Evaluation of left and right ventricular systolic and diastolic electromechanical synchrony in older people: a population-based observational study

Short title: Cardiac electromechanical synchrony in the elderly

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Summary

It is unknown whether physiological ageing also goes along with electromechanical asynchrony of contraction. Aim of the study was to evaluate synchrony of contraction in older people with (“non-healthy”) or without (”healthy”) evidence for structural cardiac disease.

In 547 persons (age 76.7 ± 5.5 years, 306 male, 241 female) recruited from a population-based cohort of the ActiFE-Um study including a random sample of people ≥ 65 years old living in the region of Ulm, Germany, various PW- and TDI-Doppler based markers for asynchrony were obtained by echocardiography.

Within a subgroup of 84 healthy subjects, at most minimal systolic and diastolic asynchrony was found. Concerning systolic asynchrony, similar observations were made within the non-healthy subgroup. However, extent of diastolic left ventricular intraventricular asynchrony and also – by tendency – diastolic interventricular asynchrony was increased in comparison to the healthy subgroup.

To conclude, no evidence that physiological ageing might go along with relevant left or right ventricular systolic or diastolic electromechanical asynchrony was found in our study. Furthermore, our population-based data support the results from other clinical studies with rather selected cohorts that structural heart diseases might go along with increased diastolic asynchrony.

Keywords: Echocardiography, asynchrony, dyssynchrony, EC coupling, ageing, population-based, elderly, older

Introduction

Cardiac morphology and function is greatly affected by aging (Chiao *et al.* 2015, Karavidas *et al.* 2010). On cellular level, the total number of cardiomyocytes decreases accompanied by simultaneous hyperplasia of the remaining myocytes and the deposition of collagen between the cells. Cardiomyocytes present a reduction both in contraction and relaxation capability as a consequence of for example alterations in calcium homeostasis. In addition, altered calcium handling may also lead to age-related changes in excitation-contraction coupling (Feridooni *et al.* 2015). The cardiac conduction system is also subjected to ageing processes resulting in a higher incidence of both brady- and tachyarrhythmias (Chow *et al.* 2012, Mirza *et al.* 2012).

Intra- and/ or interventricular electromechanical asynchrony of contraction can be a concomitant phenomenon of reduced cardiac pumping function (Carerj *et al.* 2009, Chan *et al.* 2008, Lafitte *et al.* 2006, Perez de Isla *et al.* 2008). This has also been shown in studies on cardiac resynchronization therapy (CRT) of patients with severe congestive heart failure in which echocardiographic parameters including cut-off values for evaluation of cardiac synchrony of contraction have been defined (Linde *et al.* 2012, Schuster *et al.* 2005).

We hypothesized that ageing-related alterations of excitation-contraction coupling may also result in intra- and/or interventricular electromechanical systolic or diastolic asynchrony of contraction. Therefore, aim of our study was to echocardiographically evaluate synchrony of contraction in older subjects who were recruited from a large population-based cohort.

Methods

The ActiFE study (Activity and Function in the Elderly with a focus on physical activity and co-morbidities) includes a random sample of initially 1506 people older than 65 years living in the region of Ulm, Germany, who were recruited between March 2009 and April 2010. Details have been described previously (Denkinger *et al.* 2010). Ethical approval was granted by the Ethical Committee of the University of Ulm. All participants gave written informed consent. The work described in the following has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

During the 3-years follow-up with a total participation of 834 (55.4%) subjects, cardiovascular assessment including echocardiography was performed in a total of 688 (401 male, 287 female) persons (details in **Figure 1**). The remaining subjects (17.5%) refused participation. Cardiovascular diseases and risk factors were evaluated using a standardized questionnaire. A digital 12-lead resting ECG was registered during follow-up presentation. Height and body mass were surveyed using standard equipment. Physical activity (PA, steps/ day among other parameters) was assessed by using an accelerometer (activPAL, PAL Technologies Lfd., Glasgow, UK), details have been described elsewhere (Denkinger et al. 2010).

Participants lay supine for at least five minutes before the echocardiographic examination. Thereafter, heart rate (HR) and blood pressure (RR) at rest were obtained. During echocardiography, the subject was lying in a left lateral decubitus position. By using a commercially available ultrasound system (Philips CX-50 with a S5-1 cardiac probe), all subjects were examined using a standard protocol following international guidelines (Lang *et al.* 2015). Left ventricular mass (Devereux) and left atrial volume (LAV) were calculated

according to current recommendations (Lang et al. 2015) and were indexed to BSA (calculated using Mosteller's formula). Synchrony of contraction was evaluated by analyzing 1) PW-Doppler curves within left and right ventricular outflow tract (method 1) and 2) TDI curves from three LV/RV areas (method 2, measurements were taken medial and lateral, one centimeter below mitral valve annulus and at the lateral free RV wall, one centimeter below tricuspid valve annulus, see also inset image of **Figure 2B**) (Faber *et al.* 2003, Linde et al. 2012, Perez de Isla *et al.* 2005, Perez de Isla et al. 2008, Quan *et al.* 2012, Rouleau *et al.* 2001, Yu *et al.* 2003, Yu *et al.* 2007). PW-Doppler- and TDI-curves were registered with a simultaneous superimposed ECG at a sweep speed of 100 mm/s.

For the evaluation of synchrony, both (method 1) or all three curves (method 2) were needed. However, not every single curve was obtainable in every subject due to sometimes impaired image quality. Subjects were only chosen for further analysis if evaluation of synchrony was possible by at least one of the two methods (meaning either a complete Doppler- or TDI-data set was obtainable). Therefore, 141 persons (95 male, 46 female) had to be excluded from further analyses (**Figure 1**). Subjects with known atrial fibrillation (AF), coronary artery disease (CAD), or positive anamnesis for device implantation (pacemaker or ICD) , wall motion abnormalities, reduced systolic function or diastolic function, NT-proBNP >125 pg/ml (McMurray *et al.* 2012) as well as subjects with specific impairment of the cardiac conduction system in terms of complete or incomplete left or right bundle branch block were pooled in a subgroup of persons with evidence for structural heart disease, hereafter referred to as "non-healthy". Presence of AF was evaluated by resting electrocardiogram (ECG) or anamnesis. CAD was defined as positive response to "previous myocardial infarction", "coronary heart disease", CABG or stent implantation in a

questionnaire. Reduced systolic function was assumed if LV-EF was <52% (male) or <54% (female) (Lang et al. 2015). Presence of diastolic function was defined as E/E' >15 (Nagueh *et al.* 2009). NT-proBNP was measured by Electrochemiluminescence (Elecsys 2010). Bundle branch blocks were defined according to recent recommendations (Surawicz *et al.* 2009). From the 547 persons (306 male, 241 female) analyzed in our study, 463 (264 male, 199 female) were considered not healthy according to our chosen definition. The remaining 84 persons (42 male, 42 female) were pooled in the subgroup "no evidence for structural heart disease", hereafter referred to as "healthy" (**Figure 1**).

