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Katja Buschmann*, Ryan Chaban, Anna Lena Emrich, Marwan Youssef, Angela Kornberger, Andres Beiras-Fernandez and Christian Friedrich Vahl

Septic cardiomyopathy: evidence for a reduced force-generating capacity of human atrial myocardium in acute infective endocarditis

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Abstract

Background: This study analyzes the myocardial force-generating capacity in infective endocarditis (IE) using an experimental model of isolated human atrial myocardium. *In vivo*, it is difficult to decide whether or not alterations in myocardial contractile behavior are due to secondary effects associated with infection such as an altered heart rate, alterations of preload and afterload resulting from valvular defects, and altered humoral processes. Our *in vitro* model using isolated human myocardium, in contrast, guarantees exactly defined experimental conditions with respect to preload, afterload, and contraction frequency, thus not only preventing confounding by *in vivo* determinants of contractility but also excluding effects of other factors associated with sepsis, hemodynamics, humoral influences, temperature, and medical treatment.
Methods: We analyzed right atrial trabeculae (diameter 0.3–0.5 mm, initial length 5 mm) from 32 patients undergoing aortic and/or mitral valve replacement for acute valve incompetence caused by IE and 65 controls receiving aortic and/or mitral valve replacement for nonendocarditic valve incompetence. Isometric force amplitudes and passive resting force values measured at optimal length in the two groups were compared using Student's t-test.
Results: There were no significant differences between the groups in terms of the passive resting force. The isometric force amplitude in the endocarditis group, however, was significantly lower than in the nonendocarditis group ($p = 0.001$). In the endocarditis group, the calculated active force, defined as the isometric force amplitude minus the resting force, was significantly lower ($p < 0.0001$) and the resting force/active force ratio was significantly higher ($p < 0.0001$). Using linear regression to describe the function between resting force and active force, we identified a significant difference in slope ($p < 0.0001$), with lower values found in the endocarditis group.
Conclusion: Our data suggest that the force-generating capacity of atrial myocardium is significantly reduced in patients with IE. In these patients, an elevated resting force is required to achieve a given force amplitude. It remains unclear, however, whether this is due to calcium desensitization of the contractile apparatus, presence of myocardial edema, fibrotic remodeling, disruption of contractile units, or other mechanisms.

Keywords: acute infective endocarditis; human atrial myocardium; myocardial contractility; septic cardiomyopathy.

Introduction

Infective endocarditis (IE) is an endovascular microbial infection of cardiovascular structures. The characteristic lesions consist of vegetations composed of thrombotic platelets, microorganisms (bacteria), fibrin, and inflammatory cells [1]. Despite improvements in surgical and anti-infective therapy, IE still carries a high morbidity and mortality [2]. When the aortic and/or mitral valves are destroyed by bacterial vegetations, left heart decompensation caused by volume and/or pressure overload may be present. Reflex tachycardia and depressed peripheral resistance may cause additional damage to the myocardium.

Acute IE may be defined in terms of a multifactorial systemic disease that is associated with systemic inflammatory response syndrome (SIRS) secondary to the

***Corresponding author: Katja Buschmann,** Department of Cardiothoracic and Vascular Surgery, Hospital of the Johannes Gutenberg-University Mainz, Langenbeckstraße 1, 55131 Mainz, Germany, E-mail: katja.buschmann@unimedizin-mainz.de
Ryan Chaban, Anna Lena Emrich, Marwan Youssef, Angela Kornberger, Andres Beiras-Fernandez and Christian Friedrich Vahl: Department of Cardiothoracic and Vascular Surgery, Hospital of the Johannes Gutenberg-University Mainz, Mainz, Germany

infection. SIRS is typically accompanied by arterial hypotension, caused by profound systemic vasodilatation, and multiple organ dysfunction [3]. It represents a state of acute circulatory failure characterized by persistent arterial hypotension despite adequate fluid resuscitation or tissue hypoperfusion (as evidenced by lactate levels >4 mg/dL) not explained by other causes [4].

According to Drosatos et al. [5], cardiac dysfunction plays an important role in the development of multiorgan failure caused by severe sepsis. They observed that mortality was higher in septic patients with systolic and diastolic cardiac dysfunction than in septic patients without cardiac dysfunction. The exact pathophysiological pathways underlying septic circulation patterns or septic cardiomyopathy (SCMP), however, remain to be clarified.

