PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Discovery of biomarkers for the presence and progression of left ventricular diastolic dysfunction and HEart faiLure with Preserved ejection Fraction in patients at risk for cardiovascular disease. Rationale and design of the HELPFul case-cohort study in a Dutch cardiology outpatient clinic
AUTHORS	Valstar, Gideon; Bots, Sophie; Groepenhoff, Floor; Gohar, Aisha; Rutten, Frans; Leiner, Tim; cramer, maarten jan; Teske, Arco; Suciadi, LP; Menken, Roxana; Pasterkamp, Gerard; Asselbergs, Folkert; Hofstra, Leonard; Bots, Michael; den Ruijter, Hester

VERSION 1 - REVIEW

REVIEWER	Jay N. Cohn University of Minnesota
REVIEW RETURNED	28-Dec-2018

GENERAL COMMENTS	The goal to address diagnostic predictors of HFpEF is a high priority. The authors have devised a study protocol which could improve our diagnostic criteria and allow study of appropriate interventions. However, there are some concerns that need to be addressed. The decision to draw subjects from those referred to a cardiologist from a GP would seem to focus on those with symptoms whereas the goal is to identify those without symptoms who are likely to develop heart failure. The authors need to discuss and defend this strategy. The source of the data in Tables 2 and 3 is unclear. I assume this is pilot data. If so, the BNP data is inexplicable. If BNP elevation is a prerequisite for the diagnosis of heart failure the data contradict that. Since the biomarkers are a key component of the study the authors need to clarify how they are used in diagnosis and monitoring.

REVIEWER	Simin Liu Brown University, USA
REVIEW RETURNED	04-Jan-2019

GENERAL COMMENTS	This manuscript by Valstar et al. describes a “case-cohort” study – the HELPFul study, which aims to enroll participants with high risk for cardiovascular disease (CVD) in the Netherlands, and to examine potential drivers for the progression from left ventricular diastolic dysfunction (LVDD) to heart failure (HF) and gender disparities. The authors also proposed to develop a prediction model for LVDD and HFpEF. These aims are obviously of potential clinical and public health significance. However, the rationale for the “case-cohort” design and analysis sections as described are not clear.

	Moreover, it is not clear what specific kind of information would be collected during follow-up visits. I would suggest that the manuscript be restructured to address these issues detailed below: Major comments: 1. The entire paper should be carefully edited to make it error-free. The English in the paper is adequate for review, but not for publication. i.e. please rewrite the following sentences to make them clearer: 1) "If the definitions for the diagnosis of LVDD or HFpEF change over time, also these other definitions may be considered" – line 44-45 page 5. 2) "The HFpEF population is considered heterogeneous, with the syndrome possibly developing following multiple distinct pathways." – line 52-53 page 3. Please define acronyms when first use (line 15-16 page 6 – define LAVi, LVMi, and TR velocity); Please consistently using acronyms ("EF" in line 6 page 6 should be "LVEF", keep using "HF", "CAD","CMR" instead of "heart failure", "coronary artery disease", "cardiac magnetic resonance") 2. The entire study design is confusing. The HELPFul study is said to be a "case-cohort" with an estimated case-control ratio 3:1. Cases were defined as patients at cardiac outpatient clinic with E/e' ratio >=8 and controls were patients regardless of their echo results, which indicate that both cases and controls were defined and enrolled at baseline visit!? However, the outcomes of interest were actually LVDD and HF, not E/e' ratio. For a case-cohort study, using HF as the outcome of interest, cases should be incident HF patients adjudicated during the study follow-up and controls should be randomly selected at baseline from the entire cohort (regardless of their future HF status), therefore, controls would represent the source population that gives rise to the incident cases. Referring to this HFLPFul study, E/e' ratio >=8 (the definition of cases) is more like an exposure not the outcome, and therefore, this study is a prospective cohort study and should NOT be considered as a case-cohort design. Nested case-cohort samples can be extracted from the entire cohort after getting enough HF cases. It is not clear what was the rational for enrolling participants based on their E/e' ratio and setting the "case-control ratio" to be 3:1? I would suggest that they add a figure to delineate the entire study design, from participants screening to follow-up visits. 3. For a study protocol, the power calculation should be described with details, like what are the estimated exposure frequencies, odds ratios, and annual disease incidence. Minor comments: 1. Line 11-12 page 5, what's the inclusion criteria for "cases"? E/e' >=8 or >8? 2. Line 41-42 page 4, "eccentric remodeling" should be "adverse cardiac remodeling". 3. Line 7-8 page 4, "mechanisms as men tend to have more pronounced coronary macrovascular disease in men, while women…" should be "mechanisms as men tend to have more pronounced coronary macrovascular disease, while women…".

