct: control, LV: low-volume and HV: high-volume. Statistical differences were determined by one-way ANOVA followed by Bonferroni's test. Results are expressed as mean ± SEM. *p < 0.05.*

in comparison to the Ct group (Figure 1a). However, resistance exercise at HV significantly increased the relaxation induced by insulin compared to Ct and LV groups (Figure 1a). The effect of NOS on insulin-induced vascular relaxation was evaluated in the presence of L-NAME. As shown in the Table 1, insulin-induced vasodilation was significantly reduced in Ct group; however, in both exercised groups the vasodilation was completely abolished showing a significant reversion of the curve when compared to Ct. No statistically significant differences were observed between the LV and HV groups. (Figure 1b).

Acute effect of different resistance exercise volumes on the endothelial NO synthesis

Interestingly, under basal condition, there was a significant volume-dependent increase in NO production in endothelium and smooth muscle layer (Figure 2a and 2b). After insulin stimulation, we found an enhanced endothelial NO production in Ct and LV, but not in HV when compared to their respective group under basal conditions (Figure 2a). On the other hand, in smooth muscle layer, NO fluorescence was significantly increased in all groups when compared to their respective group at baseline (Figure 2b). However, it is important to highlight that insulin-stimulation in the LV group reached similar DAF fluorescence level as the Ct group (Figure 2b).

In order to precisely evaluate the global increase in NO synthesis, the endothelial and smooth muscle layer fluorescence data were pooled and normalized to their respective group under basal conditions. The results were expressed as percentage of increase in comparison of baseline. In this experimental approach, we found a reduction in the additional production of NO in a volume-dependent way (Figure 3).

Discussion

In the present study, we demonstrated that one single bout of different volumes but same intensity of resistance exercise promotes acute endothelial adaptations in healthy animals in a volume-dependent way. The animals subjected to 15 sets / 10 repetitions (HV group) had a more pronounced vasodilatory response. In summary, our results indicate that high volume of resistance exercise promotes an improvement in the arterial relaxation induced by insulin due an enhanced NO production.

Insulin is well known to exert a crucial role in the maintenance of metabolic homeostasis; however, this hormone also plays a key role in the cardiovascular system. In vascular bed-specific endothelial cells, insulin causes a rapid and concentration-dependent increase in the production of NO through the activation of endothelial NO synthase.[16,17] In our study, low-volume of resistance exercise was not able to promote an increase in the vascular relaxation. On the other hand, high-volume of resistance exercise produced an increased insulin-induced vasodilation in superior mesenteric artery. Similarly, Mota et al.[13] observed that high-intensity resistance exercise enhanced the relaxation induced by insulin in mesenteric artery of healthy animals. Thus, we hypothesize that both, high-volume and -intensity, are linked with enhanced vascular function.

To understand the participation of NO synthase in the insulin-induced relaxation, we performed concentration-response curves for insulin in pre-incubated vascular rings with L-NAME. Our data showed that insulin-induced relaxation was fully abolished by L-NAME in all groups, reinforcing the great contribution of NO in the arterial relaxation promoted by insulin.

Table 1 – Values of Rmax obtained from concentration-response curves for insulin in mesenteric arteries before and after incubation with L-NAME

Groups	Insulin (%)	Insulin + L-NAME (%)
Control	7.66 ± 0.83	2.01 ± 0.24§
Low volume	10.77 ± 0.86	–0.36 ± 0.36§,†
High volume	18.01 ± 0.97$^{*,\#}$	–2.08 ± 0.19§,†

*The experiments were performed in the absence of L-NAME (insulin) and in the presence of 100 µM of L-NAME (L-NAME). Statistical differences were determined by one-way ANOVA followed by the Bonferroni post-hoc test. The data are expressed as mean ± SEM. *p < 0.05 vs. control, #p < 0.05 vs. low volume, § p < 0.05 vs. respective group without L-NAME and †p < 0.05 vs. control + L-NAME.*

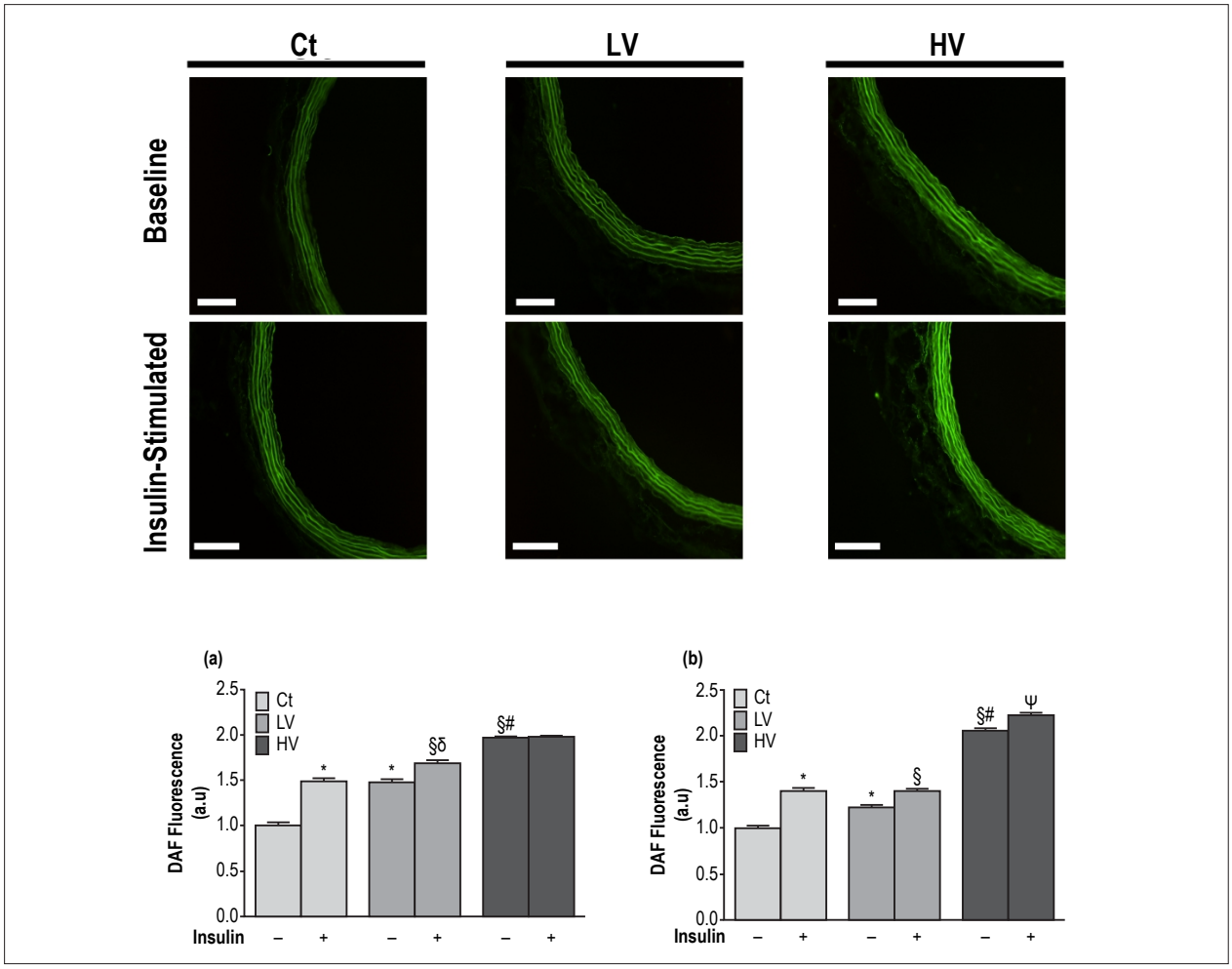

Figure 2 – *Effects of a single bout of resistance exercise in different levels of volume on endothelium-derived nitric oxide production. Detection of nitric oxide by DAF (diaminofluorescein) fluorescence at baseline and after stimulation with insulin (10 nM) (top). (a) Quantitative analyses of DAF fluorescence in endothelial layer before and after insulin stimulation; (b) quantitative analyses of DAF fluorescence in smooth muscle layer before and after insulin stimulation. Scale: 20µm. Ct: control, LV: low-volume and HV: high-volume. Statistical differences were determined by one-way ANOVA followed by Bonferroni's test. Results are expressed as mean ± SEM. *p < 0.05 vs. Ct(-); §p < 0.05 vs. LV(-); δp < 0.05 vs. Ct (+); #p < 0.05 vs. LV(+); Ψp < 0.05 vs. HV(-).*

In addition, our group has previously reported insulin-induced vasoconstriction in exercised animals via activation of MAPK/endothelin-1 pathway. This corroborates our finding on the contraction response in NO synthase inhibition in animals submitted to a single section of resistance exercise. Thus, as previously reported by our group, the functional interaction between these intracellular signaling pathways plays an essential role in the regulation of the myogenic tone in the vasculature.[12]

Our *in situ* results of NO production in superior mesenteric artery of exercised animals at different volumes demonstrated a volume-dependent increase of NO production in the

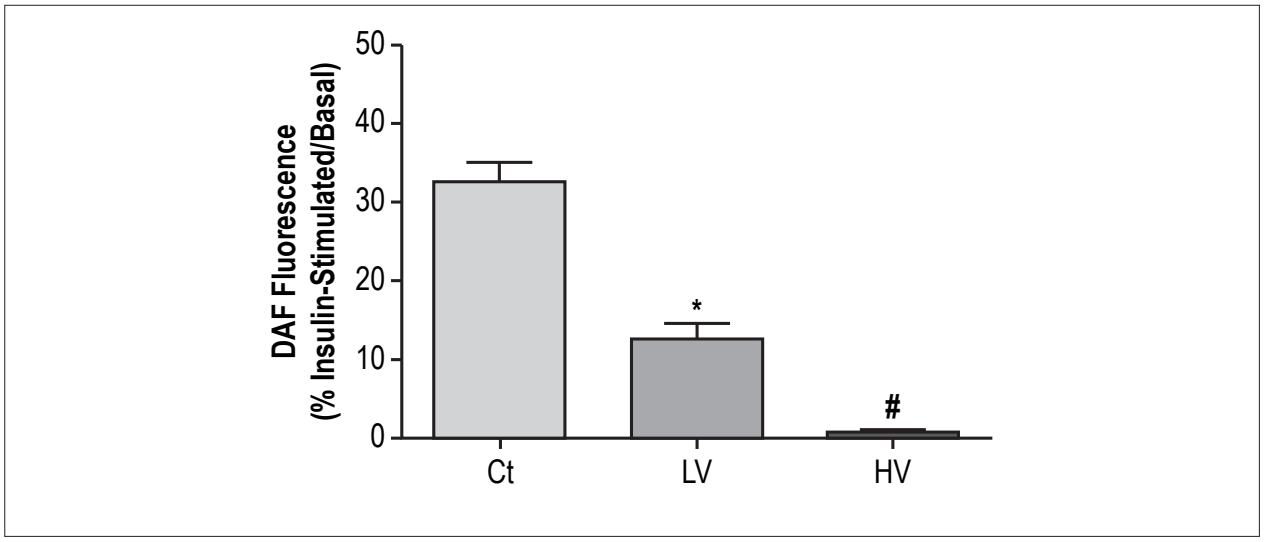

Figure 3 – *Effect of a single bout of resistance exercise in different levels of volume on additional NO synthesis in mesenteric artery stimulated by insulin (10 nmol/L). The graph shows the ratio of NO fluorescence in insulin-stimulated rings and NO fluorescence at baseline. Ct: control, LV: low-volume and HV: high-volume. Statistical differences were determined by one-way ANOVA followed by Bonferroni's test. Results are expressed as mean ± SEM. *p < 0.05 vs. Ct and #p < 0.05 vs. LV.*

endothelium and smooth muscle layers. Interestingly, in mesenteric artery rings stimulated with insulin, the additional synthesis of NO was lower in exercised animals than in the Ct group. Furthermore, our data showed that the exercised groups already had increased baseline levels of NO, and hence it is reasonable to suggest that resistance exercise might increase NO synthase activity to a sub-maximal level, preventing a substantial increase of NO synthesis in insulin-stimulated mesenteric rings.

Indeed, to exert its biological effects, endothelium-derived NO must reach the underlying smooth muscle cells.[5] Although the time-dependent diffusion rate of NO across the cell membrane is poorly understood, new molecular players have been described to be involved on the NO transport mechanisms.[18] Studies have consistently suggested that NO activates and permeates hemichannels formed by connexins (Cxs 37, 40 or 43) which are required to transfer NO from endothelial to smooth muscle cells. Therefore, differently from the HV group, in which a significant increase in vasodilation was observed, the existence of a positive trend but not significant in the LV group may be explained, at least in part, by the achievement of only suboptimal levels of NO in the smooth muscle cells. In addition, despite this mechanism was not investigated in the present study, further studies should evaluate whether resistance exercise improves gap junction channel function, and subsequently, promotes vasodilation.

Several studies using a single bout of aerobic[19] or resistance[20,21] exercise observed an enhanced vascular relaxation, suggesting an increased NO bioavailability after a session of exercise. Furthermore, the role of resistance exercise has been evaluated in the prevention and treatment of several cardiovascular diseases.[20,22] However, although the majority of the studies focus on the vascular effects of aerobic exercise, our data are the first that demonstrate the volume-dependent effect on vascular adaptations after a single bout of resistance exercise. Finally, the current findings may contribute to the establishment of safe limits of exercise for patients with endothelial dysfunction and insulin resistance.

Conclusion

In summary, we demonstrated that a single bout of resistance exercise is able to improve insulin-induced vasodilation and increase NO production in a volume-dependent manner in healthy animals. Therefore, our results suggest that vascular response to resistance exercise is directly related its volume and, hence, high-volume exercise plans should be further investigated in the treatment of cardiovascular diseases and/or maintenance of a healthy life.

Author contributions

Conception and design of the research: Mota MM, Silva TLTB, Santos MRV; Acquisition of data: Mota MM, Silva TLTB, Macedo FN, Mesquita TRR; Analysis and interpretation of the data: Mota MM, Silva TLTB, Macedo FN, Mesquita TRR; Statistical analysis: Macedo FN, Mesquita TRR; Obtaining funding: Quintans Júnior LJ, Santana-Filho VJ, Lauton-Santos S, Santos MRV; Writing of the manuscript: Macedo FN, Mesquita TRR; Critical revision of the manuscript for intellectual content: Mota MM, Silva TLTB, Macedo FN, Mesquita TRR, Quintans Júnior LJ, Santana-Filho VJ, Lauton-Santos S, Santos MRV.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Original Article

Effect of Lactation on myocardial vulnerability to ischemic insult in rats

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Abstract

Background: Cardiovascular diseases are the leading cause of mortality and long-term disability worldwide. Various studies have suggested a protective effect of lactation in reducing the risk of cardiovascular diseases.

Objective: This study was designed to assess the effects of pregnancy and lactation on the vulnerability of the myocardium to an ischemic insult.

Methods: Eighteen female rats were randomly divided into three groups: ischemia-reperfusion (IR), in which the hearts of virgin rats underwent IR (n = 6); lactating, in which the rats nursed their pups for 3 weeks and the maternal hearts were then submitted to IR (n = 6); and non-lactating, in which the pups were separated after birth and the maternal hearts were submitted to IR (n = 6). Outcome measures included heart rate (HR), left ventricular developed pressure (LVDP), rate pressure product (RPP), ratio of the infarct size to the area at risk (IS/AAR %), and ventricular arrhythmias - premature ventricular contraction (PVC) and ventricular tachycardia (VT).

Results: The IS/AAR was markedly decreased in the lactating group when compared with the non-lactating group (13.2 ± 2.5 *versus* 39.7 ± 3.5, p < 0.001) and the IR group (13.2 ± 2.5 *versus* 34.0 ± 4.7, p < 0.05). The evaluation of IR-induced ventricular arrhythmias indicated that the number of compound PVCs during ischemia, and the number and duration of VTs during ischemia and in the first 5 minutes of reperfusion in the non-lactating group were significantly (p < 0.05) higher than those in the lactating and IR groups.

Conclusion: Lactation induced early-onset cardioprotective effects, while rats that were not allowed to nurse their pups were more susceptible to myocardial IR injury. (Arq Bras Cardiol. 2017; 108(5):443-451)

Keywords: Lactation; Myocardial Infarction; Myocardial Ischemia; Parturitium.

Introduction

Coronary artery diseases are the leading cause of mortality worldwide, with about 38% of the deaths attributed to these diseases.[1] In addition to hypertension and diabetes, multiple lifestyle factors, including a high-cholesterol diet, smoking, alcohol consumption, and stress, can increase the risk of myocardial infarction.[2] Accumulating epidemiological evidence suggests that a woman's decision to breast-feed her children has a significant impact on the maternal risk of developing cardiovascular diseases.[3,4] Although national policies to promote breastfeeding have been profoundly implemented in many developed countries, the global rate of exclusive breastfeeding is below 40%.[5]

Lactation confers significant benefits to the maternal cardiovascular health.[6] Lactogenesis has a favorable effect on glucose and lipid metabolism. It increases insulin sensitivity and glucose effectiveness while reducing the risk of type 2 diabetes.[3] Nursing promotes high-density lipoprotein production and reduces triglyceride and low-density lipoprotein levels.[7,8] Consequently, lactation mobilizes the fat stores generated during pregnancy, thereby reducing the risk of cardiovascular diseases and myocardial infarction.[9] Furthermore, lactation reduces maternal blood pressure and heart rate (HR), while improving cardiac output.[10] Hanwell and Peaker[11] observed that the augmented cardiac output is directly proportional to the intensity of suckling in rats.[11] Moreover, certain hormones released during lactation, such as oxytocin, prolactin, glucocorticoids, gherlin and growth hormone, can precondition the myocardium against cardiac injury.[12,13] Centrally released endogenous oxytocin and exogenous infusion of oxytocin have been shown to protect heart against a hypoxic insult via activation of brain receptors.[14] Furthermore, oxytocin induces cardioprotection through a pathway involving mitochondrial ATP-dependent potassium channels.[15] Although, the effect of lactogenic hormones on cardiovascular health has been studied to some extent, the experimental evidence suggesting the cardioprotective role of lactation against ischemia-reperfusion (IR) injury is scarce. Thus, the current was designed to test the hypothesis that lactation reduces the myocardial vulnerability to IR injury.

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Methods

Animals

Eighteen female Sprague–Dawley rats (180–230 g) were housed in an air-conditioned colony room at 21–23°C, with a 12-hour light-dark cycle. During the experimental period, the animals had free access to food and water. The experimental protocols followed in this study conformed to the Guidelines for the Care and Use of Laboratory Animals published by National Institutes of Health (NIH Publication N°. 85-23, revised 1996) and were further approved by the institutional ethical committee at Tehran University of Medical Sciences (Tehran, Iran).

Preparation of isolated hearts

The animals were anesthetized using intraperitoneal sodium thiopental (60 mg/kg). Heparin (500 IU/kg) was also injected to prevent blood coagulation. Once the animals were anesthetized, a transabdominal incision was made, and their hearts were exposed. Following cannulation of the aorta, the heart was excised and mounted on a Langendorff apparatus. The hearts were perfused retrogradely with Krebs–Henseleit bicarbonate buffer containing (in mmol/L): $NaHCO_3$ 25; KCl 4.7; NaCl 118.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; glucose 11; $CaCl_2$ 2.5, gassed with 95% O_2 and 5% CO_2 (pH 7.35–7.45 at 37 C°). A saline-filled latex balloon was introduced into the left ventricle and inflated to yield a preload of 8-10 mmHg. The balloon was connected to a pressure transducer (Harvard, March-Hugsteten, Germany) which allowed a real time measurement of the pressures from the ventricle. Electrocardiographic recording was performed by fixation of thin electrodes on the ventricular apex and right atrium. A surgical needle (6-0 silk suture) was passed under the origin of the left anterior descending coronary artery, and the ends of the suture were passed through two plastic pipette tips to form a snare. Regional ischemia was induced by tightening the snare (30 min), and reperfusion was performed by releasing the ends of the suture (60 min). The hearts were allowed to beat spontaneously throughout the experiments.

Sample size estimation

The sample size was estimated using the Resource Equation Method.[16]

E = N - T

E = degrees of freedom (analysis of variance [ANOVA]; between 10 and 20)

N = total number of animals

T = number of treatment groups

"N" was obtained from a previously published study[17] and was adjusted accordingly to attain a valid "E".

Experimental groups

The effects of pregnancy and lactation on myocardial IR injury were studied in 18 rats randomly divided into three groups:

1. IR group (IR; n = 6): isolated hearts of virgin rats in the diestrus period underwent 30 min of regional ischemia followed by 60 min of reperfusion.
2. Lactating group (n = 6): the rats nursed their pups for 3 weeks and, after that, the maternal hearts underwent 30 min of regional ischemia followed by 60 min of reperfusion.
3. Non-lactating group (n = 6): after parturition, the rats were separated from their pups, and 3 weeks later, the maternal hearts underwent 30 min of regional ischemia followed by 60 min of reperfusion.

Hemodynamic functions

The left ventricular developed pressure (LVDP) and the HR were continuously monitored and recorded using BioLab data acquisition system. The rate pressure product (RPP) was calculated by multiplying LVDP by the HR.

Assessment of area at risk and infarct size

At the end of reperfusion, the left coronary artery was reoccluded and Evans blue (0.3–0.5 ml) dye was infused via aorta to differentiate the ischemic zone (area at risk; AAR) from the non-ischemic zone. The hearts were frozen at - 20°C and sliced into 2.0 mm traverse sections (using a stainless steel slicer matrix with 2.0 mm coronal section slice intervals) from apex to the base. The slices were incubated in 1% triphenyltetrazolium chloride (TTC in 0.1 M phosphate buffer, pH 7.4, 37 ºC) for 20 min followed by tissue fixation (10% phosphate-buffered formalin) for 24 h. TTC reacts with the viable tissue, producing a red formazan derivative which is distinct from the white necrotic area. Sections were scanned to determine non-ischemic area, AAR (ischemic area) and the infarct size (IS) by calculating the pixels occupied by each area using the Adobe PhotoShop software (Adobe Systems Seattle, WA). The AAR was expressed as a percentage of left ventricular volume for each heart. The IS was determined by using computer-aided planimetry and expressed as a percentage of the AAR.[18-20]

Assessment of ventricular arrhythmias

Ischemia-induced ventricular arrhythmias were assessed during the occlusion period and were determined in accordance with the Lambeth Conventions.[21] Ventricular tachycardia (VT) and premature ventricular contraction (PVC) including compound PVCs (such as bigeminy, couplet and salvos) were counted during ischemic period and the first 5 minutes of the reperfusion period.