To better understand the hemodynamics of a septic patient, the analogy between the heart and a hydraulic pump serving to produce the cardiac output may be helpful. The work such a pump is capable of rendering is determined by the interaction of preload, global ventricular performance, and afterload [6]. Septic shock, on the one hand, is associated with an increased heart rate and a reduced afterload resulting in an increase in cardiac output. On the other hand, there is relative hypovolemia reducing the preload and the left ventricular end-diastolic volume. Reduced myocardial stretch caused by less end-diastolic volume bates cardiac output [3].

SCMP requires some consideration as well. SCMP may be induced by IE as demonstrated by animal studies showing that the vegetation-associated host (bacteria) protease activity not only leads to proteolytic tissue damage, as evidenced by the release of extracellular matrix fragments such as fibrinogen, but also induces inflammation and myocardial cell damage [7]. In this respect, it is essential to note that SCMP can be caused not only by IE but also by any infective focus in the organism that gives rise to systemic endotoxemia [8].

The mechanisms by which sepsis causes cardiac dysfunction are not completely understood yet. Released in large quantities in patients with septic hemodynamics, circulating inflammatory cytokines such as interleukins or tumor necrosis factor-α (TNF-α) may, for example, be among the factors accounting for SCMP [5]. Clinical studies in humans investigating the pathways of SCMP are therefore subject to considerable limitations due to a variety of *in vivo* influences exerted not only by intrinsic factors but also by hemodynamic alterations caused by sepsis [3, 6, 9–13].

Recent *in vitro* experiments, on the contrary, were limited by poor availability of human myocardium. Therefore, recent work focused on analyzing the cardiodepressant effect of serum obtained from septic humans on murine [14–18] or rabbit [19, 20] myocardium.

In cardiac surgery, we frequently see patients with IE in need of valve surgery for acute and severe valve incompetence caused by infective processes at the level of the endocardium. From these patients, in whom intrinsic inflammation/SCMP is present, we obtained tissue specimens for our isolated right atrial myocardium model.

This study introduces an experimental set-up using human myocardium. As a particular advantage, this particular model eliminates the entirety of hemodynamic and other uncontrolled circumstances and allows establishing standardized measurement conditions. As atrial myocardium is supplied by diffusion from the (systemic) intra-atrial blood, the design of our model excludes all causes of myocardial dysfunction other than the intrinsic SCMP. This applies, in particular, to factors such as ischemic myocardial dysfunction secondary to coronary artery disease.

Moreover, there is some evidence that infective myocardial dysfunction in acute IE is not explained by (1) the development of endothelial injury or (2) the early development of atherosclerosis or (3) triggered by acute events caused by vulnerable plaques or the activation of the coagulation cascade [21].

In short, our model allows the analysis of the electromechanical properties of isolated right atrial myocardium under consistent oxygenation and nutrition conditions.

Cardiac electromechanical coupling (CEMC) measurement is a well-known technique that may be used to demonstrate changes in myocardial contractility [22]. Individual trabeculae all show a positive correlation between sarcomere length and generated tension, also referred to as the length-dependent active force, which underlies the Frank-Starling law [23]. The relation between the muscle length or sarcomere length, on the one hand, and the tension developed at optimal sarcomere length (with best contraction), on the other hand, is more pronounced in cardiac than in skeletal muscle [24].

In CEMC, the Frank-Starling mechanism (FSM) is defined as the myocardial contractility that depends on the preload, that is, the passive tension of the myocardium before active contraction. The greater the passive strain of the myocardial sarcomere is, the greater is the ensuing active contraction. Increasing the end-diastolic preload therefore leads to optimal muscle length for maximal active contraction force resulting from the optimal overlapping of actin and myosin filaments. The cardiac-specific sarcomeric structure is mainly responsible for the FSM [25]. The maximum force development of the muscle

is observed at optimal length. To allow comparability, all our measurements were performed at optimal length.

Materials and methods

Human atrial myocardium was obtained from 32 patients operated upon for acute IE (group E, with microbiologically proven positive blood cultures), who required urgent or emergent aortic or mitral valve surgery caused by bacterial valve destruction with acute valve insufficiency.

Our controls (group C) consisted of atrial tissue samples from 65 patients who required valve surgery for aortic and/or mitral valve incompetence caused by noninfective causes.

The gender distribution (female/male) was similar in both groups (1/3).

Age was also not different in both groups: group E with mean age of 63.4 ± 13.1 years (range 23–78 years) versus group C with mean age of 64.6 ± 8.1 years (range 42–77 years; n.s.).