	4. Line 45 page 6, “E/e” should be “E/e’ ”. 5. Line 48 page 8, “For variables provided in table 1 proper statistical ..” should be “”For variables provided in table 1, proper statistical…” Line 52-53 page 8, “To investigate interaction of sex in the case and random sample interaction terms…” should be “To investigate interaction of sex in the case and random sample, interaction terms…” 6. No limitations have been described.

VERSION 1 – AUTHOR RESPONSE

Comments of reviewers:

1) The goal to address diagnostic predictors of HFpEF is a high priority. The authors have devised a study protocol which could improve our diagnostic criteria and allow study of appropriate interventions. However, there are some concerns that need to be addressed. The decision to draw subjects from those referred to a cardiologist from a GP would seem to focus on those with symptoms whereas the goal is to identify those without symptoms who are likely to develop heart failure. The authors need to discuss and defend this strategy.

We thank the reviewer for his comments. In the Netherlands, the cardiac outpatient clinic is positioned between the general practitioner and the hospital for quick referral and fast diagnostics, which results in a population at this center with fewer symptoms and lower cardiovascular disease risk than the population often seen in a similar setting at the hospital. It is within this population where we expect large variety in diastolic function, ranging from normal diastolic function to definite diastolic dysfunction and heart failure with preserved ejection fraction (HFpEF). HFpEF is a syndrome that often presents with intermittent complaints of dyspnea and/or other less typical symptoms, which can be difficult to detect, especially in elderly patients in the community. General practitioners (GPs) are often the first clinicians that see these patients with unexplained symptoms and have the possibility to refer them for fast work-up in these cardiac outpatient clinics. Due to the efficient workflow of the cardiac outpatient clinic it is possible to perform this diagnostic work-up for 10-20 new patients each day.. There is no waiting list and patients can often be seen within days, even if their complaints are not urgent. This makes referral a fast and convenient option for the general practitioner. Due to this unique setting the source population of the HELPFul study is different from patients that are referred to the hospital. It therefore provides an opportunity to study biomarker levels and risk factors in patients that have not developed LVDD or HFpEF yet, or are still in the early stages of LVDD or HFpEF.

2) The source of the data in Tables 2 and 3 is unclear. I assume this is pilot data. If so, the BNP data is inexplicable. If BNP elevation is a prerequisite for the diagnosis of heart failure the data contradict that. Since the biomarkers are a key component of the study the authors need to clarify how they are used in diagnosis and monitoring.

The BNP measurements are included in a panel of cardiovascular biomarkers that are measured in each participant of the HELPFul study as part of research. The BNP measurements are not part of routine clinical care. Therefore they are not used for diagnosis, or for monitoring of patients by the

cardiac outpatient clinic. In patients in whom the cardiologist of the cardiac outpatient clinic considers a cardiac biomarker measurement to be indicated, e.g. troponins or natriuretic peptides, this is performed as standard care by the cardiac outpatient clinic. However these are not the biomarker measurements we report in our study protocol manuscript. We have explained this in our manuscript on page 8:

“Plasma biomarkers

BNP and hs-TnI are measured in plasma of every participant as part of the research protocol. Hs-TnI and BNP are measured using the appropriate assays on the ARCHITECT i2000 analyzer (Abbott Park, Chicago, Illinois).”

1. The in text citation for “Supplemental Table S1” is missing in your main text of your main document file. Please amend accordingly.

We thank the reviewer for his comment and have amended the citation of supplemental table S1.