Statistical analysis

All data were statistically analyzed using GraphPad InStat, version 3.06 (GraphPad Software, Inc., San Diego, CA). The data followed Gaussian distribution (Kolmogorov-Smirnov test). All results are expressed as mean ± standard error of the mean (SEM). Outcome measures between the groups were analyzed using one-way ANOVA followed by the Bonferroni *post hoc* test. For intragroup comparisons,

repeated measures ANOVA followed by the Bonferroni *post hoc* test (for selected columns) was performed. Statistical significance was defined as $p < 0.05$.

Results

Hemodynamic Parameters

Table 1 demonstrates the changes in HR, LVDP, and RPP in the IR, lactating and non-lactating groups during different periods of the experiment.

In IR group, LVDP and RPP were reduced ($p < 0.05$ and $p < 0.001$, respectively) at the end of ischemia and reperfusion period as compared with the baseline. In lactating group, LVDP and RPP reduced significantly at the end of the ischemia ($p < 0.05$) and reperfusion ($p < 0.001$) periods when compared with baseline. Furthermore, in the lactating group, the HR was markedly reduced in the reperfusion period when compared with baseline. Non-lactating animals demonstrated lower RPP during ischemia when compared with baseline ($p < 0.05$).

Intergroup analysis showed that LVDP at the end of ischemia, and RPP in the ischemia and reperfusion periods were significantly higher in the non-lactating when compared to the lactating group ($p < 0.05$). In addition, HR and LVDP in reperfusion were markedly increased in the IR and non-lactating groups, when compared with the lactating group ($p < 0.05$).

Area at risk and infarct size

As shown in Figure 1, there was no significant difference in AAR between the groups. However, the IS/AAR was significantly reduced in the lactating group as compared with the non-lactating and IR groups (13.2 ± 2.5 *versus* 39.7 ± 3.5 and 34.0 ± 4.7, respectively).

Ventricular arrhythmias

During the ischemic phase, the number of compound PVCs was statistically higher in the non-lactating group as compared with the lactating group ($p < 0.05$) (Figure 2). During the first 5 min of the reperfusion phase, the number of compound PVCs did not differ significantly between the groups (Figure 3). During ischemia and the first 5 min of reperfusion, the number of VTs was significantly lower in the lactating and IR groups as compared with the non-lactating group ($p < 0.001$) (Figure 4). In addition, the duration of VTs during ischemia and the first 5 min of reperfusion were markedly reduced in the lactating and IR groups as compared with the non-lactating group ($p < 0.01$, Figure 5).

Discussion

The current study demonstrates the effect of pregnancy and lactation on myocardial vulnerability to an ischemic insult. We observed that lactation, and not pregnancy alone, preconditioned the maternal heart against ischemia-induced myocardial infarction. Furthermore, nursing reduces the incidence and duration of ventricular arrhythmias during ischemia.

Growing evidence has indicated short-term and long-term beneficial effects of lactation on the risk factors associated with cardiovascular morbidity.[22,23] The only study indicating cardioprotective effects of lactation against IR injury was recently performed by Shekarforoush and Safari.[17] However, their study did not take into account if the cardioprotection was conferred by the pregnancy alone. In addition, these authors were unable to observe antiarrhythmic effects of lactation during myocardial ischemia.[17] In the current study, *in vitro* Langendorff model was used. Thus, the early-onset cardioprotective effects of lactation can be attributed to the intrinsic characteristics of the heart independent of the complex physiology of lactation. We observed that pregnancy increases the ischemia-induced IS and the incidence and duration of arrhythmias. Previous studies suggest that pregnancy enhances the maternal risk of cardiovascular events by increasing central fat accumulation,[24] blood pressure,[25] and insulin resistance.[26] During pregnancy, the maternal heart transforms into "a better functioning heart" and undergoes physiological hypertrophy in order to increase the cardiac pumping capacity. However, Lain et al.[26] demonstrated that the heart during late pregnancy is more susceptible to IR injury when subjected to coronary occlusion. Furthermore, during late pregnancy in mice, the IS was greater and the post-ischemic functional recovery was found to be extremely poor. Interestingly, the hemodynamic alterations and increased IS were partially restored in the post-partum mice.

The opening of mitochondrial permeability transition pore (mPTP) at the onset of reperfusion is a critical determinant of myocardial cell death.[27] In this regard, a study demonstrated

Table 1 – Hemodynamic Parameters

Groups	Baseline			Ischemia			Reperfusion		
	HR (Beats/min)	LVDP (mmHg)	RPP (mmHg x bpm)	HR (Beats/min)	LVDP (%Baseline)	RPP (%Baseline)	HR (Beats/min)	LVDP (%Baseline)	RPP (%Baseline)
IR	146.0 ± 4.9	58.4 ± 11.9	8519.6 ± 1805.2	134.6 ± 3.9	62.5 ± 8.3*	57.5 ± 7.3#	131.2 ± 5.2$	64.3 ± 8.6*$	57.4 ± 6.8#
L	138.0 ± 3.6	115.2 ± 34.2	15673.2 ± 4219.6	120.7 ± 9.1	28.3 ± 3.7*	25.2 ± 4.6*	71.7 ± 18.4	20.8 ± 7.8#	12.6 ± 7.2#
NL	148.6 ± 8.3	66.9 ± 11.9	9797.3 ± 1652.5	137.4 ± 9.7	73 ± 8.5$	67.2 ± 7.7.*$	145.4 ± 11.9$	73.1 ± 8.5$	74.0 ± 13$

** p < 0.05 versus the baseline period in the group, # p < 0.001 versus the baseline period in the group, $ p < 0.05 versus the lactating group.*
Data are presented as mean ± standard error of the mean (SEM). For LVDP, RPP and HR, the means were compared between the groups using one-way ANOVA and within the groups using repeated measure ANOVA. The post hoc test used was Bonferroni.
IR: ischemia-reperfusion; L: lactating; NL: non-lactating; LVDP: left ventricular developed pressure (mmHg); HR: heart rate (beats/min), RPP: rate pressure product (mmHg x bpm); bpm: beats per minute.

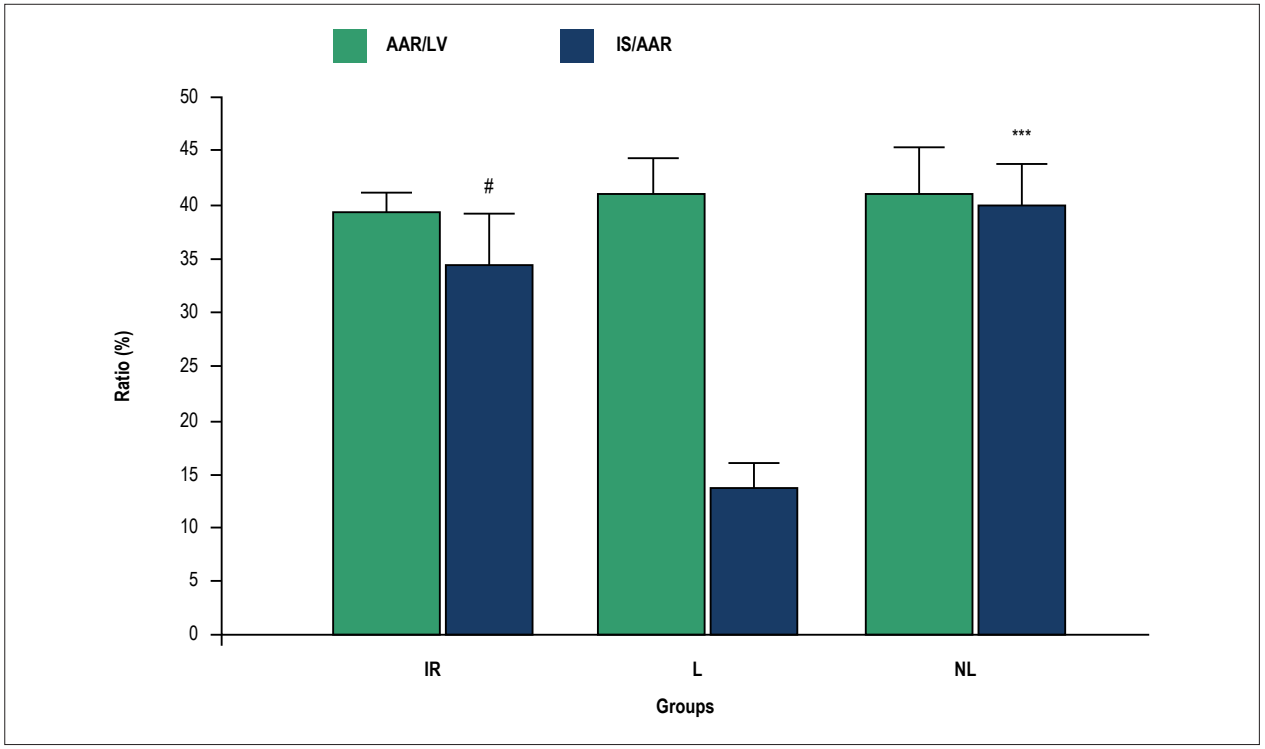

Figure 1 – *Myocardial area at risk in relation to the left ventricle (AAR/V %) and infarct size (IS/AAR %). Data are presented as mean ± S.E.M. standard error of the mean (SEM). The mean values between the groups were compared using one-way ANOVA followed by Bonferroni's post hoc test. # p < 0.05 versus the lactating group, *** p < 0.001 versus the lactating group. IR: ischemia-reperfusion; L: lactating; NL: non-lactating.*

that pregnancy lowers the threshold for the mPTP opening, which can be attributed to pregnancy-induced increase in cardiac reactive oxygen species (ROS) generation. Some authors have hypothesized that lactation may induce a resetting effect to the heart and improve pregnancy-induced alterations in cardiovascular dynamics.[3, 28]

The protective effect of lactation may be attributed to an increased metabolic expenditure of a nursing mother,[29] explaining the decrease in body mass index and cholesterol levels following lactation. In addition, initiation and maintenance of lactation involve many hormones such as oxytocin, prolactin, growth hormone, thyroxine, adrenal corticoids, and parathyroid hormone.[30] Cardiac tissue expresses a wide variety of hormone receptors, including receptors for lactogenic hormones,[31,32] and nursing-induced changes in hormonal milieu have been reported to improve the cardiovascular profiles.

Suckling is the major stimulus for the release of oxytocin from the posterior pituitary. The protective effect of oxytocin against IR injury has been previously depicted.[14] Faghihi et al.[33] demonstrated that oxytocin preconditioning reduces ischemia-induced ventricular arrhythmias by scavenging free radicals and delaying the opening of mPTP. Das and Sarkar[15] have suggested an involvement of mitochondrial ATP-sensitive potassium channels in oxytocin -induced cardioprotection. Furthermore, oxytocin has been shown to promote the release of atrial natriuretic peptide - a well-known cardioprotective hormone, which reduces the incidence of reperfusion- induced arrhythmias.[34,35] Oxytocin also exerts negative inotropic and chronotropic effects[36] which, in turn may decrease the oxygen demand of the myocardium and produce a smaller infarct following occlusion of the coronary artery. In addition to oxytocin, pretreatment with thyroid hormone has been shown to protect myocardium against lethal ischemia, in a pattern similar to that of ischemic preconditioning.[37] We observed that lactation reduced the LVDP, RPP, and HR in ischemic animals. This indicates a positive effect of nursing as it reduces myocardial oxygen consumption and improves oxygen supply to demand ratio.

Suckling does not only stimulate the release of oxytocin but also induces the secretion of prolactin (PRL) and adrenocorticotropic hormone, which are essential for galactopoesis.[38] The experimental evidence for the effect of prolactin on the cardiovascular system is quite limited. A cohort study reported the direct association of prolactin levels with endothelial dysfunction and increased risk of cardiovascular events and mortality.[39] Conversely, Krzeminski et al.[40] observed the antiarrhythmic effects of prolactin (isoform) against IR injury. A 15-day prolactin treatment markedly reduced the adrenaline-induced rise in HR, blood pressure, cardiomyocyte necrosis, and granulocyte infiltration in female rats.[41] Moreover, corticosteriods confer cardioprotection by binding to glucocorticoid receptors.[42] Glucocorticoids activate the endothelium-derived nitric oxide synthase (eNOS) and exert anti-inflammatory, antiatherogenic, and anti-ischemic effects.[43]

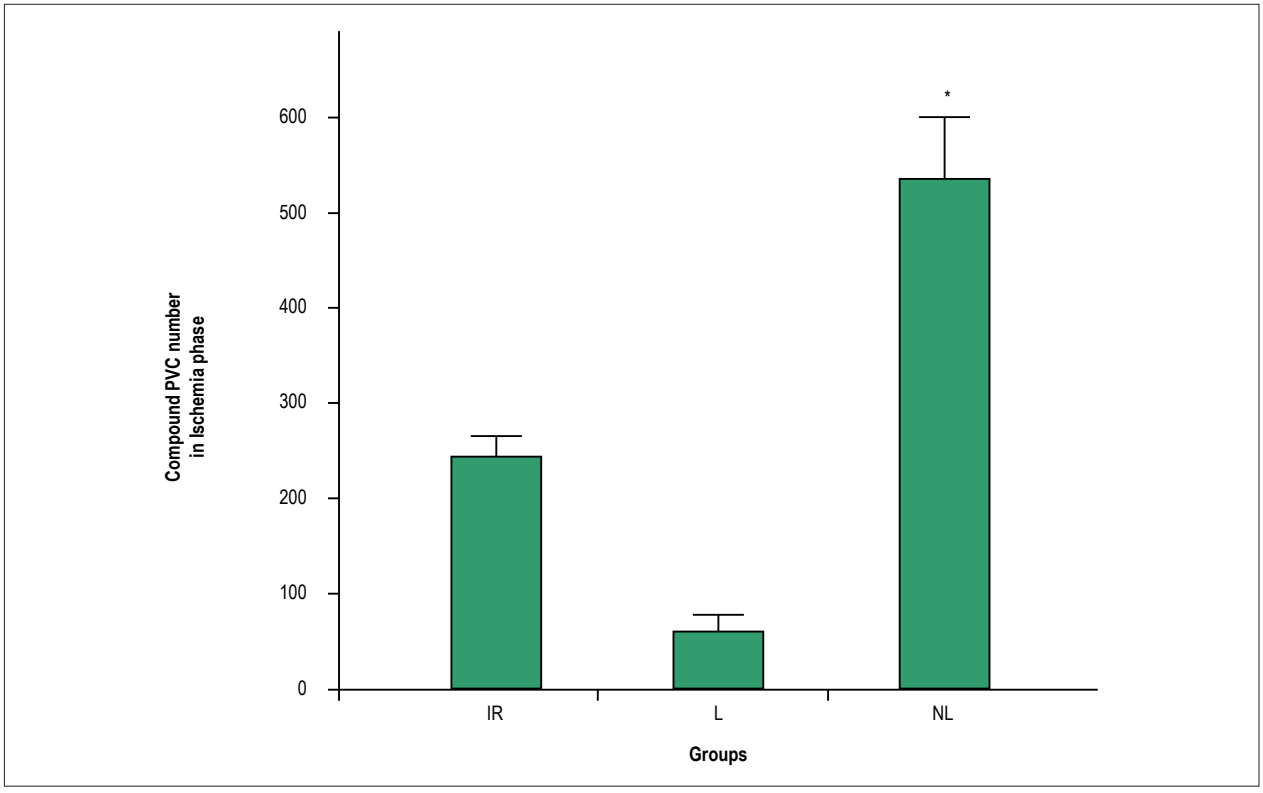

Figure 2 – *Number of compound PVCs (including bigeminy, couplet and salvos) in different groups. Data are presented as mean ± standard error of the mean (SEM). The mean values between the groups were compared using one-way ANOVA followed by Bonferroni's post hoc test. * p < 0.05 versus the lactating group. IR: ischemia-reperfusion; L: lactating; NL: non-lactating.*

The autonomic system is an important regulator of lactation and milk ejection. Vagal nerve stimulation (VNS) is essential for suckling-induced oxytocin and prolcatin release.[44] Efferent signals from the vagus nerve can inhibit the production of proinflammtory cytokines, thereby improving the pathological outcomes of diseases like sepsis, myocardial ischemia and other inflammatory disorders.[45] Interestingly, VNS has been shown to prevent reperfusion injury through inhibition of mPTP.[46]

In the current study, the protective effect of lactogenesis against IR injury can involve the potential role of lactogenic hormones, which can directly influence the cardiac dynamics via cardiac receptors. Furthermore, lactation may have improved the IR injury via VNS. A plausible confounder in this study was the emotional stress imposed on the maternal health due to the separation of the mothers from their pups. Although according to the research protocol the rats in the non-lactated group did not undergo surgery until 21 days after parturition (similar to the rats in the lactating group), the potential effect of emotional stress on cardiovascular dynamics cannot be definitely ruled out.

Conclusion

Taken together, our findings demonstrate the cardioprotective effects of lactation on maternal health, independent of pregnancy. Moreover, rats which were not allowed to breast-feed their pups, demonstrated high vulnerability to myocardial ischemic insult.

Author contributions

Conception and design of the research and Analysis and interpretation of the data: Askari S, Imani A, Sadeghipour H, Faghihi M, Edalatyzadeh Z, Choopani S, Karimi N, Fatima S; Acquisition of data: Askari S, Edalatyzadeh Z, Choopani S,; Statistical analysis: Askari S, Karimi N; Obtaining financing: Imani A; Writing of the manuscript: Askari S, Karimi N, Fatima S; Critical revision of the manuscript for intellectual contente: Fatima S.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

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Study Association

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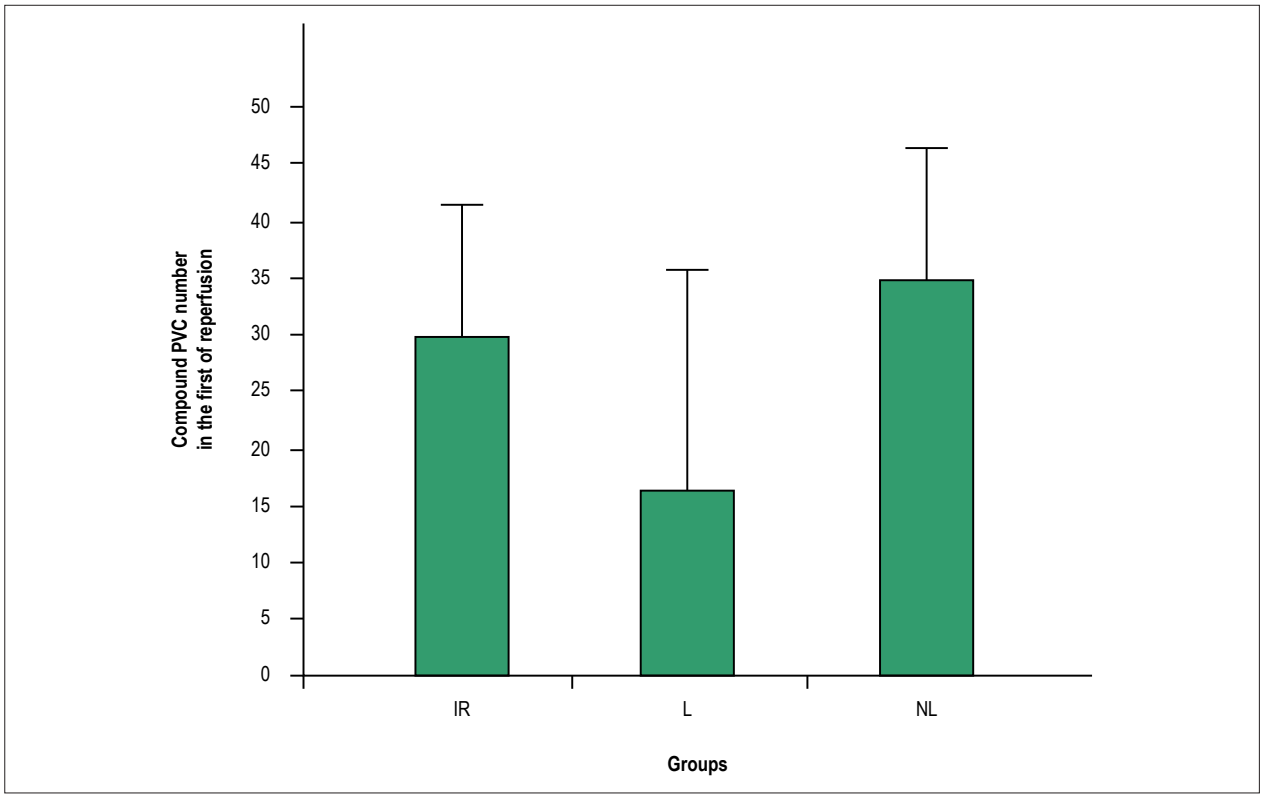

Figure 3 – *Number of compound PVCs (including bigeminy, couplet and salvos) in the first 5 min of reperfusion. Data are presented as mean ± standard error of the mean (SEM). The mean values between the groups were compared using one-way ANOVA followed by Bonferroni's post hoc test. IR: ischemia-reperfusion; L: lactating; NL: non-lactating.*