The human myocardium we used for our experiments consisted of the tips of the right atrial appendages that were removed in the course of venous cannulation for extracorporeal circulation. The study was in accordance with the institution's ethical guidelines. These myocardial tissue specimens were immediately stored in warm (37 °C) and oxygenated Krebs-Henseleit solution and transferred to our laboratory for CEMC measurement without delay. For this purpose, the right atrial tissue was prepared under the microscope to yield equally sized (0.3–0.5 × 5.0 mm) trabeculae meeting the standardized conditions for CEMC measuring using our myocardial force measurement device.

After transfer into ice-cold Krebs-Henseleit solution, the trabeculae were equilibrated in warm (37 °C) oxygenated Krebs-Henseleit solution for 30 min. Being thus prepared, the right atrial trabeculae from both groups were clamped into the connecting force transducer and vibrator of the force measurement device where they were continuously flushed with warm (37 °C) Krebs-Henseleit solution. Electrical stimulation was applied in square wave at a frequency of 60 beats/min. During electrical stimulation, the trabeculae clamped into the apparatus were prestreched, during continuous force measurement, from slack to optimal length, the latter being defined as the length at which maximum force output was achieved. At optimal length, the diameter of the fiber was measured under the microscope. The respective resting force and isometric force were recorded.

The passive resting force (mN/mm²) represents the passive force that was recorded after achieving optimal length of muscle stripe. This force was measured, as the response of the muscle stripe to the prestretching process, without application of electrical stimulation. The isometric force amplitude (mN/mm²) is the force amplitude recorded during supramaximal electrical stimulation of the muscle stripe at optimal length.

The active force (mN/mm²) is a calculated value that represents the difference between the passive resting force and the force amplitude (i.e. force amplitude minus resting force). To gain an insight into the relation between the degree of myocardial prestretching required, as expressed by the recorded passive resting force value, and the achievable active force response (force amplitude minus resting force) of the muscle stripe, the resting force/active force ratio was additionally calculated for each myocardial specimen.

Definitions

Passive resting force (mN/mm²): recorded at optimal length of muscle stripe (after prestretching process) and without application of electrical stimulation.

Force amplitude (mN/mm²): recorded during supramaximal electrical stimulation of muscle stripe at optimal length.

Active force (mN/mm²): calculated remaining active force after subtraction of the passive resting force value from the total force amplitude.

Resting force/active force: calculated ratio of the passive resting force to the active force.

Statistical analysis

Recorded and calculated EMC data from both groups were compared using Student's t-test. Additionally, linear regression analysis was performed. All statistical analyses were implemented using SPSS version 23 statistical software. $p < 0.05$ was considered statistically significant.

Results

As illustrated in Table 1, the passive resting force measured at optimal length was slightly higher in the atrial myocardium of group E than group C. However, this difference did not reach statistical significance.

Table 1: Recorded and calculated values of EMC (mean and standard deviation) for the endocarditis and control groups.

	n	Mean, mN/mm²	Standard deviation, mN/mm²
Passive resting force, mN/mm²			
Endocarditis	32	1.622	0.2612
Control	65	1.554	0.4008
p = 0.319			
Force amplitude, mN/mm²			
Endocarditis	32	4.844	0.9873
Control	65	5.812	1.3646
p = 0.001			
Active force, mN/mm²			
Endocarditis	32	3.222	0.8435
Control	65	4.258	1.0076
p < 0.0001			
Resting force/Active force			
Endocarditis	32	0.531478	0.147436
Control	65	0.367774	0.058371
p < 0.0001			

$p < 0.05$ is significant (Student's t-test).

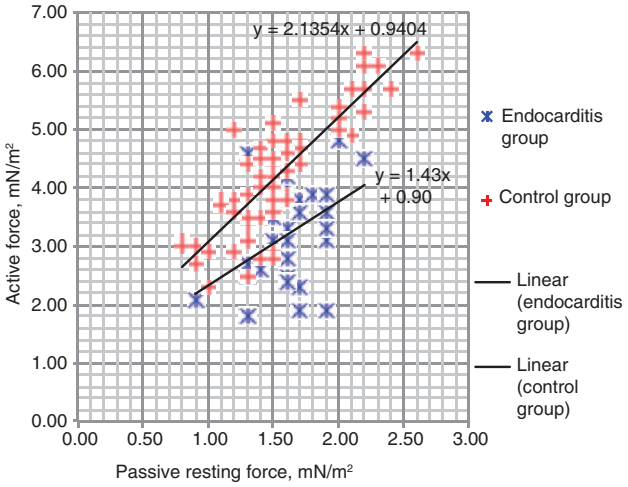

Figure 1: Active force (mN/mm²) as a function of passive resting force (mN/mm²) for the endocarditis group (n = 32; blue) and control group (n = 65; red).
All individual values form the linear regression line with significantly different slopes.

