UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS: ENDOCRINOLOGIA.

DISFUNÇÃO DIASTÓLICA NO PRÉ-DIABETES E DIABETES MELLITUS TIPO 2. AVALIAÇÃO ECOCARDIOGRÁFICA PRECOCE, COMPREENSIVA E COM PARÂMETROS AJUSTADOS PELA IDADE

TESE DE DOUTORADO

MAURO RICARDO NUNES PONTES

PORTO ALEGRE, NOVEMBRO DE 2009.

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TESE DE DOUTORADO

MAURO RICARDO NUNES PONTES

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul (UFRGS), como requisito parcial para obtenção do título de Doutor em Endocrinologia.

PORTO ALEGRE, NOVEMBRO DE 2009.

" Quem salva uma vida, salva o mundo inteiro".

TALMUD

" The very essence of cardiovascular practice is the recognition of early heart failure".

Sir Thomas Lewis.
Lewis T. Diseases of the Heart. London: MacMillan, 1933.

Dedico esse trabalho e essa realização à minha esposa, Marcela, o sol que aquece e ilumina meu caminho, tranquiliza minha alma e enche de amor o meu coração.

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LISTA DE ABREVIATURAS

A1C   Hemoglobina glicada

CMPD   Cardiomiopatia diabética

DCM   *Diabetic cardiomyopathy*

DD   Disfunção diastólica

DM   Diabetes mellitus

e GFR   Estimated glomerular filtration rate

FPG   Fasting plasma glucose

FS   Fractional shortening

HF   Heart failure

HFC   Heart failure clinic

HFPEF   Heart failure with preserved ejection fraction

HOMA   Homeostasis Model Assessment

hs CRP High sensitivity C-reactive protein

IC   Insuficiência cardíaca

IFG   Impaired fasting glucose

IGT   Impaired glucose tolerance

IR   Impaired relaxation

LAVI   Left atrial volume index

LVEDDi Left ventricular end-diastolic internal dimension

LVESDi Left ventricular end-systolic internal dimension

LVMI   Left ventricular mass index

MDRD   Modification of diet in renal disease

ND	Normal diastole
NGM	Normal glucose metabolism
NYHA	New York Heart Association
OGTT	Oral Glucose Tolerance Test
PD	Pré-Diabetes
PN	Pseudonormal
PVC	Preventive clinic
PWTd	Posterior wall thickness in diastole
RE	Restrictive
ROC	Receiver operator characteristics
RWT	Relative wall thickness
SM	Síndrome metabólica
SWTd	Septal wall thickness in diastole
TDI	Tissue Doppler imaging
TMD	Transmitral Doppler
UAER	Urinary albumin excretion rate

1. INTRODUÇÃO

O diabetes mellitus (DM) tipo 2 é uma doença metabólica com elevada taxa de complicações cardiovasculares, especialmente a doença aterosclerótica [1]. No entanto, o DM também é fator de risco para disfunção ventricular [2], e está associado com um risco 2-5 vezes maior de insuficiência cardíaca [3]. A mortalidade por insuficiência cardíaca também está aumentado no DM [4]. Esses riscos cardiovasculares aumentados se devem a associação freqüente do DM com dislipidemia, hipertensão arterial, doença coronariana e a ocorrência de uma cardiomiopatia diabética (CMPD) específica [5].

A anormalidade da função ventricular mais precoce no diabetes é usualmente a disfunção diastólica (DD) [6]. Embora autores isolados não tenham demonstrado a presença de DD em pacientes normotensos com DM inicial não complicada [7], há sólida evidência disponível apontando para a existência de DD nos pacientes com DM [8, 9, 10]. A presença de DD, mesmo em indivíduos sem DM, também é preditora de progressão para insuficiência cardíaca, e se associa com aumento da mortalidade cardiovascular [11, 12].

O diagnóstico de DD é baseado na avaliação ecocardiográfica, e pode apresentar algumas dificuldades e imprecisões importantes. A primeira delas deriva das alterações ventriculares morfofuncionais determinadas pelo envelhecimento fisiológico. No adulto jovem, o relaxamento ventricular é rápido e gera uma sucção diastólica precoce que é responsável pela maior parte do enchimento diastólico; nos idosos, o relaxamento é mais lento, e frações progressivamente maiores do enchimento ventricular ficarão na dependência da contração atrial [13]. Essas alterações modificam os valores normais dos parâmetros diastólicos ecocardiográficos de acordo com a faixa etária do paciente [14],

tornando potencialmente inadequado o uso de valores de referência fixos, não ajustados pela idade, para esses parâmetros [15].

Outra dificuldade na avaliação ecocardiográfica da DD é o uso de parâmetros incompletos para o diagnóstico. Atualmente é reconhecido que a avaliação seqüencial ("serial positioning") de múltiplos parâmetros ecocardiográficos, incluindo dados morfológicos (massa ventricular, volume atrial esquerdo), parâmetros obtidos pelo Doppler transmitral (medidas de velocidades dos fluxos de enchimento ventricular), e pelo Doppler tecidual (velocidades de relaxamento miocárdico), pode aumentar a acurácia diagnóstica da ecocardiografia [16].

Grande parte dos estudos que avaliaram a prevalência de DD no DM tem sido enviesados pela avaliação incompleta dos parâmetros de função diastólica e pelo uso de valores de referência para esses parâmetros ("pontos de corte") não ajustados pela idade [8, 17, 18], apesar de vários autores sugerirem o uso de pontos de corte específicos para a faixa etária [19, 20]. Essas limitações na avaliação ecocardiográfica da DD podem levar a uma redução da acurácia diagnóstica e possivelmente a classificação de pacientes que apresentam função diastólica normal para a idade como sendo portadores de DD [15].

Um outro aspecto que deve ser considerado é que, na história natural do DM, inclui-se uma longa fase em que a hiperglicemia se instala e evolui, gerando alterações metabólicas progressivas que finalmente levam ao DM franco [21]. Essa fase é chamada de pré-diabetes (PD), e inclui pacientes com glicemia de jejum alterada e/ou com alterações na tolerância à glicose [22]. Existe alguma evidência inicial de que modificações da função ventricular podem iniciar já nessa fase, antes mesmo do DM estabelecido [17, 23, 24]. Além disso, estudos recentes mostraram que o PD se associa a aumento da mortalidade cardiovascular e global [25, 26].

Essa cadeia de evidências sugere que seja importante, em pacientes disglicêmicos, o rastreamento de disfunção ventricular, objetivando o diagnóstico precoce, o que permitirá terapia otimizada buscando prevenir ou retardar desfechos clínicos adversos [27, 28].

A relevância clínica do tema, bem como as lacunas no conhecimento que foram acima relatadas, nos motivaram a estudá-lo; por isso desenvolvemos esse projeto de doutorado, que objetivou avaliar as relações entre disglicemia, disfunção diastólica e insuficiência cardíaca.

Este processo culminou na elaboração de três manuscritos. No primeiro, fizemos uma revisão ampla de literatura sobre as técnicas e os parâmetros ecocardiográficos disponíveis para avaliar a função diastólica em adultos, bem como as evidências dos efeitos da idade nesses parâmetros. A seguir, fizemos uma análise ponderada para obter valores de referência (pontos de corte) para cada faixa etária, e então propusemos um algoritmo prático para diagnóstico de DD usando avaliação sequencial de múltiplos parâmetros ecocardiográficos ajustados para a faixa etária do paciente.

No segundo manuscrito, um artigo original, testamos esse algoritmo de forma não invasiva em um grupo de 269 pacientes, abrangendo uma ampla gama de idades, frações de ejeção, e contemplando todo o espectro da DD, do relaxamento alterado inicial até reduções intensas de complacência ventricular.

No terceiro artigo, aplicamos esse algoritmo compreensivo e ajustado pela idade a um grupo de pacientes consecutivos encaminhados para atendimento em uma clínica de cardiologia, com o objetivo de avaliar a prevalência de disfunção diastólica em pacientes com disglicemia, e de avaliar a associação entre índices de homeostase glicêmica e marcadores ecocardiográficos de disfunção ventricular nos diferentes graus de disglicemia.

Desta forma, esperamos poder contribuir para o entendimento da cardiomiopatia diabética, esta importante complicação do DM.

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2. ARTIGO 1.

A PRACTICAL ALGORITHM TO DIAGNOSE AND GRADE DIASTOLIC DYSFUNCTION IN ADULTS, USING SERIAL POSITIONING OF MULTIPLE ECHOCARDIOGRAPHIC PARAMETERS WITH AGE-ADJUSTED REFERENCE VALUES.

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Abstract

Diastolic heart failure has been increasingly recognized as a major health burden, which increases cardiovascular morbidity and mortality. Its precursor, diastolic dysfunction (DD), can be conveniently diagnosed by Doppler echocardiography, a widely available technique. However, complexities of diastolic physiology, preload dependency of several diastolic parameters, influence of multiple haemodynamic variables, and the effect of normal aging process on myocardial relaxation make noninvasive diagnosis of DD difficult and troublesome. Additionally, it is important to distinguish alterations caused by true DD from those generated by physiological factors like aging. Echocardiographic variables used in the analysis of diastolic function were critically reviewed, including their modification caused by age, and set the stage to provide a diagnostic algorithm for characterization of diastolic function, with a combined and integrated approach to the different techniques and using age-adjusted cut-offs (reference values) for each variable.

Review

Normal left ventricular (LV) diastolic function requires the ability of the ventricle to fill with a volume that is enough to maintain a normal stroke volume while maintaining normal diastolic pressures at rest and during exercise [1]. Diastolic dysfunction (DD) refers to an impairment of LV diastolic distensibility, relaxation, stiffness or filling, regardless of whether the ejection fraction (EF) is preserved or reduced and whether the patient is symptomatic or asymptomatic [2].

There are a number of conditions (risk factors) associated to DD: older age, female gender, arterial hypertension, diabetes mellitus, obesity, hypertrophic cardiomyopathy, and infiltrative myocardial disorders [3, 4]. Thus, an asymptomatic patient with one of these conditions, and an echocardiogram showing a preserved EF and at least one LV filling abnormality, can be categorized as having DD [5]. If exercise intolerance, venous systemic congestion, pulmonary edema and dyspnea develop in such a patient, the term diastolic heart failure (DHF) or heart failure with preserved ejection fraction (HFPEF) applies [6].

In the presence of DD, 11-15% of patients older than 65 years of age will develop DHF within 5 years of diagnosis [7]. Even in the absence of overt HF, mild and moderate to severe DD are associated with a hazard ratio of 8.3 and 10.2 for all-cause mortality, respectively [8]. DD has a major impact on symptom status, functional capacity, and prognosis in both diastolic and systolic HF, irrespective of the etiology [9]. Mortality rate of DHF ranges from 5 to 8% annually [10].

It is important to perform an early echocardiographic evaluation of diastolic function in patients known to have one of the DD risk factors, in order to make early noninvasive

diagnosis. Early diagnosis allows intensive treatment of the cause of DD, aiming to prevent the development of DHF and its associated morbidity [11].

Age is known to be an important independent risk factor for DD, and several authors suggest taking it into account when evaluating diastolic function. However, no clear age-adjusted specific criteria are commonly used. The purposes of this manuscript are to review the echocardiographic criteria used to assess diastolic function in adults, to evaluate the evidence of age effect on this parameters, and to propose a practical algorithm for DD diagnosis using serial positioning of different parameters according to age-adjusted reference values.

Echocardiographic Diastolic Dysfunction Assessment. Doppler echocardiography plays a critical role in the evaluation and diagnosis of DD. It allows to measure intracardiac blood flow velocities using transmitral flow Doppler (TMD) and myocardial relaxation velocities from tissue Doppler imaging (TDI), and define the size and volume of the left atrium (LA), corrected for body surface area (LA volume index – LAVI), which is a marker of the chronic LV filling pressure [12]. Furthermore, it allows to quantify LV mass, which can be normalized by body mass index (LV mass index), providing a morphologic correlate of DD [13].

In normal sinus rhythm, diastolic flow from the LA to LV across the mitral valve has two components: early and late filling (generated by LV relaxation and LA contraction, respectively). These two flows can be assessed by transmitral Doppler, generating the so called E wave and A wave, respectively. As the velocity of blood flow across mitral valve depends on transmitral pressure gradient, E wave velocity is influenced prioritarily by the rate of early relaxation and the LA pressure. The E/A ratio gives insight on the proportion of early versus late mitral flow, which is modified in patients with DD. Hence, the TMD analysis of these two waves allows us to evaluate diastolic function in a real time. However, elevation of

LA pressure in patients with impaired relaxation can increase mitral inflow velocities, thereby masking diastolic abnormalities characterized by reduction of these early velocities. It would therefore be useful to measure velocities after a Valsalva maneuver, which reduces preload and can unmask the presence of DD [14]. During the strain phase of the maneuver, a reduction of E velocity by ≥50% of baseline or a complete reversion of E/A ratio to <1 may be useful criteria for DD [9]. However, this approach is largely qualitative, and the required diagnostic threshold varies across the studies. Even in research settings, the ability to obtain adequate data may be low, thus limiting the sensitivity of the technique.

Analysis of pulmonary vein flow velocities can be helpful in evaluating diastolic function. The velocity of systolic forward flow is inversely related to LA pressure; additionally, a late diastolic A "reversal" wave in pulmonary vein flow (corresponding to the effect of LA contraction) increases when resistance to LA forward flow increases as a result of progressive DD. However, pulmonary vein flow parameters are less sensitive for detection of disease when LV systolic function is preserved. As we now have the possibility of evaluate myocardial motion by TDI and detect the underlying LV relaxation abnormality, the usefulness of evaluating pulmonary vein flow has been minimized [15].

Tissue Doppler imaging evaluates velocity of myocardial movement (usually at the level of the mitral annulus), as the diastolic myocardial motion generates the E' wave (early diastolic myocardial velocity), and A' wave (late diastolic velocity during LA contraction). E'/A' ratio shows the relationship of myocardial velocity during early and late diastole. The E/E' ratio (early transmitral flow velocity / early annular mitral velocity) allows "correction" of the E wave for the degree of impairment in LV relaxation rate by relating it to the E' velocity (a relatively load independent index of intrinsec LV relaxation). E/E' has a strong positive correlation with LV filling pressure [16]. Although some experts indicate the average of septal

and lateral E´ velocities, the lateral portion of the mitral annulus (E'$_{LAT}$, A'$_{LAT}$) is usually preferred for TDI measurements [17], because they are less preload dependent in subjects with normal LV function, are not influenced by right ventricular diastolic function, and have more precisely defined cut-off values [9]. Additionally, E'$_{LAT}$, E'/A'$_{LAT}$ and E/E'$_{LAT}$ have the best correlation coefficients with invasively measured indexes of diastolic relaxation, LV end-diastolic pressure, and LV end-diastolic pressure-volume relations [18]. However, some experts suggest the average of septal and lateral velocities.