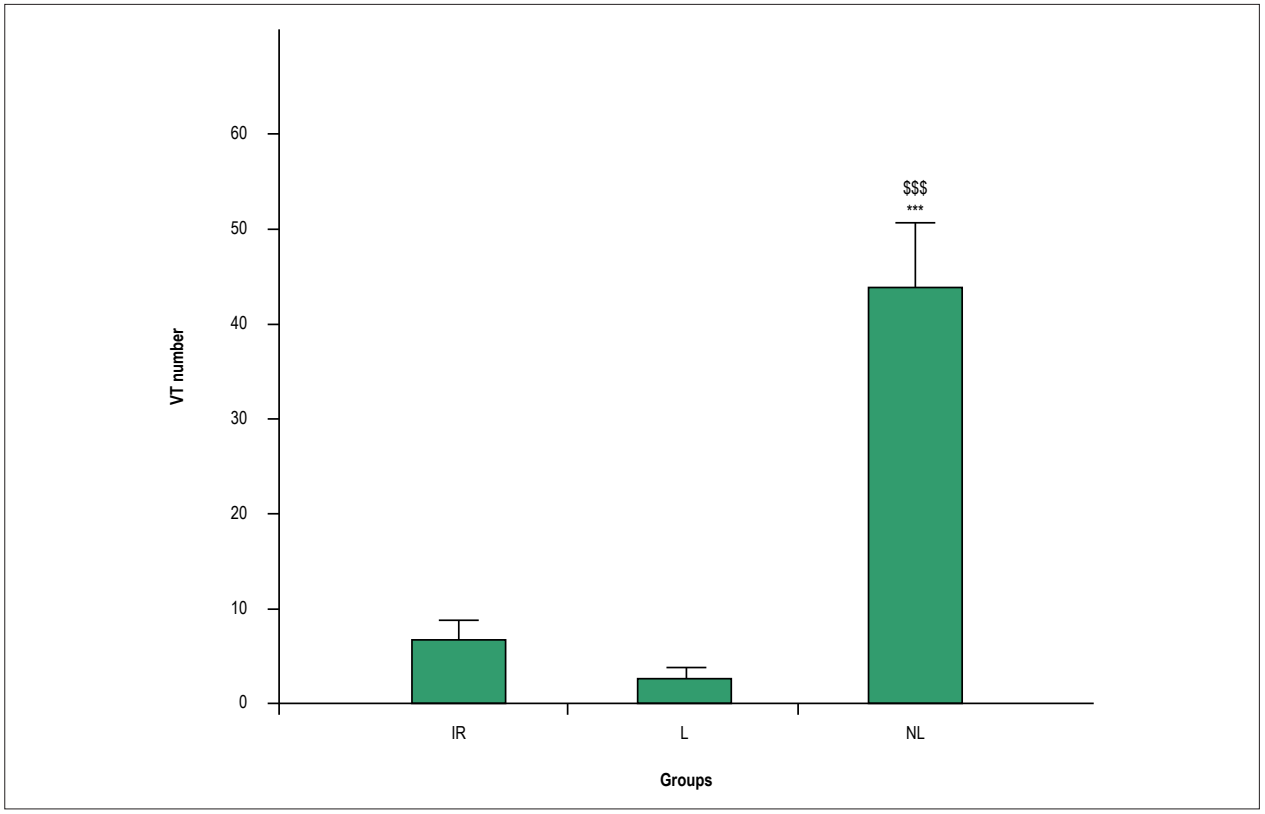

Figure 4 – *Number of ventricular tachycardia (VT) in different groups. Data are presented as mean ± standard error of the mean (SEM). The mean values between the groups were compared using one-way ANOVA followed by Bonferroni's post hoc test. *** p < 0.001 versus the lactating group; $$$ p < 0.001 versus the IR group. IR: ischemia-reperfusion; L: lactating; NL: non-lactating.*

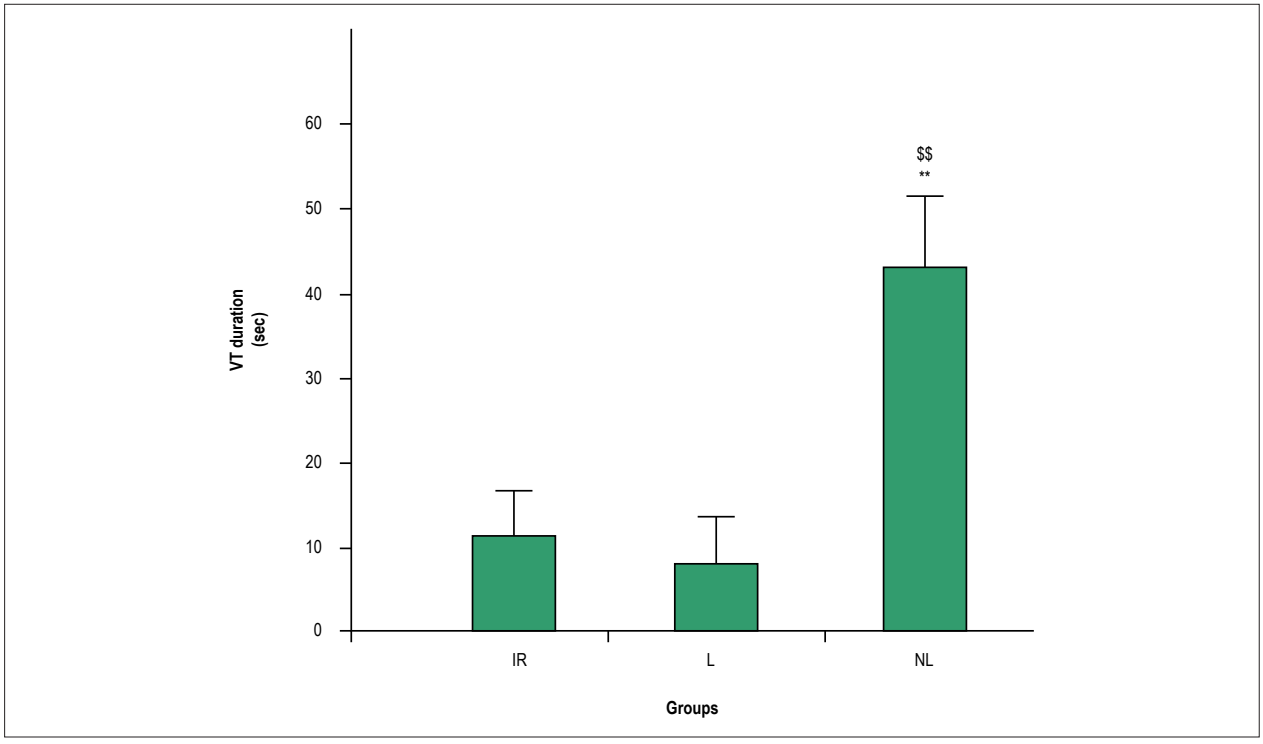

Figure 5 – *Duration of ventricular tachycardia (VT) in different groups. Data are presented as mean ± standard error of the mean (SEM). The mean values between the groups were compared using one-way ANOVA followed by Bonferroni's post hoc test. IR: ischemia-reperfusion; L: lactating; NL: non-lactating. ** p < 0.001 versus the lactating group; $$ p < 0.001 versus the IR group.*

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Assessment of Carotid Intima-Media Thickness as an Early Marker Of Vascular Damage In Hypertensive Children

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Abstract

Background: The increased carotid intima-media thickness (CIMT) correlates with the presence of atherosclerosis in adults and describes vascular abnormalities in both hypertensive children and adolescents.

Objective: To assess CIMT as an early marker of atherosclerosis and vascular damage in hypertensive children and adolescents compared with non-hypertensive controls and to evaluate the influence of gender, age, and body mass index (BMI) on CIMT on each group.

Methods: Observational cohort study. A total of 133 hypertensive subjects (male, n = 69; mean age, 10.5 ± 4 years) underwent carotid ultrasound exam for assessment of CIMT. One hundred and twenty-one non-hypertensive subjects (male, n = 64; mean age, 9.8 ± 4.1 years) were selected as controls for gender, age (± 1 year), and BMI (± 10%).

Results: There were no significant difference regarding gender (p = 0.954) and age (p = 0.067) between groups. Hypertensive subjects had higher BMI when compared to control group (p = 0.004), although within the established range of 10%. Subjects in the hypertensive group had higher CIMT values when compared to control group (0.46 ± 0.05 versus 0.42 ± 0.05 mm, respectively, p < 0.001; one-way ANOVA). Carotid IMT values were not significantly influenced by gender, age, and BMI when analyzed in both groups separately (Student's t-test for independent samples). According to the adjusted determination coefficient (R^2) only 11.7% of CIMT variations were accounted for by group variations, including age, gender, and BMI.

Conclusions: Carotid intima-media thickness was higher in hypertensive children and adolescents when compared to the control group. The presence of hypertension increased CIMT regardless of age, gender, and BMI. (Arq Bras Cardiol. 2017; 108(5):452-457)

Keywords: Child; Hypertension; Carotid Inima-Media Thickness; Biomarkers.

Introduction

Atherosclerosis is a complex multifactorial disease that begins early, as evidenced by the presence of cardiovascular risk factors developed by children and adolescents,[1] and documented by previous studies, which indicate that children and adolescents with obesity, dyslipidemia, high blood pressure, and inadequate glucose metabolism have increased risk of developing atherosclerosis in adulthood.[2] Additionally, the increased carotid intima-media thickness (CIMT) correlates with the presence of atherosclerosis in adults and describes vascular abnormalities in both hypertensive children and adolescents.[3] Lande et al.[4] reported that hypertensive children and adolescents with increased CIMT correlated with more severe hypertension assessed by ambulatory blood pressure monitoring, when compared to a control group. Their findings also showed that CIMT is increased in children with primary hypertension, regardless of the effects of obesity. Also, children with end-stage chronic kidney disease (ESCKD) have significantly increased blood pressure levels and CIMT[5-10]. However, CIMT also increases as a physiological reaction of the vessel when adapting to the age-dependent rise in blood pressure in children and adolescents.[11] In fact, CIMT changes could reflect non-atherosclerotic and adaptive responses to aging and mechanical stress.[11,12] CIMT seems to coincide with the normal development of children and increases with age, as it does in adults. The objective of the present study was to assess CIMT as an early marker of atherosclerosis and vascular damage in hypertensive children and adolescents compared with non-hypertensive subjects, controlling for age, gender, and body mass index (BMI) and to evaluate the influence of these variables (gender, age, and BMI) on CIMT in each group.

Methods

Patients

We selected 148 consecutive hypertensive children regularly followed at the hypertension outpatient clinic of the

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Pediatric Nephrology Clinic. All subjects had office systolic and/or diastolic BP ≥ 95th percentile for gender and height on ≥ 3 occasions (office hypertension). Hypertension was confirmed by 24 hour ambulatory blood pressure monitoring (ABPM), defined as average daytime and/or nighttime BP ≥ 95th percentile for gender and height according to the ABPM pediatric norms.[13] Each child had their height and weight measured at the time of their appointments. Body mass index (BMI) was calculated using the standard formula.[14] Children were considered overweight or obese when they had BMI ≥ 85th and 95th percentile, respectively, for age and gender.[15,16] Patients' blood and urine samples were collected between 1 week before and one week after the appointment, for assessment of serum glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TGC), and basal insulin. Subjects were classified as having diabetes when treated for insulin-dependent or non-insulin-dependent diabetes or having elevated fasting glucose levels (≥ 126 mg/dL). The use of lipid-lowering drugs or the presence of TC > 200 mg/dL, HDL-C < 40 mg/dL, LDL-C > 100 mg/dL or TGC > 150 mg/dL was recorded.[17] Subjects also underwent echocardiogram and electrocardiogram (EKG) exams. Exclusion criteria included children with no blood and urine samples, unconfirmed arterial hypertension, and children with diabetes, dyslipidemia, metabolic syndrome, with ESCKD or any other systemic disease. Children who had both essential and secondary hypertension were included in the study. For the control group, we selected 200 consecutive healthy children and adolescents who underwent echocardiography for assessment of an innocent cardiac murmur referred to the study by a private pediatrician. This population was selected among patients from the private health care system. *Systolic blood pressure* (SBP) *and diastolic blood pressure* (DBP) were measured with appropriate cuff sizes according to arm size in the sitting position twice on both arms after a 5 minute-rest before the echo exam. Control and hypertensive subjects were controlled for gender, age (± 1 year), and BMI (± 10%). Exclusion criteria in the control group were children diagnosed with diabetes, dyslipidemia, hypertension, metabolic syndrome, and any systemic disease, according to information provided by their parents or private pediatrician. Each child had their height, weight, and blood pressure measured at the time of their echocardiogram. Children were not sedated before exams. Children who refused to undergo the ultrasound examination and a proper or complete examination, such as very young children, were excluded from the study. The institutional ethical committee approval was obtained for the study. The legal representative of each child provided written informed consent before examination. Children over 10 years of age also signed a consent form.

Blood sample analysis of hypertensive children

Venous blood was collected after overnight fasting. Standard techniques were used to determine serum glucose, TC, HDL-C, LDL-C, TGC, and basal insulin. Information about control children's blood sample analysis was provided solely by their parents and their private pediatrician.

Ultrasound measurements

Carotid IMT measurements were made using high-resolution B-mode ultrasonography (Philips Medical Systems' HD11 platform) with a broadband width linear array transducer (L 3–12 MHz). Sonography and readings were conducted by a trained and certified sonographer. The subjects were examined in the supine position with an extended neck and the probe in the antero-lateral position. On longitudinal 2D ultrasound images of the carotid artery, the near wall and the far wall were displayed as 2 echogenic lines (the adventitia and intima), separated by the hypoechoic media. The distance between the leading edge of the first bright line of the far wall (lumen-intima interface) and the leading edge of the second bright line (media-adventitia interface) was defined as the CIMT. For this study, we measured the CIMT on the distal 10 mm of the far wall of both the right and left common carotid artery. After zooming and freezing the image, we manually measured the CIMT using electronic calipers. Five measurements were recorded on each side and the average of these measurements was used for the final CIMT analyses, according to the Brazilian Cardiovascular Imaging Department Task Force for Carotid Ultrasound[18] and Association for European Paediatric Cardiology.[19]

Statistical analysis

Quantitative variables were described by mean and standard deviation. Qualitative variables were shown as frequencies and percentages. Kolmogorov-Smirnov test was used to assess the normality of the distribution. The Chi-Square test was used to compare qualitative variables between groups. Quantitative variables were compared using the one-way analysis of variance (ANOVA) model and the least significant difference (LSD) for multiple comparisons. For independent samples, two groups were compared using Student's *t*-test. Pearson's correlation coefficient was used to evaluate the linear association between two quantitative variables. A p-value of < 0.05 indicated statistical significance. The sample size calculation was not performed at the present study, since there are no normative values for CIM in healthy children and adolescents. No systematic random sampling was used. The subjects in both groups were chosen by convenience. Data were analyzed with the SPSS v. 20.0 computer program.

Results

Fifteen hypertensive children and adolescents were excluded from the study for not having undergone lab tests. A total of 133 hypertensive children and adolescents (male, n = 69; mean age, 10.5 ± 4 years) underwent carotid ultrasound exam. All these subjects were undergoing antihypertensive therapy. All hypertensive children and adolescents had normal TC (152 ± 36 mg/dL), normal HDL-C (46 ± 13 mg/dL), normal LDL-C (84 ± 25 mg/dL), normal TGC (86 ± 44 mg/dL), normal fasting glucose (86 ± 10 mg/dL), and normal basal insulin (10 ± 4 mIU/L). The authors identified secondary hypertension in 58 children, of which causes included coartaction of the aorta, reflux nephropathy, ectopic kidney, polycystic kidney disease, chronic pyelonephritis, renal artery stenosis, solitary kidney, and renal atrophy.

None of these children were undergoing dialysis treatment. There were no significant differences between children with and without identified secondary hypertension (p = 0,55). None of these children had left ventricular hypertrophy on echocardiogram or EKG alterations. Sixty-four (48%) subjects were within normal BMI range, 33 (24.8%) were considered obese, 33 (24.8%) were considered overweight, and 3 (2.25%) were considered thin. As for the children and adolescents in the control group, 79 subjects were excluded from the study for presenting a metabolic disorder (such as diabetes or dyslipidemia) or any systemic disease according to reported information or because the BMI showed a difference > 10% for age and gender. One hundred and one children and adolescents (males, n = 64; mean age, 9.8 ± 4.1 years) were selected as controls for gender, age, and BMI for the hypertensive group. Sixty-seven (55%) were within normal BMI range, 26 (21%) were obese, 23 (19%) were considered overweight, and 5 (4.1%) were thin. All these subjects had normal echocardiogram results. There were no significant differences regarding gender (p = 0.954) and age (p = 0.067) between groups. Hypertensive subjects had higher BMI when compared to control group (p = 0.004), although within the established range of 10%. Carotid intima-media thickness was higher in hypertensive children when compared to control group (0.46 ± 0.05 versus 0.42 ± 0.05 mm, respectively, p < 0.001; Table 1; Figure 1). Carotid IMT values were not significantly influenced by age, gender, and BMI when analyzed in the 2 groups separately (Figure 2). After multiple linear regression analysis, the increase in CIMT remained independently associated to hypertension (p < 0.001). According to the adjusted determination coefficient (R^2), only 11.7% of CIMT variations are accounted for by the variations of each group including age, gender, and BMI.

Discussion

Children with primary hypertension are usually overweight and obese, a fact which makes it difficult to separate the effect of blood pressure from the metabolic disturbances.[20] According to our findings, 49.6% of the hypertensive children and 40% of the children in the control group were obese or overweight, a factor that would tend to overshadow any potential difference in the groups because of hypertension. However, the present study confirmed that hypertensive children had higher values of CIMT when compared to the control ones regardless of

Table 1 – Basal characteristics of the study population

	CG	HG	p value
Gender (N/%)			
Male	64(52.9%)	69 (51.9%)	
Female	57 (47.1%)	64 (48.1%)	0.954
Age (years; mean ± SD)	9.8 ± 4.1	10.5 ± 4	0.162
BMI (kg/m^2; mean ± SD)	19.9 ± 4.4	21.9 ± 6.3	0.004
CIMT (mm; mean ± SD)	0.42 ± 0.05	0.46 ± 0.05	< 0.001*

*CG: control group; HG: hypertension group; BMI: body mass index; CIMT: carotid intima-media thickness; SD: standard deviation; * Student's t-test for independent samples.*

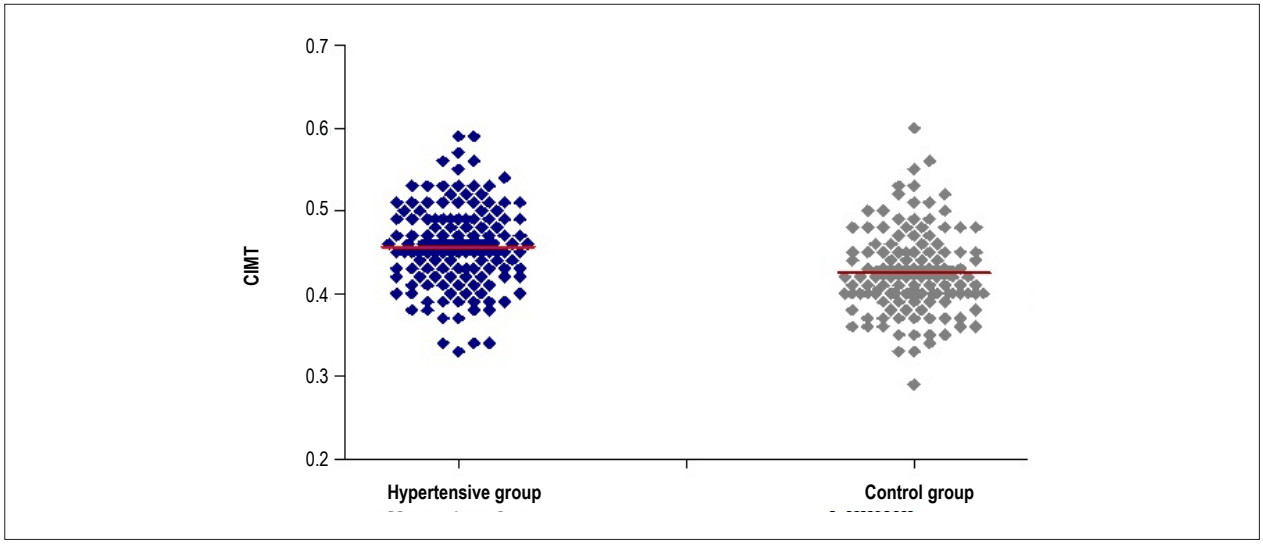

Figure 1 – *Carotid intima-media thickness (CIMT) values between hypertensive group (HG) and control group (CG).*

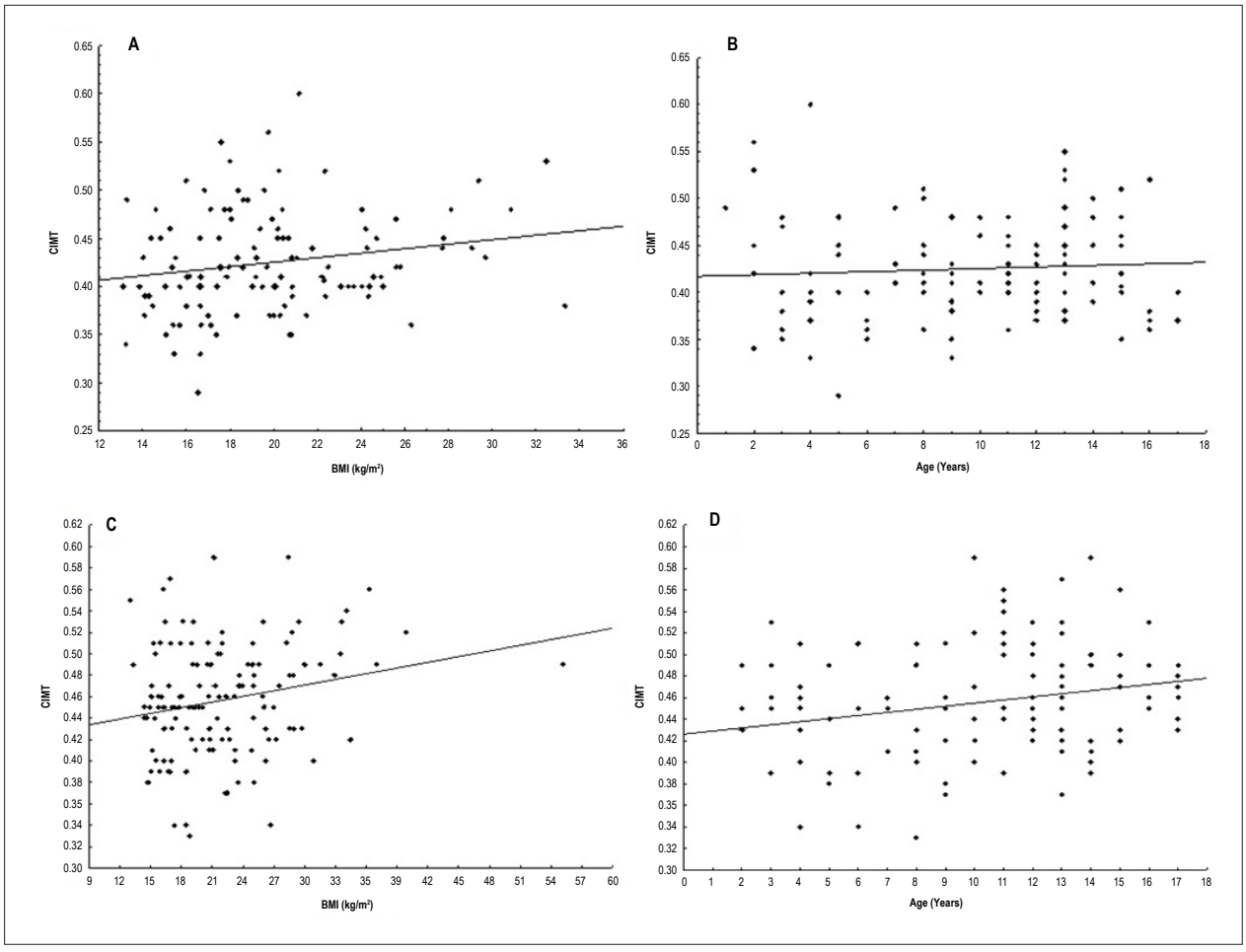

Figure 2 – *Panels A and B) Correlation between carotid intima-media thickness (CIMT) with age and body mass index (BMI) in hypertensive group.Panels C and D) Correlation between carotid intima-media thickness (CIMT) with age and body mass index (BMI) in control group.*

age, gender, and BMI. This finding confirms the studies by Lande et al[4] and provides evidence that hypertension can lead to vascular abnormalities in childhood, regardless of obesity. Availability of normative CIMT data for children is limited. In the study by Le et al,[21] child CIMT was compared against the percentile charts available for an ethnicity- and gender-matched 45-year-old adult population to determine the vascular age. They assessed nonobese children with familial dyslipidemia and obese children with multiple atherosclerosis-promoting risk factors such as high triglyceride, high total and LDL cholesterol, low HDL cholesterol, high blood pressure, and high insulin levels. Vascular age was similar in both groups. In the present study, we evaluated only children with hypertension and excluded children with other atherosclerosis-promoting risk factors. CIMT is considered a reflection of multiple risk factors; however, primary contributors to intima-media thickening are age and hypertension.[22-24] The presence of hypertension significantly increases CIMT values due to the hypertrophy of the media layer of the vessel wall.[25] Previous studies[26-33] concluded that a normal carotid arterial wall is unaffected by age or gender until approximately 18 years, after which age, there is diffuse progressive intimal thickening. Hence, in hypertensive children and adolescents, CIMT reflects a physiological reaction of the vessel to adapt the age-dependent rise in blood pressure, plus the effects of hypertension itself. However, hypertension seems to be on the rise with the increase in childhood overweight and obesity. The prevalence of obesity in children is increasing, and thus, inducing an increase in metabolic syndrome of these children. Obesity is associated to several risk factors for cardiovascular disease in adulthood and to other chronic diseases, such as dyslipidemia, hyperinsulinemia, hypertension, and early atherosclerosis.[2,16,20,34-38] In this regard, any study that aims to evaluate a specific measurement in children and adolescents, such as CIMT, should consider BMI and match this population for gender and age, as performed in the present study.