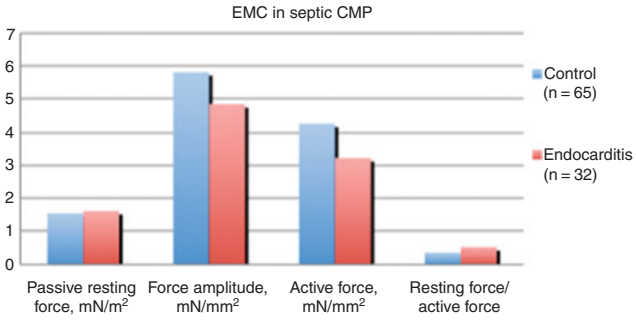

Figure 2: Mean values of EMC measured [passive resting force (mN/mm²) and force amplitude (mN/mm²)] and calculated values [active force (mN/mm²) and ratio of resting force/active force] for the endocarditis group (n = 32; red) and control group (n = 65; blue).

In Figure 1, the active force is shown as a function of the passive resting force. A linear regression line was fitted to the data for better illustration of the linear function of the relationship between the passive resting force required and the active force response in the myocardium. The slope of the linear function differed significantly between groups E and C ($p < 0.0001$). The fact that the linear function obtained for group E is less steep than that for group C indicates that it is not possible to achieve the same level of active force response in the myocardium of patients with IE than in the myocardium of the control group.

Figure 2 provides an overview of the basic findings in terms of the mean passive resting force and force values measured as well as the active force values and resting force/active force ratios calculated for groups E and C. Statistically significant differences between the mean force amplitude values measured and the active force values and resting force/active force ratios calculated for the two groups are represented in Table 1.

Statistical analysis (Table 1) did not yield a significantly elevated passive resting force in group E, although there seems to be a trend toward a higher passive resting force in patients with IE and SCMP. Interestingly, however, the force amplitude is significantly reduced in endocarditis myocardium (4.8 vs. 5.8 mN/mm²) in SCMP. Additionally, the calculated active force was significantly lower (3.2 vs. 4.3 mN/mm²) and the passive resting force/active force ratio was significantly higher (0.53 vs. 0.37 mN/mm²) in group E.

Discussion

In view of the fact that IE is a highly complex disease in the course of which a variety of factors, including valve insufficiency and sepsis, exert their respective influences on hemodynamics, our study design compares the force-generating capacity of human atrial myocardium out of patients showing acute valve insufficiency (group C) with atrial myocardium out of patients showing acute valve insufficiency caused by bacterial vegetations (group E) in the intention to observe only the "septic" effect on myocardium.

The isolated atrial myocardium model introduces an experimental set-up that not only uses human myocardial tissue but also enables us to perform highly standardized measurements of myocardial function in the setting of SCMP. Under *in vivo* conditions, preload, afterload, and frequency are determined by the inflammatory process of endocarditis, so that it is not possible to make a differentiation between the hemodynamic septic effect (caused by vasodilatation) and indeed a reduced cardiac contractility (SCMP). Thus, the *in vitro* model of isolated human myocardium is adequate to compare the hemodynamics related to valve insufficiency (group C) with an additionally endocarditis (group E) under exactly defined conditions of preload, afterload, and frequency. Under clinical conditions, a comparison of both groups is not possible because there is no way of standardized differentiation.

Based on tissue specimens from a large patient population, our current analysis yielded three important observations: in the atrial myocardium of patients operated upon for IE, (1) the extent of force generation at optimal length was reduced, (2) the passive resting force required to achieve a given force amplitude was elevated, and (3) the passive resting force at optimal length was not significantly altered.

The passive resting force at optimal length is determined by several factors, with the parallel and serial elastic units of the muscle contributing in their capacity as muscular elements. Moreover, with any alteration of the quality and mass of connective tissue, fibrosis (perimysial or endomysial fibrosis) may be able to alter the passive resting force. On a molecular level, the number of adherent cross-bridges between the contractile units plays an important role similar to that of permanently fixed cross-bridges (latch bridges). However, as the passive resting force is similar in both groups, there is no direct evidence for alterations on the connective tissue level (inflammatory fibrosis). On the contrary, our current experiments do not exclude the possibility that the mass of connective tissue per square millimeter may be reduced. After all, an increased number of permanently fixed cross-bridges could compensate for a decreased amount of connective tissue. However, in the current literature, there is no evidence for a latch-bridge mechanism in the human myocardium of patients with IE.