DD can be categorized in three grades according to its severity: Grade I (impaired relaxation), Grade II (pseudonormal pattern) and Grade III (restrictive pattern)[14]. The usual presentation of DD is characterized by an initial and progressive impairment in LV relaxation followed by a superimposed decline in LV compliance that results in the need for increased LA pressures to maintain LV filling and cardiac output.

Transmitral flow Doppler pattern of impaired LV relaxation is an early sign of DD. It is characterized by reduced early and increased late diastolic flow (reduced E, increased A, reverted E/A ratio) and a similar alteration in myocardial velocities (reduced E' and increased A' velocities, reverted E'/A' ratio) (impaired relaxation) [9, 14] . The more advanced grade (restrictive pattern), manifested by predominant early diastolic filling and rapid velocity deceleration, as well as marked reduction of myocardial velocities and increase in filling pressure (increased E wave, reduced deceleration time of E wave, reduced E' and increased E/E'), has the greatest prognostic impact. The intermediary (pseudonormal) pattern occurs when LV filling pressure rises to maintain normal cardiac output and increases ("normalizes") the early filling reduced by impaired relaxation (normalized E wave, E deceleration time and E/A ratio) [19].

Pseudonormal and normal patterns can hardly be distinguished on the basis of transmitral flow alone, because of its preload dependency [1, 8, 19]. In this situation, accurate detection of grade II DD can be achieved by integration of clinical information, application of additional techniques, analyzing the effect of maneuvers of preload modification (e.g. Valsalva maneuver), the pulmonary venous flow, and measurement of LA and LV sizes and morphologies. The use of tissue Doppler is very helpful in this differentiation, as E' is reduced, and E/E' ratio is usually increased, unmasking the elevation of LV filling pressure [20-24].

Studies reporting the prevalence of DD in community-based settings applied distinct criteria to diagnose DD, consequently finding discrepant results. Usually, the more comprehensive the criteria, the more prevalent is DD. In a Norwegian population in the Tromso Study (3022 patients), using only TMD, the prevalence of DD varies from 2 to 15% [25]. In the European-based sample MONICA Augsburg, which also used only TMD, overall prevalence of diastolic abnormality was 11.1%, rising to 15.8% among those older than 65 years [26]. In the Strong Heart Study, again using isolated TMD parameters for the diagnosis of DD, prevalence was 19% [27]. In another community-based study of 2042 subjects, Redfield et al used a more comprehensive transmitral and tissue Doppler analysis and found a prevalence of 28% [8]. In a recently published survey of 1275 subjects, using mitral inflow, pulmonary venous flow and TDI parameters, the prevalence of any DD was nearly 35%, and moderate to severe DD was around 7% [28]. The more recent study, just published, evaluated 539 patients using mitral inflow, pulmonary vein flow by pulsed-wave Doppler, and mitral annular velocities, showing an overall prevalence of LV DD as high as 27% [29].

As a conclusion, it seems clear the critical importance of performing sophisticated testing and comprehensive evaluation for the diagnosis of DD , using data gathered on LA

volume, LV mass, tissue Doppler velocity, measurement of filling pressure (E/Em), in addition to transmitral flow data [30, 31].

Effects of age on the indexes of diastolic filling. In the young adult, LV relaxation is rapid, generating an early diastolic suction effect that results in around 90% of filling occurring in early diastole. In middle age, LV relaxation slows, early LV filling decreases, and the contribution of LA contraction increases to about 30%. By age 65, further impairment of relaxation has occurred and up to 50% of flow may occur in late diastole [14]. These modifications determined by age have a significant impact on the normal values for each echocardiographic parameter. So, the unadjusted cut-off values commonly used to define DD in a population may be inadequate for the evaluation of elderly patients, potentially leading to misclassification [32].

As a consequence, several authors acknowledge that age is one of the strongest determinants of E/A, E', and E/E', and suggest that age-dependent cut-off values should be taken into consideration when classifying patients as having DD [9, 14, 32-34].

There are a number of studies which evaluated normal, non-ischemic, non-hypertensive populations using transmitral Doppler and tissue Doppler imaging, and established age-stratified normal (reference) values for diastolic echocardiographic parameters in these population-based settings [33, 35-38] . The use of these reference cut-offs has the potential of minimizing the possible mistake of classifying an elderly normal patient as having DD just because of using a cut-off value that is inadequate for his/her age.

Diastolic Dysfunction according to age-adjusted criteria. Some authors, acknowledging the influence of age in the diastolic function, used age-adjusted reference values for the diagnosis of DD, in a variety of clinical settings. Herkner et al. studied patients with severe arterial hypertension, in order to evaluate the prevalence of DD. They calculated a Z score

(standardized normal deviation from the mean value of patient age group), with a normal value of 0 ± 2. With this strategy of correction by age, the authors showed a lower prevalence of DD by TMD. They stated that "use of a single Doppler E/A ratio threshold value has a weak diagnostic power to detect age-independent changes in mitral flow patterns" [39].

In 2003 Vinereanu et al evaluated the prevalence of DD in diabetic patients comparing with age- and sex-matched nondiabetic controls, correcting different TMD diastolic parameters for age <50 years or >50 years. They used an Em cut-off value that was not age-stratified. Using these criteria, they still found a high prevalence of DD in their diabetic patients [40]. In 2007, Fuentes et al., assessing the prevalence of DD in patients with and without metabolic syndrome, used two levels of age stratification for E/A (above and below 55 years), E' (corrected by using 3 different age groups), and noncorrected deceleration time [41]. They found that metabolic syndrome was associated with increase in LV mass and impairment of LV relaxation as evaluated by TMD and TDI . These authors have the virtue of taking into account age-adjusted DD reference values and cut-offs to evaluate the prevalence of DD in different clinical situations. However, they used distinct and arbitrary criteria, and did not use all technical resources now available, which probably would decrease the accuracy of their evaluations. Also, there was great variability in the age groups selected for analysis, preventing standardization of criteria.

The availability of an algorithm which uses serial positioning of different variables to diagnose and grade DD (including TMD, TDI, anatomical parameters and evaluation of filling pressure) while taking into account previously validated age-adjusted cut-off values for these variables, would be very useful, allowing standardization and reproducibility of parameters, preventing misclassification of patients, and providing an accurate framework for use in population studies.

Development of an algorithm for diastolic dysfunction diagnosis and grading. There are a number of authors who presented algorithms, sets of DD criteria and/or reference ranges for Doppler echocardiographic parameters of diastolic function in healthy subjects [9, 15, 16, 23, 33, 42-51]. Although they attempt to reach an important goal (systematization of diagnosis), there is a reason for criticism: most of them use mitral flow parameters only, providing low accuracy for the diagnosis of DD [33, 42-48]. Some of them are also limited because few patients were enrolled [47], or less sensitive parameters to separate normal from pseudonormal pattern were used [52]. Some of them were created to be applied in the setting of diastolic heart failure, instead of DD [51, 53]. The more accurate ones incorporated remodeling parameters (e.g. LA volume and LV mass measurement) and/or noninvasive surrogates of LV filling pressure (e.g. E/E') [49, 54].

A guideline document containing recommendations for the evaluation of LV diastolic function by echocardiography was recently published [13]. It contains a comprehensive review of physiology, atrioventricular correlates of DD, techniques and significance of measurements of different DD parameters. It also provides algorithms for the estimation of LV filling pressures in patients with normal and depressed EF, and proposed an approach to grade DD, using TMD, TDI, LA volume and filling pressure. However, the main limitation of this practical approach is common to all of the algorithms: there are no consistently age-adjusted cut-offs, so the same reference values are used for all age groups. This is potentially a source of error, allowing misclassification of older patients.

Additionally, eight of these algorithms were reviewed and tested for concordance in classifying patients with or without DD. These analyses showed a very poor concordance between measures, with up to a 16-fold difference in the prevalence of DD when distinct algorithms were applied to an external cohort of patients [55].

METHODS

In order to overcome the limitations of previous algorithms, an algorithm was established, based in a consensus between a cardiologist (MRNP) and an expert in echocardiography (LCD), as a tool to standardize the diagnosis and grading of DD.

The development of this consensus algorithm was based on a critical review of literature. An eletronic search in MEDLINE database was conducted for studies describing age specific ranges for conventional Doppler and tissue Doppler measures of diastolic function in healthy people since 1990, followed by a manual search of textbooks and review articles, scrutinising their reference lists. A search in the Cochrane Reviews database was also performed. The same methodology was used to search studies describing correlations and reference values for LVMI and LAVI. In this way, we identified previous studies which validated each individual echocardiographic parameter for detection of DD [9, 14, 25, 32, 35, 37, 56-66].

Each parameter was incorporated into the algorithm to provide a comprehensive non-invasive assessment of diastolic filling of the heart, which takes into consideration at the same time TMD, TDI, noninvasive evaluation of filling pressure (E/E'), and atrioventricular remodeling which is a hallmark of DD and DHF (eg. LV hypertrophy and increases of LA volume indexed by body surface area) [16, 19, 67-69].

Table 1 shows the proposed criteria for the diagnosis of DD. Figure 1 shows the proposed algorithm for grading the intensity of DD.

RESULTS.

We are proposing an algorithm for diagnosis of DD with some important features: first, the inclusion of morphological parameters in the analyses of diastolic function. This approach

was suggested by the strong correlation between atrioventricular remodeling indices and DD parameters; in fact, inclusion of such parameters in the diastolic evaluation allows a more complete and refined diagnostic characterization [70] .

Second, we include a comprehensive evaluation of diastolic function, using parameters derived from distinct techniques in serial positioning. The main reason is the fact that distinction of normal versus pseudonormal filling is difficult using the preload-dependent mitral inflow pattern alone and in most patients other parameters will be necessary to complete the assessment [49]. Additionally, this strategy overcomes concerns of low specificity of each parameter for stand-alone diagnosis of LV DD.

Third, we used age-adjusted cut-offs (published reference values) for each Doppler parameter. These reference values were obtained from a priori definition of cut points for this continuous echocardiographic variables, based on the distribution of this measurements in previously studied randomly selected noninstitutionalized samples of the general population free of clinically detectable cardiovascular disease [14]. Such a classification system for partitioning values exceeding reference limits was previously validated in a Framingham Sub study [71]. Published values for the 95% confidence limits of the Doppler variables stratified by age were taken [19, 29] and submitted to a weighted metaanalysis to construct tables of practical cut point limits for LV DD for each decade of adult life (Table 1), based in these studies which included more than 5000 subjects [9, 14, 16, 25, 32, 35, 37, 56-66].

DISCUSSION.

Diagnosis of Diastolic Dysfunction (Table 1). We consider DD present when at least two (out of five) criteria from table 1 (obtained by distinct techniques) were fulfilled, according to age-specific reference values when they apply.

Assessment of LV diastolic function requires a meticulous and systematic approach. Each variable was considered altered in an individual patient when it exceeded the 95% confidence interval (95% CI) of the normal range for that age, using published values [33, 35, 37, 57]. From TMD evaluation, inversion of E/A ratio according to age-adjusted cut-offs is the cornerstone of initial pathophysiology of DD. From tissue Doppler interrogation, reduction of E' velocity (or E'/A' inversion) provides relatively preload-independent information to support the presence of DD. In case of discrepancy, additional information provided by evaluation of filling pressure obtained from E/Em ratio would be useful. These techniques are also affected by aging, therefore age-adjusted cut-offs are also important.

Doppler variables should always be viewed in the context of LA and LV size and function [72]. Studies using modern equipments and measurement techniques have shown that LAVI is not associated with age [73, 74]. A study showed that a normal indexed LA volume for men and women is 21 ml/m^2, with 32 ml/m^2 being the 90% upper confidence limit of the 95th percentile (nearly 2 SD from the mean) [75]. A prospective study showed that LAVI ≥32 ml/m^2 has a sensitivity of 67% and a specificity of nearly 100% for detection of DD [20]. An observational meta-analyses of eight studies including 5806 patients without baseline cardiac disease have shown that LAVI ≥32 ml/m^2 is an independent predictor of hard outcomes [76]. In a cohort study in an elderly population (1160 patients) LAVI ≥32 ml/m^2 was a strong independent predictor of cardiovascular events, with higher predictive value than other echocardiographic indices such as LVMI or LV DD by mitral flow Doppler [77]. Therefore, the cut point for LAVI ≥32ml/m^2 was incorporated to the algorithm based in epidemiological, populational and prognostic reasons.

In contrast to earlier findings, more recent studies using modern technology and strict measurement methods showed lower values of LVMI [78] (confirmed by autopsy studies)

[79], and no association with age [80, 81]. In an echo sub-study of the Strong Heart Study, authors specifically stated that "it does not seem necessary to adjust partition values for age to recognize elevated LVMI" [81].

In this context of LA and LV measurements, patients with preserved systolic function and normal LA size will almost certainly have normal filling pressure and diastolic function. Since the presence of LV hypertrophy (LV mass, indexed for body size, exceeding the upper 95% confidence interval of normal for individual patient sex and age group) [57] increases the degree of diagnostic certainty, it was used as confirmatory and supportive evidence in our algorithm [82]. So, the mitral inflow and annular velocities, in conjunction with morphological findings, represent the most efficient tools for evaluating diastolic function.

Diastolic Dysfunction Grading (Figure 1). It has been previously demonstrated that TMD velocities show an evolution over time with the impairment of LV function [83-85], in a progression that unveils three patterns:

Impaired relaxation (DD Grade I). DD is directly related to the reduction of early LV relaxation (E' decreases), which becomes apparent at an early stage of LV dysfunction [86]. This reduction decreases early diastolic filling, and an abnormal relaxation pattern is seen on the mitral flow velocity curve, consisting of a low E velocity and a delay in E deceleration time. This delay is partially compensated by a more vigorous end-diastolic atrial contraction (higher A velocity), and, hence, the E/A ratio is reduced [15]. LA pressure remains relatively normal at rest in this early stage of DD (normal LAVI and E/E'), and patients may have symptoms only with exertion. However, even this mild degree of diastolic dysfunction places patients at increased risk for adverse cardiovascular events [8].

Pseudonormal pattern (DD Grade II). With further impairment of diastole, atrial pressure increases, and the relative difference between atrial and ventricular pressures decreases. As

a consequence, E deceleration time shortens, E velocity and E/A ratio will now increase, and the mitral flow velocity profile may appear normal. However, E' velocity will remain reduced, identifying the underlying LV relaxation abnormality. A reduced Em velocity, increased E/E' ratio, and increased LA volume can, therefore, be readily used to discriminate an individual with normal versus grade II (pseudonormal) diastolic dysfunction [16, 23].