Study limitations

The present study has some important limitations identified as (a) inclusion of both essential and secondary hypertension in the hypertensive group; (b) lack of ABPM in the control group and (c) lack of blood samples in the control group. We included children with essential and secondary hypertension. However, we are not certain if the impact of early hypertension, as in secondary causes, will induce higher CIMT in the future when compared to essential hypertension, which usually begins in older children. Moreover, a possible correction of the secondary cause may influence the CIMT measurements. ABPM was not performed in the control subjects to confirm normotension. Finally, we did not request blood and urine samples from the subjects in the control group. These children were selected from the private health care system at a private cardiology clinic and we only obtained information about their blood sample analysis reported by their parents and their private pediatrician.

Conclusions

Carotid intima-media thickness was higher in hypertensive children and adolescents when compared to the control group. The presence of hypertension increased CIMT regardless of age, gender, and BMI in both hypertensive and non-hypertensive children and adolescents.

Author contributions

Conception and design of the research: Baroncini LAV, Sylvestre LC, Pecoits Filho R; Acquisition of data and Writing of the manuscript: Baroncini LAV, Sylvestre LC, Baroncini CV; Analysis and interpretation of the data: Baroncini LAV, Baroncini CV, Pecoits Filho R; Statistical analysis and Critical revision of the manuscript for intellectual content: Baroncini LAV.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Myocardial Viability on Cardiac Magnetic Resonance

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Abstract

The study of myocardial viability is of great importance in the orientation and management of patients requiring myocardial revascularization or angioplasty. The technique of delayed enhancement (DE) is accurate and has transformed the study of viability into an easy test, not only for the detection of fibrosis but also as a binary test detecting what is viable or not. On DE, fibrosis equal to or greater than 50% of the segmental area is considered as non-viable, whereas that below 50% is considered viable. During the same evaluation, cardiac magnetic resonance (CMR) may also use other techniques for functional and perfusion studies to obtain a global evaluation of ischemic heart disease. This study aims to highlight the current concepts and broadly emphasize the use of CMR as a method that over the last 20 years has become a reference in the detection of infarction and assessment of myocardial viability.

Introduction

Cardiac magnetic resonance (CMR) has been established as a method to detect myocardial infarction. Using a quick protocol, we are able to obtain information on anatomy, function, tissue characterization, perfusion, and viability, with excellent spatial resolution and image quality. CMR uses different techniques to assess viability, and the technique of delayed enhancement, *per se,* is currently a reference standard for this purpose.

The precise determination of myocardial muscle with or without viability is of extreme importance in the management of a patient with cardiac dysfunction. Viable muscle has a potential for contractile recovery and, therefore, a patient with ischemic cardiomyopathy and ventricular dysfunction may improve his functional capacity after myocardial revascularization[1,2] and, consequently, have improved survival.[3,4]

The identification of infarcted muscle, even in silent (occult) infarction, is important because this tissue can be a substrate for ventricular tachyarrhythmia,[5,6] becoming one of the most important causes of sudden death.

Keywords

Myocardial Infarction / metabolism; Myocardial Revascularization; Tissue Survival; Magnetic Resonance Spectroscopy; Angioplasty.

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CMR presents a state of continuous development, with new tools added at each year to improve this already powerful technology.

This article aims to highlight the current concepts and, in a general way, emphasize the use of CMR as a method that has become over the past 20 years a reference in the detection of myocardial infarction and assessment of viability.

The pathophysiology of myocardial infarction by CMR

CMR's high resolution has transported the concept of transmural extent of infarction from the established theory of experimental studies into the clinical reality.[7] As illustrated (Figure 1), the duration of the myocardial ischemia is the greatest determinant of the transmural extent of infarction. In a canine model, a coronary occlusion shorter than 20 minutes promotes change in regional contractility without permanent injury or myocardial infarction. The infarction itself develops later and always takes place from the subendocardium to the epicardium. The subendocardium is the most metabolically vulnerable region and one that requires a higher level of oxygenation. After 3 – 6 hours of coronary occlusion, the infarction reaches its transmural extent if reperfusion does not occur.[7]

Other factors can modulate the transmurality of an infarction and its size. Most of these factors are associated with an increased demand of oxygen to the myocyte. In patients with hypertension, tachycardia, or high levels of circulating catecholamines, we can observe an accelerating effect in the establishment of the infarction, causing lesions larger than those in patients without these conditions. In situations involving the level of oxygenation to the myocyte such as anemia, hypoxia, or carbon monoxide poisoning, we will more often also have the establishment of sudden and larger infarctions. In the presence of collaterals, the opposite will occur as a protective effect: the presence of collaterals may reduce the size of the infarction. The same happens when subsequent and short attacks occur in a situation known as precondition (ischemic preconditioning).[8,9]

Drug treatments (beta-blockers), thrombolysis, and/or adequate reperfusion by angioplasty can modify the relationship between the duration of the coronary occlusion and the final size of the infarction. The current problem is that many studies have demonstrated this in animal models, but have failed to demonstrate the same in clinical studies.

We agree with Arai[7] when he questions the final results of these studies since the reduction of the infarction was measured in the animal model as a fraction of the area at risk. The area at risk is a portion of the myocardium that was hypoperfused during coronary occlusion but did not "die" (infarcted). Some studies have attempted to use sestamibi in the emergency room to assess the area at risk,[10] but were unable to do so. We consider this outcome as a

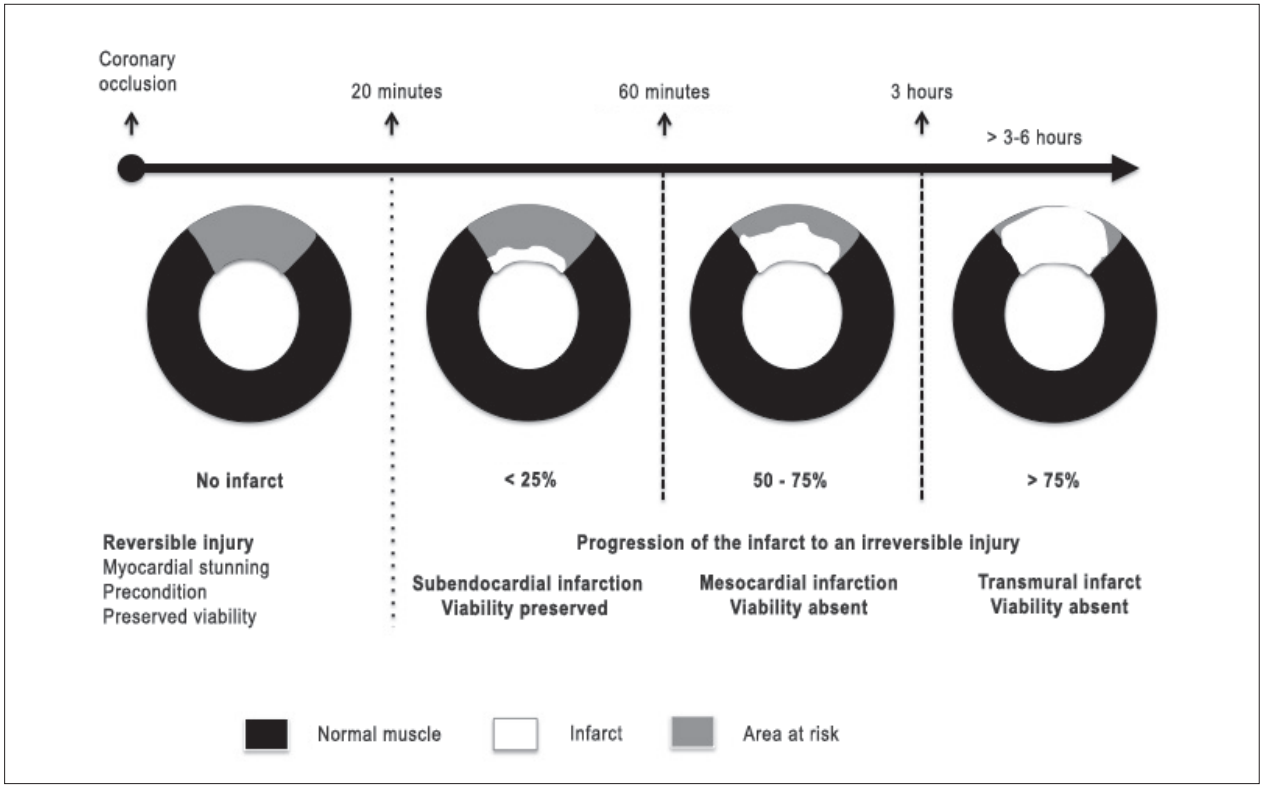

Figure 1 – *Ischemia duration is the major determinant of the infarct size and its transmural extent. Modified from Arai et al.*[7]

methodological problem. CMR should be used in this clinical situation, since it does not interfere with the decay of the radiotracer throughout week and time and may, in addition to using the technique of delayed enhancement to quantify the size of the infarction, resort to the T2 technique (edema) in order to assess the area at risk.[11]

In addition to evidence of the existence of some level of myocardial regeneration after acute myocardial infarction,[12] the final setting after an injury induced by hypoxia in the long term is the replacement of the tissue by fibrosis, defined as replacement fibrosis. In this fibrosis, the area of the infarction is replaced by a scar containing collagen, lacking proteins or structures required for a normal segmental contraction. However, the process of regeneration and the infarct size may influence the possibility of the infarction developing changes in the segmental contractility. In other words, a small myocardial infarction (< 25% of the segment area) probably will not lead to changes in myocardial contractility; on the other hand, a large myocardial infarction (> 75% of the segment area) will promote segmental or regional hypokinesia/akinesia. Thus, the detection of the infarct as present or absent is essential but equally important is the infarct size. Therefore, this replacement and the absence of the tissular actin-myosin mechanism promote loss of contraction and diastolic properties,[13] which may or may not be macroscopically undetectable by the contractile capacity of neighboring tissues and by the regeneration induced in the segment.

The most important thing is that CMR, with the technique of delayed enhancement, is able to detect myocardial infarction (fibrosis) not detected by clinical evaluation or other methods, such as ECG, echocardiography, and scintiscan.[14,15] This knowledge is a differential, because even very small areas with delayed positive enhancement, such as approximately 1% of the left ventricular mass, have large prognostic implications.[15-17]

Myocardial viability

The detection of viable myocardium reflects the presence of living myocytes, and this does not depend on the existence or not of contractile dysfunction or responsiveness of the muscle to external stimuli. Therefore, it is already well established the disconnection between viable myocardium and its pattern of contractility, with several studies demonstrating that the viable muscle could be hypokinetic by chronic cardiomyopathy due to hypoperfusion or acute ischemic cardiomyopathy.[18-20]

The study of myocardial viability is recommended for patients with ischemic cardiomyopathy, and the interest is to know whether a possible revascularization procedure will promote improvement in left ventricular systolic function. In this case, the potential for improvement in contractility will depend on two conditions: first, the muscle must be alive (absence of delayed enhancement); second, the muscle must be ischemic, being this the mechanism of dysfunction. Therefore, myocardial viability is said to be present when a hypokinetic or akinetic muscle features areas without necrosis whose coronary supply is known to be reduced. With this, we imagine that a procedure that restores the blood flow is one that removes the infarct from a hibernating state.[21]

The terms "stunned" and "hibernating" myocardium are used to describe this phenomenon of a condition that is viable, albeit dysfunctional. The reversible phenomenon of the stunned myocardium is identified when the contractile dysfunction develops during acute and intense ischemia, persisting even after restoration of the coronary flow, typically for a period of days to weeks. The reversible phenomenon of the hibernating myocardium, in turn, is identified when the contractile dysfunction takes place during chronic ischemia that is not strong enough to cause cellular necrosis. In this case, the phenomenon fits into the hypothetical concept of pathophysiologic mechanisms capable of inducing adjustment in perfusion-contraction coupling, at a very low level of both, reducing its contractility due to low oxygenation, and avoiding its death.[22-25]

Also noteworthy was the demonstration, as early as 1998, of the CMR ability to detect and quantify the area of microvascular obstruction (no-reflow) associated with an acute myocardial infarction.[26] The microvascular obstruction is a marker of severe myocardial injury, which is also associated with worse prognosis after acute myocardial infarction.[27]

Unfortunately, the myocardial viability is still highly associated with the detection of segmental or regional contractile dysfunction and is still widely used in the reasoning of clinical cardiologists when they think about the potential of contractile recovery, most likely because the echocardiography is the method most commonly used in clinical practice. With this, we demonstrate in Table 1 the conditions that can cause myocardial dysfunction and are able, in some way, to mask or make difficult a diagnosis of viable myocardium.

The absence of myocardial viability is the most frequent consequence of a coronary occlusion leading to myocardial infarction. Within this context, a series of parameters may be used to detect whether an infarction indeed occurred and how much of the infarcted territory can be saved. In a review, Kaul[28] summarized the most accurate markers of infarction, classifying them from less to more precise (Figure 2). For example, the isolated presence of myocardial contractility alteration does not provide information about the presence or absence of infarction, because the hibernating or stunned muscle may be viable but hypokinetic. At the other extreme, the macroscopic and precise identification that magnetic resonance can offer us, with its abilities of tissue characterization, measurement of size, and transmural extent of infarction (delayed enhancement), allows us a better characterization of myocardial viability.

Table 1 – Comparison between conditions that may lead to regional myocardial dysfunction

	Nontransmural Myocardial Infarction	Transmural Myocardial Infarction	Stunned myocardium	Hibernated myocardium	Post-infarction remodeling	Nonischemic cardiomyopathies
Perfusion	Normal or reduced depending on the existence or not of adequate reperfusion or microvascular obstruction	Normal or reduced depending on the existence or not of adequate reperfusion or microvascular obstruction	Normal by definition	Reduced by definition	Normal	Normal
Function	Normal	Reduced	Reduced but reversible with perfusion restoration (hours or weeks)	Reduced but reversible with perfusion restoration (may take months to recover)	Reduced	Normal or reduced (depending on the percentage of the affected area)
Metabolism	Normal or reduced (low FDG uptake)	Reduced (low FDG uptake)	Not reduced (high FDG uptake)	Not reduced (high FDG uptake, perfusion-metabolism mismatch)	Probably normal	Normal or reduced (low FDG uptake)
Histology	Replacement fibrosis	Replacement fibrosis	Normal myocytes	May be normal or present a certain degree of differentiation of the myocytes, with loss and disorganization of cellular elements	Hypertrophy, dilation, and architectural distortion of myocardial fibers	Replacement fibrosis
Delayed Enhancement	Delayed enhancement in the subendocardium (< 50% of the area of the segment). Usually in a coronary territory, unless it has multiple infarctions or the patient has undergone surgery with graft placement	Transmural delayed enhancement that may compromise from the subendocardium to the epicardium (> 50% of the area of the segment). Usually in a coronary territory, unless it has multiple infarctions or the patient has undergone surgery with graft placement	Normal, unless there is a combination of stunned myocardium and myocardial infarction	Normal, unless there is a combination of hibernated myocardium and myocardial infarction	Negative (myocardial dysfunction remote to a large infarction) and, therefore, viable.	Variable, best identified as mesocardial, epicardial, diffuse, or even negative

Table modified from Arai.[7]

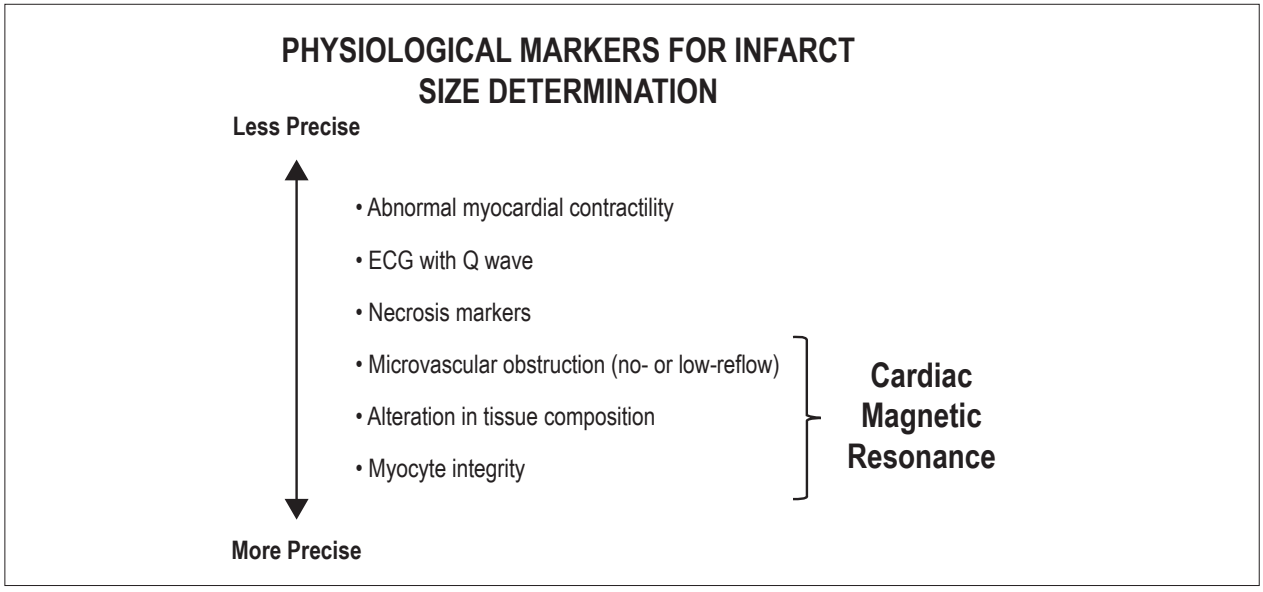

Figure 2 – *Clinical and physiological markers for determination of the infarct size. Modified from Kaul.[28]*

Technique of magnetic resonance for evaluation of viability

Several techniques of magnetic resonance imaging can be used for the evaluation of myocardial viability, some still without clinical applicability.

- Spectroscopy can be used to evaluate the cellular metabolites and analyze whether the integrity of the myocytes is present or not.[29] Sodium imaging by magnetic resonance, in turn, may also be a method to differentiate viable from infarcted muscle[30] and was recently used in volunteers for evaluation of viable muscle with 3-Tesla magnetic resonance. These two techniques have primary limitations in current days due to low signal-to-noise ratio, low spatial resolution, and exceedingly long time for acquisition.
- The use of T1 and T2 images and maps can assist us in the evaluation of myocardial edema and infarction and, in some situations in the areas at risk.[11,31,32] However, these techniques also have some limitations, the main one related to the fact that changes in T1 or T2 are not specific to detect viability and possible potential for recovery of contractility.
- The use of cine magnetic resonance (cine MRI) can assist in the assessment of segmental and global contractility, or even parietal segmental thickness. These data continue to be of great importance, but there are studies, such as that of Perrone-Filardi et al.,[33] that have demonstrated that viable muscle may be present in segments with significant parietal thinning. We may also use stressor agents such as dobutamine,[34,35] adenosine,[36] regadenoson,[36] and dipyridamole[37,38] to assess contractile (cine MRI) or perfusional (first-pass perfusion) alterations, respectively, or even perform combined protocols for multimodal assessment of the myocardium.
- Even with all of the sequences described above, the gold standard in magnetic resonance for the assessment of myocardial viability is the delayed myocardial enhancement study, as discussed below.