The isometric force amplitude was recorded based on a standardized protocol [26], applying supramaximal stimulation, which means that we provoked the maximum achievable force response after prestretching to optimal length. In group E, the maximal force amplitude was significantly lower than in group C. This suggests (a) a reduction of contractile units contributing to force generation per square millimeter or (b) a reduced force output per contractile unit. A reduced number of contractile units per square millimeter may be the result of myocardial edema or destruction of contractile units. The destruction of contractile units in this respect may be functional (i.e. after a history of overstretching) or morphologic [i.e. as a function of insufficient supply (infarction)]. Clinically, these findings translate into an impairment of the systolic function.

The calculated resting force/active force ratio was significantly higher in group E than in group C, which indicates that in SCMP an elevated preload (resting force) is required to achieve a given active force amplitude. On the muscle preparation level, this means that there seems to be a diastolic dysfunction.

In summary, our data suggest that IE causes a severe impairment of systolic and diastolic function. Thus, we conclude that the FSM is impaired in SCMP.

The need for a higher preload (with a higher resting force), corresponding to a higher prestretching tension to achieve optimal length for maximal force development, in group E is in keeping with the FSM, stipulating the need for the optimal overlap of actin and myosin filaments in the sarcomeric structure [25] to generate maximum force. With a trend toward an elevated passive resting force in SCMP, there seems to be an attenuated diastolic dysfunction perhaps caused by fibrotic remodeling and potentially leading to an impaired systolic function caused by reduced prestretching of the muscle as a further consequence.

Therefore, we assume that SCMP has no harmful effect on the sarcomeric structure that is responsible for the maximum force development by actin and myosin interaction after optimal prestretching [25].

The diastolic dysfunction in SCMP seems to be caused by myocardial stiffness due to fibrotic remodeling of the cytoskeleton and the extracellular matrix of the myocardium. This is in keeping with recent studies reporting fibrotic tissue modifications caused by inflammation and cytokines [27]. This correlation between cytokines such as TNF-α and interleukin-10, on the one hand, and an impaired cardiac function and elevated mortality, on the other hand, has been already proven.

It is well possible that the quantity of sarcomeres in a muscle stripe of standardized muscle thickness could be reduced due to edema/swelling of myocytes or swelling of the extracellular structure. Edema could therefore be a reason for a significantly lower active force in SCMP. Of note, histopathological postmortem observations of patients who died of septic shock showed interstitial fibrosis in 100% and interstitial edema in 90% of the patients [28].

Sepsis is a circulatory compromise with microcirculatory alterations and mitochondrial damage, and all these factors reduce cellular energy production. This is the origin of sepsis-induced myocardial dysfunction mediated by cytokines [11]. This state seems to be reversible [17] in survivors, so that the myocardium may in fact experience a hibernation-like nonfunctional condition during severe sepsis. To reduce myocardial oxygen consumption, β-blocker Ivabradin and insulin are recommended as therapy strategy.

We assume that any infectious agent initially causes inflammation with circulating cytokines induced by endotoxemia. Sepsis and its hemodynamic changes including a reduced afterload caused by vascular nitric oxide (NO) result in the release of cytokines such as TNF-α and interleukins [29]. As it is also well known, TNF-α in its turn stimulates macrophages to produce NO [30]. Elevated NO and increased reactive oxygen species (ROS) lead first to myocardial hibernation and reduced contractility [29] and second to fibrotic remodeling [31–33] by tipping the balance between ROS and antioxidant enzymes.

The early use of antibiotics to keep inflammatory mediators from exerting their full effects may be considered recommendable. Critical care monitoring of troponin

and B-type natriuretic peptide (BNP) is crucial for the detection of SCMP, which in the course of time will result in cardiac failure and left ventricular dilatation [23].

Nevertheless, an elimination of the septic focus (bacterial vegetation) in good time by early operation should be recommended [34].

Limitations

The study protocol was very simple and focused on mechanical performance. Additional histopathological data of the preparations are not available. Although the current data suggest an impairment of myocardial function in atrial myocardium, it is likely that similar effects could be observed in ventricular myocardium, although this cannot be derived with a sufficient degree of certainty from our experiments, which were implemented using atrial myocardium only. In this respect, the differences between atrial and ventricular myocardium in terms of their blood supply, microcirculation, and pressure conditions as well as other characteristics must be considered.

Furthermore, the proven cardiac depressant effect is not independently observed in septic patients, as patients with IE undergoing cardiac surgery (as an essential requirement to obtain human myocardium for our experimental set-up) mostly also present with valve insufficiency.