Synchrony analysis was performed offline. By using PW-Doppler curves (**Figure 2A)**, the time intervals from the beginning of the QRS-complex to the onset (T-AVo/ T-PVo), peak (T-AVp, T-PVp) and end (T-AVe, T-PVe) were measured both for the left and the right ventricle (Linde et al. 2012, Yu *et al.* 2009). Concerning TDI measurements (**Figure 2B**), the time interval from the beginning of the QRS complex to maximum velocity of the S-wave was classically used for evaluation of systolic asynchrony in cardiac resynchronization studies (Faber et al. 2003, Linde et al. 2012). However, a clear peaking of the S-wave is often hard to detect, especially in subjects with reduced systolic contraction velocities (Perez de Isla et al. 2005, Perez de Isla et al. 2008). This situation resulted in substantial measurement errors in those studies (Chung *et al.* 2008) and is at least partly held responsible for the still suboptimal response prediction in CRT-therapy (Yu et al. 2009). As similar problems were also expected in our geriatric cohort, we decided to evaluate the time intervals from beginning of the QRS complex to the beginning (T-EjctoX) and, the end (T-EjcteX) of ejection as done in other studies (Linde et al. 2012, Perez de Isla et al. 2005, Perez de Isla et al. 2008). For the evaluation of diastolic synchrony, the time intervals from the beginning of the QRS

complex to the beginning (T-EmoX) and peak (T-EmpX) of the Em-wave and, respectively, peak (T-AmpX) of the Am-wave were measured in every left/right ventricular area. As the duration of ventricular diastole depends on the heart rate, all diastolic measurements were normalized to the heart rate at the moment of registration of the respective TDI curve (Quan et al. 2012, Schuster et al. 2005, Yu et al. 2007). By using these measurements, the actual parameters for the subject-specific evaluation of systolic and diastolic (early (analyses of Em) and late (analyses of Am)) LV/RV inter-, LV intra- and RV intraventricular synchrony were calculated (Faber et al. 2003, Linde et al. 2012). **Table 1** gives an overview of the performed calculations.

To the best of our knowledge, no clear “normal values” for cardiac (a)synchrony in healthy subjects exist. In order to evaluate relevancy of asynchrony two provisional approaches in terms of model-like considerations were chosen. First (= definition 1), calculated synchrony parameters were compared with established echocardiographic parameters used as a part of medical indication for cardiac resynchronization therapy. Here, a cut-off of 40 ms (Yu et al. 2009) is suggested for PW-Doppler based evaluation (equivalent in our study to the clinically-used parameter: InterSys-1) of potential interventricular asynchrony. InterSys-2-4 are not used clinically but again, a cut-off of 40 ms was assumed virtually. Concerning systolic TDI-parameters, a cut-off of 65 ms (Yu et al. 2009) for septal to lateral delay is used clinically and this cut-off value was provisionally transferred also to the other systolic TDI synchrony markers. To the best of our knowledge, no cut-off values for the diastolic TDI parameters have been defined. Second (= definition 2), both in the healthy and non-healthy group, systolic or diastolic asynchrony was defined to be potentially relevant if in

a subject a respective parameter exceeded a cut-off defined as mean value of the healthy group ± 2 standard deviations.

Reproducibility of echocardiographic asynchrony parameters is known to be problematic (Fraser *et al.* 2003, Mandysova *et al.* 2008, Vinereanu *et al.* 1999). To assess intraobserver variability of systolic parameters, T-AVo, T-PVo, T-Ejcto-1 and T-Ejcto-2 and consecutively InterSys-1and LV-IntraSys-1 were assessed twice in all subjects of the subgroup of healthy people. In order to also evaluate reproducibility in non-healthy subgroup, T-Emp1$_c$ and T-Emp2$_c$ and consecutively LV-IntraDia-2 were also assessed twice in randomly selected 10% of all subjects of this group. These two procedures were done accordingly by a second observer for evaluation of interobserver variability. Time interval between the two assessments for intraobserver variability was > 1 year. Based on these data, inter- and intraobserver variability have been evaluated with the following statistical approaches: 1.) comparison of mean value (paired t-test), 2.) intraclass correlation coefficient (ICC, two-way mixed model, absolute agreement) (Gisev *et al.* 2013), 3.) Coefficient of variance (COV) calculated as a percentage: standard deviation of the difference multiplied by 100 and divided by mean value of the two measurements (Synek 2008), and 4.) Bland-Altman analysis (Giavarina 2015).

SPSS 21 was used for statistical analysis. Data are presented as means with standard deviation and 95% confidence interval. Normality of the data was verified with the Shapiro-Wilk test. The continuous variables did show normal distribution and therefore, (paired) student's t-test or ANOVA/ least significant difference-Bonferroni Test were used for group comparison. Finally, statistical differences of nominal data were examined using Fishers-exact test. Two-sided p-values <0.05 were considered to be statistically significant.

Results

Table 2 gives an overview of the subgroup's characteristics including clinical, anthropometric and PA data, echo quality and standard echocardiographic parameters.

Persons without evidence for structural heart disease

Evaluation of interventricular systolic and diastolic synchrony

Measured PW-Doppler parameters of systolic interventricular synchrony of healthy persons (viz. without evidence for structural heart disease according to our definition, see methods) are given in **Table 3A.** On average, the onset of left ventricular ejection was slightly earlier than that of the right ventricle. Time to peak ejection velocity was also reached earlier in the left ventricle. The end of left ventricular ejection was also earlier by tendency. Total duration of ejection did not differ between both ventricles.

The subject-specifically calculated PW-Doppler parameters for the evaluation of actual systolic interventricular synchrony are shown in **Table 4A**. Consistent with the above-mentioned averages of the measured parameters, Intersys1-3 speak for a minimal interventricular systolic asynchrony.

TDI curves assessed in area 2 and 3 (see methods and **Figure** 1) are usable for the evaluation of both systolic and diastolic interventricular synchrony. Contrarily to the PW-Doppler measurements, average time from the beginning of the QRS-complex to ejection-onset (T-Ejco) and -end (T-Ejce) did not differ between basal right and left ventricular free wall (**Table 3B**). However, TDI-parameters for interventricular synchrony (Intersys-5 and -6, **Table 4A**) also speak for a minimal interventricular systolic asynchrony.

Measured TDI parameters for evaluation of diastolic interventricular synchrony are presented in **Table 3C**. On average, left ventricular diastole began later (T-EmoX$_c$) than in the right ventricle, whereas peaks of early diastolic velocities (T-EmpX$_c$) occurred simultaneously. Peak velocity of atrial enddiastolic contraction (T-AmpX$_c$) was also reached later in the left than in the right ventricle. The calculated parameters for the actual evaluation of interventricular diastolic synchrony (**Table 4A**) pointed to a minimally asynchronous beginning of early/ late diastolic velocity increase (InterDia-1 and -3) and also peaking of early diastolic velocities (InterDia-2).

Evaluation of left ventricular intraventricular systolic and diastolic synchrony

TDI-curves or area 1 and 2 (**Figure 1**) were used for evaluating the left ventricular intraventricular systolic and diastolic synchrony. On average, ejection onset of interventricular septum was earlier than that of left ventricular free wall, whereas ejection ended simultaneously (**Table 3B**). According to the calculated parameters (LV-IntraSys-1 and -2, **Table 4B**), a minimal left ventricular intraventricular systolic asynchrony was observed.