Delayed myocardial enhancement

Studies of delayed myocardial enhancement using T1-weighted magnetic resonance techniques have been reported in the literature since the mid-1980s.[39] This approach is simple and based on the assumption that infarcted tissue or tissue with increased extracellular space accumulates gadolinium and appears with increased signal on magnetic resonance images (hyperintense signal), mainly in the images acquired 10 minutes after infusion of the contrast medium.

Initially, the shades of gray and white representing the normal and infarcted muscle overlapped due to the inability of the old sequences in detecting lesions with mild to moderate contrast accumulation. In the late 1990s and early 2000s, Kim et al.[40] and Simonetti et al.[41] developed a technique able to override gray muscle (normal), highlighting the muscle that accumulates the contrast (infarcted muscle). This technique is currently used on a large scale and is part of all protocols of cardiac examination by magnetic resonance. This has enabled high-resolution images of acute and chronic infarction, demonstrating a high correlation with histopathological studies and virtually equal measurement of myocardial size and viability (myocytes).[40-42]

Image acquisition and use of intravenous contrast

The acquisition of delayed contrast-enhanced images is relatively simple and does not require pharmacological or physical stress. Using only a peripheral venous line, we can perform infusion of the intravenous contrast medium gadolinium. After obtaining the scout images, we perform

a global and segmental functional study of the right and left ventricle with the cine MRI technique (see specific section). We can, at this moment, infuse gadolinium 0.10 or 0.20 mmol/kg and, after approximately 10 minutes, acquire images of viability (delayed enhancement) of the myocardium in the short, long two-chamber, and outflow axes, as the acquisition of the cine MRI images. Each acquisition of delayed enhancement takes approximately 10 seconds and one apnea, with the entire examination taking an average of 30 minutes (Figure 3).

Delayed enhancement is a technique that can be acquired in different ways, such as 2D, 3D, gradient-echo or inversion-recovery techniques, in addition to those performed in apnea or free breathing.[43-45] The best technique is chosen and adapted depending on the clinical condition of the patient, the manufacturer of the available equipment, and the experience of the local group.

In practice, an assessment of delayed enhancement (viability) with a short and objective protocol can also be carried out. With this, we are able to abolish the use of cine MRI images and infuse gadolinium alone, acquiring the delayed contrast-enhanced images, which can take only 15 – 20 minutes to be carried out.

Another important factor is the knowledge about the different contrasts used. We currently have approximately 10 different types of gadolinium on the market, and many of them have not been tested for cardiac use, even though they are routinely used.[46] The most frequently used ones are gadopentetate dimeglumine, gadodiamide, gadoversetamide, and gadoterate meglumine. There is an increasing need for the administration of lower doses of these contrasts to avoid possible adverse effects (Table 2).[46] In this case, we must always carry out the CMR with the principle of the lowest possible dose in order to establish a diagnosis.

Quantification of myocardial infarction and prediction of improvement in contractility (viability)

Clinical and animals studies have shown that areas with high signal intensity in the technique of delayed enhancement are highly reproducible when compared with the pathology, especially if the inversion time is properly used. In an animal model, Amado et al.[47] demonstrated a close correlation between the histopathology and the technique of delayed enhancement ($r^2 = 0.94$, $p < 0.001$). These findings have also been identified with high reproducibility in the clinical setting in acute[48] and chronic[49] infarction.

The assessment of viability by magnetic resonance may be performed by a dichotomous approach, strengthened by the Brazilian guidelines.[27] In accordance with the clinical definition, viability is deemed as present when below 50% of the area of the affected segment, and absent when greater than 50%. We know that this is a categorization of an almost linear phenomenon and that the smaller the necrosis, the greater the probability of improvement in contractility of the segment after revascularization. The reverse is also true, since the greater the necrosis, the smaller the probability of contractile recovery after revascularization.

In addition to assessment and quantification of fibrosis/global viability, CMR routinely evaluates the potential of contractile recovery in a segmental manner, attempting to characterize the 17 segments of the left ventricle. We divide the groups of quantification of delayed enhancement into five, according to the probability of contractile recovery of the studied segment (Figure 4):[21,50]

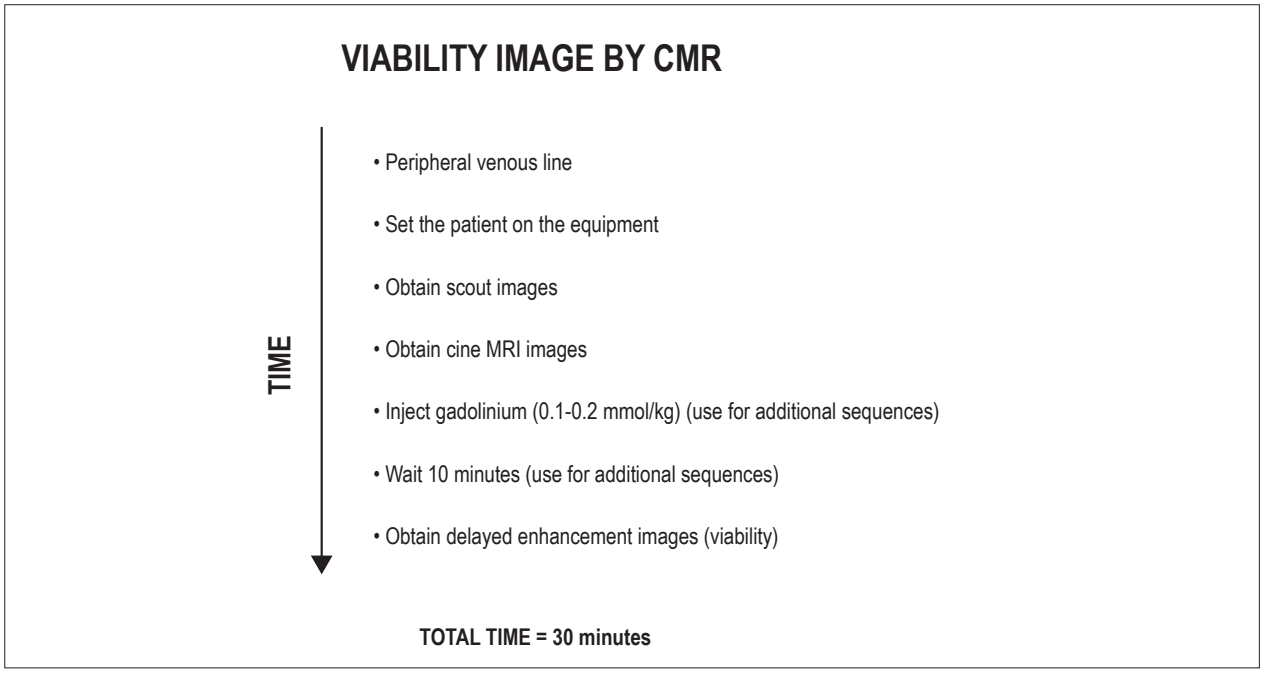

Figure 3 – *Example of acquisition steps from a protocol for viability/infarction by magnetic resonance. Modified from Weinsaft et al.[20]*

Table 2 – Gadolinium-based contrasts currently used

Agent	Name	Manufacturer	Recommended dose
Gadopentetate Dimeglumine (Gd-DTPA2)	Magnevist®	Bayer Healthcare	0.1 mmol/kg (0.2 mL/Kg)
Gadodiamide (Gd-DTPA-BMA)	Omniscan®	GE Healthcare	0.1 mmol/kg (0.2 mL/Kg) 0.05 mmol/kg (0.1 mL/Kg)§
Gadoversetamide (Gd-DTPA-BMEA)	OptiMark®	Mallinckrodt	0.1 mmol/kg (0.2 mL/Kg)
Gadoteridol	ProHance®	Bracco Diagnostics	0.1 mmol/kg (0.2 mL/Kg) Additional dose of 0.2 mmol/kg (0.4 mL/Kg)
Gadobenate Dimeglumine (Gd-BOPTA)	MultiHance®	Bracco Diagnostics	0.1 mmol/kg (0.2 mL/Kg)
Gadobutrol (Gd-DO3A-butrol)	Gadavist® Gadovist®	Bayer Healthcare	0.1 mmol/kg (0.1 mL/Kg)
Gadofosveset trisodium	Ablavar®	Lantheus Medcl	0.03 mmol/kg (0.12 mL/Kg)
Gadoxetate disodium	Eovist® Primovist®	Bayer Healthcare	0.025 mmol/kg (0.1 mL/Kg)
Gadobenate Dimeglumine (Gd-DTPA- Dimeglumine)	Viewgam®	M.R. Pharma S.A. / Alko do Brasil	0.1 mmol/kg (0.2 mL/Kg)
Gadoterate Meglumine (Gd-HP-DOTA)	Dotarem® Artirem®	Guerbet	0.1 mmol/kg (0.2 mL/Kg)

Other agents have been previously tested but are currently not available in the market: Mangafodipir Trisodium (Teslascan®; GE Healthcare) and Ferumoxides (Feridex®; Amag Pharms). Modified from Nacif et al.[46]

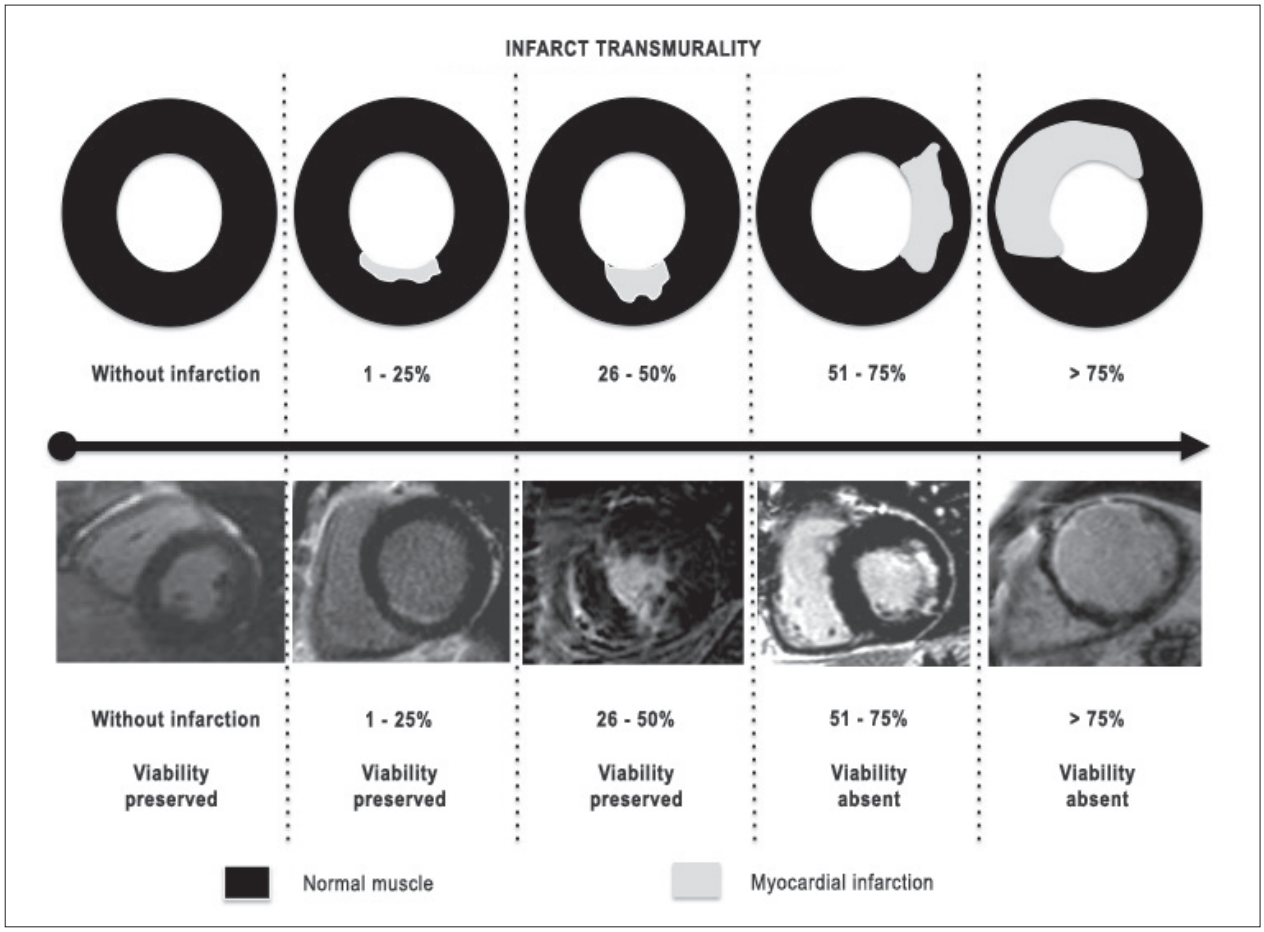

Figure 4 – *Examples of five different groups of quantification of delayed enhancement, noting that for magnetic resonance, the quantification occurs in a continuum and the potential of viability should be discussed and not just treated as a present or absent dichotomous variable.*

- The first is the myocardium without any delayed enhancement, *i.e.*, zero fibrosis/infarction, with a high probability (around 80%) of contractile improvement.
- The second comprises a group with 1 – 25% of the area of the segment with delayed enhancement. In this group, the probability of improvement decreases to 60%.
- The third group includes between 26 – 50% of delayed enhancement, compromising the cardiac muscle, with a probability of improvement in contractility after revascularization of around 40%.
- The fourth group has 51 – 75% of compromised muscle and may present an improvement in contractility in up to 10% of the cases. In this group, we believe that the decision between revascularization and clinical treatment should be widely discussed. The risks of angioplasty or surgery may outweigh the benefits of revascularization, and this depends greatly on the experience and structure of each institution.
- The fifth group comprises those having more than 75% of the area of the myocardial segment compromised, with the potential of contractile recovery of less than 1%.

In addition to this segmental evaluation, we must consider a probabilistic issue related to a certain degree of biological uncertainty, but which we can apply to the 17 segments of the left ventricle. This should be done because the importance will lie in the degree of global systolic functional improvement of the left ventricular ejection fraction. In this way, the improvement in the global function after revascularization may be predicted with great accuracy when considering the threshold of at least 10 viable or normal segments among the 17 segments of the left ventricle according to the classification of the American Heart Association (AHA).[51]

Medical report of magnetic resonance

The report of a study of myocardial viability must necessarily include the measurement of the left ventricular mass, quantify the area of fibrosis and the transmural extent of infarction, and identify the 17 segments of the left ventricle, according to the studies of Cerqueira et al.,[52] in order to facilitate the correlation with other imaging methods.

There are several ways to quantify myocardial fibrosis, all of which with scientific value, with some applications being more accurate than others. We encourage the description in the report of the method used to quantify the fibrosis, which may be visual (qualitative)[53] or by means of a manual software (planimetry),[47] semiautomatic (with manual correction)[54] or automatic (without any manual correction).[55,56]

As already well studied and characterized by the MESA (*Multi-Ethnic Study of Atherosclerosis*) study in the work by Rizzi et al.,[57] several techniques are mostly used to quantify the infarction. We have the visual technique, planimetry (manual), the techniques of standard deviation,[54] full-width half maximum (FWHM),[58] and the possible correction of the image noise.[59] We must remember that all scientific studies exclude images with low technical quality and with artifacts from breathing or acquisition of images, not being possible the analysis by these techniques. Of course, the semiautomatic analysis with manual correction and discard of artifacts becomes the method of choice for quantifying fibrosis and infarction in the day-to-day clinical setting.[56,58,60-68]

Based on the information described above, we believe that a good way of visualizing such data in the medical reports is the use of polar maps – Bull's Eyes (Figure 5) – to facilitate the explanation of the findings identified by a medical specialist and the understanding of the attending physician.

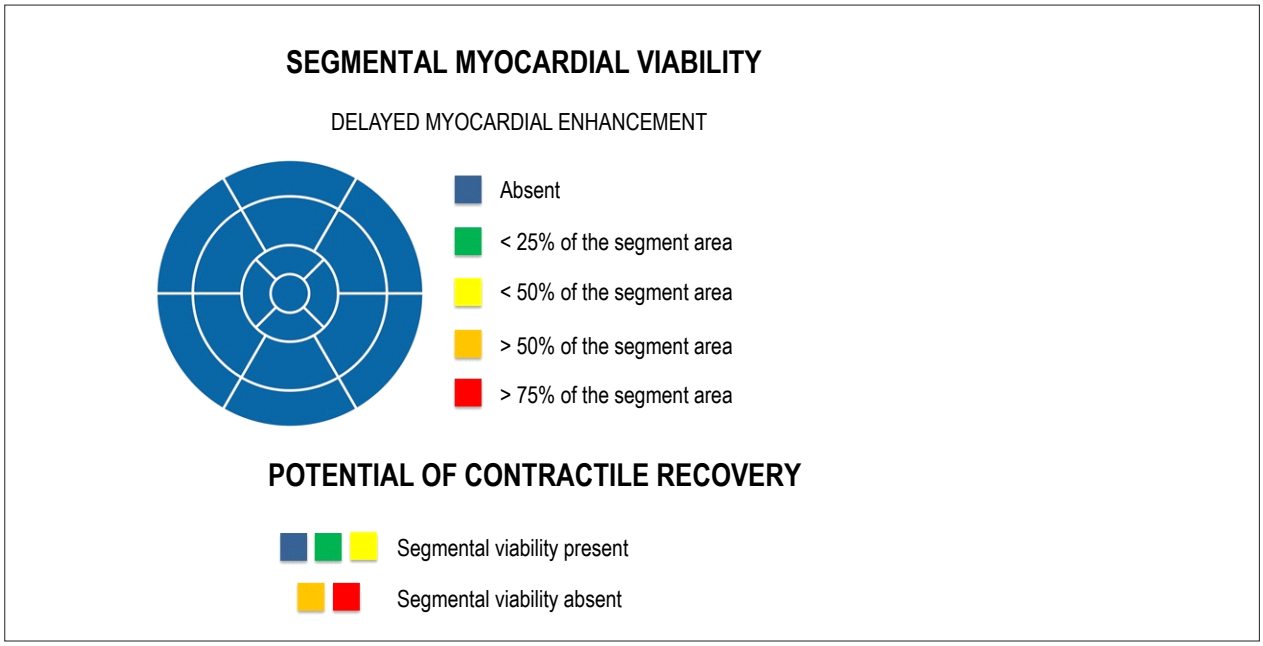

Figure 5 – *Example of a polar map that can be used in medical reports.*

Ischemic acute myocardial disease

In the setting of acute illness, rapid myocardial reperfusion prevents the death of viable muscle and improves the ejection fraction and the long-term prognosis.[31,69] Even after a successful reperfusion, myocardial dysfunction may persist. It is important to distinguish whether this is related to the necrosis or to myocardium stunning. The differentiation between these two clinical situations is of great importance because a stunned muscle must have a significant functional and clinical improvement after coronary reperfusion, either by angioplasty or surgery.[20]

CMR has the ability to differentiate these two clinical situations using the technique of delayed enhancement. This concept was very well studied in the work of Choi et al.,[70] in which all patients underwent delayed enhancement up to 7 days after the infarction and a second examination between 8 to 12 weeks. In this study, the absence or presence of small foci of delayed enhancement on the analysis of the transmural extent of infarction was able to significantly predict segmental and global functional improvement ($p < 0.001$). Other studies had very similar results, and we believe that the use of CMR in post-infarction must be routinely encouraged.[71,72]

In addition to the ability to characterize the infarction, the technique of delayed enhancement is able to modify in practice some diagnosis in our day-to-day clinical setting. As an example, it is relatively common for patients with suspected infarction and normal catheterization to have a definitive etiologic diagnosis demonstrated by CMR, such as myocarditis, vasospasm with reperfusion or, in other cases, small myocardial infarctions caused by occlusion of small vessels undetected in the first analysis on catheterization (in various situations, the report of the catheterization had to be changed after CMR).[21] In Figure 6, we suggest an algorithm for the use of delayed enhancement in the setting of acute myocardial infarction.

Chronic ischemic myocardial disease

For over 14 years, magnetic resonance with the technique of delayed enhancement has been used in patients with chronic ischemic disease. The work of Kim et al.[50] demonstrated the importance of this technique in predicting segmental and global functional improvement after revascularization by surgery or angioplasty.

The transmural extent of infarction is currently of high efficacy to identify those patients who will or will not respond to revascularization. CMR must be used routinely in centers that have this technology.

In a study published by Schvartzman et al.,[73] the inverse relationship between the transmural extent and functional recovery after revascularization was significant ($p < 0.002$).

Based on these data, we consider as not being important the use of cutoff values, as currently used, dichotomizing the viability over 50% of the transmural extent as non-viable muscle and results inferior to that as viable muscle. We believe that this is not physiological and may harm some patients who could respond to revascularization even when more than 50% of the transmural extent is affected, since each segment may have an interdependent microcirculation with a certain degree of functional improvement.

Chronic cardiac failure

When we think of treatment for ischemic disease, we must also include chronic cases of patients with established cardiac failure who depend on the optimization of the clinical treatment.

The use of delayed enhancement and measurement of the transmural extent of infarction has been demonstrated to be a great predictor of response to clinical treatment. In the work by Bello et al.,[74] magnetic resonance was used in patients with chronic cardiac failure and an ejection fraction of 26 ± 11% before and after 6 months of therapy with beta-blockers. These authors demonstrated improvement in myocardial remodeling and global and segmental function of viable segments.

Viability and the STICH study

One of the major criticisms in academia regarding the design of the STICH (*Surgical Treatment for Ischemic Heart Failure*) study was the lack of use of magnetic resonance imaging in the identification of myocardial viability since this is a method currently proved to have great reproducibility and increased accuracy. The study randomized patients with ischemic cardiomyopathy who randomly underwent myocardial revascularization or clinical treatment. This scientific design prevents the confusion between medical decision and clinical condition of the patients, and has a great statistical ability to identify the real benefit of choosing between one or other therapy. Unfortunately, even using methods known in the literature such as stress echocardiography and myocardial scintigraphy, the study of viability by these two methods had no ability to identify patients who would benefit from the therapy. Therefore, the STICH study was a negative study for the concept of analysis of viability, which left open the possibility of this hypothesis being tested by magnetic resonance.[21]

The viability by CMR has been tested, and the results were very different from the STICH study. One of the main studies published in the Journal of the American College of Cardiology[75] showed that the viability, as detected by CMR, was of great importance in differentiating the group with ischemic cardiomyopathy and severe dysfunction of the left ventricle who would benefit from myocardial revascularization. Currently, CMR should be performed in all patients with ischemic cardiomyopathy with left ventricular dysfunction for characterization of myocardial viability.[27]

Conclusions

Magnetic resonance is able to assess the myocardial viability through a series of different techniques and methods. These techniques can assess metabolic, functional, and morphological alterations and tissue characteristics, in addition to evaluating cellular viability.