Perspectives

The data argue for the presence of a significant cardiodepressant effect on atrial myocardium associated with the occurrence of endocarditis. There are several candidates that may contribute to the pathophysiological and pathoanatomical chain leading to reduced contractility. However, based on the current data, no decision in favor of one or some of these factors is justified.

Author Statement

Research Funding: Authors state no funding involved. Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals obtained in this study. Ethical approval: The research related to human use complied with all the relevant national regulations and institutional policies, was performed in accordance to the tenets of the Helsinki Declaration, and has been approved by the author's institutional review board or equivalent committee.

Author Contributions

Katja Buschmann: Formal analysis; Resources; Writing (original draft); Writing (review and editing). Ryan Chaban: Formal analysis. Anna Lena Emrich: Writing (review and editing). Marwan Youssef: Formal analysis. Angela Kornberger: Writing (original draft). Andres Beiras-Fernandez: Formal analysis; Supervision; Writing (original draft). Christian Friedrich Vahl: Funding acquisition; Methodology; Writing (original draft); Writing (review and editing).

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Supplemental Material: The article (DOI: 10.1515/iss-2016-0202) offers reviewer assessments as supplementary material.

Reviewer Assessment **Open Access**

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***Corresponding author: Katja Buschmann,**
Department of Cardiothoracic and Vascular Surgery, Hospital of the Johannes Gutenberg-University Mainz, Langenbeckstraße 1, 55131 Mainz, Germany, E-mail: katja.buschmann@unimedizin-mainz.de

Reviewers' Comments to Original Submission

Reviewer 1: Kwabena Frimpong-Boateng

Dec 11, 2016

Reviewer Recommendation Term:	Revise with Major Modifications
Overall Reviewer Manuscript Rating:	50

Custom Review Questions	**Response**
Is the subject area appropriate for you?	5 - High/Yes
Does the title clearly reflect the paper's content?	3
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	3
Are the results/conclusions justified?	2
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	2
Are units and terminology used correctly?	2
Is the number of cases adequate?	4
Are the experimental methods/clinical studies adequate?	2
Is the length appropriate in relation to the content?	3
Does the reader get new insights from the article?	3
Please rate the practical significance.	2
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	3
Please rate the appropriateness of the figures and tables.	3

Please rate the appropriateness of the figures and tables.	3
Please rate the appropriateness of the references.	3
Please evaluate the writing style and use of language.	5 - High/Yes
Please judge the overall scientific quality of the manuscript.	2
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:

This is an interesting paper on the use of human atrial myocardium to investigate the mechanisms underlying the well documented phenomenon of sepsis-induced myocardial depression, so-called septic cardiomyopathy (SCMP).

The paper is well-written for the most part but there are several issues the authors must address:

1. The study utilizes intraoperative specimens taken from the right atrial appendages of 32 patients with infective endocarditis requiring urgent surgical intervention (mitral and/or aortic valve replacements) and compares them with 65 control patients needing similar operations but without endocarditis. No mention is however made of whether the authors sought and obtained informed consent from these 97 subjects or whether institutional ethical approval was obtained to conduct the study.

2. The authors indicated that the patients and controls were similar with regards to gender. Age is ostensibly another important confounder. Older patients would be expected to have age-related changes in contractility independent from the surgical indication. Did the authors control for age in the study? Similarly, one is left to assume that these patients were all adults; this ought to be stated if that was case.

3. The title of the paper is: SEPTIC CARDIOMYOPATHY: EVIDENCE FOR A REDUCED FORCE GENERATING CAPACITY OF HUMAN ATRIAL MYOCARDIUM IN ACUTE INFECTIVE ENDOCARDITIS. The authors seem to have assumed that septic cardiomyopathy is somewhat interchangeable with infective endocarditis. This is not so. Septic cardiomyopathy is an undisputed manifestation of sepsis especially when accompanied by shock but the same cannot be said about infective endocarditis: SIRS is the usual trigger of SCMP and is always present in sepsis but not invariable in infective endocarditis. The implication is one can investigate the myocardial depression in septic cardiomyopathy because it is always present; however, myocardial depression is not always present in infective endocarditis unless there is accompanying SIRS. The authors may wish to amend the title to reflect this fact.

4. Following up on 3 above; the Methods section of the study needs to clarify if indeed all the 32 patients with IE satisfied current criteria for establishment of the diagnosis of SIRS, Sepsis, or Septic Shock.