Concerning the diastole, on average, no differences of the beginning of increase and peaking of early diastolic velocities (T-EmoX$_c$ and T-EmpX$_c$, **Table 3c**) within interventricular septum and the left ventricular free wall were detectable. Contrarily, the peak of late atrial velocity was reached earlier within the interventricular septum than in the left ventricular free wall (T-AmpX$_c$, **Table 3c**) on average.

Calculated parameters (LV-IntraDia-1-3, see **Table 4E**) indicated a minimal intraventricular asynchrony of the early and late left ventricular diastole.

Evaluation of right ventricular intraventricular systolic and diastolic synchrony

Finally, right ventricular intraventricular systolic and diastolic synchrony was evaluated by analyzing the TDI curves of area 1 and 3 (**Figure 1**). Comparable to the left ventricle, the onset of ejection was detectable earlier in the interventricular septum than in the right ventricular free wall, whereas it ended simultaneously (**Table 3B**) on average. Calculated parameters pointed to a minimal (RV-IntraSys-1 and -2, **Table 4C**) right ventricular systolic interventricular asynchrony.

Differently to the left ventricle, on average, the beginning of the increase of early diastolic velocity within the interventricular septum was earlier than within the basal right ventricular free wall, while its peaking occurred simultaneously just like the peaking of late enddiastolic velocity did (**Table 3B**). Calculated measures (RV-InterDia-1-3) speak for a minimal right ventricular interventricular diastolic asynchrony (**Table 4F**).

Influence of sex and QRS duration and relevancy of asynchrony

T-PVo (♂: 79±16 ms vs. ♀: 83±15 ms, p=0.021), T-PVe (♂: 376±25 ms vs. ♀: 386±26 ms, p=0.005), T-Ejce1 (♂: 383±31 ms vs. ♀: 404±29 ms, p=0.002), T-Ejce2 (♂: 390±30 ms vs. ♀: 410±28 ms, p=0.005), T-Emo3$_c$ (♂: 477±50 ms vs. ♀: 523±42 ms, p<0.001) and T-Emp3$_c$ (♂: 586±61 ms vs. ♀: 626±53 ms, p=0.008) slightly differed between male and female persons. However, none of the calculated asynchrony parameters showed significant sex differences. Only LV-IntraSys-2 did show a small but significant correlation with QRS duration (r=0.26, p=0.020).

Table 5 gives an overview of the amount of healthy subjects fulfilling the respective criterions of asynchrony according to our definitions (see methods). With the exception of Intersys-2, less than 5% of all healthy subjects met a respective criterion according to definition 1 (**Table 5A**). Concerning definition 2 (**Table 5B**), also only a small amount of

subjects (at most 8.2% depending on the respective parameter) exhibited relevant asynchrony.

Asynchrony in subjects with evidence for structural heart disease

To get an idea of potential effects of structural heart disease on cardiac synchrony in the elderly, the hitherto presented analyses were again performed in the subgroup of non-healthy subjects according to our definition (see methods). For reasons of clarity, only the calculated variables of asynchrony are presented (**Table 4**). No significant differences of all calculated parameters of systolic interventricular asynchrony, LV intraventricular asynchrony and RV intraventricular asynchrony were observed (**Table 4A-C**). InterDia-1-3 pointed to an increase of diastolic interventricular asynchrony in comparison to healthy subjects at least by tendency (**Table 4D**). LV-IntraDia-1-3 gave evidence for a significant increase of early but not late diastolic LV intraventricular asynchrony as a consequence of structural heart disease (**Table 4E**). Minimal diastolic RV intraventricular asynchrony did not differ between the healthy and non-healthy group (**Table 4F**).

InterSys-2 (♂: 35±25 ms vs. ♀: 41±30 ms, p=0.018), InterSys-5 (♂: -1±26 ms vs. ♀: 8±25 ms, p=0.003) and RV-IntraSys-1 (♂: 16±14 ms vs. ♀: 13±10 ms, p=0.013) revealed minimal but significant sex-differences. InterSys-1 ($r=0.35$, $p<0.001$), InterSys-3 ($r=0.30$, $p<0.001$) and RV-IntraSys-1 ($r=0.28$, $p<0.001$) slightly correlated with QRS duration.

Percentage of non-healthy persons fulfilling a respective literature criterion did not differ from the percentage of healthy persons (**Table 5A**). A similar situation was also observed concerning our analyses in which our self-defined (definition 2, see methods) cut-off values for systolic asynchrony were used (**Table 5B**). However, in good concordance with the above mentioned significant differences of LV-IntraDia-1 and LV-IntraDia-2, cut-off

values of these parameters for early LV intraventricular diastolic asynchrony were significantly exceeded by more often by non-healthy than by healthy subjects.

Inter- and Intraobserver variability

Analyses of reproducibility are presented in **Table 6**. Concerning interobserver variability, mean values of all exemplarily evaluated systolic parameters did not differ statistically, whereas means of the measured but not calculated diastolic parameters of observer 2 were slightly smaller than in observer 1. Intraclass correlation coefficients (COV) revealed mostly barely acceptable (COV 0.7-0.8) to good (COV 0.8-0.9; measured systolic variables) and excellent (COV > 0.9; measured diastolic variables) interrater variability. However, COVs of the calculated variables were consecutively lower with a large confidence interval including inacceptable values (COV<0.7) indicating problematic reproducibility of theses variables in the same way as the comparably high limits of agreements (LOM) of the Bland-Altman analyses. Similar results were found for intraobserver variability as also shown in **Table 6**.

Discussion

In our cross-sectional study, synchrony of cardiac contraction was evaluated in a population-based geriatric cohort. Both in the subgroups of healthy and non-healthy subjects according to our definitions, mean values of our calculated parameters of synchrony were generally small and only a minority of subjects fulfilled defined asynchrony criteria. This suggests that both systolic and diastolic asynchrony of contraction were not relevant in our cohort. Within the subgroup of non-healthy subjects, most variables of diastolic inter- and LV intraventricular asynchrony were slightly increased in comparison to the subgroup of healthy

subjects indicating that structural heart diseases might go along with an increase of diastolic asynchrony.