Restrictive pattern (DD Grade III). In the later stages of DD, LV compliance is greatly reduced, early mitral inflow is very rapid (increased E velocity), and there is very little contribution to LV filling from atrial contraction (small A wave). E/A ratio is very high, and there is rapid equilibration of LA and LV pressures (short deceleration time). A low E', an increase in E/E' and high LA volume confirms a reduction in LV diastolic function and an increase in LV filling pressure. Clinically, restrictive pattern is associated with the "sickest" ventricles and is predictive of mortality, particularly if it is not reversible [87].

Accurate diagnosis and grading of DD is very important, because each incremental stage of DD carries an associated increase in cardiovascular morbidity and mortality, and correlates with overall prognosis in heart failure [8, 60, 88, 89].

Perspectives. This proposed algorithm should be evaluated for internal consistency, tested against other algorithms commonly used and ideally it should be submitted to a comparative simultaneous Doppler-conductance catheter study [18]. However, it is tempting to believe that this algorithm would reduce the misclassification of patients because of their age and the use of inadequate reference values, and it will also be more accurate in any age group, because of the serial positioning of echocardiographic parameters. Nevertheless, it can be difficult to predict the final effect of the application of the algorithm in the prevalence of DD in general population. The observed prevalence, however, surely will be closest to the true one.

Conclusions

Diastolic abnormalities of the LV are associated with significant morbidity and adverse outcomes. Assessment of real myocardial diastolic properties is complex and elusive; however, clinicallly meaningful information regarding diastolic function can be obtained noninvasively. The assessment (and interpretation) of diastolic parameters and LV filling pressures continues to evolve. Using a stepwise, evidence-based, age-stratified algorithmic approach involving easily obtainable morphological and Doppler echocardiographic variables as well as an evaluation of LV filling pressures, detection and grading of DD can be performed in most patients, in a way that may add accuracy and precision over and above previous non age-adjusted algorithms. The main goal must be the early and reliable identification of DD in asymptomatic patients, providing an opportunity to manage the underlying etiology adequately to prevent progression to heart failure and to reduce adverse outcomes.

Competing interests

The author(s) declare that they have no conflicts of interest.

Authors' contributions

MRNP participated in the design of the study, reviewed the papers, drafted the manuscript and helped in the design of the algorithm.

LCD helped to review the papers, participated in the drafting of manuscript and provided expertise in echocardiography to design the algorithm.

LHC participated in the design of the study, participated in study coordination and reviewed the manuscript for important intellectual content.

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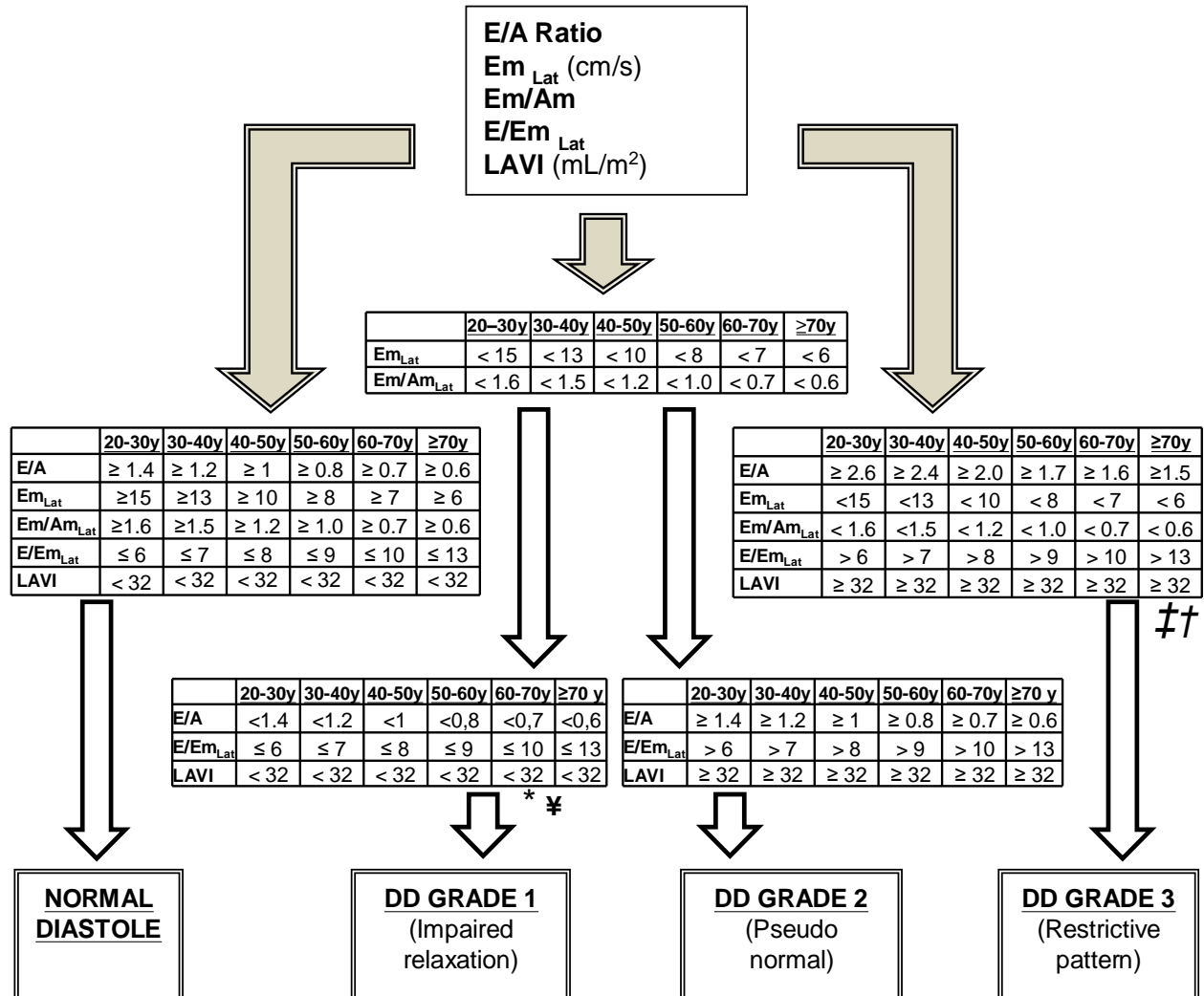
Figure 1

E/A Ratio
Em $_{Lat}$ (cm/s)
Em/Am
E/Em $_{Lat}$
LAVI (mL/m^2)

	20–30y	30-40y	40-50y	50-60y	60-70y	≥70y
Em$_{Lat}$	< 15	< 13	< 10	< 8	< 7	< 6
Em/Am$_{Lat}$	< 1.6	< 1.5	< 1.2	< 1.0	< 0.7	< 0.6

	20-30y	30-40y	40-50y	50-60y	60-70y	≥70y
E/A	≥ 1.4	≥ 1.2	≥ 1	≥ 0.8	≥ 0.7	≥ 0.6
Em$_{Lat}$	≥15	≥13	≥ 10	≥ 8	≥ 7	≥ 6
Em/Am$_{Lat}$	≥1.6	≥1.5	≥ 1.2	≥ 1.0	≥ 0.7	≥ 0.6
E/Em$_{Lat}$	≤ 6	≤ 7	≤ 8	≤ 9	≤ 10	≤ 13
LAVI	< 32	< 32	< 32	< 32	< 32	< 32

	20-30y	30-40y	40-50y	50-60y	60-70y	≥70y
E/A	≥ 2.6	≥ 2.4	≥ 2.0	≥ 1.7	≥ 1.6	≥1.5
Em$_{Lat}$	<15	<13	< 10	< 8	< 7	< 6
Em/Am$_{Lat}$	< 1.6	<1.5	< 1.2	< 1.0	< 0.7	< 0.6
E/Em$_{Lat}$	> 6	> 7	> 8	> 9	> 10	> 13
LAVI	≥ 32	≥ 32	≥ 32	≥ 32	≥ 32	≥ 32

‡†

	20-30y	30-40y	40-50y	50-60y	60-70y	≥70 y
E/A	<1.4	<1.2	<1	<0,8	<0,7	<0,6
E/Em$_{Lat}$	≤ 6	≤ 7	≤ 8	≤ 9	≤ 10	≤ 13
LAVI	< 32	< 32	< 32	< 32	< 32	< 32

* ¥

	20-30y	30-40y	40-50y	50-60y	60-70y	≥70 y
E/A	≥ 1.4	≥ 1.2	≥ 1	≥ 0.8	≥ 0.7	≥ 0.6
E/Em$_{Lat}$	> 6	> 7	> 8	> 9	> 10	> 13
LAVI	≥ 32	≥ 32	≥ 32	≥ 32	≥ 32	≥ 32

NORMAL DIASTOLE

DD GRADE 1 (Impaired relaxation)

DD GRADE 2 (Pseudo normal)

DD GRADE 3 (Restrictive pattern)

Figure 1 - Diastolic dysfunction: Grading algorithm according to age

LV hypertrophy (LVMI >95g/m2♀ >115g/m2♂ is a supportive criterion for DD grading.

* Some authors describe DD grade Ia (impaired relaxation with mild elevations of E/Em)

¥ Athletes can have an increase in LAVI without DD.

† A restrictive pattern on TMD with normal Em and E/Em suggests pericardial disease.

‡ Em/Am can return to normal in severe restrictive DD, as atrial contraction deteriorates.

Table 1

Table 1. Diastolic dysfunction – Diagnostic criteria by age *

	Age Range					
	20 – 30y	**30 – 40y**	**40 – 50y**	**50 – 60y**	**60 – 70y**	**≥70y**
1. TMD: E/A [A]	<1.4/≥2.6	<1.2/≥2.4	<1/≥2.0	<0.8/≥1.7	<0.7/≥1.6	<0.6/≥1.5
2. TDI: <u>Either</u> E´ Lat (cm/s)	< 15	< 13	< 10	< 8	< 7	< 6
<u>Or</u> E´/A´ Lat [B]	< 1.6	< 1.5	< 1.2	< 1.0	< 0.7	< 0.6
3. E/E´ Lat (ratio E / Em) [C]	> 6	> 7	> 8	> 9	> 10	> 13
4. LAVI (mL/m^2) [D]	≥ 32	≥ 32	≥ 32	≥ 32	≥ 32	≥ 32
5. LVMI (g/m^2) [E]	>95♀ >115♂	>95♀ >115♂	>95♀ >115♂	>95♀ >115♂	>95♀ >115♂	>95♀ >115♂

* Patients are required to have two out of five criteria to be diagnosed with DD.

Reference values and cut-offs based on weighted metaanalyses of references: **<u>A</u>**: 14, 25, 29, 33, 35, 37, 63, 64; **<u>B</u>**: 16, 35, 37, 58-60, 62-66; **<u>C</u>**: 29, 35, 59, 63, 64; **<u>D</u>**: 20, 56, 70-72; **<u>E</u>**: 56, 73-76.

Using <u>septal TDI</u>, the following cut-offs should be used for DD diagnosis [9, 32, 35, 72, 73, 74]

Em Septal(cm/s): 20-30y: <12   30-40y: <10   40-50y: <7   50-60y: <6   60-70y: <5   ≥70y: < 5

E/Em Septal: 20-30y: >7   30-40y: >8   40-50y: >10 50-60y: >12 60-70y: >13 ≥70y: >16

Em/Am Septal: 20-30y:<1.3   30-40y: <1.2 40-50y: <1.0 50-60y: <0.8 60-70y: <0.7 ≥70y: <0.6

E/A=early mitral flow velocity/ late mitral flow velocity ratio; Em$_{Lat}$=Lateral early annular velocity; Am$_{Lat}$=Lateral late annular velocity   Em/Am= annular early / late mitral velocity ratio. Em$_{Lat}$=Lateral early annular velocity E/Em= early mitral flow velocity/ lateral early annular velocity. LAVI= left atrial volume index; LVMI= left ventricular mass index.

3. ARTIGO 2.

NONINVASIVE VALIDATION OF AN AGE-ADJUSTED ALGORITHM FOR DIAGNOSIS AND GRADING OF DIASTOLIC DYSFUNCTION

Short title: **Testing an age-adjusted algorithm for diastolic dysfunction**

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Running head: **"**Age-adjusted algorithm for diastolic dysfunction"

Abstract

Echocardiographic diagnosis of diastolic dysfunction (DD) can be biased by alterations generated by the physiological aging process. Many authors suggest the use of age-specific cut-off points for echocardiographic parameters, but there is no age-adjusted algorithm for DD. To test noninvasively and to define usefulness of an age-adjusted algorithm for diagnosis and grading of DD, 269 patients [41% male, 60 ± 12 years (range 21 – 89)] underwent a standardized questionnaire and a comprehensive echocardiogram. LV mass index (LVMI) and left atrial volume index (LAVI), transmitral Doppler (TMD) early (E) and late (A) peak flow velocity, E/A ratio, tissue Doppler (TDI) early (E´) and late (A´) mitral annular velocity, E´/A´ and E/E´ ratios were obtained. A significant, clinically relevant correlation with age was found for TMD and TDI variables, in opposition to LVMI and LAVI. Patients with DD (n = 194, 72 %) were of similar age, had higher LVMI, LAVI, and E/E´, lower EF and E´ velocity, and worse NYHA class than patients without DD. LVMI, LAVI and E/E´ steadily increased, and E´ decreased, as DD progressed. TMD variables showed bimodal behaviour, "pseudonormalizing " as filling pressures increased. The age-adjusted algorithm showed high discriminatory power and performed better than 2 other well-known algorithms, either in patients with EF <50% or >50%. In conclusion, this comprehensive age-adjusted algorithm consistently separates patients with normal and abnormal diastolic function, adequately classifies DD according to severity, and prevents the confounding effect of physiological aging process. It can be used in clinical practice and research, increasing accuracy for diagnosis and grading of DD.

INTRODUCTION

Diastolic heart failure (HF) is responsible for 40-50 % of the cardiovascular morbidity and mortality associated with HF [1]. Diastolic dysfunction (DD) is its precursor, and can be conveniently diagnosed by Doppler echocardiography. However, the complexity of diastolic physiology, preload dependency of Doppler parameters, and the influence of the aging process on myocardial relaxation make noninvasive diagnosis of DD difficult and sometimes inaccurate [2]. Early and precise noninvasive DD diagnosis, allowing tailored treatment, is essential to prevent development of diastolic HF and to decrease its associated morbidity and mortality [3].

The normal aging process has a major influence on diastolic function, leading to a reduction in left ventricular (LV) relaxation velocity and early diastolic filling, and to a progressive increase in late diastolic filling [4]. These modifications have an impact on the normal values for each echocardiographic parameter. The unadjusted cut-off values commonly used to define DD may be inadequate to evaluate elderly patients, potentially leading to DD misclassification [5]. As a consequence, several authors have suggested that age-dependent cut-off values should be taken into account when diagnosing DD [6-8]. However, no clear age-adjusted criteria for DD diagnosis are commonly used.