5. The authors indicate in their introduction that „the atrium is supplied by diffusion from the (systemic) intra-atrial blood“. This cannot be entirely true. The right atrium, including the auricular appendage receives arterial blood supply from branches of the right coronary artery in most individuals. It is not possible that a working cardiac muscle, even if it is atrial, can be adequately supplied by diffusion from intra-atrial venous blood. Indeed the right atrium, including the auricular appendage, receives blood supply from the right coronary artery (RCA). Within millimeters after emerging from the aorta, the right coronary artery gives off two branches, the conus artery which runs to the right ventricular outflow tract, and the atrial branch which gives off the SA nodal artery, which runs along the anterior right atrium to the superior vena cava, encircling it before reaching the SA node. If it is accepted that the atrial wall receives arterial blood supply then the following assumption made by the authors in the design of their experiments will be questionable: „Our model excludes all causes of myocardial dysfunction other than intrinsic SCMP. This applies in particular to factors such as ischemic myocardial dysfunction secondary to coronary artery disease“.

6. In the section on Discussion, the authors state „The isometric force amplitude was recorded based on a standardized protocol applying supramaximal stimulation …“ without describing or citing a reference for this protocol. The reference should be indicated.

7. In the same paragraph referred to in 5 above, the authors suggest ‚a reduced number of contractile units per square millimeter‘ may be responsible for the contractile defect. Of note, this study only investigated the electromechanical aspects of the myocardial depression. No histologic data was provided. The study therefore does not provide any direct evidence for this claim. If they have evidence from the work of others, they may wish to provide that otherwise the argument is unwarranted. Others have given very cogent reasons for this effect - impaired contractile response to calcium reversed by the calcium sensitizer levosimendan; myocardial cGMP and NO metabolic derangement induced by circulating TNF-a and IL-1a; etc. If they have contrary or additional suggestions, they need to support them.

8. In paragraph 5 of the discussion, the authors state that there seems to be diastolic dysfunction in these patients, but others have already both systolic and diastolic dysfunction involving the LV especially and not sparing the RV. Milrinone and dobutamine apparently improve the systolic dysfunction but not the diastolic dysfunction. Levosimendan, a new calcium sensitizer was shown to reverse both systolic and diastolic dysfunction in in vitro studies.

9. In paragraph 6 of discussion, the authors again interchange infective endocarditis with SCMP when they state „In summary our data suggest that IE causes a severe impairment of systolic and diastolic function. And thus we conclude that the FSM is impaired in SCMP“. They need to clarify this as it is the SIRS element in IE that is the cause of myocardial depression, not just the IE per se.

10. In paragraph 7 of discussion, the authors imply that diastolic dysfunction is attributable to fibrotic remodeling occurring in SCMP. Again the authors need to show the evidence for this. It is a well noted characteristic of septic cardiomyopathy that it is acute and the myocardial depression is reversible in those who survive it. Some workers reported that LV ejection fraction is normalized in a few days [see Chest. 1999 Nov; 116(5):1354-9]. Also, the authors are suggesting that the systolic dysfunction results from the same hemodynamic effect (the preload defect) that their model has eliminated in this study! The work of many investigators suggest the contractile defect is intrinsic to the myocardium and results from humoral factors e.g. TNF-a, IL-1-a. (Please check: Parrillo et al. A circulating myocardial depressant substance in humans with septic shock. Septic shock patients with a reduced ejection fraction have a circulating factor that depresses in vitro myocardial cell performance. J Clin Invest. 1985 Oct; 76(4):1539-53) and (Hollenberg et al. Tumor necrosis factor depress myocardial cell function: results using an in vitro assay of myocyte performance. Clin Res. 1989; 37:528-534).

11. The issues with diastolic dysfunction and fibrotic remodeling are recurrent. The references given to support the assertion do not seem to support the idea. E.g. the paper cited in ref 27 is on cystic fibrosis and not on fibrosis in septic cardiomyopathy. Ref 28 reports on septic shock patients who died, a very different group from those who survive. The changes reported may not be generalizable to those who survive septic shock, whose circulatory insufficiency is usually reversible in a few days (Chest. 1999 Nov; 116(5):1354-9). I have gone through ref 21 and did not see where it was stated that „Elevated nitric oxide and increased reactive oxygen species (ROS) lead firstly to myocardial hibernation and reduced contractility and secondly, after 7-10 days [21], to fibrotic remodeling by tipping the balance between ROS and antioxidant enzymes.“ The authors need to verify these references and make the corrections as appropriate.
12. In the conclusion it is stated „In conclusion, and based on our data, we first of all recommend anti-edematous treatment to prevent a further reduction of contractile units per mm2 in SCMP.“ But the data presented shows no demonstration of the reduction of contractile units, which is a histologic feature not investigated in this study. At this stage, this assertion is only a hypothesis that needs investigation, no conclusions yet to be drawn. Their study shows convincingly the myocardial depression occurring in sepsis using a model of human atrial myocardium and their findings in this regard ought to be pointed out; they have not investigated the mechanisms underlying the myocardial depression and thus conclusions based on the mechanisms do not appear justifiable.
13. Finally, the authors made no comment on whether other groups have performed similar in vitro studies using human myocardium and what their findings were. If no such studies are available, they need to point that out as well.