For the purpose of the study we used strict criteria to define “healthy” persons without evidence for structural heart disease. NT-proBNP was an important parameter using the recommended cut-off of 125 pg/ml for diagnosis of heart failure in a non-acute setting (McMurray et al. 2012). In this way, any hemodynamically relevant valvular heart diseases (Bergler-Klein *et al.* 2014, Moura *et al.* 2008, Troughton *et al.* 2009) and diseases of pulmonary circulation (Galie *et al.* 2016) were adequately ruled out. We did not exclude subjects with arterial hypertension or diabetes mellitus per se. For example, the diagnosis “atrial hypertension” according to our definition subsumes subjects from both extremes in terms of properly treated arterial hypertension without any secondary complications on the one side and longstanding, untreated hypertensive subjects with severe secondary cardiac structural alteration on the other side. The same applies to diabetes mellitus and diabetic cardiomyopathy. The final common pathway of left atrial volume and/or pressure overload caused by these two diseases but also by other various factors in an individual subject is an severity-dependent increased release of BNP (Mahadavan *et al.* 2014). Therefore, hypertensive/diabetic cardiomyopathy (as a potential consequence of insufficiently treated underlying disease which may affect echocardiographic measures) were also adequately ruled out by normal NT-proBNP (Bergler-Klein et al. 2014, Galie et al. 2016, Moura et al. 2008, Santos *et al.* 2014, Troughton and Richards 2009). As a results of our strict criteria, about 88 % of all subjects from our initial cohort were pooled in the non-healthy subgroup.

A relevant amount of the absolute value of our measured parameters represents myocardial conduction of excitation. The longer systolic TDI time intervals of both the left and right ventricular free wall in comparison to the interventricular septum with close

proximity to the specific cardiac conduction system (AV node) were in line with this findings. As cardiac size differs between men and women (Lang et al. 2015), one might also expect differences in measured parameters (males > females) due to relatively longer conduction times in larger hearts. However, almost none of the measured parameters exhibited significant sex differences and if they did, time intervals were longer in female subjects. This might be explained by known sex-specific differences of EC-coupling (Parks *et al.* 2013). Ultimately, these differences had no consequence for the actual evaluation of cardiac synchrony. Almost none of the calculated parameters of asynchrony correlated with QRS duration which is rather not surprising as QRS duration was normal by definition within this subgroup. As EC-coupling is also affected by cardiac afterload (Janssen 2010), a potential dependency of our evaluated asynchrony parameters and blood pressure at the time of echocardiographic measurement might exist. However, we were not able to evaluate this relation appropriately in our cohort, as the range of the observed blood pressure values during echocardiography within our cohort was too small. Due to similar reasons, namely the limited age span of people >65 in our study, we also could not closer examine the potential association of asynchrony and age.

Studies evaluating synchrony of contraction in “healthy” subjects are scarce. To the best of our knowledge, our study is even the first one concerning a geriatric cohort. Quan et al. (Quan et al. 2012) studied 88 “healthy subjects” (40 ± 15 years, 48% male). In accordance to our data, the authors also reported of earlier peaking of systolic outflow in the left (T-AVp) than in the right (T-PVp) ventricle with simultaneous ending. Contrarily, no difference of the beginning of systolic outflow between left and right ventricle was observed. Comprehensive systolic and diastolic asynchrony TDI data was presented by Yu et al. (Yu et al. 2003) in a study which included 106 „healthy subjects” (64.3 ± 9.5 years, 60 % male).

Here, time to isovolumic contraction and time to peak systolic contraction (as a difference to T-Ejcto and T-Ejce in our study) and time to peak early and late diastolic velocities, respectively, (equivalent to T-EmpX and T-Ampx) were measured, whereby no correction for heart rate of the diastolic measures was used. The authors found that systole and diastole within the left ventricle were “highly synchronized” but the time to isovolumic and peak systolic velocities of the right ventricle were – comparable to our data with reservations – slightly delayed. In a study by Sadeghian et al., the time to peak systolic velocity of basal septal and lateral left ventricle was measured in 65 “healthy young adult volunteers” (30.1 ± 7.5 years, 52% male) and also septal to lateral delay (equivalent to LV-IntraSys-1 and -2 with reservations) was calculated (Sadeghian *et al.* 2010). Mean values of time to peak systolic velocities of basal septal and lateral ventricle differed by ≈ 45 ms and calculated mean septal to lateral delay was 55 ± 39 ms. A similar septal to lateral delay (median 66 ms, by measuring time to peak systolic velocities as well) was reported by Miyazaki et al. (Miyazaki *et al.* 2008) in a study including 40 “healthy subjects” (age 47 ± 15 years, 60% male). So did Ng et al. in a further study (58 ± 40 ms, 122 “healthy” subjects, 44 ± 13 years, 53% male) (Ng *et al.* 2008). These results are contrary to ours with only a minimal left ventricular intraventricular systolic asynchrony. The mentioned methodical problems of measuring the time to peak velocity interval might be responsible for this fact. In the last study mentioned (Ng et al. 2008), also a septal to lateral delay of early peak diastolic velocity (equivalent to LV-IntraDia-1) of 15 ± 15 ms was reported. This is in concordance to our results. Yu et al. (Yu et al. 2007) evaluated left ventricular intraventricular systolic and diastolic asynchrony via TDI in “100 healthy volunteers recruited from the community” (64.2 ± 9.4 years, 71% male) by using a twelve segmental model. No details concerning the time intervals from beginning of QRS complex to peak systolic and, respectively, peak early myocardial velocities were given but a mean

maximum difference of 54 ± 23 ms (systolic) and 63 ± 25 ms (diastolic) between the time intervals of the different segments indicated a left ventricular intraventricular systolic and diastolic asynchrony in healthy individuals. With reservations, both the systolic and diastolic extent of asynchrony seemed to be slightly bigger than in our study. Again, the use of the problematic systolic peak velocities might be an explanation for the discrepancy concerning systolic asynchrony. The differences concerning diastolic asynchrony remain unclear. By using a six segmental model of the left ventricle, a mean maximum difference (again no specific time intervals were reported) of time to peak systolic and early diastolic velocity of 12 ± 10 ms and, respectively, 10 ± 9 ms in a cohort of 35 "healthy adults" were reported by Wang et al. (Wang *et al.* 2007). Therefore, again with the above-mentioned reservations, extent of left ventricular intraventricular systolic and early diastolic asynchrony was comparable to our cohort with geriatric subjects.

To the best of our knowledge, no population-based studies evaluating cardiac synchrony are available yet. In our subgroup with non-healthy subjects no differences of the evaluated systolic synchrony parameters in comparison to the subgroup with healthy subjects were observed. The percentage of subjects with potentially relevant systolic asynchrony according to our definitions also did not differ. However, extent of diastolic left ventricular intraventricular asynchrony and also – by tendency – diastolic interventricular asynchrony was increased in comparison to the healthy subgroup. Yu et al. reported of the presence of diastolic asynchrony in a cohort with heart failure patients despite narrow QRS-complex (Yu et al. 2007). As especially diastolic heart failure has a known increased prevalence in the elderly, our observations therefore seem to be conclusive.

The results of our analyses concerning reproducibility are the major limitation of our study. "Classical" echocardiographic synchrony markers obtained by PW- and TDI-Doppler

were analyzed in our study. Comparable with other studies (Fraser et al. 2003, Mandysova et al. 2008, Vinereanu et al. 1999), particularly the reproducibility of the calculated variables was at least partly problematic so that our results have to be interpreted with caution. It is noteworthy but not apologetic that other similar studies (Miyazaki et al. 2008, Ng et al. 2008, Quan et al. 2012, Sadeghian et al. 2010, Wang et al. 2007, Yu et al. 2003, Yu et al. 2007) also did not pay adequate attention to this important issue. Meanwhile, imaging modalities like for example strain analysis (Gorcsan *et al.* 2012) with better reproducibility are available which will be included in echocardiographic data acquisition of the next follow-up of our cohort. As another limitation, it has to be noticed that our "non-healthy" group is extremely heterogeneous concerning clinical characteristics and therefore, this may hide some potentially significant differences of synchrony in particular non-healthy subgroups.