The purpose of this paper was to test noninvasively and to determine the usefulness of a previously proposed algorithm (Algorithm A) for diagnosis and grading of DD [9]. Its two main proposed features are: first, serial positioning of different echocardiographic parameters; second, the use of age-adjusted cut-offs and reference values for each Doppler parameter.

METHODS

Study sample. Outpatients were consecutively enrolled between April 2006 and September 2008, from two distinct populations: PVC sample, 139 patients without known heart disease, from a preventive cardiology clinic; HFC sample, 130 patients referred for evaluation in a heart failure clinic. Patients with connective tissue disease, congenital heart disease, aortic disease, moderate/severe valvular heart disease, atrial fibrillation, hepatic disease or malignancy were excluded. All subjects provided written and informed consent. The study was performed in accordance with the Second Helsinki Declaration and ethical approval was obtained from the local hospital ethics committee.

Clinical evaluation. Patients underwent an interview and clinical examination to record demographic and anthropometric data. They were weighted in light clothes without shoes, and height was recorded. BMI was calculated as weight (kilograms)/height (meters^2). Valvular heart disease was defined as at least moderate stenosis or regurgitation of mitral and/or aortic valve. HF was diagnosed using Framingham criteria, and graded by the New York Heart Association (NYHA) system.

Echocardiography. All patients underwent transthoracic echocardiogram performed by a single certified sonographer cardiologist who was blinded to the patients' clinical data, using commercially available equipment (GE Vingmed System Five) according to a standardized protocol [10]. Two-dimensional (2D) and color Doppler imaging were performed to screen for valvular stenosis or regurgitation. Measurements were performed online, and all parameters were determined using the average of ≥3 heart beats. Septal (SWTd) and posterior wall (PWTd) thicknesses, LV end systolic and end diastolic internal dimensions (LVESDi and LVEDDi) were measured using M-mode or 2-dimensional echo, and LV mass was calculated. LV mass index (LVMI) was calculated by indexing for body surface area [10].

Left ventricular hypertrophy was defined as a LV mass index >95 g/m^2 for women and >115 g/m^2 for men[10]. Relative wall thickness (RWT) was calculated by the formula RWT = (2 x PWTd)/ LVEDDi, and was considered increased if >0.42. LV remodeling patterns were defined as concentric remodeling (normal LVMI with increased RWT), concentric hypertrophy (increased LVMI with increased RWT), eccentric hypertrophy (increased LVMI with normal RWT) or absence of LV remodeling [10].

Left atrial volume was measured by biplane Simpson´s method [11], and subsequently indexed by BMI. Ejection fraction (EF) was determined using the modified Simpson´s method. Transmitral flow Doppler (TMD) velocities were recorded using pulsed-wave Doppler, with the sample-volume at the tip of mitral valve leaflets in the apical 4-chamber view. Mitral inflow measurements included peak velocities of early (E) and late (A) diastolic filling, the E/A ratio, and deceleration time of E (DT). For the tissue Doppler imaging (TDI) sample-volume was placed at the lateral mitral annulus. Measurements were performed for early (E´) and late (A´) annular velocities. Ratios of E´/A´ and E/E´ were calculated [12].

Intraobserver variability was assessed in 10 randomly selected subjects, and expressed as the mean percent error, derived as the absolute difference between 2 measurements taken on the same patient by the same operator in two distinct occasions, divided by the mean value of the difference [13]. Mean absolute differences for intraobserver variability were 2.0 ± 0.4 % (range 1.5 - 2.6%) for transmitral flow indices, and 1.3 ± 0.3% (range 0.8 - 1.7%) for pulsed wave tissue Doppler variables.

Diagnosis and grading of DD. Patients were evaluated using an algorithm recently proposed by our group (**algorithm A**) [9], which incorporates serial positioning of parameters of atrioventricular remodeling (LAVI, LVMI), mitral flow Doppler (E/A), tissue Doppler (E´, E´/A´) and a surrogate for LV end-diastolic pressure (E/E´). Additionally, the algorithm takes

into account the effects of age in diastolic function, using age-adjusted reference values as cut off points.

Diastolic function was categorized in 4 stages (grades of DD): normal diastolic function (ND), impaired relaxation (IR), pseudonormal patttern (PN), and restrictive (RE) pattern [14].

Internal consistency of the algorithm. The concordance between the diagnosis and grading of DD and the clinical and echocardiographic parameters was assessed, to corroborate the internal consistency of the age-adjusted algorithm. Correlation between age and echocardiographic parameters was tested, and the characteristics of patients with and without DD were compared and contrasted. Subsequently, clinical characteristics of patients were evaluated with respect to their DD grade. Next, TMD and TDI variables were tested to evaluate if they presented the expected behavior (bimodal and unimodal, respectively, according to their preload dependency)[15]. Finally, the algorithm´s discriminating value was tested using oneway ANOVA, calculating the F statistics, and comparing it with the calculated F critical value at 95% probability level [16, 17, 18].

External consistency of the algorithm. External validity of the age-adjusted algorithm was tested by comparison with two well-known algorithms: algorithm **B**, that uses unadjusted values for mitral and pulmonary venous inflow parameters and fixed E/E´ cut off of 10 [19]; and algorithm **C**, that suggests distinct approaches for patients with and without systolic dysfunction as determined by EF < or >50% [20].

Both alternative algorithms were used to diagnose and grade DD in the study sample, and compared to the age-adjusted algorithm. The prevalence of DD and the prevalence of each grade of DD were defined according to each of the algorithms. Subsequently, a test was performed to find out whether the age-adjusted algorithm could adjust for the effect of age on the prevalence of DD when compared to the alternative algorithms.

Clinical and echocardiographic characteristics of patients with a discordant diagnosis in the three algorithms were compared to determine if these characteristics support the classification made by the age-adjusted algorithm.

Statistical analysis. Continuous variables were presented as mean ± standard deviation (SD). Categorical variables are displayed as numbers and percentages. Comparisons between subjects from PVC and HFC samples, as well as subjects with normal and abnormal diastolic function were performed using independent samples t test (or Mann-Whitney´s test when assumptions for the t tests were not satisfied) for continuous variables and chi-square tests for categorical variables. The discriminating value of age-adjusted algorithm for DD grading was tested comparing the mean E´ and mean E/E´ in the four grades of DD using the Oneway ANOVA. Posthoc tests included Bonferroni, Levene´s test for homogeneity of variances, Welch and Brown-Forsythe statistics and correction of Games-Howell when they apply. Corrected F statistics were calculated for E´ and E/E´, and compared with the calculated F critical value at a 95% probability level.

McNemar´s test was used to determine if there were differences in diagnostic conclusions using different algorithms to diagnose any grade of DD. The strenght of association between general characteristics and age was tested using the Spearman rank correlation test. All hypothesis testing were two-sided, and $p < 0.05$ was considered significanct. Results were analyzed using software SPSS version 15.0 (SPSS Inc., Chicago, Illinois, 2006).

RESULTS

Clinical and echocardiographic variables. The complete study sample included 269 patients (41% male, 60 ± 12 years [range 21 – 89 years], body mass index 28 ± 4 kg/m^2, EF

64 ± 12% [range 23% – 88%], LVMI 126 ± 42 g/m^2, LAVI 37 ± 10 ml/m^2. Mean E wave was 76 ± 20 cm/s, A wave was 74 ± 23 cm/s, E/A ratio was 1.15 ± 0.60, E´ 8.2 ± 3.0 cm/s, A´ 10.0 ± 2.7 cm/s, E´/A´ ratio 0.84 ± 0.32, and E/E´ ratio 10.8 ± 6.1.

Patients originating from PVC and HFC samples were of similar age (61 ± 15 versus 59 ± 9 years, p = 0.24) and BMI (28 ± 3 versus 28 ± 4 kg/m^2, p = 0.64). However, patients from the HFC sample had lower EF (59 ± 15 vs. 69 ± 7 %, p <0.001), and higher heart rate (80 ± 13 vs 74 ± 10 bpm, p = 0.021), LVMI (147 ± 44 vs.106 ± 28 g/m^2, p <0.001) and LAVI (40 ± 11 vs. 33 ± 8 ml/m^2, p <0.01) than subjects from the PVC sample. HFC subjects also had lower myocardial relaxation velocities (E', 6.3 ± 2.2 vs. 10.0 ± 2.6 cm/s and A', 8.7 ± 2.4 vs.11.2 ± 2.5 cm/s, p <0.001), higher E/A ratio (1.28 ± 0.81 vs. 1.02 ± 0.28, p <0.001) and higher E/E' ratio (14.3 ± 6.9 vs. 7.5 ± 2.4, p <0.001) than patients from the PVC sample, indicating that their hearts were more severely affected, with a higher prevalence of clinical HF and LV dysfunction. The complete study sample comprises patients with a wide range of EF, ages (spanning from the third to ninth decade), and covers the entire spectrum of diastolic LV dysfunction, from slow LV relaxation to high diastolic LV stiffness. This population presents the characteristics which are considered ideal for the validation of a proposed diagnostic flowchart [21].

Age and echocardiographic parameter correlations. No significant correlation was found between EF and age. Negative correlations were found for age and E/A ratio (r = - 0.315), E´ (r = - 0.448) and E´/A´ ratio (r = - 0.398) (p <0.001 for all correlations). A positive correlation was found for age and E/E´ (r = 0.316, p <0.001). These findings support the use of age-adjusted cut-offs for those variables.

LVMI and LAVI both showed a weak, although significant, correlation with age (r = 0.165, p = 0.007, and r = 0.203, p = 0.01, respectively). However, age was responsible for

only 2.7% and 4% of the variability of these parameters, respectively, supporting the use of fixed cut-offs for these parameters in the algorithm.

Characteristics of patients with diastolic dysfunction. Applying the age-adjusted algorithm to the entire population, patients were classified as ND (n = 75, 28%) or DD (n = 194, 72%) (Table 1). Patients with DD were of similar age (59 ± 13 vs. 60 ± 9 years, p = 0.850) and higher heart rate (77 ± 11 vs. 73 ± 10 bpm, p = 0.049), BMI (28.7 ± 3.9 vs. 27.5 ± 3.2 kg/m^2, p = 0.017), LVMI (133 ± 42 vs. 108 ± 37 g/m^2, p <0.001), LAVI (38.3 ± 10.4 vs. 32.8 ± 8.3 mL/m^2, p <0.001) and E/E´ ratio (12.2 ± 6.6 vs. 7.0 ± 2.0, p <0.001) when compared to patients with ND. They also presented lower EF (62 ± 13 vs. 67 ± 9%, p = 0.005) and E´ velocity (7.1 ± 2.5 vs. 10.9 ± 2.5 cm/s, p <0.001), and significantly worst NYHA class (DD: class I 53, class II 21, class III 20, class IV 6% vs. ND: class I 93, class II 4, class III 3, class IV 0%, p for trend <0.001), compared to ND subjects.

LAVI was increased in 74% of the DD patients, but also was increased in 44% of ND patients. LVMI was increased in 78% of DD patients and in 48% of ND patients. These numbers corroborate the notion that we cannot use LAVI and LVMI as isolated criteria for the diagnosis of DD, because of high number of false-positive [22].

DD graded according to the age-adjusted algorithm showed ND, IR, PN and RE in 75 (28%), 95 (35%), 72 (27%) and 27 (10%) patients, respectively (Table 1). PN patients were slightly younger than RE patients. The RE group had the highest mean heart rate and the lowest EF, A and A'. TMD parameters (E velocity and E/A ratio) presented the expected bimodal aspect, with significant reduction in the IR group and "pseudonormalization" as DD progresses (Figure 1). TDI parameters showed the expected preload independency; E´ progressively and steadily decreased (and E/E' progressively increased) from ND to IR, PN and RE groups.

Discriminatory power of the algorithm. The overall discriminatory power for DD grading of the age-adjusted algorithm A was tested using oneway ANOVA and comparing corrected F statistics for E´ and E/E´ with the calculated critical value for F at 95% probability level for these two variables (which was 2.64). The algorithm had an observed value of corrected F much higher than critical value, for E´ [$F_{(3,265)} = 77$] and for E/E´[$F_{(3,265)} = 61$, $p < 0.001$ for both variables against F critical value]. These high F values indicate that the age-adjusted algorithm can discriminate accurately the grades of diastolic dysfunction and separate precisely mean E´ and mean E/E´ for patients with progressive DD.

DD prevalence according to each algorithm. DD prevalence with age-adjusted algorithm was 72%; with algorithm B 67% ($p = 0.124$ vs. algorithm A); and with algorithm C, 38% ($p < 0.001$ vs. algorithm A). In patients with EF ≥50%, DD was present in 30% according to algorithm A, in 63% according to algorithm B ($p = 0.067$ vs. algorithm A) and in 30% according to algorithm C. In patients with EF <50%, DD was present in 91%, 97% and 100%, using algorithms A, B and C respectively (all p values >0.05).

The prevalence of each grade of DD with algorithm A was as follows: 28% ND, 35% IR, 27% PN, 10% RE; with algorithm B, 33% ND, 32% IR, 23% PN, 12% RE; with algorithm C, 62% ND, 14% IR, 13% PN, 11% RE. These comparisons suggest that algorithm A has a greater ability to detect IR and PN than algorithm B and C ($p < 0.001$ for both comparisons).

Discriminatory value of the algorithms across gradings of DD. The prevalence of age-adjusted increase in E/E´ was 0% in ND, 15% in IR, 100% in PN, and 100% in RE patients, according to algorithm A; 14% in ND, 25% in IR, 75% in PN, and 100% in RE, according to algorithm B; and 23% in ND, 40% in IR, 82% in PN, and 100% in RE according to algorithm C.

Adjustment for age effect in the prevalence of DD. Using algorithm A, mean age was similar in patients with and without DD (59 ± 13 vs. 60 ± 9 years, $p = 0.85$); using algorithm B, mean age was significantly greater in patients with DD than ND patients (62 ± 12 vs. 55 ± 10 years, $p = 0.009$); the same occurred using algorithm C (66 ± 12 vs. 56 ± 11 years, $p < 0.001$). Patients with DD only according to algorithm B were older than those who had DD according to both algorithms (68 ± 7 vs 52 ± 12 years, $p < 0.001$). Likewise, the mean age of patients who had DD according to algorithm C (but not algorithm A) was greater than patients with DD by both algorithms (68 ± 6 vs 54 ± 11 years, $p = 0.008$).

Categorizing age groups by decade, the prevalence of DD using algorithm A was not influenced by age group either in patients with EF <50 % ($p = 0.719$) or in patients with EF $\geq$ 50% ($p = 0.139$). Otherwise, the prevalence of DD using algorithm C was influenced by age in the group of patients with EF $\geq$50 % ($p < 0.001$, Cramer´s V 0.467, indicating that age was responsible for 22 % of prevalence of DD in this group). In patients with EF <50 %, a measure of association was impossible to compute (prevalence of DD in this group is 100 % according to algorithm C).