This manuscript by Valstar et al. describes a “case-cohort” study – the HELPFul study, which aims to enroll participants with high risk for cardiovascular disease (CVD) in the Netherlands, and to examine potential drivers for the progression from left ventricular diastolic dysfunction (LVDD) to heart failure (HF) and gender disparities. The authors also proposed to develop a prediction model for LVDD and HFpEF. These aims are obviously of potential clinical and public health significance.

We thank the reviewer for his comments.

However, the rationale for the “case-cohort” design and analysis sections as described are not clear. Moreover, it is not clear what specific kind of information would be collected during follow-up visits. I would suggest that the manuscript be restructured to address these issues detailed below:

We thank the reviewer for pointing out these inconsistencies, and have now more clearly described what we mean with case control, and for clarity have changed the section on the study design on page 6 and included a flow chart. We have added the following:

“Study design

HELPFul is a single center, prospective, case-cohort study conducted at a cardiac outpatient clinic in Utrecht, the Netherlands. All patients aged 45 years and older without previous cardiac interventions or congenital heart disease who are referred by the general practitioner (GP) to this outpatient clinic

are eligible for inclusion. On three of the four inclusion days, only patients with elevated filling pressures, defined as an E/e' ≥ 8.0 are eligible for inclusion. On the fourth day, 25% of all patients attending that day are invited to participate regardless of their echocardiography results (Table 1). The case-cohort design results in a group of 'cases' that have slightly elevated filling pressures, of whom a percentage may eventually deteriorate in diastolic function. Part of the patients in the case group may also already have LVDD. The random sample will reflect the distribution of exposure and also serve as a pool for the selection of healthy controls. With a case-cohort design the distribution of LVDD and HFpEF in the source population is accurately reflected in the random sample, while simultaneously creating a pool for the selection of controls[32]. A flow chart of the study design and procedures is presented in supplemental figure S2."'

We have also extended the section on follow-up to describe better what information will be collected, and we have added this to the methods section on page 8-9;

"All participants will be followed up for occurrence of any cardiac event (fatal and non-fatal myocardial infarction, proven unstable angina, coronary revascularization, hospitalization for heart failure, sudden death and death from any cause) by means of

1. linkage with regional (Julius General Practitioners Network)[41] and national registries (National Hospital Discharge Registry and Statistics Netherlands (i.e. National Causes of Death Registry))[42].

2. questionnaires sent through e-mail or letter 2 years after enrolment in HELPFul, after which a yearly questionnaire will be sent. The questionnaires will enquire after status of symptoms of cardiovascular disease and specifically of heart failure and hospitalization for cardiac disease."

Major comments:

1. The entire paper should be carefully edited to make it error-free. The English

in the paper is adequate for review, but not for publication.

We thank the reviewer for his suggestion and edited the manuscript carefully to improve the use of English for publication.

i.e. please rewrite the following sentences to make them clearer:

1) "If the definitions for the diagnosis of LVDD or HFpEF change over

time, also these other definitions may be considered" – line 44-45 page 5.

We have changed the sentence to:

If the diagnostic criteria for LVDD or HFpEF change over time we will incorporate these new criteria in the classification of LVDD or HFpEF in the HELPFul study population.

2) "The HFpEF population is considered heterogeneous, with the

syndrome possibly developing following multiple distinct pathways." – line 52-53 page 3.

We have deleted this sentence in the current manuscript.

3) Please define acronyms when first use (line 15-16 page 6 – define LAVi, LVMi, and TR velocity);

We have scrutinized the paper and all acronyms are first defined before the abbreviation is used.

4) Please consistently using acronyms ("EF" in line 6 page 6 should be "LVEF", keep using "HF", "CAD","CMR" instead of "heart failure", "coronary artery disease", "cardiac magnetic resonance")

We have scrutinized the paper and all acronyms are now used consistently.