Conclusion

Despite known age-related alterations of cardiac conduction system and excitation-contraction coupling, only a minimal extent of asynchrony of contraction was found in our geriatric cohort both in the subgroups of healthy and non-healthy.

To conclude, no evidence that physiological ageing might go along with relevant left or right ventricular systolic or diastolic electromechanical asynchrony was found. However, our population-based data support the results from other studies with rather selected cohorts that structural heart diseases might go along with increased diastolic asynchrony.

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Figure 1: Summary of inclusion/ exclusion procedure.

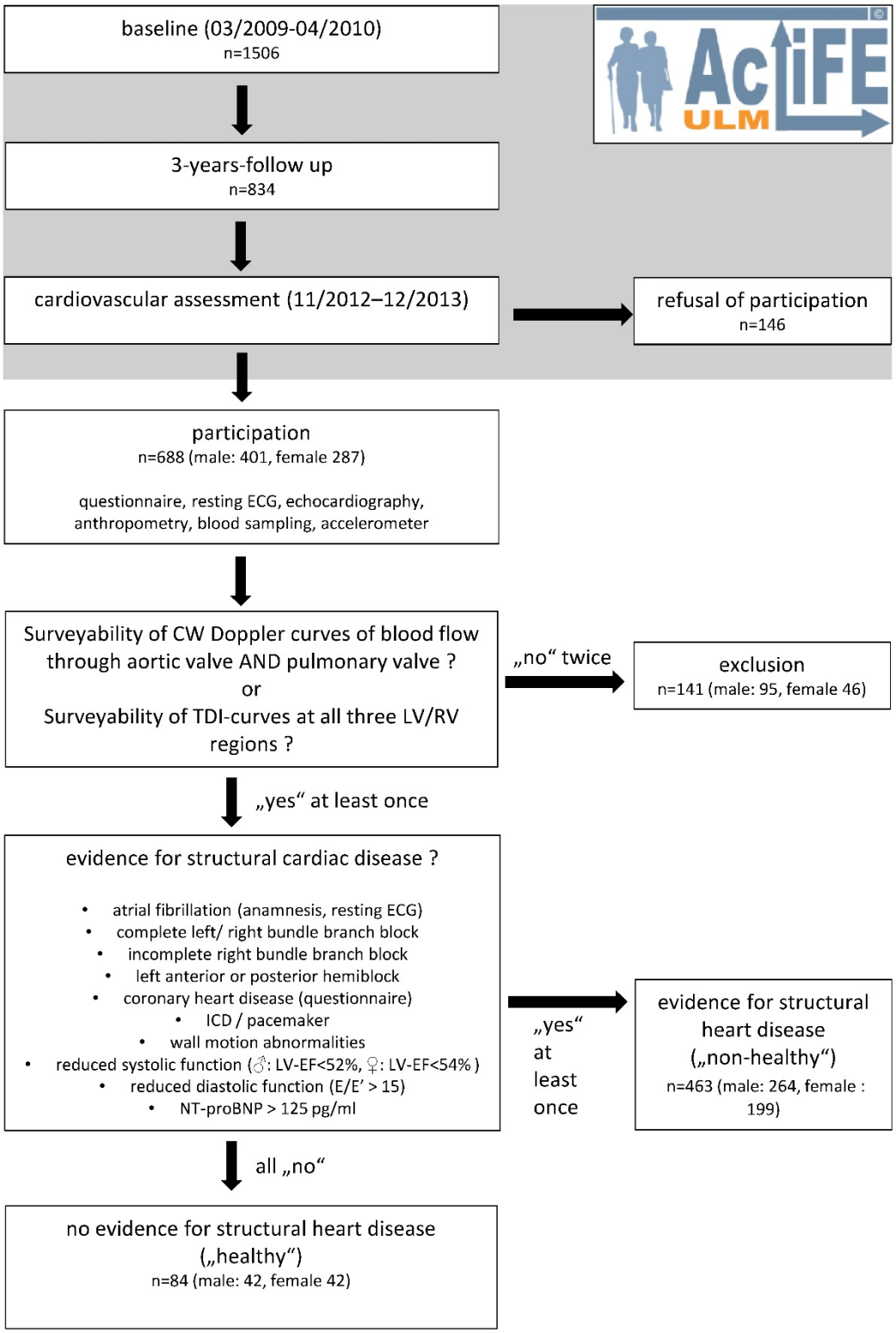

Figure 2: (A) PW- and (B) TDI-curves with measured asynchrony parameters. T-AVo/p/e and T-PVo/p/e: time from beginning of QRS complex (bQRS) to onset (o), peak (p) and end (e) of blood flow within left (AVo)/ right ventricular (PVo) outflow tract. T-Ejco/e 1-3: time from bQRS complex to onset (o) and end (e) of ejection, measured at three different myocardial sites (see inset): 1 = interventricular septum), 2 = left ventricular free wall 1 cm blow mitral valve annulus, 3 = right ventricular free wall 1 cm below tricuspid valve annulus. T-Emo/ p 1-3: Time from bQRS complex to onset (o) and peak (p) of early diastolic myocardial velocity (Em) measured at sites 1-3. T-Amp 1-3: Time from bQRS complex to peak (p) of late diastolic myocardial velocity (Am) measured at sites 1-3.

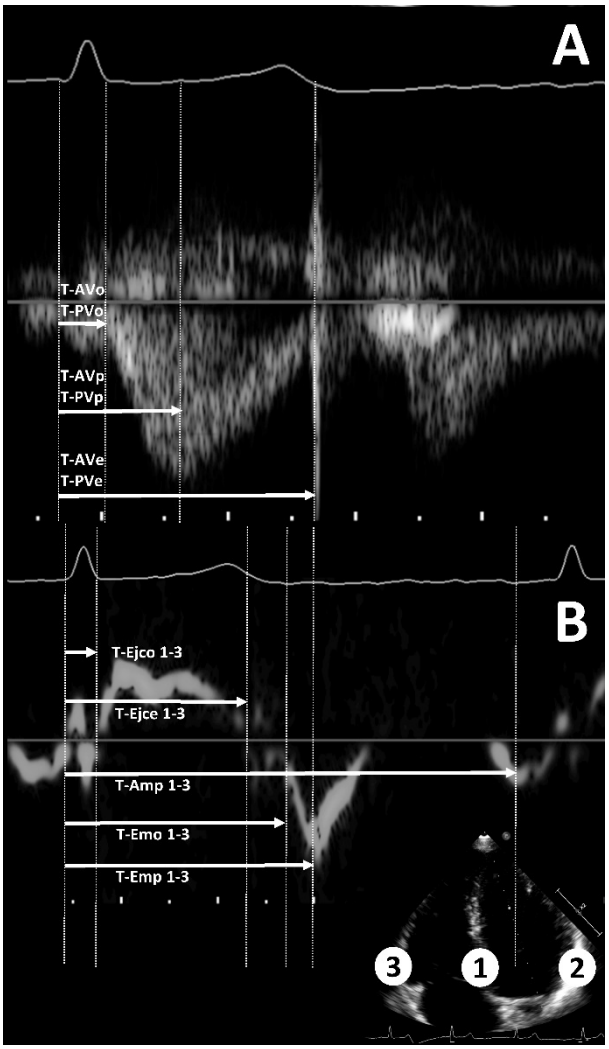

Table 1: Calculated measures for synchrony. See **Figure 2** for abbreviations of the measured parameters of synchrony.