Comparison of discordant patients. Clinical and echocardiographic characteristics of patients diagnosed discordantly by the different algorithms was used to determine their consistency. Table 2 shows the comparison of patients with a discordant classification according to algorithms A and B ($n = 61$). Patients with DD only according to algorithm B (but not algorithm A), when compared to patients with DD according to both algorithms, were older, less likely to come from the HFC sample (the sicker patients), had a more favorable NYHA classification, higher mean E´ and E´/A´, and lower mean E/E´. Patients with DD only according to algorithm A, when compared to patients classified as normal diastolic function

by both algorithms, tended to be younger, were more likely to come from the HFC sample, and had lower mean values of E´ and E´/A´. They also had higher mean values of E/E´, LAVI, LVMI, and had a higher prevalence of prognostically worse forms of LV remodeling.

Table 3 shows the comparison of patients with discordant classification according to algorithms A and C (n = 104). DD patients according to algorithm C (but not to algorithm A), when compared to patients with DD according to both algorithms, were less likely to come from the HFC sample (the sicker patients), had a more favorable NYHA classification and remodeling patterns, had a higher mean E´ and E´/A´, and lower mean E/E´. Patients with DD only according to algorithm A, when compared to patients classified as ND by algorithms A and C, were younger, more likely to come from the HFC sample, and had greater LVMI and higher prevalence of worse forms of LV remodeling and NYHA class. They also had lower mean values of E´ and E´/A´, and higher mean values of E/E´.

In the subgroup with EF ≥50 %, patients with DD only according to algorithm A had a lower mean E´ (8.9 ± 1.9 vs 11.1 ± 2.4 cm/s) and E´/A´ (0.79 ± 0.23 vs 1.14 ± 0.34 cm/s), and a higher E/E´ ratio (8.5 ± 2.3 vs 6.9 ± 1.8, p <0.001 for the 3 comparisons) compared to patients classified as ND according to both algorithms. In the subgroup with EF <50 %, patients with DD only according to algorithm C had a greater mean E´ (9.3 ± 4.1 vs 4.8 ± 1.5 cm/s, p <0.001) and E´/A´ (1.17 ± 0.41 vs 0.69 ± 0.19 cm/s, p = 0.001), and a smaller E/E´ ratio (8.3 ± 3.6 vs 20.7 ± 8.3, p = 0.017) compared to patients classified as having DD by both algorithms.

DISCUSSION

In the present study, an algorithm for diagnosis and grading of DD using serial positioning of multiple echocardiographic parameters and age-adjusted reference values for each Doppler parameter was tested noninvasively in a wide range sample. The results indicate that this algorithm has good discriminatory power for diagnosis and grading of DD, prevents the confounding effect of the physiological aging process in the detection of DD, precluding misdiagnosis, and performs favorably when compared to two other well-known algorithms, either in patients with or without systolic dysfunction.

Serial positioning. Evaluation of diastolic function is a difficult task because of the multiple influences onr LV filling, the limitations of each echocardiographic parameter, and the effect of various physiologic determinants including the normal aging process. In order to overcome these limitations, many authors suggest a comprehensive evaluation of diastolic function using different techniques and parameters [23]. In agreement with this recommendation, the serial positioning of different parameters was introduced in a recently proposed diagnostic flowchart, because of concerns about low specificity for stand-alone diagnosis of DD, specially for the E/E´ ratio [21, 24]. In the proposed age-adjusted algorithm [9], serial positioning of distinct diastolic parameters was also used. This strategy proved valuable, and yielded an increased performance for the diagnosis of DD.

Accordingly, Doppler transmitral pattern associated with TDI variables and E/E´, plus evaluation of LAVI and LVMI, when looked at in combination, were sufficiently reliable to recognize abnormal diastolic function, to grade and follow the progression of DD from abnormal relaxation to a pseudonormal and restrictive pattern. Furthermore, serial analysis of these complementary parameters allowed predicting LV filling pressure [25].

Effect of the aging process. The normal aging process have a significant impact on diastolic parameters, by means of intrinsec myocardial abnormalities, independent of ischemia and LV hypertrophy [26]. As a consequence, all measurements of long-axis diastolic function and mitral inflow velocities need to be corrected by age [27]. Therefore, age-dependent cut-offs should be taken into account when classifying patients as having DD [7, 28]. The unadjusted cut-offs commonly used to define DD may be inadequate for the evaluation of elderly patients, potentially leading to misclassification and overdiagnosis [27].

In the present study, with the use of an age-adjusted algorithm, mean age of patients with and without DD was similar, differently from other 2 algorithms. Also, the prevalence of DD using this algorithm was not influenced by age group neither in patients with EF <50% nor in patients with EF ≥50%. With the use of alternative algorithms, the prevalence of DD was influenced by age, which was responsible for 22% of prevalence of DD in patients without systolic dysfunction. These findings suggest that the age-adjusted algorithm can isolate and adjust for the effect of age on DD prevalence. This is an important property of the algorithm, and suggests that it can prevent misdiagnosis due to the use of fixed cut-off values.

Internal consistency of the age-adjusted algorithm. In order to demonstrate internal consistency, each echocardiographic parameter used in the algorithm was correlated with age, to show its influence in diastolic function. Correlations between age and parameters from TMD, TDI and the E/E´ ratio were found as previously demonstrated by a number of authors [7, 29-32]. In contrast to previous reports [33-36], both LAVI and LVMI showed a weak (although significant) correlation with age. However, age was responsible for only 3 - 4% of the variability of these parameters in our patients, supporting the use of fixed cut-offs for this

parameters in the algorithm. This is in accordance with an echo substudy of the Strong Heart Study, in which the authors found and specifically stated that it does not seem necessary to adjust partition values for age to recognize elevated LVMI [37].

Moreover, clinical and echocardiographic variables of patients with and without DD according to the age-adjusted algorithm were compared. Patients with DD were younger, had higher heart rate, LVMI and LAVI, as well as lower EF. E´ velocity was significantly lower, and E/E´ was significantly higher than in patients with normal diastolic function. All these findings are consistent with the presence of DD, suggesting an adequate diagnostic power of age-adjusted algorithm.

Subsequently, characteristics of patients within each grade of DD were analyzed. The RE group had the highest mean heart rate and the lowest EF, A wave and A´ wave, reflecting the fact that they were sicker patients and the final failure of LA contraction to compensate for the severe, longstanding increase in LV filling pressures. For LVMI, LAVI, and E/E´, the behaviour was exactly as expected; patients with ND had similar values as compared to IR, then increasing steadily and significantly as DD worsened until the highest values in the RE patients. TMD variables (E velocity and E/A ratio) presented the expected bimodal aspect, with significant reduction in the IR group and "pseudonormalization" as DD progresses. This behavior illustrates the "U-shaped" conformation of TMD variables [15]. E´ and E´/A´ ratio showed preload independence, decreasing progressively from ND to IR to PN and RE. All parameters behaved exactly as expected if a accurate discriminatory power for classification and grading of DD by the age-adjusted algorithm is to be believed.

Comparison with other algorithms. External consistency of algorithm A was tested comparing the prevalence and grading of DD with the prevalence and grading obtained by the use of two other algorithms (B and C). The prevalence of DD was greater with algorithm

A than algorithm B and C, and it was higher than previously published community-based studies, which report a prevalence of 27-35% using a comprehensive, non-age adjusted transmitral and tissue Doppler analysis (with or without pulmonary venous flow) [1, 15, 38]. In a more selected, hypertensive population from a tertiary referral clinic, and using the algorithm of the Canadian Consensus guidelines [4], the prevalence of DD was 59% [39], which is still lower than our population (72%) because HF patients were excluded in that study.

The discriminatory value of the 3 algorithms across the entire range of DD was assessed by the analysis of E/E´, which is is a surrogate for LV end-diastolic pressure; if DD grading algorithm is accurate, E/E´ is expected to rise in all patients in the PN and RE groups, which are characterized by high preload. It can rise in a subgroup of patients with impaired relaxation, and is expected to stay below the age-specific normal cut-off point in patients with normal diastolic function[11]. DD groups classified by algorithm A behave exactly as described; the prevalence of age-specific elevation of E/E´ was 0% in ND, 15% in IR, 100% in PN, and 100% in RE. However, DD groups classified by algorithms B and C have some inconsistent values; prevalence of age-specific elevation of E/E´ is 14% and 23% in ND groups; and of less than 100% (75% and 82% respectively) in PN patients. These values consistently indicate a higher discriminatory power of algorithm A over algorithms B and C.

Subsequently, the characteristics of discordant patients according to the distinct algorithms were compared to determine their consistency. Patients classified as having DD by algorithm A (and as normal by B or C) had lower myocardial velocities, higher diastolic pressure and atrioventricular remodeling. When algorithm A classified patients as normal (in opposition to B and C), they had higher myocardial velocities and lower diastolic pressure. In both cases, echocardiographic characteristics support the diagnosis made by algorithm A, irrespective of systolic function.

Limitations. The results of the present study should be evaluated under the scope of its potential limitations. First and more importantly, it is a noninvasive evaluation, and patients were classified based on multiple validated clinical and echocardiographic parameters, without invasive confirmatory measurements. However, in accordance with the present study, there are several other studies which evaluated DD noninvasively using a similar strategy [40-41]. Previous studies have stated the validity of this combination of parameters in selected groups of patients [21, 40]. Even so, an important perspective is to proceed with an invasive validation of the age-adjusted algorithm in a study using the conductance catheter approach to confirm the performance of the proposed algorithm [42, 43].

Another potential limitation is that myocardial velocities were measured only in the lateral portion of the mitral annulus. It was probably an accurate measure of global LV diastolic function, since our patients had no significant regional asynergy in this wall. Additionally, a number of authors also preferred the lateral annulus because they are less preload dependent in subjects with normal LV function, are not influenced by right ventricular diastolic function, and showed the best correlation coefficients with invasively measured indexes of diastolic relaxation and filling pressures [6, 43, 44].

The age-adjusted algorithm does not include evaluation of E deceleration time (DT), pulmonary venous flow analysis, and transmitral Doppler during a Valsalva maneuver. These techniques are sometimes difficult and demanding for the patient, and they have a high variability and low percentage of adequate measurements. Additionally, the proposed algorithm had the same strategy for patients with and without reduction of EF, as suggested by others [1]. It is reassuring that the algorithm did better than the alternative ones, that use different strategies for patients with EF < or > 50%.

Preload dependency of E´ $_{lat}$ may occur even in severely affected ventricles, particularly at the extremes of higher filling pressures. In young patients with dilated cardiomyopathy, occasionally a pattern of severe DD in TMD evaluation with (false) normal E´ can be seen, a pitfall which does not occur with the use of septal mitral annulus [45]. In the present study, this situation presented in only two patients. Despite this failure of TDI to recognize severely reduced intrinsic myocardial relaxation, both patients were adequately classified by the algorithm, based on the other parameters.

In summary, the proposed age-adjusted algorithm consistently separates patients with normal and abnormal diastolic function and adequately grades DD. Compared to other two well-known algorithms, it performs well and prevents the confounding effect of the physiological aging process. The proposed algorithm provides a useful tool that can be used in daily practice and in clinical trials, potentially avoiding misdiagnosis in younger and elderly patients, and increasing the precision and accuracy of DD diagnosis.

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FIGURE 1
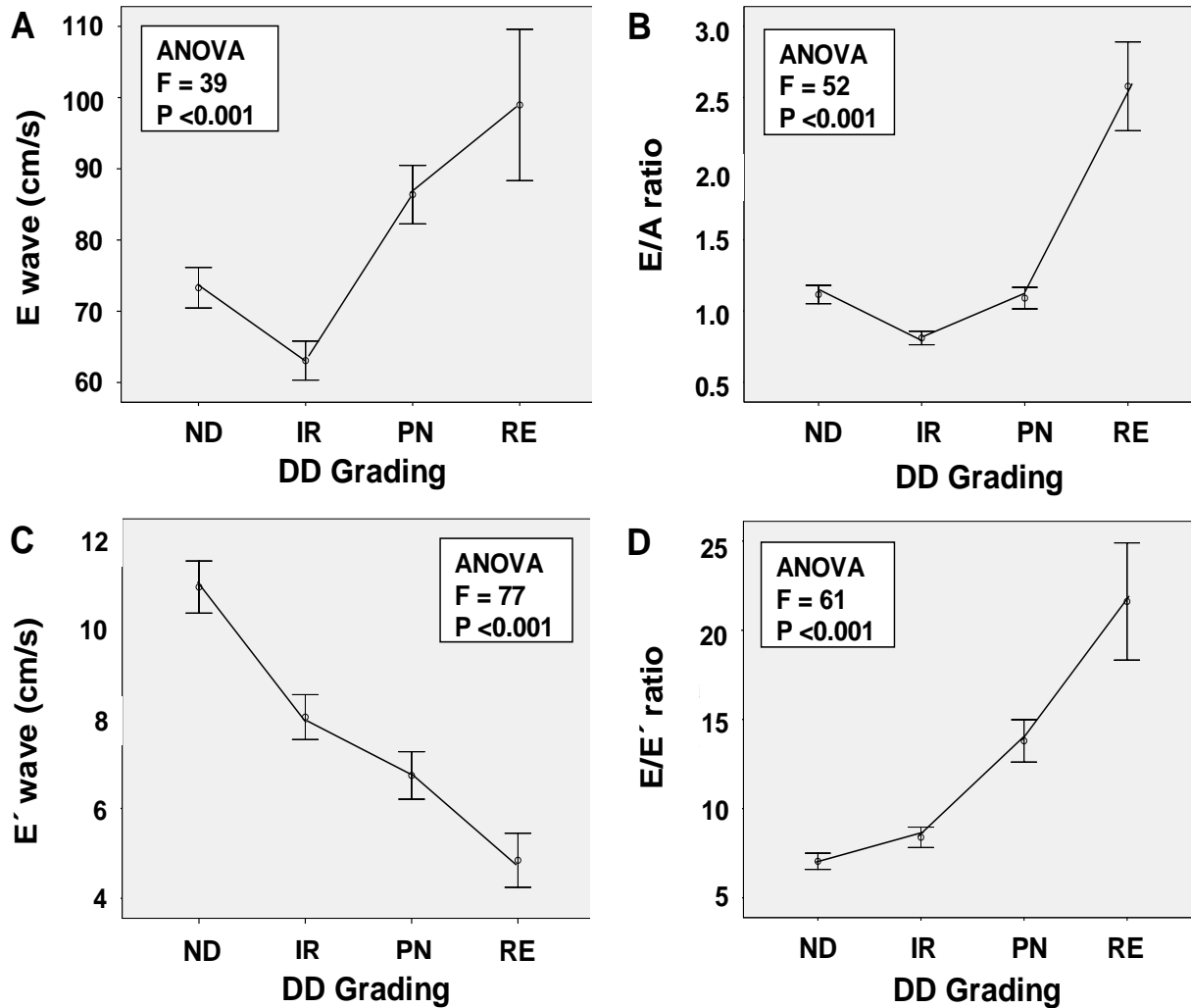
Figure 1. Distribution of Doppler parameters across grades of diastolic dysfunction. Transmitral flow Doppler parameters (**A** and **B**) showed bimodal behavior, decreasing in subjects with impaired relaxation and increasing in subjects with increased atrial pressures (PN and RE). Tissue Doppler parameters (**C** and **D**) showed unimodal behavior (E´ steadily increased and E/E´ decreased as DD worsened). ND = normal diastole; IR = impaired relaxation; PN = pseudonormal; RE = restrictive pattern; DD = diastolic dysfunction.