2. The entire study design is confusing. The HELPFul study is said to be a "case-cohort" with an estimated case-control ratio 3:1. Cases were defined as patients at cardiac outpatient clinic with E/e' ratio >=8 and controls were patients regardless of their echo results, which indicate that both cases and controls were defined and enrolled at baseline visit!? However, the outcomes of interest were actually LVDD and HF, not E/e' ratio. For a case-cohort study, using HF as the outcome of interest, cases should be incident HF patients adjudicated during the study follow-up and controls should be randomly selected at baseline from the entire cohort (regardless of their future HF status), therefore, controls would represent the source population that gives rise to the incident cases. Referring to this HFLPFul study, E/e' ratio >=8 (the definition of cases) is more like an exposure not the outcome, and therefore, this study is a prospective cohort study and should NOT be considered as a case-cohort design. Nested case-cohort samples can be

extracted from the entire cohort after getting enough HF cases.

We thank the reviewer for expressing his concerns, and we realized that we have used the phrasing of both “case-cohort” and “case-control” in the manuscript which has caused the confusion. We will explain this better:

Case cohort: Characteristic of a case-cohort study is the prospective sampling of both cases and a random sample of the source population. In the HELPFul study both cases and the random sample is selected at baseline (T0) from the source population. We do not select those with established LVDD as cases, because until now most studies focus on patients with established LVDD or HFpEF. As a consequence, relatively little is known about patients with slightly elevated filling pressures. For this reason we have chosen this as the initial outcome for selection of cases. Our aim is to be able to compare participants who have developed elevated filling pressures, i.e. E/e’ ≥ 8, (cases) with non-cases (E/e’ ≤ 8). Therefore elevated filling pressures, reflected by E/e’ ≥ 8, is the definition of the outcome for cases in our case-cohort design. Generally an E/e’ ≥ 8.0 is not considered to be left ventricular diastolic dysfunction (LVDD), this is usually defined as E/e’ ≥ 13-15 and/or other objective echocardiographic evidence of LVDD. This is the outcome we have defined in our methods for LVDD. We expected the case group to be enriched for patients with established LVDD, making this sampling method more efficient for the purpose of recruiting patients with established LVDD. In this scenario the reviewer is again correct that it is similar to a nested case-control as selection of cases and controls is performed retrospectively from an existing prospective cohort study. The design allows for investigation of multiple stages of diastolic dysfunction. A second advantage is that it provides direct estimates of absolute risk, and not only risk ratios, because the random sample reflects the distribution of exposure of the source population and approximates the true incidence of cases in the source population. For determining the added value of biomarkers in clinical practice, absolute risk estimates are crucial for both caregivers and patients.

Case-control: For biomarker pilot studies we want to compare differences in biomarker levels in those with LVDD or HFpEF, now defined as a ‘case’, to (matched) controls without LVDD. For these pilot studies LVDD will be defined by a current diagnostic algorithm for LVDD, for instance the criteria defined by the ESC 2016 guidelines for heart failure. The controls will be selected from the random sample (subcohort).

It is not clear what was the rational for enrolling participants based on their

E/e’ ratio and setting the “case-control ratio” to be 3:1?

We have explained the rational for enrolling participants based on their E/e’ ratio above in more detail.

Case-control ratio of 3:1 is indeed confusing and was used erroneously and has been changed in the revised manuscript. When selecting cases and controls from the case-cohort for biomarker measurements we expect the sampling ratio to be 1:3. However this depends on the multiple factors, such as the research question and costs. We have included an example of a sample size estimation in the revised manuscript, page 9. Recruitment of participants in the case-cohort study was expected to be around 1:1.

I would suggest that they add a figure to delineate the entire study design,

from participants screening to follow-up visits.

We thank the reviewer for his suggestion and we have added a flowchart of the entire study design.

3. For a study protocol, the power calculation should be described with details,

like what are the estimated exposure frequencies, odds ratios, and annual

disease incidence.