The technique most widely used and with the greatest potential for clinical use is delayed myocardial enhancement. This technique is able to identify in a simple and objective way areas of hyperintense signal in the myocardium after administration of the contrast agent, with excellent histologic correlation to characterize areas with infarction/fibrosis.

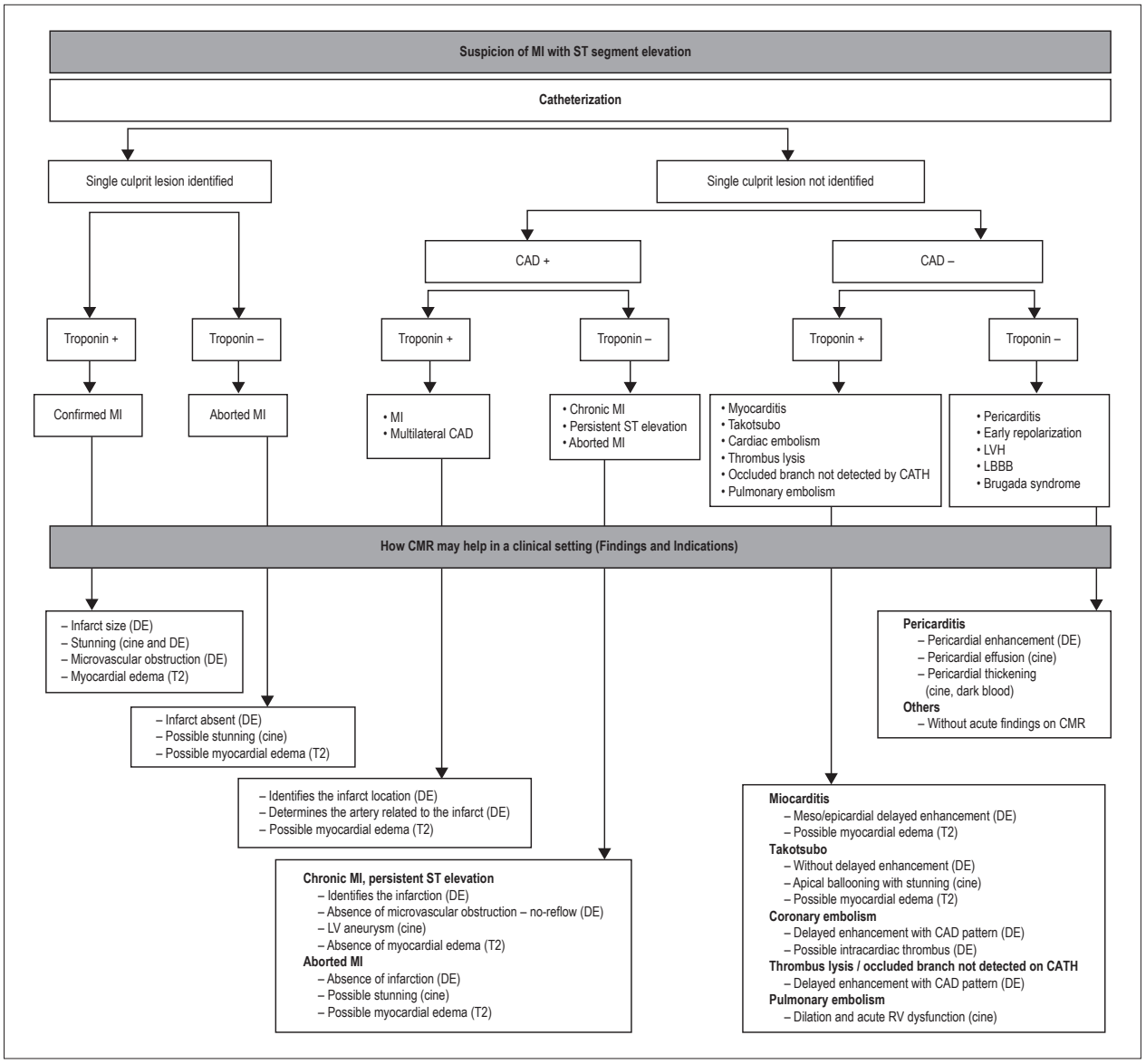

Figure 6 – *Algorithm for magnetic resonance use in patients with suspected acute myocardial infarction with ST-segment elevation. Based on the presence or absence of signs on cardiac catheterization defining the diagnosis of coronary artery disease and its location and the presence of alterations in markers of myocardial necrosis, the findings from the CMR may lead to a definitive diagnosis of myocardial injury. MI: myocardial infarction; CAD: coronary artery disease; CMR: cardiac magnetic resonance; CATH: catheterization; DE: delayed enhancement; LV: left ventricle; RV: right ventricle. Modified from Kim et al.*[50]

The technique of delayed enhancement can evaluate myocardial viability not only by a dichotomization between absent and present but also by an almost linear continuum based on the ability of each tissue to recover the contractile capacity.

In addition to the viability, the delayed myocardial enhancement has the ability to detect occult infarcts, characterize lesions that increase markers of myocardial necrosis, and establish an etiological diagnosis of cardiomyopathy, and it may predict an arrhythmogenic potential and risk of death in patients with ischemic or nonischemic myocardiopathy.

Author contributions

Conception and design of the research and Obtaining funding: Nacif MS; Acquisition of data: Souto ALM, Nacif MS; Writing of the manuscript: Souto ALM, Souto RM, Nacif MS; Critical revision of the manuscript for intellectual content: Souto ALM, Souto RM, Teixeira ICR, Nacif MS.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Prediction Models for Decision-Making on Chagas Disease

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To investigate the relationship between future or unknown outcomes and baseline health states among people with specific conditions, prediction models are an interesting strategy used to assist diagnosis, prognosis and treatment.[1] They estimate the likelihood of clinical events taking into account clinical relevant measures and complementary tests.[2] These predictors and their importance vary between the different events of interest and their prediction ability varies when considered singly or in combination with other predictors.[2] They may facilitate simple and direct comparisons of risks, individualize treatment regimens, and may refine prognosis stratification of patients, especially when many prognostic factors are known. Models have to be simple, easy to use and lead the clinician to make decisions which are more likely to bring benefit to the patients.

The World Health Organization estimates that 7-8 million people worldwide are infected with T.cruzi.[3] Chagas disease is endemic in Latin America and the immigration pattern in the past years has made this disease an important health issue in many countries. In United States, more than 300,000 individuals might be infected[4] and one study estimated that 3.5% of immigrants to Canada from Latin America were infected.[5] Physicians should be able to recognize signs and symptoms of Chagas disease as globalization increase the burden of this disease in non-Latin American countries, where vector transmission is unlikely to occur.[5]

Chagas disease has a chronic and persistent inflammation of the myocardium that leads to destruction of cardiomyocytes, arrhythmias, and embolic events, which are the leading causes of death. The intensity and aggression of Chagas disease differ substantially from that observed in other cardiomyopathies and these factors are responsible for the worse prognosis.[6] Because of its unique clinical and pathological characteristics a decision making based on other cardiomyopathies parameters might not offer all the potential benefit to patients, therefore improvements/adaptations on this knowledge are required. However, in the field of Chagas disease, there are few risk prediction models to assist decision making.

Keywords

Chagas Disease; Chagas Cardiomyopathy; Decision Support Techniques; Decision Making.

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Here, we provide comments and a brief discussion regarding the prediction models on Chagas disease field currently available in the literature. In 2016, Brasil et.al.[7] developed and validated a diagnostic decision support tool to decide about proceed or not to diagnostic investigations for chronic Chagas disease. The following predictors were identified: sex, age, referral from blood bank, history of living in a rural area, recognizing the kissing bug from pictures, systemic hypertension, number of siblings with Chagas disease, number of relatives with history of stroke, electrocardiogram with low voltage, anterior superior divisional block, pathologic Q wave, right bundle branch block, and extrasystoles. This model was developed and temporally validated in a single center study, with very good discrimination and calibration performance in both samples. Therefore it could be recommended in ordinary use in diagnostic investigation despite its impact is not yet known.

The second model in discussion is the one to predict severe or moderate systolic dysfunction in Chagas disease.[8] It was used based on the following clinical, electrocardiographic and radiologic data: sex, chest X-ray, right bundle branch block, anterior superior divisional block, ventricular extra systole, pathologic Q-wave, primarily ventricular repolarization alterations, left bundle branch block, and pacemaker rhythm. The validation was in a rural cohort of patients with Chagas disease, randomly selected and submitted to clinical, electrocardiogram and echocardiographic evaluation. A normal electrocardiogram excluded the presence of moderate or severe dysfunction, not requiring the application of statistical models in 43% of this population. This tool can be widely used, including rural areas, since it needs simple clinical, electrocardiographic and radiological data. It can provide the decision to start specific treatment to heart failure until echocardiography is not available, identifying patients who may have benefit from this early treatment. It was validated in a fully independent sample, and it had good performance in both cohorts. Thus it can be recommended for ordinary use in urban and rural populations.

In 2006, Rassi et.al.[9] developed and validated a risk score for predicting overall death in Chagas' heart disease. It was found that six clinical features were important in predicting death: NYHA class III or IV, cardiomegaly on chest radiography, segmental or global wall-motion abnormalities on echocardiography, nonsustained ventricular tachycardia on Holter monitoring, low QRS voltage on electrocardiography, and male sex. This model was developed and validated in a fully independent concurrent cohort and its performance in both cohorts was good. However, this model needs several complementary tests to estimate individual risk (e.g. Holter monitoring), and

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it does not evaluate the left ventricle ejection fraction, a known strong prognostic predictor in Chagas heart disease.[10] Additionally, it is difficult to make decision from its estimates as Chagas disease has three main death mechanisms that require completely different treatment approaches (i.e. stroke, sudden death, and heart failure).

Another interesting model studied the risk of sudden death in chronic Chagas' heart disease.[11] Four independent predictors were identified, each of which was assigned a number of points proportional to its regression coefficients: QT-interval dispersion, syncope, ventricular extrasystoles and severe dysfunction of the left ventricle. The risk scores for each patient were divided in three groups: low risk, intermediate, and high risk. This study showed that a simple model can predict sudden death with a good clinical relevance of the model with C statistic score of 0.84. Highlighting Chagas disease unique characteristics, the QT-interval dispersion was the strongest predictor of sudden-death in Chagas heart disease, which is not common in other etiologies. Unfortunately, this model was not yet been externally validated, and it requires QT-dispersion, which is not easily measured. Therefore it cannot be recommended for ordinary practice and its applicability depends on the setting. Another research group conducted the "SEARCH-RIO study" that evaluated electrocardiogram, signal-averaged electrocardiogram, and Holter monitoring variables in chronic Chagas disease as predictors of cardiac death and new onset ventricular tachycardia as a composite outcome.[12] This long term follow-up developed a risk stratification score showing that electrical markers are independent predictors of adverse outcome. The electrical markers were: abnormal Q-wave, previous ventricular tachycardia episodes, 24-h standard deviation of normal RR intervals < 100 ms, and positive intraventricular electrical transients on signal-averaged electrocardiogram. The study had a good relevance with C-statistic of 0.89, but was not externally validated. Additionally, the model's composite outcome makes decision more complicated, and the requirement of Holter monitoring makes its applicability setting dependent. Therefore it cannot be recommended for ordinary practice.

Sousa et.al.[13] studied the risk and benefit of prevention strategies of cardioembolic ischemic stroke in Chagas disease. The factors that increased the risk of an event were: systolic dysfunction, apical aneurysm, primary alteration of ventricular repolarization and age > 48 years. Based on the analysis, four risk groups were defined to the rate of events in these groups. The suggestion is to use of warfarin for high-risk patients (score 4 or 5), acetylsalicylic acid or warfarin for those with moderate risk (score 3), acetylsalicylic acid or no intervention for the low risk group (score 2) and no prophylaxis for the very low risk group (score 0 or 1). This model was developed in a very large sample, and it has a very good performance. However, with the availability of new anticoagulants, the applicability of this model is setting dependent. Additionally, it was not yet validated externally, thus cannot be recommended for ordinary practice.

Benznidazole is the main trypanocidal drug used to treat Chagas disease. This drug is recommended (Class I) as trypanocidal treatment in the acute phase of Chagas disease, congenital Chagas disease, chronic phase of Chagas disease in children aged ≤ 12 years, organ donor with Chagas disease, and reactivation antiparasitic treatment in coinfection Chagas/HIV.[10] More than 30% of patients treated may present adverse drug reactions.[14] There is a prediction model to identify patients with high risk to develop adverse reactions to benznidazole and to identify the risk of requiring benznidazole interruption due to adverse reactions.[15] It was found that female sex, graduation from elementary school, and white and mulatto races were considered to predict overall adverse drug reactions and treatment discontinuation. This model was developed in a small sample; it has a moderate discrimination and a good calibration performance. However, it was not yet externally validated.

The use of clinical prediction models can be an interesting strategy to assist diagnosis, prognosis and treatment decision making. However, the user must be concerned with the applicability of the model for the patient under care. All the mentioned models were developed in urban cohorts; therefore these samples resemble in many aspects the populations of migrants with Chagas disease in non-endemic countries. Even if some models presented are not validated and cannot be widely used, they raise a consciousness of which clinical aspects health-care providers should concern with when assessing patients with Chagas disease. Nevertheless, the correct interpretation and application of Chagas disease prediction models remains a challenge to clinical decision-making. To fulfill the purpose of facilitating these models use, we turn available online calculators concerning the prediction models in the following link: http://shiny.ipec.fiocruz.br:3838/pedrobrasil/. It´s important to remind that this website for the calculation of risk prediction scores are not intended to replace the currently available guidelines for chronic Chagas disease health care, instead they are intended to complement, facilitate the application of current recommendations, improving medical decision making and ultimately bring more benefit to patients with Chagas disease.

Updating the existing models and providing new ones can be useful for several purposes in the field of Chagas disease. This raises the question of models that are likely to bring benefit, such as: how to predict the progression of indeterminate form for cardiac and digestive forms, models for cardiac transplant indication; one that can predict which patient would have benefit with benznidazole treatment; and a model in the field of cardiac rehabilitation to predict who will have benefits. In light of the personalized medicine era, further research is needed to reach individual predictions, where genetic or innate biomarkers can play bigger roles, as well as making these prediction instruments friendlier.

Author contributions

Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Mendes FSNS and Brasil PEAA.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Case 2/2017 – 56-Year-Old Male with Refractory Heart Failure, Systemic Arterial Hypertension and Aortic Valve Stenosis That Led to Heart Transplantation

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The patient is a 56-year-old male, born and living in São Paulo, hospitalized due to heart failure decompensation and submitted to heart transplantation.

His symptoms started at the age of 51 years, with dyspnea on moderate exertion, which evolved in 3 months to dyspnea on minimum exertion and orthopnea, and wheeze. The patient required hospitalization for clinical compensation. After discharge, he was referred for treatment at InCor. His discharge prescription included daily furosemide 40 mg, captopril 100 mg, spironolactone 25 mg, and aminophylline 200 mg.

The patient smoked (40 pack-years) and had systemic arterial hypertension. His parents had died due to stroke.

On his first medical visit, one month after that hospitalization, his complaints remained similar to those at the time of hospitalization.

His physical examination (Jul 23, 2008) revealed: weight, 73.6 kg; height, 1.58 m; body mass index, 29.5 kg/m^2; pulse rate, 76 bpm; right upper limb and right lower limb blood pressures, 148/96 mm Hg and 150/100 mmHg, respectively. His lung auscultation showed no crepitant rales, and his heart auscultation revealed low cardiac sounds with no murmur. His abdominal exam was normal. There was neither lower limb edema, nor signs of increased jugular venous pressure, and his pulses were palpable and symmetrical.

The X ray revealed marked cardiomegaly.

The ECG (Jul 18, 2008) showed: sinus rhythm; heart rate, 67 bpm; PR, 163 ms; QRS duration, 96 ms; QTc, 455 ms; overload of left chambers and secondary changes of ventricular repolarization (Figure1).

His laboratory tests were as follows: hemoglobin, 14.4 g/dL; hematocrit, 44%; red blood cells, 5,000,000/mm^3; leukocytes, 11,400/m^3; uric acid, 9 mg/dL; glucose, 105 mg/dL; creatinine, 1.05 mg/dL; total cholesterol, 266 mg/dL; HDL-C, 35 mg/dL; LDL-C, 153 mg/dL; triglycerides, 412 mg/dL; potassium, 4.6 mEq/L; sodium, 139 mEq/L; and normal urinalysis.

The following diagnoses were established: hypertensive heart disease, obesity, glucose intolerance, hypertriglyceridemia and hyperuricemia.

The echocardiogram (Dec 2, 2008) revealed the following diameters: aorta, 37 mm; left atrium, 44 mm; and left ventricle (diastole/systole), 68/57 mm. The ejection fraction (Teicholz) was 33%, and there was marked diffuse hypokinesia. The septal and posterior wall thickness was 10 mm. The aortic valve showed mild fibrocalcification of its leaflets, maximum and mean transvalvular gradients of 27 mmHg and 17 mmHg, respectively, with estimated valvular area of 1.4 cm^2, mild stenosis being then considered.

Myocardial MIBI 99mTc scintigraphy with dobutamine (Jan 2009) revealed mild fixed low uptake in the inferior wall, and dilatation and diffuse hypokinesia of the left ventricle with ejection fraction of 27% (Figure 2).

The ECG at peak administration of dobutamine, with heart rate of 166 bpm, revealed ST-segment depression, attributed to previous repolarization changes, consequent to left ventricular hypertrophy (Figures 3 and 4).

Diuretic dynamic renal scintigraphy with DTPA 99mTc showed no change in renal perfusion, clearance or size.

Spirometry revealed mild obstructive disorder, which improved with bronchodilator use.

The patient progressed with dyspnea on moderate exertion and an episode of syncope preceded by chest pain. Coronary cineangiography showed no obstructive lesion. The left circumflex artery was small and the right coronary artery was dominant (Dec 15, 2009). The following drugs were prescribed: daily enalapril, 40 mg; carvedilol, 12.5 mg; furosemide, 40 mg; spironolactone, 25 mg; propatylnitrate, 30 mg; simvastatin, 40 mg; acetylsalicylic acid, 100 mg; and salbutamol, 6 mg.

The patient sought the emergency unit because of dyspnea worsening for 15 days, being then on minimum exertion and orthopnea, with lower limb edema and episodes of chest pain, some of which lasted longer in the last 3 days. He related that worsening to interruption of the medication.

The physical examination (Jan 21, 2014) revealed: pulse rate, 84 bpm; blood pressure, 100/70 mm Hg; normal lung and heart auscultations; normal abdominal exam; and lower limb edema, +++/4+.

The chest X ray (Jan 21, 2014) showed increased pulmonary vascular bed and global cardiomegaly (Figure 5).

Keywords

Heart Failure; Hypertension; Aortic Valve Stenosis; Heart Transplantation.