TABLES

Table 1. Clinical and echocardiographic characteristics of patients within each grade of diastolic dysfunction.

	Normal Diastole (N = 75)	Impaired Relaxation (N = 95)	Pseudo Normal (N = 72)	Restrictive Pattern (N = 27)	p
Age	60 ± 9 [ab]	61 ± 12 [ab]	57 ± 15 [a]	64 ± 11 [b]	0.029
Gender (Female n%)	42 (56) [a]	64 (67) [a]	45 (63) [a]	9 (33) [b]	0.013
BMI (kg/m^2)	27.5 ± 3.2	28.7 ± 4.5	28.7 ± 3.3	29 ± 2.9	0.104
Heart rate (bpm)	73 ± 10 [a]	77 ± 9 [a]	74 ± 12 [a]	86 ± 17 [b]	0.008
LVMI (g/m^2)	108 ± 37 [a]	115 ± 27 [a]	137 ± 43 [b]	182 ± 38 [c]	<0.001
LAVI (ml/m^2)	32.8 ± 8.3 [a]	33.3 ± 6.3 [a]	40.0 ± 9.1 [b]	51.0 ± 12.2 [c]	<0.001
Ejection fraction (%)	67 ± 9 [a]	67 ± 8 [a]	64 ± 11 [a]	42 ± 13 [b]	<0.001
E wave (cm/s)	73 ± 12 [a]	63 ± 13 [b]	86 ± 17 [c]	99 ± 27 [d]	<0.001 [*]
A wave (cm/s)	69 ± 17 [a]	81 ± 19 [b]	84 ± 24 [b]	39 ± 8 [c]	<0.001 [*]
E/A ratio	1.1 ± 0.3 [a]	0.8 ± 0.2 [b]	1.1 ± 0.3 [a]	2.6 ± 0.8 [c]	<0.001 [*]
E´ wave (cm/s)	10.9 ± 2.5 [a]	8.0 ± 2.4 [b]	6.7 ± 2.2 [c]	4.8 ± 1.5 [d]	<0.001
A´ wave (cm/s)	10.1 ± 2.2 [a]	11.5 ± 2.7 [a]	9.2 ± 2.3 [a]	7.0 ± 2.2 [b]	<0.001
E´/A´ ratio	1.1 ± 0.3 [a]	0.7 ± 0.2 [b]	0.8 ± 0.3 [b]	0.7 ± 0.2 [b]	<0.001
E/E´ ratio	7.0 ± 2.0 [a]	8.4 ± 2.8 [a]	13.8 ± 5.0 [b]	21.6 ± 8.3 [c]	<0.001

BMI= body mass index; LVMI= left ventricular mass index; LAVI= left atrial volume index; E= early transmitral Doppler blood flow; A= late transmitral Doppler blood flow; E/A= ratio of early and late transmitral Doppler blood flow; E´= early annular tissue Doppler velocity; A´= late annular tissue Doppler velocity; E´/A´= ratio of early and late annular tissue Doppler velocity ; E/E´= ratio of early transmitral Doppler blood flow and early annular tissue Doppler velocity;.

* "U-shaped" configuration, with "pseudonormalization" of transmitral flow parameters [elevation (E, E/A) or reduction (A)] in the stages characterized by preload compensation.

Table 2. Characteristics of the 61 patients with discordant classification according to algorithms A and B.

	Diastolic Dysfunction			Diastolic Dysfunction		
	Present according to A and B (n = 157)	**Present only according toB** (n = 24)	**p**	**Absent according to A and B** (n = 51)	**Present only according to A** (n = 37)	**p**
Age (years)	61 ± 12	68 ± 7	<0.001	57 ± 8	52 ± 12	0.06
E´ (cm/s)	6.5 ± 2.2	8.6 ± 1.4	<0.001	12.0 ± 2.1	9.6 ± 2.0	<0.001
E´/A´	0.7 ± 0.2	0.9 ± 0.2	<0.001	1.2 ± 0.3	0.9 ± 0.3	<0.001
E/E´	13.2 ± 6.9	8.5 ± 1.9	<0.001	6.4 ± 1.6	8.1 ± 1.6	<0.001
LVMI (g/m^2)	137 ± 44	133 ± 39	0.969	96 ± 30	114 ± 25	0.004
LAVI (ml/m^2)	39.5 ± 10.8	38.8 ± 9.7	0.989	29.9 ± 5.8	33.2 ± 6.2	0.012
HFC sample (%)	66	34	<0.001	6	41	<0.001
NYHA class (%)						
I	46	79	<0.001	100	81	<0.001
II	22	13		0	19	
III	25	8		0	0	
IV	7	0		0	0	
LV Remodeling (%)			<0.001			<0.001
Normal	11	17		45	14	
Concentric remodeling	10	4		22	16	
Eccentric hypertrophy	33	25		25	30	
Concentric hypertrophy	46	54		8	40	

E´= early annular tissue Doppler velocity; E´/A´= ratio of early and late annular tissue Doppler velocity ; E/E´= ratio of early transmitral Doppler blood flow and early annular tissue Doppler velocity; LVMI= left ventricular mass index; LAVI= left atrial volume index; HFC= heart failure clinic; NYHA= New York Heart Association;.

Table 3. Characteristics of 104 patients with discordant classification according to algorithms A and C.

	Diastolic Dysfunction			Diastolic Dysfunction		
	Present according to A and C (n = 96)	Present only according toC (n = 6)	p	Absent according to A and C (n = 69)	Present only according to A (n = 98)	p
Age (years)	65 ± 12	68 ± 6	0.875	60 ± 9	54 ± 11	0.007
E´ (cm/s)	5.2 ± 1.4	8.7 ± 2.9	<0.001	11.1 ± 2.4	8.9 ± 1.9	<0.001
E´/A´	0.7 ± 0.2	1.0 ± 0.3	<0.001	1.1 ± 0.3	0.8 ± 0.2	<0.001
E/E´	16.1 ± 7.2	7.9 ± 3.2	0.002	6.9 ± 1.8	8.5 ± 2.3	<0.001
LVMI (g/m^2)	150 ± 42	175 ± 38	0.642	102 ± 30	115 ± 34	0.041
LAVI (ml/m^2)	43.6 ± 10.9	40.6 ± 11.3	0.916	32.1 ± 7.7	33.1 ± 6.3	0.818
HFC sample (%)	66 %	34%	<0.001	10 %	38%	<0.001
NYHA class (%)						
I	46	79	<0.001	96	78	<0.001
II	22	13		4	19	
III	25	8		0	3	
IV	7	0		0	0	
LV Remodeling (%)						
Normal	6	0	0.001	39	17	<0.001
Concentric remodeling	4	0		17	41	
Eccentric hypertrophy	41	67		22	17	
Concentric hypertrophy	49	33		22	25	

E´= early annular tissue Doppler velocity; E´/A´= ratio of early and late annular tissue Doppler velocity ; E/E´= ratio of early transmitral Doppler blood flow and early annular tissue Doppler velocity; LVMI= left ventricular mass index; LAVI= left atrial volume index; HFC= heart failure clinic; NYHA= New York Heart Association.

4. ARTIGO 3.

Diastolic Dysfunction is Already Present in Pre-Diabetes.

Short running title: Diastolic dysfunction in prediabetes and diabetes

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ABSTRACT

OBJECTIVE. To assess the prevalence of LV diastolic dysfunction (DD) in patients with pre-diabetes (PD) and type 2 diabetes (DM), and to evaluate association between indices of glucose homeostasis and echocardiographic markers of DD.

RESEARCH DESIGN AND METHODS. Outpatients from a preventive clinic were classified as normal glucose metabolism (NGM), PD, and DM. LV function was assessed by echocardiography; measurements included left atrial volume index (LAVI), early diastolic mitral inflow (E), peak systolic (Sm´), and early diastolic (E´) myocardial velocities, and E/E´ ratio, a surrogate for LV end-diastolic pressure. DD was evaluated using an algorithm with multiple parameters and age-adjusted reference values, and classified as normal diastole (ND), impaired relaxation (IR), pseudonormal (PN), and restrictive (RE) pattern.

RESULTS. One hundred and forty patients were included (41% NGM, 42% PD, 17% DM). Systolic function was similar in all groups. Comparing to NGM, PD and DM subjects had increasingly higher LAVI and E/E´, and lower E´. Prevalence of DD increased from NGM to PD and DM (37%, 58%, and 83%, p for trend <0.001). On multivariable logistic regression, PD (OR 2.5 [1.1-5.7], p = 0.038) and DM (OR 9.7 [2.6-33.1], p = 0.001) were independently associated with DD after adjustment for confounding factors. There were linear associations between 2h plasma glucose and E´, and between A1C and E/E´; these associations were retained when DM patients were excluded from the analysis.

CONCLUSIONS. PD and DM are independently associated with DD, and there is a linear association between glycemic variables and DD parameters which encompasses the nondiabetic range. These findings may warrant early screening of LV function in patients with pre-diabetes.

Type 2 diabetes mellitus (DM) is a well-established risk factor for left ventricular (LV) dysfunction, and it is associated with a two- to fivefold increased risk of heart failure (HF) [1, 2]. This risk excess can be explained by the frequent DM association with dyslipidemia, arterial hypertension, coronary artery disease (CAD), and the occurrence of a specific diabetic cardiomyopathy (DCM) [3]. The earlier LV abnormality in DCM is diastolic dysfunction (DD) [4], which can predict progression to HF, even in this early stage. DD itself is associated with high mortality [3,5].

The natural history of DM includes a long phase on which dysglycemia increases, generating progressive metabolic derangements which ultimately progress to DM [6]. This phase is called prediabetes (PD) [7]. Surprisingly, the prevalence and impact of LV dysfunction in this early stage of dysglycemia is less well studied, although there are some evidence suggesting that DD possibly begins before DM is established [8, 9].

We hypothesized that the diastolic function parameters correlate with glycemic indexes in a continuum manner, and that DD could begin in the prediabetic stage of dysglycemia. Therefore, the aims of this study were to evaluate the prevalence of DD in patients with prediabetes; and to evaluate the association between the indexes of glucose homeostasis and the echocardiographic markers of LV function in the different degrees of dysglycemia.

RESEARCH DESIGN AND METHODS.

A cross-sectional study was performed in a consecutive sample of patients (≥45 yrs) without known heart disease attending an outpatient preventive cardiology facility at the Lutheran University of Brazil, between April 2006 and November 2008. Patients with ischemic heart disease, connective tissue disease, congenital heart disease, aortic disease,

moderate or severe valvular heart disease, atrial fibrillation or other arrhytmia that preclude adequate echocardiographic evaluation of DD were excluded. Patients with hepatic disease, malignancy, type 1 DM, serum creatinine ≥1.5 mg/dL or estimated glomerular filtration rate (eGFR) ≤60 ml/min/1.73 m^2 were also excluded. Ischemic heart disease was defined as a history of hospital admission because of chest pain, percutaneous coronary intervention, coronary artery bypass grafting, major ischemic alterations on the electrocardiogram as defined by the Minnesota codes 1.1-3, or ischemic response on treadmill exercise test and/or nuclear scintigraphy. Valvular heart disease was defined as stenosis or regurgitation of mitral and/or aortic valve. Metabolic syndrome was defined according to National Cholesterol Education Program Adult Treatment Panel III criteria [10]. All subjects provided written and informed consent. The institutional ethical review board of the participating institutions approved the protocol. The study was conducted in accordance with the declaration of Helsinki.

Clinical and laboratory evaluation. Patients underwent an interview and clinical examination to record demographic and anthropometric data. They were weighted in light clothes without shoes, and height was recorded. Body mass index (BMI) was calculated as weight (kilograms)/height (meters^2). Waist circumference was measured with a tape in horizontal plane around the abdomen at the level of the iliac crest, at the end of a normal expiration. Hip circumference was measured at the widest part of lower body, and the waist-hip ratio was calculated. Office blood pressure was measured with a mercury sphigmomanometer, using nondominant arm and with the patient in a sitting position, after a 10 min rest. The mean of two measurements was considered. The patient was categorized as hypertensive based on use of antihypertensive drugs and/or office blood pressure ≥140/90 mmHg.

Fasting plasma glucose (FPG) was measured by the glucose-peroxidase colorimetric enzymatic method (Biodiagnostica). Oral glucose tolerance test (OGTT) was performed measuring plasma glucose 2h after a 75g glucose load. Postprandial (PP) glucose was measured 2h after a standard mixed meal. Glycated hemoglobin (A1C) was measured by a high-performance liquid chromatography system (normal range 4-6%; Merck-Hitachi 9100). High sensitivity C reactive protein (hs-CRP) and urinary albumin excretion rate (UAER) were measured by immunoturbidimetry. Creatinine was measured by the Jaffé method; uric acid and the lipid profile by a colorimetric method. LDL cholesterol was calculated using the Friedewald formula. GFR was estimated using the Modification of Diet in Renal Disease (MDRD) formula: 186 x [plasma creatinine(mg/dL)$^{-1.154}$ x age (years)$^{-0.203}$ x (1.212 if black) x (0.742 if female) [11]. Plasma insulin was measured by radioimunoassay. Homeostasis Model Assessment (HOMA-ir) was used as an index of insulin resistance (HOMA-ir= plasma insulin (mU/mL) X FPG (mg/dL) X 0.0555 / 22.5), and HOMA-beta (%) as an index of insulin secretion (HOMA-Beta= 20 X Insulin (mU/mL) / [FPG (mg/dL) X 0.0555] – 3.5); they were not calculated in subjects using insulin (n = 2). Glycemic categories were defined by baseline FPG and 2-h glucose at OGTT. Subjects were classified in the following groups: normal glucose metabolism (NGM) (FPG <100 mg/dL, and 2 h plasma glucose <140 mg/dL); PD, elevated FPG (100-125 mg/dL) or 2h plasma glucose (140-199 mg/dL); or DM (FPG ≥126 mg/dl or 2h plasma glucose ≥200mg/dl, or previous use of antidiabetic medications) [12].