	calculation
systolic asynchrony	
interventricular asynchrony	
InterSys-1	|(T-AVo) - (T-PVo)|
InterSys-2	|(T-AVp) - (T-PVp)|
InterSys-3	|(T-AVe) - (T-PVe)|
InterSys-4	|(T-AVe - T-AVo) - (T-PVe - T-PVo)|
InterSys-5	|(T-Ejco2) - (T-Ejco3)|
InterSys-6	|(T-Ejce2) - (T-Ejce3)|
LV intraventricular asynchrony[*]	
LV-IntraSys-1	|(T-Ejco1) - (T-Ejco2)|
LV-IntraSys-2	|(T-Ejce1) - (T-Ejce2)|
RV intraventricular asynchrony	
RV-IntraSys-1	|(T-Ejco1) - (T-Ejco3)|
RV-IntraSys-2	|(T-Ejce1) - (T-Ejce3)|
diastolic asynchrony	
interventricular asynchrony	
InterDia-1	$\|(T\text{-}Emo2_c) - (T\text{-}Emo3_c)\|$
InterDia-2	$\|(T\text{-}Emp2_c) - (T\text{-}Emp3_c)\|$
InterDia-3	$\|(T\text{-}Amp2_c) - (T\text{-}Amp3_c)\|$
LV intraventricular asynchrony	
LV-IntraDia-1	$\|(T\text{-}Emo1_c) - (T\text{-}Emo2_c)\|$
LV-IntraDia-2	$\|(T\text{-}Emp1_c) - (T\text{-}Emp2_c)\|$
LV-IntraDia-3	$\|(T\text{-}Amp1_c) - (T\text{-}Amp2_c)\|$
RV intraventricular asynchrony	
RV-IntraDia-1	$\|(T\text{-}Emo1_c) - (T\text{-}Emo3_c)\|$
RV-IntraDia-2	$\|(T\text{-}Emp1_c) - (T\text{-}Emp3_c)\|$
RV-IntraDia-3	$\|(T\text{-}Am12_c) - (T\text{-}Amp3_c)\|$

$_c$ = corrected for heart rate: measure * 1000/cycle length [ms]

Table 2: Clinical characteristics of the included subjects.

	healthy Male (n=42) mean (SD)	Female (n=42) mean (SD)	p-value ♂ vs. ♀	non-healthy Male (n=264) mean (SD)	Female (n=199) mean (SD)	p-value ♂ vs. ♀	p-value (♂) healthy vs. non-healthy	p-value (♀) healthy vs. non-healthy
clinical characteristics								
Age (years)	74.9 (5.1)	73.6 (3.2)	0.474[*1]	77.8 (5.8)	76.3 (5.5)	**0.005**[*1]	**0.001**[*1]	**0.005**[*1]
blood pressure systolic (mmHg)	139 (17)	132 (18)	0.072[*1]	136 (19)	138 (20)	0.485[*1]	0.371[*1]	0.075[*1]
blood pressure diastolic (mmHg)	76 (9)	72 (8)	**0.018**[*1]	74 (9)	73 (9)	0.260[*1]	0.171[*1]	0.375[*1]
heart rate at rest (bpm)	67 (9)	67 (8)	0.922[*1]	64 (11)	67 (10)	**<0.001**[*1]	**0.028**[*1]	0.547[*1]
body mass index (kg/m²)	27.6 (3.1)	26.2 (4.7)	**0.047**[*1]	27.5 (3.6)	27.0 (4.4)	0.126[*1]	0.721[*1]	0.254[*1]
waist circumference (cm)	101.9 (9.0)	91.2 (11.3)	**<0.001**[*1]	102.5 (10.7)	93.5 (11.6)	**<0.001**[*1]	0.852[*1]	0.300[*1]
serum NT-proBNP (mg/dl)[*3]	65 (46)	89 (54)	**0.011**[*1]	177 (294)	198 (207)	0.202[*1]	**<0.001**[*1]	**<0.001**[*1]
steps/ 24 h[*4]	9541 (2949)	8380 (2792)	**0.049**[*1]	7896 (3537	8171 (3262)	0.156[*1]	**<0.001**[*1]	0.632[*1]
	prevalence (%)	prevalence (%)		prevalence (%)	prevalence (%)			
cardiovascular risk factors								
coronary heart disease				25.0	10.1	**<0.001**[*2]	**<0.001**[*2]	**0.029**[*2]
atrial fibrillation				13.3	8.5	0.137[*2]	**0.007**[*2]	**0.049**[*2]
hypertension (%)	64.3	64.3	1.000[*2]	71.6	73.4	1.000[*2]	0.364[*2]	0.259[*2]
dyslipidaemia (%)	26.2	47.6	**0.030**[*2]	31.4	37.7	**0.030**[*2]	0.846[*2]	0.463[*2]
current smoking (%)	4.8	7.1	1.000[*2]	5.1	1.0	1.000[*2]	1.00[*2]	0.040[*2]
diabetes (%)	19.0	11.9	0.548[*2]	15.6	10.7	0.548[*2]	0.651[*2]	0.787[*2]
standard echocardiographic parameters								
Aortic root (mm)	34 (4)	29 (4)	**<0.001**[*1]	34 (4)	29 (4)	**<0.001**[*1]	0.910[*1]	0.575[*1]
LAVI (ml/m²)[*5]	23.0 (7.7)	18.6 (5.3)	**0.017**[*1]	24.3 (9.8)	21.3 (8.5)	**0.008**[*1]	0.667[*1]	0.117[*1]
ISVD (mm)	11 (2)	10 (2)	**0.018**[*1]	12 (2)	11 (2)	**<0.001**[*1]	**0.034**[*1]	0.107[*1]
LVEDD (mm)	52 (5)	47 (5)	**<0.001**[*1]	52 (6)	48 (6)	**<0.001**[*1]	0.486[*1]	0.588[*1]