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Anatomopathological Session

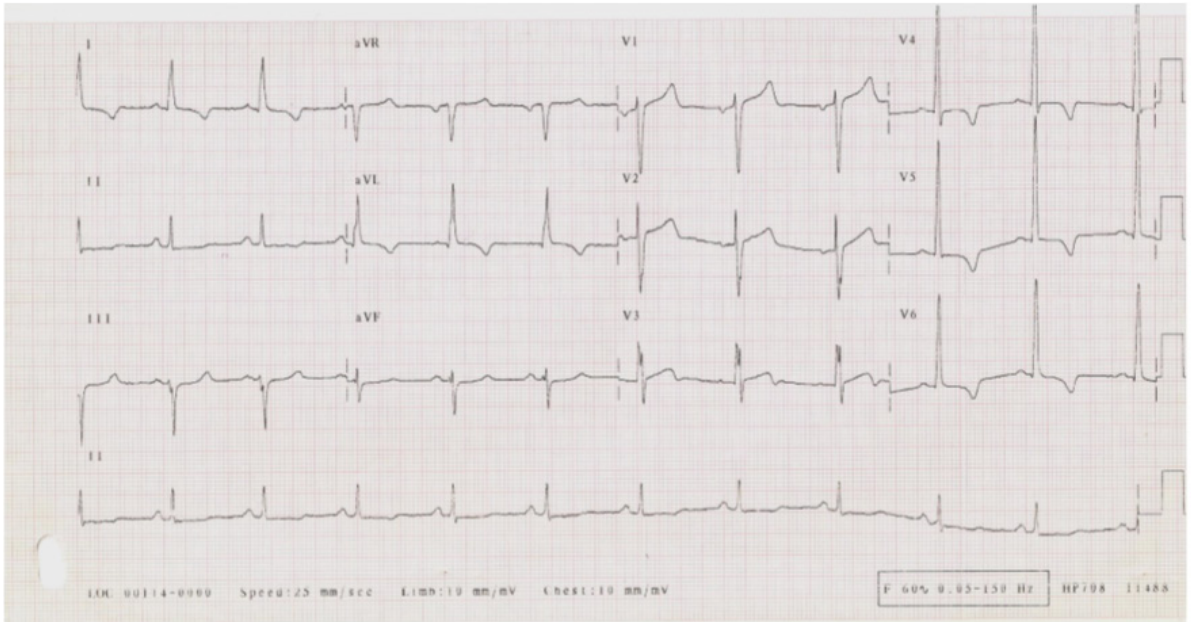

Figure 1 – *ECG: Sinus rhythm, left atrial and left ventricular overload with strain.*

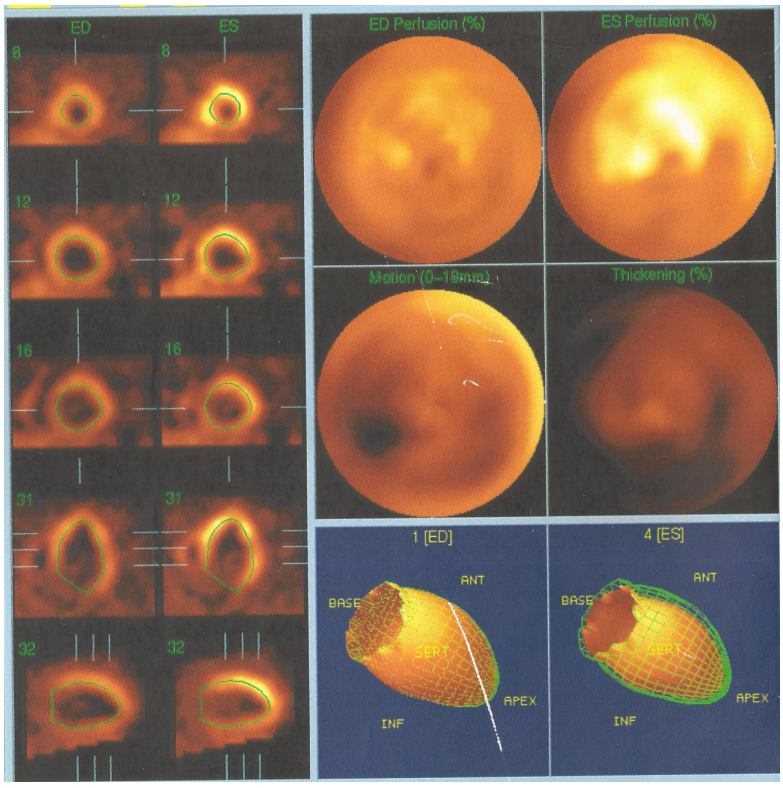

Figure 2 – *Nuclear ventriculography (gated SPECT scan): Diffuse left ventricular hypokinesia.*

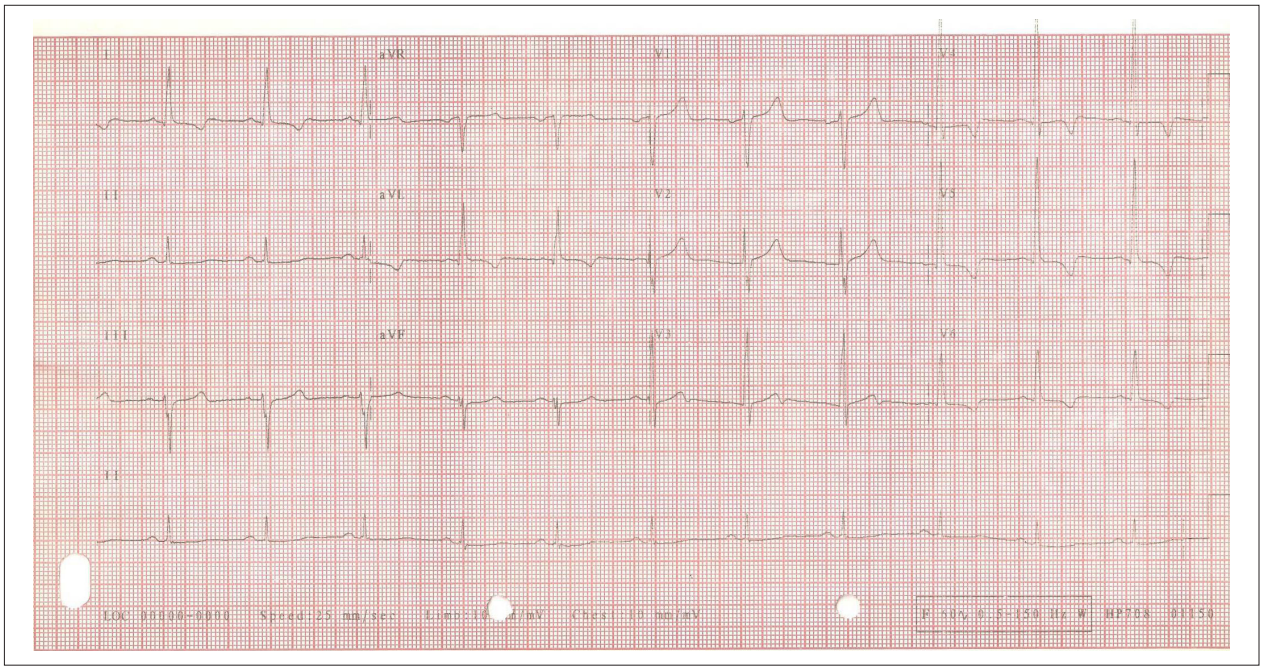

Figure 3 – *Resting ECG: Ventricular repolarization changes (left leads: inverted T waves).*

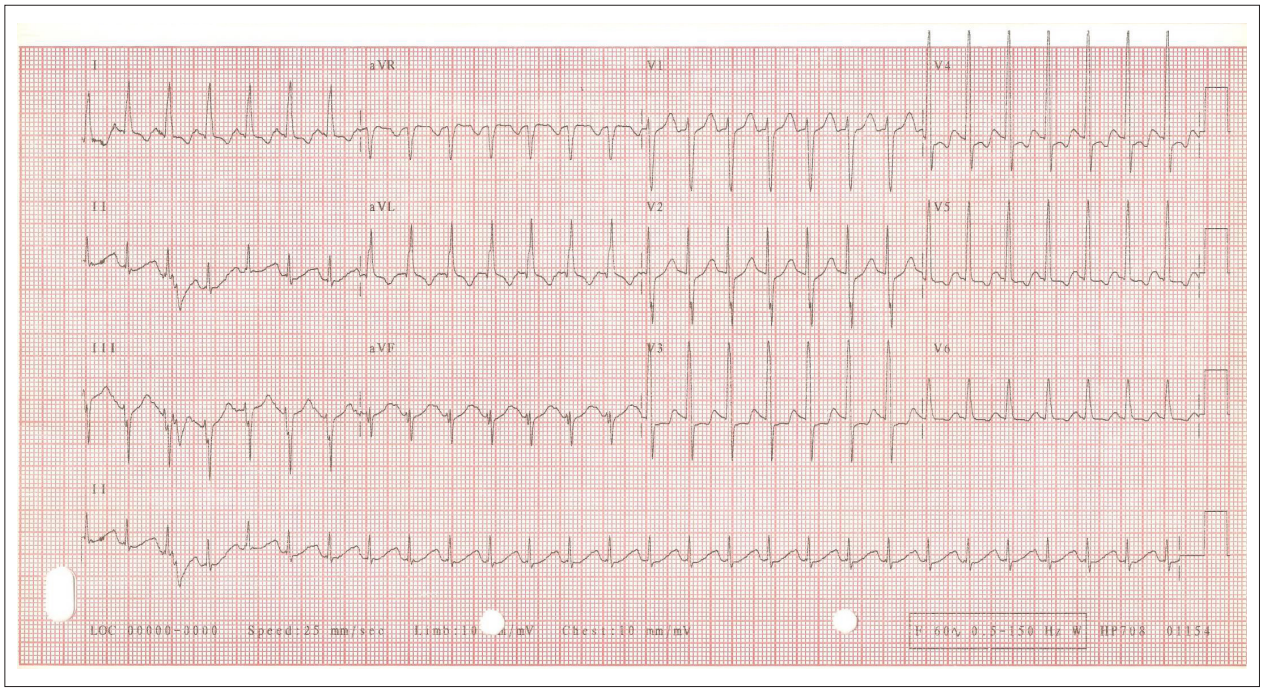

Figure 4 – *ECG at peak exertion: Heart rate of 167 bpm, ST-segment depression of 1 mm.*

The laboratory tests revealed: increased levels of myocardial lesion markers (CK MB, 12.43 ng/mL; troponin I, 0.38 ng/mL), which decreased in the following measurements; red blood cells, 5,300,000/mm^3; hemoglobin, 15 g/dL; hematocrit, 49%; leukocytes, 7,540/mm^3 (61% neutrophils, 1% eosinophils, 1% basophils, 32% lymphocytes, and 5% monocytes); platelets, 183,000/mm^3; urea, 108 mg/dL; creatinine, 1.73 mg mg/dL (glomerular filtration 44 mL/min/1.73 m^2); TSH, 7 μUI/mL; sodium, 134 mEq/L; potassium, 4.0 mEq/L; PT (INR), 1.5; and APTT (rel), 1.07.

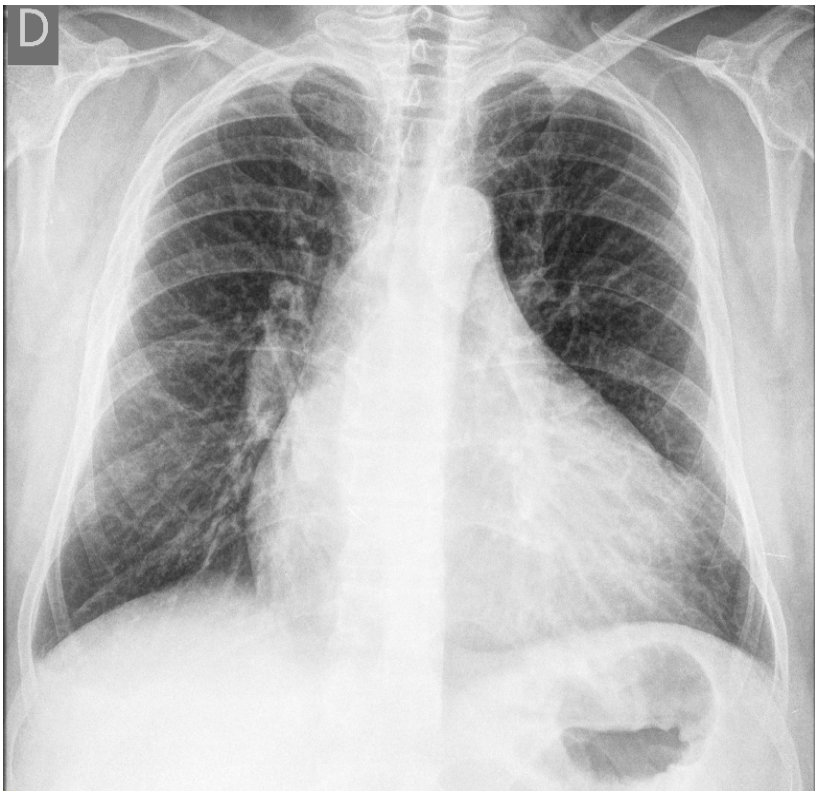

Figure 5 – *Chest X ray (PA). Pulmonary fields with signs of congestion: vascular bed inversion and apparent fissure; marked global cardiomegaly.*

The new echocardiographic assessment (Jan 27, 2014) showed the following diameters: aorta, 35 mm; left atrium, 55 mm; right ventricle, 34 mm; left ventricle (diastole/systole), 76/72 mm. Ejection fraction was 20%, and septal and posterior wall thickness was 9 mm. The left ventricle showed marked diffuse hypokinesia, and the right ventricle, moderate hypokinesia. The aortic valve was moderately fibrocalcific, with reduced mobility of its leaflets, and maximum and mean transvalvular gradients of 28 mm Hg and 18 mm Hg, respectively. The estimated systolic pulmonary pressure was 50 mm Hg.

One week after admission, the laboratory reassessment revealed worse kidney function with creatinine of 2.24 mg/dL (glomerular filtration of 32 mL/min/1.73m^2) and urea of 119 mg/dL.

The patient had pneumonia, arterial hypotension and low cardiac output, the last two persisting even after pneumonia treatment. The patient received vasoactive amines and intra-aortic balloon for circulatory support. Heart transplantation was indicated.

His new laboratory assessment 1.5 month after admission revealed: hemoglobin, 11.6 g/dL; hematocrit, 36%; leukocytes, 9,280/m^3; platelets, 90,000/mm^3; urea, 77 mg/dL; creatinine, 1.44 mg/dL (glomerular filtration 54 mL/min/1.73 m^2); AST, 39 U/L; ALT, 26 U/L; alkaline fosfatase, 142 U/L; gamma GT, 332 U/L.

The patient underwent orthotopic heart transplantation (Mar 18, 2014).

Clinical aspects

The 56-year-old male patient, who smoked and had arterial hypertension and hypertriglyceridemia, developed heart failure with dilatation of heart chambers and severe systolic dysfunction. His echocardiography showed low-gradient aortic stenosis.

According to the Brazilian Society of Cardiology III Guideline on Heart Failure, a patient with signs and symptoms of heart failure should undergo some tests to characterize the heart failure, such as resting electrocardiography, chest X ray, echocardiography and BNP measurement. Then, laboratory and invasive tests, such as coronary cineangiography and cardiac biopsy, should be performed for etiological diagnosis.[1]

In the case here reported, there were neither epidemiological data suggestive of Chagas disease, nor conduction disorders usually found in that disease, such as right bundle-branch block and left anterior block, whose prevalence is three times greater than that in the general population.[2,3]

Another etiology to remember is rheumatic heart disease, because mild aortic stenosis was detected on echocardiography. In rheumatic disease, the mitral valve is most commonly affected, followed by double mitral-aortic impairment and isolated aortic valve impairment. Although there was no report

of an acute rheumatic fever episode during childhood, that often passes unnoticed by the patients with that heart valve disease. Against that diagnosis is the age of clinical manifestation, usually around 30 years, although the age may range from 20 years to 50 years.[4,5]

Persistent rheumatic carditis can be the cause of heart failure with ventricular dilatation. However, the age group is younger, from 5 years to 20 years, being a cause of diagnostic confusion with infective endocarditis and not with heart failure etiology.[6]

There were risk factors for coronary heart disease, such as arterial hypertension, low HDL-cholesterol levels, hypertriglyceridemia and smoking, predictors of acute ischemic coronary events at a younger age.[7,8]

The present patient had a history of neither acute myocardial infarction nor typical angina. The echocardiogram revealed no segment deficiency of contractility, but identified diffuse hypokinesia. Scintigraphy evidenced a mild fixed uptake reduction in the inferior wall, which is not rare in patients with dilated cardiomyopathy.[9]

Even adding the changes in scintigraphy to the electrocardiographic ones, the later attributable to left ventricular overload signs, the chances of ischemic cardiomyopathy would be low. However, one should rule out the diagnosis of any treatable cause of heart failure, such as coronary artery disease. Considering our patient's clinical findings, coronary cineangiography was properly indicated, although it resulted normal.

In addition, non-rheumatic aortic valve stenosis could explain all the patient's clinical findings. Although the aortic valve stenosis is considered to have hemodynamic repercussion if the valvular area is equal to or smaller than 1 cm^2 and the mean gradient is greater than 40 mm Hg, the clinical entity called low-flow, low-gradient aortic stenosis has been recently increasingly studied. Its most common presentation is marked left ventricular dilatation and very low ejection fraction, and some authors have reported its prevalence ranging from 5% to 10% of patients with marked aortic valve stenosis. The prognosis is very poor, with 3-year mortality of 50% for patients on drug treatment, and of 6% to 30% for patients undergoing surgery. Thus, the precise assessment of the grade of stenosis and of myocardial dysfunction is essential to determine those patients' treatment. When the diagnosis is uncertain, some diagnostic methods can be used, such as stress Doppler echocardiography with dobutamine to assess low flow reserve (lack of minimal 20% increase in left ventricular systolic volume). Another diagnostic method is to measure the calcium score of the heart valve on cardiac tomography, usually greater than 1650 Agastston.[10]

Against that diagnosis is the patient's heart valvular area greater than 1.2 cm^2 and his age, because, on average, individuals with that type of stenosis are older than 70 years.[11]

Because the patient has arterial hypertension, hypertensive heart disease cannot be ruled out as responsible for the patient's clinical findings.

In the Framingham study, in 91% of the cases with heart failure, arterial hypertension preceded that condition. Hypertension doubled the incidence of heart failure in men and tripled it in women. In Brazil, arterial hypertension associated with coronary artery disease is the most frequent cause of heart failure.[12]

The incidence of heart failure is proportional to blood pressure levels, to age and to hypertension duration. Blood pressure control can decrease the incidence of heart failure by 50%.[13,14]

Untreated arterial hypertension causes changes in the sympathetic and renin-angiotensin-aldosterone systems, which lead to hypertrophy, followed by myocyte apoptosis and autophagy, proliferation of fibroblasts, interstitial collagen accumulation, and, eventually, adverse remodeling and pump failure.[15-18]

The diagnosis of dilated cardiomyopathy is limited in the present case, because of the presence of known causes of heart failure, such as arterial hypertension and aortic valve disease.

Regarding the final cardiac decompensation, it is worth noting its major causes: drug non-adherence, cardiac arrhythmias, and disease progression and complications, such as pulmonary infection or thromboembolism. In the present case, there was infection, and neither thromboembolism nor disease progression can be ruled out. (Desidério Favarato, MD)

Diagnostic hypothesis: heart failure due to cardiopathy with ventricular dilatation. Etiology: hypertensive heart disease or aortic valve disease, non-rheumatic aortic stenosis. (Desidério Favarato, MD)

Anatomopathological examination

The formalin-fixed explanted heart missed part of the left atrium and weighed 580 g. It showed dilatation of the ventricular cavities, and focal areas of fibrosis (Figure 6A). The microscopic study evidenced hypertrophy of myocardial fibers and interstitial fibrosis (Figure 6B). On gross examination, the aortic valve was thickened and calcified, without commissural fusion (Figure 7A). The other valves, pericardium and coronary arteries showed no significant changes. After decalcification, the microscopic study of the aortic valve evidenced fibrous thickening, dense calcification and no inflammatory signs (Figure 7B). **(Paulo Sampaio Gutierrez, MD)**

Anatomopathological diagnoses:

- Calcified aortic valve disease
- Hypertensive cardiomyopathy
- Dilatation of the cardiac chambers with hypertrophy of myocardial fibers and areas of interstitial fibrosis **(Paulo Sampaio Gutierrez, MD)**

Comment

The major question in this case is the cause of heart failure, in particular the causative role aortic valve disease might have played.

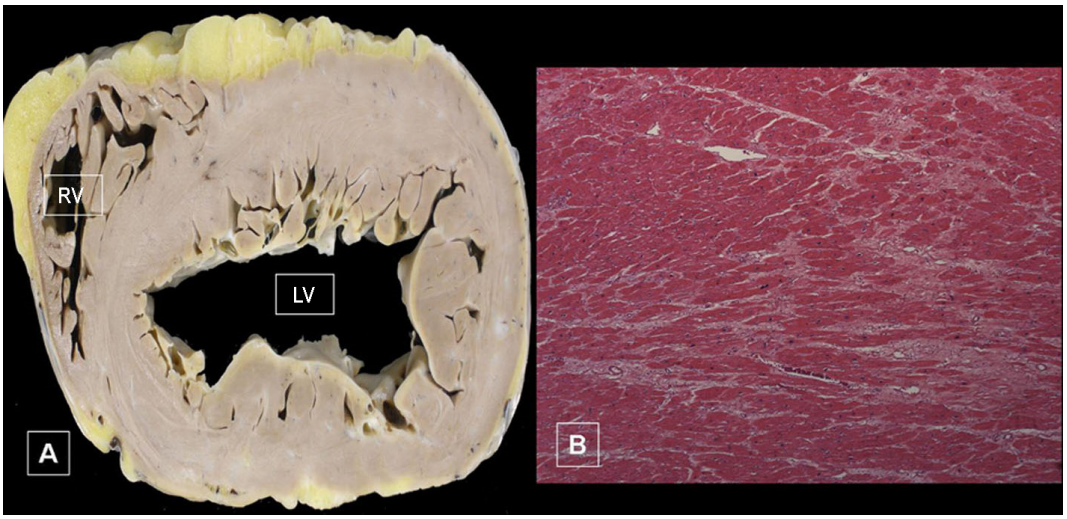

Figure 6 – *On gross examination, cross-section of the heart (distorted by fixation) (A) at ventricular level, showing dilatation of the cavities, with normal to mildly increased wall thickness. Note the presence of small white areas in the left ventricular wall, corresponding to fibrosis, also identified on the microscopic exam (B) as lighter areas amidst the darkly stained myocardium. (Hematoxylin-Eosin; x5). RV: right ventricle; LV: left ventricle.*

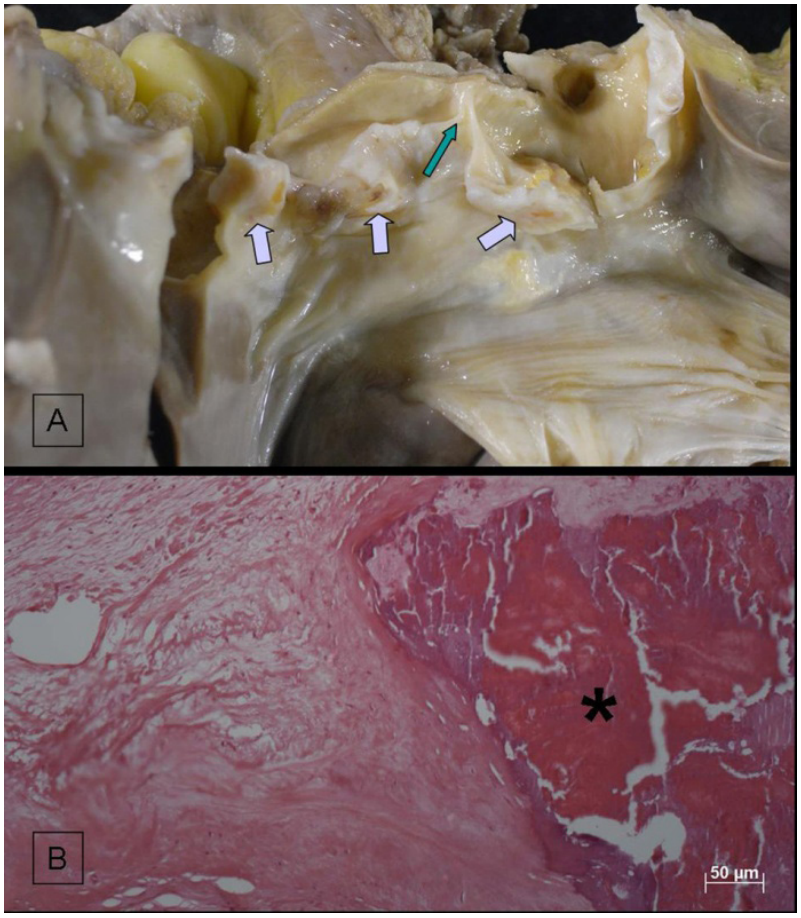

Figure 7 – *A) Opened aortic valve showing calcification nodules in its three leaflets (purple arrows). There is no commissural fusion (green arrow). B) Microscopic section of the aortic valve with areas of dense calcification (asterisk). Note the absence of inflammatory cells. (Hematoxylin-Eosin; x5).*

The anatomopathological exam is not ideal to assess heart valve dysfunction, because the heart is analyzed without movement and stiffened by fixation. However, the significant calcification suggests that heart valve disease might have contributed significantly to heart failure. On the other hand, the patient is hypertensive, and, thus, hypertensive cardiomyopathy should not be ruled out.