Echocardiographic evaluation: All patients underwent transthoracic echocardiogram performed by a single certified sonographer cardiologist who was blinded to the subjects´ clinical characteristics, using a commercially available equipment (GE Vingmed System Five, 2,5 Mhz transducer) according to a standardized protocol [13]. Two-dimensional (2D) and color Doppler imaging were performed to screen for valvular stenosis or regurgitation.

Measurements were made on line, and all parameters were determined using the average of ≥3 heart beats. Septal (SWTd) and posterior wall (PWTd) thicknesses, LV end systolic (LVESDi) and end diastolic (LVEDDi) internal dimensions were measured using M-mode or 2-dimensional echo, allowing calculation of LV mass indexed to body surface area (LVMI) [13]. Left ventricular hypertrophy was defined as a LVMI >95 g/m^2 for women and >115 g/m^2 for men [17]. Relative wall thickness (RWT) was calculated by the formula RWT = (2 X PWTd) / LVEDDi, and was considered increased if >0.42. LV remodeling patterns were defined as concentric remodeling (normal LVMI with increased RWT), concentric hypertrophy (increased LVMI with increased RWT), eccentric hypertrophy (increased LVMI with normal RWT) or absence of LV remodeling [13]. Left atrial volume index (LAVI) was obtained by measuring left atrium by biplane Simpson´s method [14], and subsequently indexing by BMI. Transmitral flow Doppler (TMD) velocities [peak velocities of early (E) and late (A) diastolic filling, the E/A ratio, and deceleration time of E (DT)] were recorded with the sample-volume in the tip of mitral valve leaflets in the apical four-chamber view [15]. For the pulsed-wave tissue Doppler imaging (TDI) sample-volume was placed at the lateral mitral annulus, and measurements included peak systolic (Sm´), early (E´), and late (A´) diastolic annular velocities. E´/A´ and E/E´ ratios were calculated [16].

Systolic function was evaluated by ejection fraction (EF) measured by modified Simpson´s method, fractional shortening (FS, calculated as LVEDDi minus LVESDi normalized for LVEDDi), and Sm´. Systolic dysfunction was defined as EF <50%, FS <27%/ < 25% (for women and men, respectively), or Sm´ below age-specific cut-off value (20-30y: <10 cm/s; 30-40y: <9.5 cm/s; 40-50y: <9 cm/s; 50-60y: <8 cm/s; >60y: <7.6 cm/s) [17, 18, 19]. Diastolic function was evaluated using an age-adjusted algorithm which incorporates serial positioning of multiple diastolic parameters from echocardiography [20]. Diastolic dysfunction

was categorized in four grades: normal diastolic function (ND), impaired relaxation (IR), pseudonormal patttern (PN), and restrictive (RE) pattern [21].

Intraobserver variability was assessed in 10 randomly selected subjects, and expressed as the mean percent error, derived as the absolute difference between two measurements taken on the same patient by the same operator in two distinct occasions, divided by the mean value of the difference [22]. Mean absolute differences for intraobserver variability were 2.0 ± 0.4% (range 1.5% to 2.6%) for transmitral flow indices, and 1.3 ± 0.3% (range 0.8% to 1.7%) for pulsed wave tissue Doppler variables.

Statistical analysis. Categorical variables were expressed as frequencies and percentages, and quantitative variables as mean ± standard deviation (SD) or median (interquartiles 25%-75%) unless otherwise specified. Data analysis were performed in two steps defined *a priori*. First, the patients were categorized according the glycemic groups in NGD, PD and DM and the echocardiograph parameters and diastolic function indexes were evaluated. In this setting, the association of PD with DD could be evaluated. Second, selected glycemic parameters were correlated with E' and E/E' in a continuous way, without groups categorization. These parameters reflect the diastolic function. This analysis was performed for all subjects and also after excluding those with DM, therefore, the effect of glycemic variables in diastolic function of non-diabetic patients could be evaluated.

Group comparisons were made by chisquare test (categorical data) or ANOVA followed by Bonferroni test, Levene´s test for homogeneity of variances, Welch and Brown-Forsythe statistics and correction of Games-Howell when they apply. Nonparametric group comparisons were made using Kruskal-Wallis H test. Multivariate logistic regression modeling was used to assess the association between glycemic category and the presence of DD, with adjustment for relevant clinical covariates and for all independent variables which

had a univariate significance level of p <0.10. Event number per independent variable ratio of 10:1 was respected. Importance of the contribution of PD and DM for the regression model was tested with Wald statistics; the presence of multicolinearity and statistical stability of the model were tested evaluating the correlation between groups and constant, and the magnitude of standard error for each variable. Model discrimination was tested by calculating the overall accuracy of the model to predict the outcome, and by calculating the area under the ROC (receiver operating characteristics) curve. Goodness of fit was tested by the Hosmer-Lemeshow test [23, 24]. Associations between selected glycemic variables and diastolic parameters were estimated using Spearman Rank correlation test, in all patients and subsequently excluding patients with DM. All tests were two-sided and were considered significant at a level of 0.05. All analysis were conducted with SPSS software, version 15.0 (SPSS Inc, Stanford USA). All authors have full access to the data, have read and agreed to the manuscript as written, and take responsability for its integrity.

RESULTS.

General characteristics of the study subjects. The study group was composed of 140 patients (34% men, mean age 59 ± 9 years [range 45.5 – 80.9 years], BMI = 28.5 ± 4.4 kg/m^2). The prevalence of hypertension in the whole sample was 79%. No patient had ischemic heart disease or symptomatic HF. Patients were classified as NGM (57 patients, 41 %), PD (59 patients, 42%), or DM, (24 patients, 17%). Subjects in the DM group had a median DM duration of 4.2 years (2.7 – 4.7 years).

Baseline characteristics according to category of glycemic homeostasis. Patients from groups PD and DM, when compared to NGM group, had a larger waist and a

higher prevalence of metabolic syndrome (Table 1). The mean levels of hs-CRP and uric acid were higher, and HDL-cholesterol progressively lower from NGM to PD to DM. Pacients in the DM group had higher BMI and UAER than NGM and PD groups. There were no significant differences on gender, systolic and diastolic blood pressure, hypertension prevalence, heart rate, total cholesterol and triglycerides among groups. There were no differences in the use of cardiac medications. As expected, DM patients use more antidiabetic drugs (Metformin, 71%; sulphonylureas, 37%; insulin, 8%; glitazones, 4%; acarbose, 4%).

All variables associated with glycemic homeostasis progressively increased from NGM to PD and DM (fasting glucose: 87 ± 7 vs. 101 ± 10 vs. 151 ± 56 mg/dL; 2h plasma glucose: 105 ± 21 vs. 146 ± 29 vs. 235 ± 50 mg/dL; PP glucose: 88 ± 13 vs. 97 ± 16 vs. 152 ± 92 mg/dL; A1C 5.6% ± 0.5 vs. 5.9% ± 0.5 vs. 7.3% ± 1.3, p <0.001 for all comparisons). All variables reflecting insulin homeostasis showed similar results [fasting insulin 7.3 (5.1 – 11.4) vs. 9.8 (6.8 – 16.0) vs. 12.9 (9.3 – 23.6), χ^2 19.9, p <0.001; HOMA-ir 1.6 (1.1 – 2.5) vs. 2.6 (1.7 – 3.9) vs. 4.8 (3.4 – 7.6), χ^2 7.8, p = 0.021; HOMA-beta 108 (74 – 176) vs. 106 (70 – 162) vs. 74 (47 – 125), χ^2 43.9, p <0.001, respectively for NGM, PD and DM group, p<0.001].

Echocardiographic measurements. All three systolic parameters were similar across groups; accordingly, the prevalence of systolic dysfunction was also similar among the three groups (Table 2). Compared to NGM group, patients from PD and DM groups had a higher mean LAVI. LV concentric hypertrophy was present in 21% vs. 27% vs. 46% of patients from NGM, PD and DM groups, respectively (p = NS). All other conventional echocardiographic parameters, as well as the frequency of adverse LV remodeling, were comparable in the three groups. Transmitral diastolic flow variables had similar values in the

three groups, except for A wave, which was higher in PD group compared to both other groups (p <0.001 PD vs. NGM and DM groups). In contrast, tissue Doppler diastolic velocities (E´ and E´/A´) were progressively lower as dysglycemia progresses from NGM to PD and to DM groups. LV end diastolic pressure index (E/E´ ratio), a well-known indicator of DD, was significantly higher in PD and DM groups compared to NGM group.

Prevalence of DD in each category of glycemic homeostasis. DD prevalence increased steadily across the three groups of dysglycemia (Figure 1A), from 37% in the NGM group, to 58% in the PD group, and 83% in the DM group (p for trend <0.001). The severity of DD also increased as dysglycemia became worse; the prevalence of IR and PN patterns were progressively higher as dysglycemia progresses from NGM to PD to DM (Figure 1B). These findings were not significantly influenced by gender, as prevalence of DD both in male and female subjects were similar (data not shown).

Multivariable analysis. In multivariate logistic regression, PD and DM were independently associated with DD after adjustment for waist, systolic blood pressure, presence of metabolic syndrome, hs-CRP, UAER, serum creatinine and HDL cholesterol (Table 3). In this model, correlation between group and constant was low (-0.027), and the magnitude of standard error for each variable was quite low (0.048 to 1.031), suggesting that there was no significant multicolinearity and that regression model is stable. Overall accuracy of the model to predict subjects having the outcome (with a predicted probability of 0.5 or greater) was 66% (sensitivity 84%, specificity 76%, positive and negative predictive value 62% and 70% respectively), and the area under the ROC curve was 0.751, indicating a good model discrimination; optimal sensitivity was 65% and optimal specificity was 76% The Hosmer-Lemeshow goodness-of-fit test resulted in a chi-square of 7.565 (p = 0.477),

indicating that the observed and predicted probabilities closely match and the model adequately fits.

Associations between glycemic variables and diastolic parameters. Correlations between 2h OGTT plasma glucose and A1c with E' and E/E´ were tested. Figure 2A shows a significant moderate negative correlation between 2h plasma glucose and E' (r = - 0.311, p <0.001). This association was also present in the nondiabetic range, as the diabetic patients were excluded (r = - 0.262, p = 0.005, Figure 2.B). A significant moderate positive correlation between A1C and E/E´ was observed (r = 0.282, p = 0.001, Figure 2.C), which was also retained when patients with DM were excluded (r = 0.273, p = 0.003, Figure 2.D).

DISCUSSION.

In this study, the prevalence and severity of DD was higher in prediabetes, and even higher in type 2 diabetes, as compared to euglycemia. These differences were independent of age, gender, systolic function, LV hypertrophy, blood pressure, BMI, waist, metabolic syndrome, CAD, hs-CRP, medications in use, and albuminuria. PD and type 2 DM had also higher values of LAVI and diastolic pressures (as indicated by higher E/E´ ratio), and lower myocardial diastolic TDI velocities. These Doppler parameters correlated linearly with glycemic variables, even in the nondiabetic range.

Traditional Doppler assessment of diastolic function has been semiquantitative, with a poor concordance, and requires important improvement [25]. Proposed refinements include a combined approach, including age-adjusted parameters and new modalities of M-mode and tissue Doppler in addition to the already used TMD criteria [26, 27]. In this context, studies evaluating the DD prevalence in DM patients had conflicting results. Isolated TMD-based evaluation are likely to find lower prevalence (~ 47%) because of lower sensitivity [8, 28]. Other

authors were not able to find increased prevalence of DD in asymptomatic normotensive DM subjects, although they found an increase in the A wave, that could reflect incipient DD [29]. TDI-based studies have found a prevalence as high as 75% of DD in DM [30]. The present study found DD in 83% of DM group, which was a higher value than most previous studies and probably reflects a better diagnostic performance and absence of misclassification with the use of age-adjusted cut-off points [27]; however, the study sample is not large enough to make conclusive remarks.

Studies in animals and humans indicated that structural and functional cardiovascular alterations are already occurring in prediabetic state [31, 32]. An increase in LV hypertrophy prevalence was previously demonstrated in prediabetes [9]. It is well-known that DD is a predictor of heart failure [33]. The possible link between prediabetes, DD and heart failure was suggested by a study which demonstrated a higher risk of new HF in patients with higher baseline fasting glucose [34]. Finally, there is evidence that PD is associated with an increase in cardiovascular and all-cause mortality [35], independently of progression to established DM [36]. However, there is no previous study with a comprehensive, age-adjusted echocardiographic evaluation, confirming higher prevalence of DD in prediabetes [8]. Only one group, using limited TDI criteria, detected a correlation between parameters of DD and glucose tolerance [37]. The present study showed a linear inverse correlation between 2h-plasma glucose and E´ (as well as between A1C and E/E´), which is sustained across the nondiabetic range; and a high prevalence of DD in PD subjects (58%). These findings may provide a missing link in the chain which leads from early dysglycemia to heart failure and increased mortality.

This study was unable to show increased LV hypertrophy, adverse remodeling, and systolic dysfunction in PD and DM groups. A number of studies reported an elevated

frequency of LV hypertrophy and asymptomatic systolic dysfunction in people with DM [8, 9, 33], although reports in PD patients are less conclusive [38]. Discordance with our findings can be explained by several factors, including rigid exclusion criteria in our study, probably selecting dysglycemic patients without comorbidities and complications, unlikely to present systolic dysfunction and LV hypertrophy; early alterations that are likely to be found only with stress echocardiography or LV strain measurements [39, 40]; less than four years of DM duration (which is the time point when Sm′ begins to decline) [41]; finally, an increase in radial contractility can initially compensate for reduced longitudinal myocardial function, resulting in a normal global contractility [42].

TMD variables (E, E/A) were not different in both dysglycemic groups when compared to NGM. These variables are greatly influenced by the preload; therefore, they underestimate the prevalence of DD, and cannot be used for standalone diagnosis [16]. In this study there was an increase of A wave in both PD and DM, replicating findings of other groups in type 1 and type 2 DM [27, 43] and indicating early DD, unmasked by the need to increase the atrial contribution to LV filling. This is consistent with the increase in LAVI which was shown in DM group. This alteration is a well known indication of chronicity and severity of DD [44], and predicts adverse cardiovascular events [45]. Additionally, there was an increase in LAVI also in the PD group in our study, corroborating the presence of DD in this group.

TDI Doppler diastolic velocities (E′, E′/A′) steadily decreased from NGM to PD and DM, reflecting their accuracy and preload independency. These findings are supported by the results of other groups [46], which showed that DM is associated with a decreased E′/A′ (<1). Better performance of TDI is partially explained by the fact that E′ and E′/A′ correlate linearly with the worsening of DD, and does not present "pseudonormalization" [47].