We agree with the reviewer, and have decided to change this section of the manuscript and add an example of what we based the power calculation on, page 9 :

“Case-control selection within the case-cohort

For the case-cohort, we consider cases to be patients with echocardiographic-defined E/e’>8. Cohort refers to the total population sample (including those with E/e’>8). In this way we create a case-cohort design. For a nested diagnostic case-control study, we take samples from this case-cohort study in which the cases are patients with HFpEF, and controls (no HFpEF) are sampled from the cohort. For the power calculations, we use the 'Harrell's rule of thumb' applicable to diagnostic research. For the aforementioned calculation, we speculate that around 15% of the patients will be diagnosed with HFpEF; the true cases for the nested case-control design. Thus we can evaluate one diagnostic predictor in multivariable logistic regression analysis per 10 HFpEF cases. As we aim to analyse at least 15 determinants/biomarkers, we therefore would need at least 150 patients with HFpEF. Hence we require the inclusion of around 1000 patients in total.”

Minor comments:

1. Line 11-12 page 5, what’s the inclusion criteria for “cases”? E/e’ >=8 or >8?

The inclusion criteria is E/e’ of 8 or higher, we therefore changed it to E/e’ ≥ 8.0.

2. Line 41-42 page 4, “eccentric remodeling” should be “adverse cardiac

remodeling”.

In this case eccentric remodeling refers to hypertrophy with thinning of the left ventricular wall, which is typical of heart failure with reduced ejection fraction. We would therefore argue that the use of eccentric remodeling best describes what we intended to convey in this sentence. We therefore would like to keep this phrasing.

3. Line 7-8 page 4, “mechanisms as men tend to have more pronounced

coronary macrovascular disease in men, while women…" should be

"mechanisms as men tend to have more pronounced coronary macrovascular

disease, while women…".

We thank the reviewer for noticing and removed the recommended part; 'in men'.

4. Line 45 page 6, "E/e" should be "E/e' ".

We thank the reviewer for noticing and changed it to E/e'.

5. Line 48 page 8, "For variables provided in table 1 proper statistical .." should

be ""For variables provided in table 1, proper statistical…"

We thank the reviewer for noticing and changed this to: 'in table 1, proper..'.

Line 52-53 page 8, "To investigate interaction of sex in the case and random

sample interaction terms…" should be "To investigate interaction of sex in the

case and random sample, interaction terms…"

We thank the reviewer for noticing and changed this to: 'random sample, interaction..'.

6. No limitations have been described.

We have included the following limitations on page 12:

"Limitations

1. The cases as defined in the study design are not necessarily patients with LVDD or HFpEF by current diagnostic criteria, but were selected for having slightly elevated filling pressures on echocardiography. As explained in the discussion, there is a knowledge gap on which patients with a possible early form of LVDD will eventually progress towards actual LVDD. Therefore we consider sampling these patients to investigate biomarker levels and factors involved in progression to LVDD of high value. However we are sampling relatively many participants with no definite LVDD or HFpEF by current diagnostic criteria. This however will only lead to a lower efficiency for selection of patients with LVDD or HFpEF, whereas it does provide the opportunity to study patients most at risk for eventually developing LVDD or HFpEF efficiently.

2. Our study population has a high prevalence of hypertension, but a low prevalence of chronic inflammatory comorbidities that are associated with LVDD and HFpEF, such as diabetes mellitus and overweight. Furthermore chest pain is a common complaint, hinting toward the importance of possible microvascular dysfunction in this study population. The external validity could be affected when the generalizability to the population at risk of developing LVDD or HFpEF in the community is lower. However hypertension and CMD are known to be important in the development of LVDD and HFpEF. Therefore our study population is representative of for instance community based elderly of 65 years and older. Even so prudence is appropriate when generalizing results to community based or hospital based patients with LVDD or HFpEF, particularly in the context of chronic inflammatory comorbidities.

3. Patients are referred to this center when the GP considers referral to be indicated. However the cardiology outpatient clinic does not record the indication for referral in their electronic patient database. Therefore we have no data on the indication for referral. As mentioned previously patients that are referred often do not have acute/severe complaints of cardiac problems. This could lead to referral bias, which is a form of selection bias usually affecting the comparison of cases and controls and generalizability of the results. Due to our study design we do not expect referral bias to be a problem, because cases and participants of the random sample are selected from the same source population. Furthermore, if future results are generalized within the context of the study we do not see a problem with external validity."