LV-EF (%)	66 (8)	67 (7)	0.737[*1]	62 (11)	64 (10)	0.054[*1]	0.042[*1]	0.289[*1]
LVMI (g)	109 (27)	93 (25)	**0.012**[*1]	117 (32)	100 (24)	**<0.001**[*1]	0.198[*1]	0.145[*1]
LV-E/A	0.83 (0.22)	0.86 (0.23)	0.694[*1]	0.85 (0.249)	0.88 (0.30)	0.397[*1]	0.805[*1]	0.968[*1]
LV-E/E'	8.8 (2.3)	9.7 (2.3)	0.088[*1]	11.5 (3.2)	12.0 (3)	0.138[*1]	**0.008**[*1]	0.119[*1]
TAPSE (mm)	27 (4)	24 (5)	**0.011**[*1]	25 (5)	25 (5)	0.153[*1]	0.055[*1]	0.688[*1]
resting ECG								
P wave duration (ms)	114 (15)	112 (12)	0.205[*1]	111 (19)	109 (18)	0.185[*1]	0.287[*1]	0.286[*1]
PQ interval (ms)	172 (29)	169 (22)	0.434[*1]	190 (38)	173 (35)	**<0.001**[*1]	**0.001**[*1]	0.471[*1]
QRS duration (ms)	95 (8)	91 (9)	**0.014**[*1]	106 (21)	94 (16)	**<0.001**[*1]	**0.002**[*1]	0.932[*1]
QT (ms)	400 (30)	413 (31)	**0.017**[*1]	421 (34)	411 (45)	**0.005**[*1]	**<0.001**[*1]	0.529[*1]
QTc (ms)	425 (25)	435 (21)	**0.019**[*1]	436 (40)	433 (53)	0.669[*1]	**0.002**[*1]	0.717[*1]

[*1]= Mann-Whitney U-test, *[2] = Fisher's exact test, *[3]=data presented as median (interquartile range). [*4] = data available from (male/female) 39/40 healthy and 253/190 non-healthy subjects, [*5] = data available from (male/ female) 39/32 healthy and 209/ 161 non-healthy subjects.

Table 3: Measured and calculated parameters of synchrony in the subgroup of healthy subjects according to our definition.

			T-XVo			Δ o_p[*4]			T-XVp			Δ p_e[*5]			T-XVe			Δ o_e[*6]	
A	measured parameters systolic synchrony	PW Doppler	n	mean (SD)	CI	n	mean (SD)	CI	n	mean (SD)	CI	n	mean (SD)	CI	n	mean (SD)	CI	n	mean (SD)	CI
		Aortic valve	8 0 7	75 (13)	72; 78	8 0 7	83 (16)	80; 87 101;	8 0 7	158 (21)	153; 163 181;	8 0 7	215 (26)	209; 221 187;	8 0 7	373 (28)	367; 379 375;	8 0 7	298 (26)	292; 304 294;
		Pulmonary valve	4	80 (15)	77; 84	4	106 (21)	111	4	187 (25)	192	4	194 (30)	201	4	381 (26)	387	4	300 (28)	307
		p-value[*1]		**0.018**			**<0.001**			**<0.001**			**<0.001**			0.061			0.057	

			T-EjcoX			Δ o_e[*6]			T-EjceX		
B		TDI	n	mean (SD)	CI	n	mean (SD)	CI	n	mean (SD)	CI
		septum (TDI 1)	8 3	76 (15)	73; 79	8 3	318 (31)	311; 325	8 3	394 (32)	387; 401
		LV lateral free wall (TDI 2)	8 1	87 (20)	82; 91	8 1	313 (31)	306; 320	8 1	400 (30)	393; 407
		RV lateral free wall (TDI 3)	7 4	85 (16)	81; 89	7 4	316 (33)	308; 324	7 4	401 (34)	393; 409
		p-value[*2]		**<0.001**			0.631			0.327	
		p-value posthoc 1 vs 2[*3]		**<0.001**							
		p-value posthoc 1 vs 3[*3]		**0.004**							
		p-value posthoc 2 vs 3[*3]		1.000							

			T-EmoX$_c$[*7]			Δ o_p[*4]			T-EmpX$_c$[*7]			T-AmpX$_c$[*7]		
C	measured parameters diastolic synchrony	TDI	n	mean (SD)	CI	n	mean (SD)	CI	n	mean (SD)	CI	n	mean (SD)	CI
		septum (TDI 1)	8 3	532 (56)	520; 544	8 3	73 (16)	69; 76	8 3	605 (61)	592; 618	8 3	922 (33)	915; 930
		LV lateral free wall (TDI 2)	8 1	525 (51)	513; 536	8 1	68 (16)	64; 71	8 1	592 (56)	580; 605	8 1	939 (36)	931; 947
		RV lateral free wall (TDI 3)	7 4	499 (51)	487; 511	7 4	106 (26)	100; 112	7 4	606 (60)	592; 619	7 4	920 (39)	911; 929
		p-value[*2]		**<0.001**			**<0.001**			0.285			**0.002**	
		p-value posthoc 1 vs 2[*3]		1.000			0.327						**0.010**	

	p-value posthoc 1 vs 3[*3]	**<0.001**	**<0.001**	1.000
	p-value posthoc 2 vs 3[*3]	**0.010**	**<0.001**	**0.003**

All measures are given in ms. [*1] paired t-test, [*2] ANOVA, [*3] post-hoc, [*4] Difference between onset and peak, [*5] Difference between peak and end, *6 Difference between onset and end, [*7] X = area of measure, $_c$ = corrected for heaart rate: measure * 1000/cycle length [ms]. SD = standard deviation, CI = 95% confidence interval (lower limit; upper limit), Sys = systolic, dia = diastolic, LV = left ventricle, RV = right ventricle.

Table 4: Calculated parameters for systolic and diastolic asynchrony in healthy and non-healthy subjects.

	healthy			non-healthy			
	n	mean (SD)	CI (5; 95)	n	mean (SD)	CI (5; 95)	p-value[*1]
systolic asynchrony[*2]							
A: interventricular asynchrony							
InterSys-1	70	13 (11)	11; 16	386	17 (16)	15; 19	0.148
InterSys-2	70	34 (22)	28; 39	386	37 (26)	34; 39	0.510
InterSys-3	70	16 (12)	13; 19	386	20 (18)	18; 22	0.200
InterSys-4	70	17 (11)	14; 20	386	20 (17)	18; 22	0.394
InterSys-5	71	16 (12)	14; 19	289	21 (18)	18, 23	0.116
InterSys-6	71	20 (17)	17; 24	289	23 (19)	21; 25	0.342
B: LV intraventricular asynchrony							
LV-IntraSys-1	80	16 (13)	13; 19	305	18 (18)	16; 20	0.873
LV-IntraSys-2	80	14 (12)	11; 16	305	17 (14)	15; 18	0.132
C: RV intraventricular asynchrony							
RV-IntraSys-1	72	12 (9)	10; 15	342	15 (12)	14; 16	0.330
RV-IntraSys-2	72	21 (16)	17; 25	342	22 (17)	20; 23	0.753
diastolic asynchrony[*2]							
D: interventricular asynchrony							
InterDia-1	71	38 (29)	31; 45	289	47 (35)	43; 51	0.053
InterDia-2	71	33 (28)	27; 40	289	43 (34)	39; 47	**0.020**
InterDia-3	71	32 (30)	25; 39	289	39 (33)	35; 43	0.075
E: LV intraventricular asynchrony							
LV-IntraDia-1	80	23 (21)	19; 28	305	36 (32)	32; 40	**0.002**
LV-IntraDia-2	80	24 (21)	19; 28	305	35 (34)	31; 38	**0.020**
LV-IntraDia-3	80	27 (22)	22; 32	305	34 (37)	30; 38	0.109
F: RV intraventricular asynchrony							
RV-IntraDia-1	73	45 (28)	39; 52	342	45 (28)	39; 52	0.803
RV-IntraDia-2	73	36 (27)	30; 42	342	39 (32)	36; 43	0.692
RV-IntraDia-3	73	26 (22)	21; 31	342	28 (31)	25; 32	0.738

SD = standard deviation, CI = 95% confidence interval (lower limit; upper limit). LV = left ventricle, RV = right ventricle. [*1] U-test. *[2] all measures in ms.