Patients from DM group had an increase in the E/E´ratio, indicating higher LV end diastolic pressure generated by DD. This finding replicates the results of other groups, which also found increase in E/E´ in patients with DM, independent of albuminuria [48, 49]. Additionally, the present study showed an increase in E/E´ also in PD patients. To our knowledge, this is a previously unrecognized alteration in PD. Possible explanations included a prolonged time of untreated hyperglycemia, generating myocardial damage, DD and consequently increase in E/E´ .

In conclusion, prediabetes and type 2 diabetes were associated with increased prevalence and severity of DD even after adjustment for all relevant confounding factors. LAVI and LV diastolic pressure were higher and myocardial diastolic TDI velocities were lower in these groups. These diastolic parameters were linearly correlated with glycemic variables, even in the nondiabetic range. These findings warrant screening for DD in early stages of impaired glycemic metabolism.

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LEGENDS.

Figure 1. Prevalence of DD according to group. A. DD prevalence across categories of glycemic status. B. Prevalence of each DD grade across categories of glycemic status.

Figure 2. Linear associations between glycemic parameters and diastolic variables. A. Relation between early diastolic mitral annular velocity (E´) and 2-hour plasma glucose in all patients. B. Relation between E´ and 2-hour plasma glucose excluding diabetic patients. C. Relation between ratio of early diastolic mitral flow (E) and mitral annular velocity ratio (E/E´) and A1C in all patients. D. Relation between early diastolic mitral flow and mitral annular velocity ratio (E/E´) and A1C excluding diabetic patients.
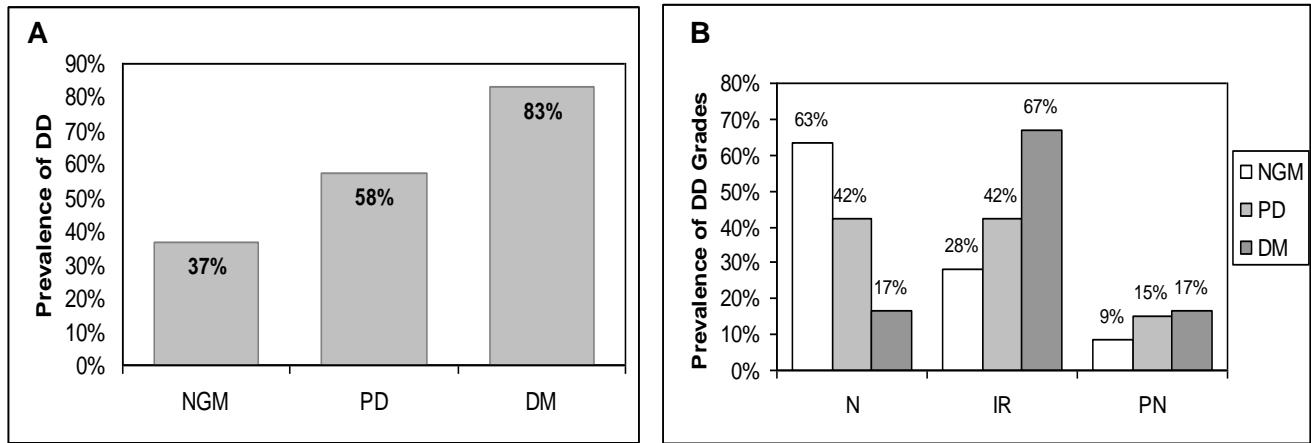
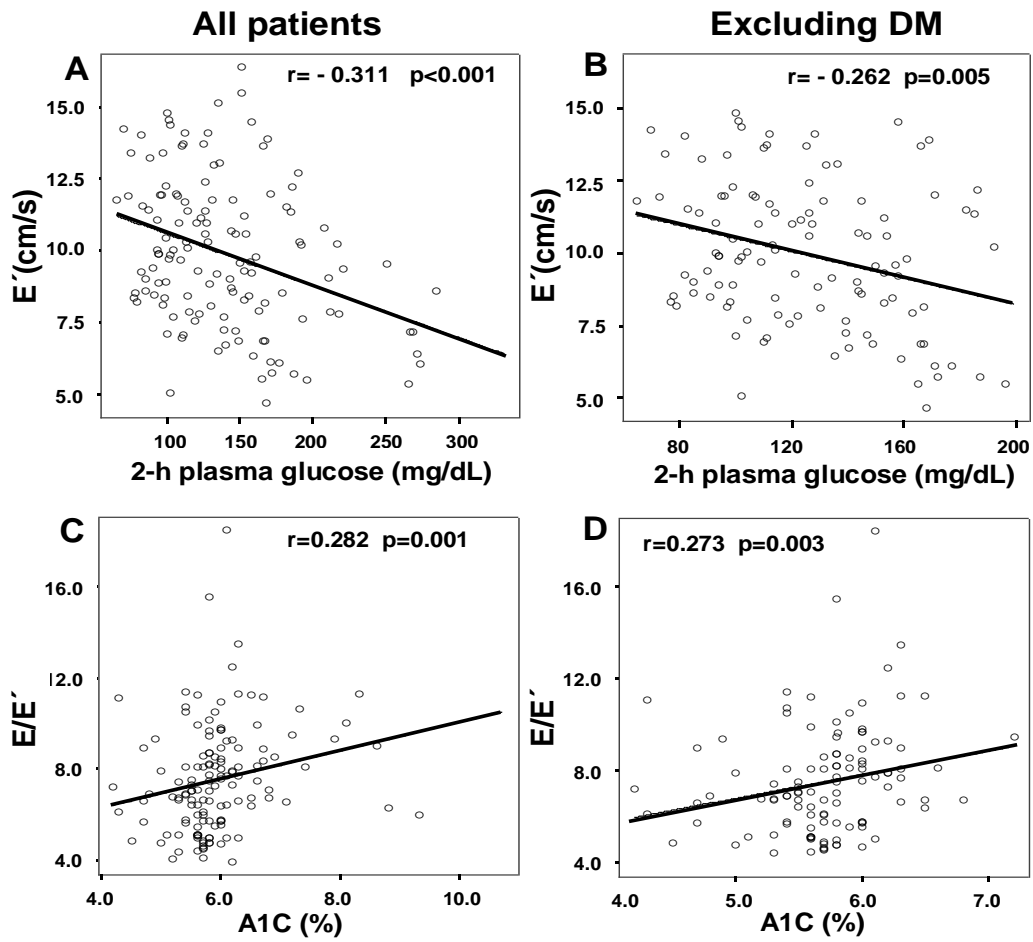**FIGURES**.

Figure 1. Prevalence of DD according to group.

Figure 2 - Linear associations between glycemic parameters and diastolic variables.

TABLES.

Table 1 – Subgroup characteristics according to categories of glycemic status

	Normal Glucose Metabolism (n = 57)	Prediabetes (n = 59)	Diabetes Mellitus (n= 24)	p
n	57	59	24	
Age (years)	58 ± 8	60 ± 9	59 ± 10	0.126
Male n (%)	16 (28)	21 (36)	11 (46)	0.295
BMI (kg/m^2)	27.6 ± 3.9	28.4 ± 3.8	30.9 ± 5.8†*	0.005
Systolic BP (mmHg)	137 ± 15	143 ± 18	143 ± 19	0.075
Diastolic BP (mmHg)	88 ± 9	86 ± 9	86 ± 11	0.803
Waist (cm)	95 ± 11	99 ± 11‡	105 ± 12§	0.01
Metabolic syndrome n(%)	20 (35)	47 (80)¶	21 (88)¶	<0.001
Hypertension n(%)	43 (75)	49 (83)	19 (79)	0.600
Creatinine (mg/dL)	0.86 ± 0.18	0.94 ± 0.19	0.94 ± 0.24	0.060
UAER (mg/24h)	20.9 ± 4.9	11.5 ± 11.9	30.2 ± 31.7‡‖	0.001
eGFR (mL/min/1.73 m^2)	103 ± 29	94 ± 25	94 ± 25	0.131
Total cholesterol (mg/dL)	223 ± 45	215 ± 44	200 ± 47	0.130
HDL cholesterol (mg/dL)	53 ± 12	46 ± 14*	42 ± 9 ¶	0.001
hs-CRP (mg/dL)	2.5 ± 2.1	4.9 ± 5.0*	4.4 ± 4.6‡	0.002
Uric acid (mg/dl)	5.2 ± 1.2	5.6 ± 1.5‡	6.0 ± 1.7‡	0.036
Drug use n (%)				
RAS inhibitors	25 (44)	30 (50)	17 (70)	0.100
Beta blockers	18 (33)	18 (33)	8 (33)	0.926
Calcium channel blockers	1 (2)	5 (8)	2 (8)	0.247
Diuretics	23 (41)	28 (47)	11 (45)	0.733
Statins	21 (37)	32 (54)	14 (58)	0.100

Data are means ± SD or median (interquartile range), unless otherwise indicated. * p<0.01 vs. NGM; † p<0.01 vs. PD; ‡ p<0.05 vs. NGM; § p=0.01 vs. NGM; ‖ p=0.001 vs PD; ¶ p<0.001 vs. NGM. BP, blood pressure; UAE, urinary albumin excretion; GFR, glomerular filtration rate; hs-CRP, high sensitivity C-reactive protein;

Table 2 - Echocardiographic variables according to categories of glycemic status

	Normal Glucose Metabolism (n = 57)	Prediabetes (n = 59)	Diabetes Mellitus (n= 24)	p
Conventional measurements				
LAVI (mL/m^2)	31.3 ± 6.9	35.3 ± 7.8 *	34.6 ± 8.5 *	0.017
LVEDD (cm)	4.6 ± 0.5	4.7 ± 0.5	4.7 ± 0.5	0.611
LVESD (cm)	2.8 ± 0.4	2.9 ± 0.4	2.8 ± 0.4	0.107
LVMI (g/m^2)	101 ± 31	110 ± 25	109 ± 29	0.217
Systolic parameters				
Ejection fraction (%)	69 ± 6	68 ± 6	71 ± 7	0.114
Fractional shortening (%)	39 ± 6	38 ± 5	41 ± 7	0.109
Sm´ (cm/s)	9.7 ± 2.1	9.3 ± 2.3	9.9 ± 2.5	0.419
Diastolic variables (Flow Doppler)				
E (cm/s)	69.3 ± 14.6	73.5 ± 15.3	68.1 ± 12.9	0.190
A (cm/s)	65.6 ± 14.9	78.5 ± 19.6‡	73.7 ± 16.2	0.110
E/A ratio	1.09 ± 0.28	0.98 ± 0.28	0.96 ± 0.28	0.070
DT E (ms)	246 ± 56	246 ± 56	255 ± 43	0.783
Diastolic variables (Tissue Doppler)				
E´ (cm/s)	10.7 ± 2.5	9.6 ± 2.7*	9.1 ± 2.9†	0.016
A´ (cm/s)	10.7 ± 2.4	11.5 ± 2.8	12.0 ± 2.8	0.098
E´/A´ (cm/s)	1.04 ± 0.35	0.88 ± 0.31†	0.80 ± 0.28 ‡§	0.002
E/E´ ratio	6.8 ± 1.9	8.1 ± 2.7‡	7.8 ± 1.9†	0.005

Data are mean ± SD or median (interquartile range), unless otherwise indicated. * $p < 0.05$ vs NGM; † $p < 0.01$ vs. NGM; ‡ $p < 0.001$ vs. NGM; § $p < 0.01$ vs PD. LAVI, left atrial volume index; LVEDDi, left ventricular end-diastolic internal dimension; LVESDi, left ventricular end systolic internal dimension; LVMI, left ventricular mass index; Sm´, systolic mitral annular velocity; E, early diastolic inflow velocity; A, late diastolic inflow velocity; E/A, ratio of early to late diastolic inflow velocity; DT E, E deceleration time; E´, early diastolic mitral annular velocity; A´, late diastolic mitral annular velocity; E´/A´, ratio of early to late diastolic mitral annular velocity; E/E´, ratio of early diastolic inflow velocity to early diastolic mitral annular velocity.

Table 3. Multivariable Logistic Regression evaluating association between categories of glucose metabolism and diastolic dysfunction.

GROUP	n	DD n	DD (%)	OR	CI 95%	P	Wald Statistic	Adjusted OR *	CI 95%	P
Normal Glucose Metabolism	57	21	(37)	1	-	-	-	-	-	-
Pre-Diabetes	59	34	(58)	2.3	1.1 - 4.9	0.026	4.29	2.5	1.1 - 5.7	0.038
Diabetes Mellitus	24	20	(83)	8.6	2.6 - 28.5	<0.001	11.37	9.7	2.6 - 33.1	0.001

* Adjusted OR: Odds Ratio from Multivariate Logistic Regression modeling adjusting for the following variables: systolic blood pressure, waist, prevalence of metabolic syndrome, hs-CRP, urinary albumin excretion rate, creatinine, HDL cholesterol. NGM, normal glucose metabolism; PD, pre-diabetes; DM, type 2 diabetes mellitus.

5. CONCLUSÕES E PERSPECTIVAS.

A partir dos trabalhos realizados foi possível propor um algoritmo para diagnóstico da disfunção diastólica (DD) baseado na avaliação seqüencial de múltiplos parâmetros ecocardiográficos e com pontos de corte ajustados pela idade para cada um desses parâmetros. Cada um desses parâmetros foi incorporado ao algoritmo, de forma a obter uma abordagem compreensiva, não invasiva da função diastólica. Também foi possível demonstrar, de forma não invasiva, que esse algoritmo diferencia com precisão pacientes com e sem DD; classifica adequadamente os pacientes de acordo com a severidade da DD; tem um desempenho melhor do que outros dois algoritmos bem conhecidos; e evita os viéses gerados pelas alterações fisiológicas inerentes à idade do paciente.

Utilizando este algoritmo em um grupo de pacientes divididos de acordo com a presença de disglicemia, conseguimos demonstrar que pacientes com pré-diabetes e diabetes apresentam maior prevalência e maior gravidade de DD que os pacientes normoglicêmicos. Este achado foi independente de possíveis fatores de confusão, sendo corroborado pela presença de correlação linear entre parâmetros de função diastólica e variáveis glicêmicas, que se manteve significativa mesmo na faixa normoglicêmica, e pelas alterações de parâmetros diastólicos (redução das velocidades de relaxamento miocárdico diastólico, aumento do volume atrial esquerdo e da pressão diastólica ventricular esquerda) nos pacientes com PD e DM.   .

Esses achados podem indicar a necessidade de realizar rastreamento ecocardiográfico de DD em fases precoces de disglicemia. Estudos prospectivos de intervenção serão necessários para confirmar se essa abordagem diagnóstica precoce,

associada a tratamento intensivo nos pacientes em que for demonstrada a DD, trará efetiva redução de desfechos adversos.

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