Thus, both processes – systemic arterial hypertension and calcified aortic valve disease – should be considered to play a role in the installation of cardiac dysfunction.

Regarding the cause of valve heart disease, the lack of a clinical history, of mitral valve impairment, of aortic valve commissural fusion and of inflammatory cells on the microscopic study indicates this is not consequent to rheumatic disease. The diagnosis to be considered is "degenerative" valve disease, and it is worth noting, however, that the patient's age is under the age group in which that lesion usually causes symptoms sufficiently severe to require surgery: in a series,[19] less than 6% of the men with tricuspid aortic valve (as our patient) underwent surgery before the age of 60 years, and none before the age of 50, when our patient already had, on echocardiography, moderate stenosis. A European multicenter study has reported a mean age of 69 years for patients with aortic stenosis.[20] **(Paulo Sampaio Gutierrez, MD)**

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Myocardial Deformation by Echocardiogram after Transcatheter Aortic Valve Implantation

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Introduction

Myocardial deformation (strain) analysis may be done through echocardiography from data obtained by tissue doppler technique or two-dimensional images from speckle tracking, allowing the calculation of longitudinal, circumferential and radial deformations of myocardial fibers.[1,2] Myocardial strain analysis has recently been used in the evaluation of regional myocardial movement and function and in the systolic time-to-peak calculation which studies cardiac synchronicity and myocardial electromechanical coupling.[1] In clinical practice, conventional echocardiographic investigation is helpful in detecting global myocardial dysfunction and alterations in ventricular segmental contractility. In cardiotoxicity from the use of chemotherapeutics, we may find alterations in cardiac mechanics with no modification of the left ventricle ejection fraction (LVEF). Thus, the analysis of myocardial deformation brings relevant information in the analysis of regional ventricular systolic dysfunction in subclinical conditions.[2]

Similarly, previous investigations have shown that the study of myocardial deformation is able to detect sudden and early alterations in systolic function of valve disease patients, even before they present LVEF modifications.[1,3,4] This phenomenon is due to a significant decrease in myocardial deformation in three different spatial planes, triggering deformation modifications in the longitudinal, circumferential and radial axes.[5] In patients who present reduced longitudinal myocardial deformation, we can observe radial deformation that is superior to normality parameters, which may result in preserved ventricular function, when estimated by LVEF. Other applications of myocardial deformation include amyloidosis, hypertrophic cardiomyopathy, right ventricular dysfunction, athlete's heart, cardiac dyssynchrony and valve diseases (mitral insufficiency and aortic stenosis).[2]

Transcatheter aortic valve implantation (TAVI) appeared as a new option for the treatment of inoperable aortic stenosis or high surgical risk patients.[6] The use of myocardial deformation with echocardiography in the immediate evaluation of aortic stenosis patients undergoing TAVE is underexplored.

Case 1

Patient, female, 79 years old, with atrial fibrillation and pulmonary hypertension, and decreased functional capacity in the last year. Three-dimensional transesophageal echocardiography showed presence of aortic valve stenosis with maximum transvalvular gradient of 67 mmHg and mean of 39 mmHg, valve area of 0.5 cm^2, and LVEF (Simpson's method) of 60%. Pre-TAVI strain analysis was -14% and after implantation of aortic prosthesis Sapien XT of 23 mm (Edwards Lifescience, USA) immediate improvement of the myocardial deformation to -20% was observed.

Case 2

Patient, male, 81 years old, with cirrhosis from hepatitis B and hypothyroidism, symptomatic, functional class II (NYHA) from aortic valve stenosis. Implantation of aortic valve Sapien XT of 26 mm, with immediate improvement of the strain from -15% to -22%. Myocardial dyssynchrony evaluation with analysis through pre TAVI systolic time-to-peak calculation method went from 132 ms to 65 ms after the procedure.

Case 3

Patient, male, 77 years old, with aortic stenosis for 10 years, with recent worsening of functional class. Echocardiogram showed maximum transvalvular gradient of 87 mmHg and mean of 49 mmHg, valve area of 0.7 cm^2 and LVEF of 57%. Implantation of aortic valve Sapien XT, 23 mm, with immediate normalization of the pre-procedure myocardial strain from -12% to -20% after the procedure (Figure 1).

Case 4

Patient, female, 74 years old, with aortic stenosis for 20 years, functional class II (NYHA). Echocardiogram showed maximum transvalvular gradient of 65 mmHg and mean of 38 mmHg, valve area of 0.6 cm^2 and LVEF of 28%. Implantation of aortic valve Sapiens XT, 23 mm, with immediate improvement of the myocardial strain from -10% to -13% and LVEF improvement to 34% (Figure 2).

Discussion

Aortic valve stenosis evolution results in pressure overload in the myocardial wall, leading to increased cardiac thickness. Ventricular hypertrophy progresses as aortic stenosis becomes more significant, an adaptation mechanism for ventricular

Keywords

Myocardium/physiopathology; Echocardiography. Doppler; Transcatheter Aortic Valve Replacement, Aortic Valve Stenosis.

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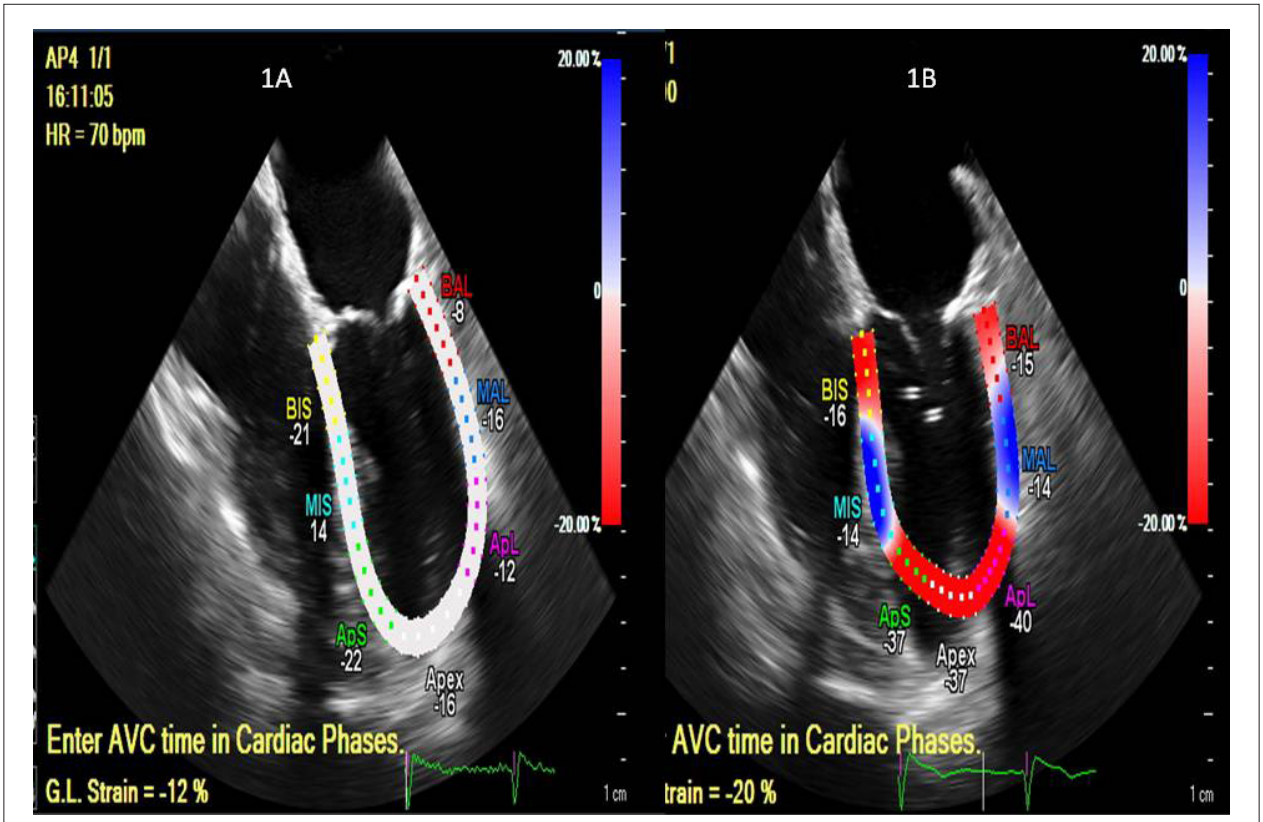

Figure 1 – *Global longitudinal strain before (Figure 1A: -12%, VN < -20%) and after (Figure 1B: -20%) percutaneous implantation of aortic prosthesis. Case 3 patient.*

function maintenance. However, this compensatory mechanism is finite, triggers an increase in left ventricular volume (positive remodeling) and a decrease in ejection fraction over time.[4]

Lancellotti et al.,[5] in studies using echocardiography, observed the following alterations in patients with aortic stenosis: 1) asymptomatic patient with significant aortic stenosis presents expressive increase in global left ventricular afterload; 2) Ventricular afterload increase negatively affects myocardial function, especially in the axial deformation, despite the normal ejection fraction; 3) Elevated afterload in low-flow aortic stenosis patients, when systemic arterial compliance is reduced; 4) The state of low-flow is related to a compromised diastolic function and reduction of myocardial deformation. Longitudinal, radial and circumferential myocardial deformations are significantly compromised in patients with elevated afterload. In an initial stage of aortic valve stenosis, however, there is only a reduction in the longitudinal myocardial deformation, mainly related to alterations in subendocardial cardiac fibers. Radial and circumferential deformations may be, paradoxically, vicarious in this period. Subsequently, with the progression of aortic stenosis severity, we now have a reduction of the radial and circumferential deformation due to modifications of the fibers of the mid-myocardial layer which present circumferential spatial alignment.

Current guidelines recommend aortic valve replacement in symptomatic or asymptomatic aortic valve stenosis patients with reduction of the ejection fraction.[6,7] When compared to patients with preserved systolic function, those with reduced ejection fraction present less favorable clinical evolution after valve repair.[4] In patients with preserved ventricular function, myocardial deformation analysis allows early detection of subclinical alterations of myocardial mechanics, which could, after the procedure, positively result in longer survival and better quality of life.

In a study by Delgado et al.,[4] aortic valve replacement surgery improved the analysed parameters through speckle tracking, even though the ejection fraction remained unaltered. Additionally, these authors report an improvement in myocardial deformation after valve replacement. That solidifies the ability of myocardial deformation analysis to detect sudden alterations in systolic function in patients with severe aortic stenosis.

In another study, Bauer et al.,[3] who assessed 8 patients submitted to TAVI, showed improvement in global and regional systolic function of the left ventricle, even in patients with reduced ejection fraction.

Similarly, Sebastian et al.[6] observed an improvement of myocardial mechanics in patients submitted to TAVI 12 months after the procedure. Becker et al.[7] showed a positive response in myocardial deformation 7 days and 6 months after TAVI. Specifically, there was an improvement in the ejection fraction

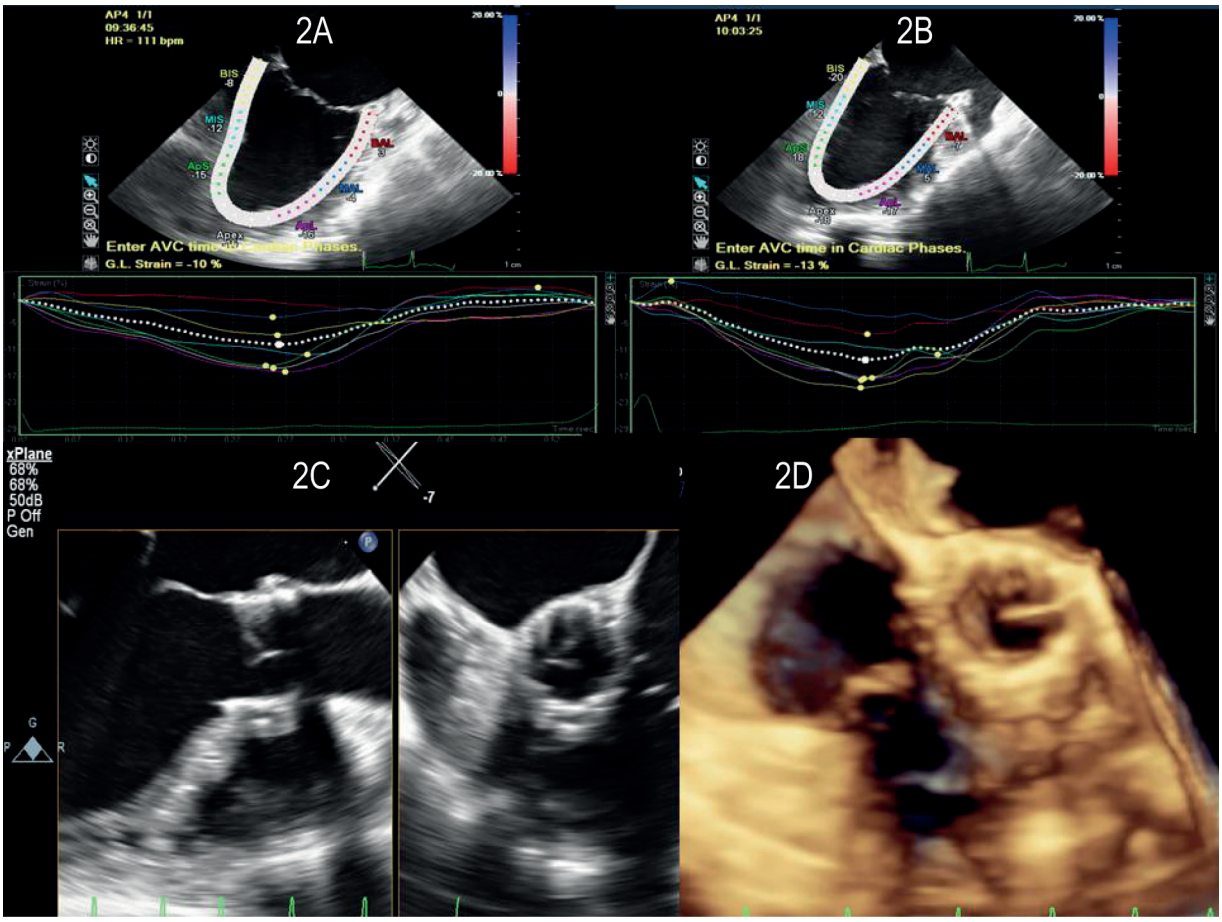

Figure 2 – *Myocardial strain analysis before (Figure 2A : -10%) and after (Figure 2B: -13%) percutaneous implantation of aortic prosthesis. Figure 2C: demonstration with two-dimensional transesophageal echocardiography of implanted aortic prosthesis, left figure (longitudinal plane), right figure (transverse plane). Figure 2D: demonstration with three-dimensional transesophageal echocardiograph of the implanted aortic prosthesis, en face. Case 4 patient.*

of 51±6% pre-TAVI to 54±4% and 57±3%, 7 days and 6 months after the procedure. For circumferential myocardial deformation analysis, there was an improvement of -14.9±1 pre-TAVI to -16.1±1.2 and -17.3±1.5 in 7 days and 6 months after TAVI, respectively.

In a similar way, in the present 4-case report, we observed significant improvement of myocardial deformation analysis immediately after TAVI. Three patients presented preserved LVEF, and one (Case 4), presented significant ventricular dysfunction, with improvements observed in LVEF and longitudinal myocardial deformation, immediately after the procedure. These data confirm that left ventricular function in valvulopathies directly depends on the afterload, and immediate relief of this variable may positively influence clinical outcomes. The causal relation between immediate recovery of myocardial deformation after TAVI – even in patients with preserved left ventricular function – and the benefits in symptoms and morbidity and mortality should be explored in future studies.

Author contributions

Conception and design of the research: Vieira MLC, Caixeta AM. Acquisition of data: Stangenhaus C. Analysis and interpretation of the data: Stangenhaus C. Writing of the manuscript: Stangenhaus C. Critical revision of the manuscript for intellectual content: Stangenhaus C, Vieira MLC, Fischer CH, Nunes Filho ACB, Perin MA, Caixeta AM.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Three-Dimensional Printing Model-Guided Percutaneous Closure of Atrial Septal Defect

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A 32-year-old female with a 2-year history of chest distress was admitted to our department due to exacerbation for 3 days. On physical examination, we found fixed splitting second heart sounds on the patient's pulmonic area. An echocardiography was performed and showed a 15-mm atrial septal defect of the inferior vena cava type. After obtaining the patient's consent, a three-dimensional printing cardiac model was printed out. We tried various sizes of ASD occluders on the model to completely cover the defect, which indicated that a 28-mm occluder was appropriate. Thus, we placed a 28-mm ASD occluder during the operation and succeeded after only one attempt. The patient was re-assessed by echocardiography, which showed a favorable position of the ASD occluder without any left-to-right shunt.

Three-dimensional printing (3D printing) is a new technology that converts two-dimensional medical images into a tangible object, allowing not only a comprehensive view of the cardiac anatomical structures but also preoperative simulation to choose the optimal size of ASD occluder. Although it has been applied to orthopedics, general surgery and so on, the use of 3D printing in cardiology is still at its infancy. Our case showed the feasibility of using a 3D printing cardiac model to guide the percutaneous closure of ASD. It is likely to increase the success rate and reduce the operation time for interventional cardiology, especially the complex ASD cases, and more studies should be carried out to extend its fields of application.

Keywords

Heart Septal Defects, Atrial / surgery; Echocardiography / methods; Imaging, Three-Dimensional.

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Conception and design of the research: Luo H; Acquisition of data: Luo H, Xu Y; Analysis and interpretation of the data: Wang Z, Liu Y; Writing of the manuscript: Luo H; Critical revision of the manuscript for intellectual content: Liu Y, Gao C.

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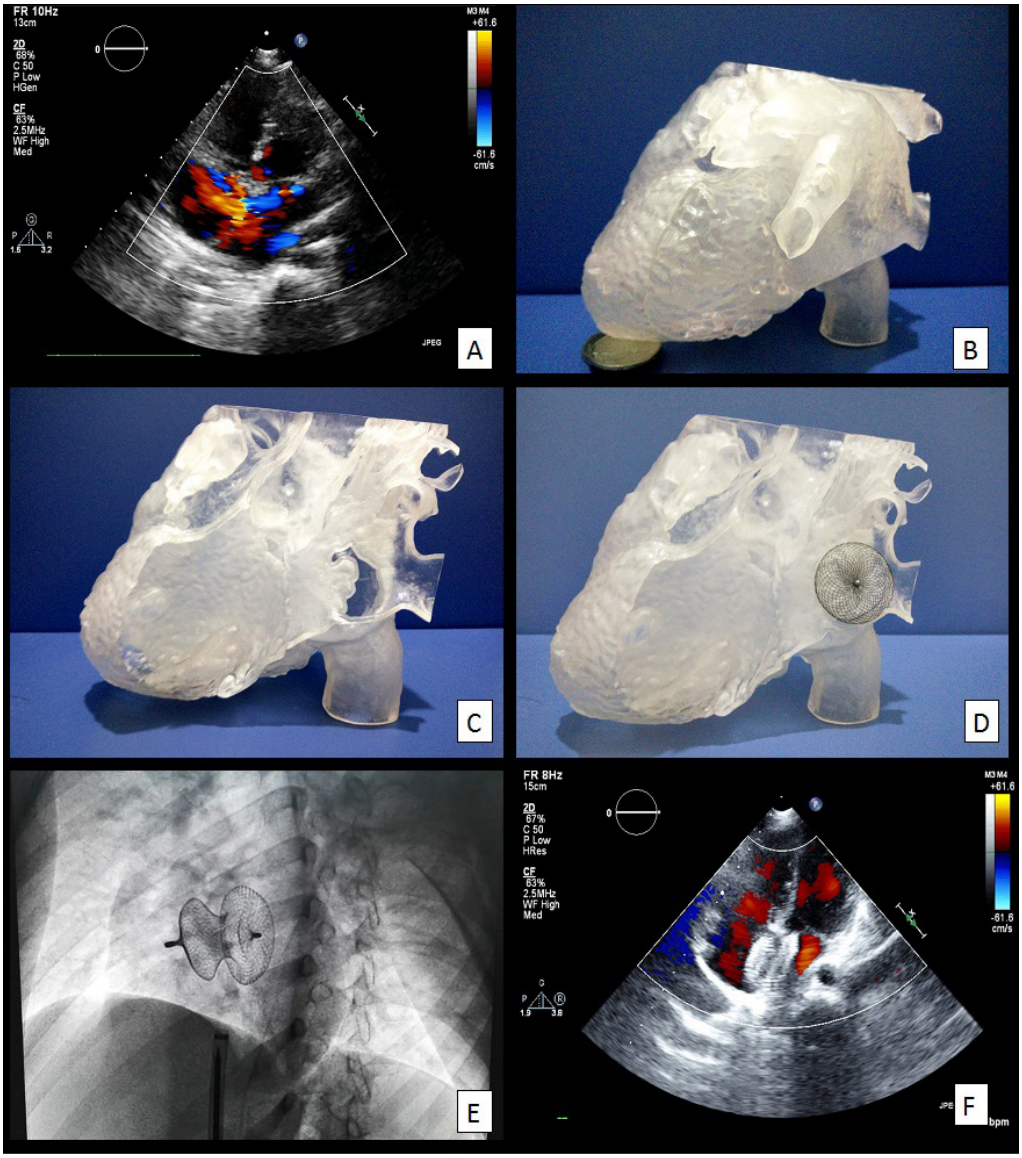

Figure 1 – *Echocardiographic apical four-chamber axis view showing a 15-mm ASD with left-to-right shunt (A). Three-dimensional printing cardiac model in whole view (B) or being separated to show the ASD (C). A 28-mm occluder was placed to completely cover the ASD (D). Intraoperative placement of a 28-mm ASD occluder after one trial (E). Postoperative echocardiographic apical four-chamber axis view showing no left-to-right shunt (F).*