Table 5: Clinical relevance of calculated synchrony parameters.

		A						B					
			no structural heart disease		structural heart disease				no structural heart disease		structural heart disease		
		criterion*[1]	n	fulfilled [%]	n	fulfilled [%]	p-value	criterion*[2]	n	fulfilled [%]	n	fulfilled [%]	p-value
systolic asynchrony	Intersys-1	≥ 40 ms	0/ 70	0.0	25/ 386	6.5	0.021	≥ 35	5/ 70	7.1	40/ 386	10.4	0.516
	Intersys-2	≥ 40 ms	26/ 70	37.1	161/ 386	41.7	0.511	≥ 78	2/ 70	2.9	29/ 386	7.5	0.200
	Intersys-3	≥ 40 ms	3/ 70	4.3	49/ 386	12.7	0.041	≥ 40	3/ 70	4.3	49/ 386	12.7	0.041
	Intersys-4	≥ 40 ms	3/ 70	4.3	42/ 386	10.9	0.124	≥ 39	3/ 70	4.3	43/ 386	11.1	0.087
	Intersys-5	≥ 65 ms	0/ 71	0.0	14/ 289	4.8	0.081	≥ 40	5/ 71	7.0	38/ 289	13.1	0.219
	Intersys-6	≥ 65 ms	1/ 71	1.2	14/ 289	4.8	0.320	≥ 54	4/ 71	5.6	20/ 289	6.9	1.000
	LV-IntraSys-1	≥ 65 ms	0/ 80	0.0	14/ 305	4.6	0.085	≥ 42	5/ 80	6.3	37/ 305	12.1	0.160
	LV-IntraSys-2	≥ 65 ms	0/ 80	0.0	5/ 305	1.6	0.588	≥ 38	3/ 80	3.8	27/ 305	8.9	0.162
	RV-IntraSys-1	≥ 65 ms	0/ 72	0.0	2/ 342	0.6	1.000	≥ 30	4/ 72	5.6	47/ 342	13.5	0.073
	RV-IntraSys-2	≥ 65 ms	0/ 72	0.0	16/ 342	4.7	0.087	≥ 53	6/ 72	8.2	26/ 342	7.6	0.811
diastolic asynchrony	InterDia-1							≥ 96	5/ 71	7.0	28/ 289	9.7	0.647
	InterDia-2							≥ 89	3/ 71	4.2	34/ 289	11.8	0.079
	InterDia-3							≥ 92	2/ 71	2.8	20/ 289	6.9	0.272
	LV-IntraDia-1							≥ 65	2/ 80	2.5	49/ 305	16.1	**0.001**
	LV-IntraDia-2							≥ 66	3/ 80	3.8	46/ 305	15.1	**0.004**
	LV-IntraDia-|							≥ 71	6/ 80	7.5	23/ 305	7.5	1.000
	RV-IntraDia-1							≥ 101	2/ 73	2.7	30/ 342	8..8	0.092
	RV-IntraDia-2							≥ 90	2/ 73	2.7	21/ 342	6.1	0.397
	RV-IntraDia-3							≥ 70	4/ 73	5.5	23/ 242	6.7	1.000

*[1] (Yu et al. 2009), *[2] cut-offs: ≥ mean ± 2 standard deviation.

Table 6: Analyses of reproducibility**.** Please note that evaluation of reproducibility of systolic and diastolic parameters have been performed in two separate subgroups (see methods).

	observer 1		observer 2	Interobserver variability (O1A1 vs. O2)						Intraobserver variability (O1A1 v.s O1A2)					
	assessment 1	assessment 2						Bland-Altman						Bland-Altman	
	mean (SD)	mean (SD)	mean (SD)	p-value[*1]	ICC	CI (5; 95)	COV (%)	md	LOM	p-value[*1]	ICC	CI (5; 95)	COV (%)	md	LOM
systolic synchrony															
T-AVo	75 (13)	76 (14)	76 (13)	0.382	0.757	0.622; 0.844	10.9	-1.1	-24.0; 21.7	0.155	0.887	0.824; 0.927	8.1	-1.4	-18.3; 15.6
T-PVo	81 (15)	86 (16)	82 (14)	0.269	0.874	0.801; 0.921	8.4	-1.3	-20.2; 17.7	<0.001	0.878	0.719; 0.937	8.9	-5.3	-23.2; 12.6
T-Ecto1	76 (15)	75 (13)	77 (13)	0.610	0.849	0.766; 0.902	9.1	-0.6	-20.0; 18.9	0.059	0.877	0.808; 0.920	8.6	1.9	-15.7; 19.5
T-Ejcto2	87 (20)	86 (18)	88 (17)	0.438	0.799	0.687; 0.870	12.1	-1.3	-30.8; 28.2	0.586	0.911	0.862; 0.943	8.7	0.6	-20.3; 21.6
InterSys-1	13 (11)	14 (11)	12 (10)	0.124	0.728	0.564; 0.830	53.8	1.8	-16.7; 20.2	0.625	0.623	0.392; 0.766	58.0	-0.7	-22.8; 21.4
LV-IntraSys1	16 (13)	14 (13)	15 (12)	0.584	0.787	0.667; 0.863	46.8	0.6	-19.4; 20.7	0.169	0.740	0.595, 0.833	55.9	1.8	-21.0; 24.6
diastolic synchrony															
T-Emp1	590 (75)	592 (71)	581 (72)	0.012	0.977	0.952; 0.988	2.7	8.6	-32.1; 49.2	0.782	0.994	0.988; 0.997	1.4	0.5	-22.3; 23.3
T-Emp2	586 (74)	588 (80)	576 (77)	0.026	0.965	0.962; 0.982	3.4	0.7	-72.5; 73.9	0.307	0.987	0.975; 0.993	2.1	3.0	-32.0; 38.0
LV-IntraDia-2	28 (29)	35 (38)	28 (25)	0.956	0.733	0.472; 0.864	61.4	0.2	-49.1; 48.7	0.231	0.641	0.303; 0.801	78.2	-7.1	-75.5: 61.4

O = observer, A = assessment. SD = standard deviation, ICC = intraclass correlation coefficient, CI = 95% confidence interval (lower limit; upper limit), COV = coefficient of variation, md = mean difference, LOM = limits of agreement (lower; upper). [*1] paired t-test.