Reviewer 2: anonymous

Jan 10, 2017

Reviewer Recommendation Term:	Reject
Overall Reviewer Manuscript Rating:	40

Custom Review Questions	**Response**
Is the subject area appropriate for you?	4
Does the title clearly reflect the paper's content?	5 - High/Yes
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	4
Are the results/conclusions justified?	3
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	3
Are units and terminology used correctly?	3
Is the number of cases adequate?	2
Are the experimental methods/clinical studies adequate?	2
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	3
Please rate the practical significance.	2
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	3
Please rate the appropriateness of the figures and tables.	4
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	1 - Low/No
Please judge the overall scientific quality of the manuscript.	2
Are you willing to review the revision of this manuscript?	No: Not good enough

Comments to Authors:
Interesting study with severe linmitations.
Ethics are not adressed.
Why do authors recommend anti-edema treatment in infective endocarditis?
Authors have given no proof that edema causes a decline active force.

Reviewer 3: anonymous

Feb 08, 2017

Reviewer Recommendation Term:	Revise with Major Modifications
Overall Reviewer Manuscript Rating:	85

Custom Review Questions	**Response**
Is the subject area appropriate for you?	4
Does the title clearly reflect the paper's content?	4
Does the abstract clearly reflect the paper's content?	3
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	1 - Low/No
Are the results/conclusions justified?	4
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	3
Are units and terminology used correctly?	5 - High/Yes
Is the number of cases adequate?	5 - High/Yes
Are the experimental methods/clinical studies adequate?	4
Is the length appropriate in relation to the content?	1 - Low/No
Does the reader get new insights from the article?	4
Please rate the practical significance.	4
Please rate the accuracy of methods.	4
Please rate the statistical evaluation and quality control.	2
Please rate the appropriateness of the figures and tables.	3
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	5 - High/Yes
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:

This well written paper deals with myocardial function in patients with septic cardiomyopathy. On a cellular level, the authors describe a severely reduced function of atrial myocardium in patients with endocarditis.

I have several comments:

1. The abstract is too long and not structured.
2. The introduction is far too long.
3. The authors assume that endocaritis patients suffer from septic cardiomyopathy. This is not automatically true: I have seen a lot of patients with endocarditis without suppressed cardiac function, and I have seen many patients with a real septic cardiac malfunction. How can the authors know that cardiac malfunction really occurred in those patients?

4 At least part of the conclusion is speculative and not based on the results of this study: The authors have not shown that edema is the reason for atrial myocardial dysfunction. Therefore, the recommendation of anti-edematous treatment is speculative.

5. Statistics: the electromechanical coupling results are compared with a student´s t-test. If the values are normally, that would be ok. If the values are not normally distributed (Kolmogorov-Smirnov-test), then we would need a median and a range or interquartile range.

Authors' Response to Reviewer Comments

Feb 20, 2017

Reviewer 1:

1. The study was in accordance with the institution's ethical guidelines.
All patients that sign for admission give their permission that residual material may be used for scientific purpose. The rigth atrial myocardium is standardized excised at the auricle for the venouse canulation of extracorporal circulation.
2. Both groups were also similar regarding age. We deleted that Table in our last version of the manuscript and missed to mention it in the text. Sorry for that. Table 1: Characteristics of patients. Group E (Endocarditis) and Group C (Control).
3. With the title „SCMP: Evidence for a reduced force generating capacity ....in acute infective endocarditis" we do not want to assume that infective endocarditis is interchangeable with septic cardiomyopathy. The SCMP is a huge syndrome with many influences, of course, and is also at least not visible in every infective endocarditis. All patients of group E had to underwent emergent surgery caused by beginning shock. First we might think that this shock in patients with acute valve insufficiency caused by endocarditis is primarily cardiogen induced, but in our study - under exclusion of hemodynamic influences of valve insufficiency - we proof that there is also a not too less influence of septic cardiomyopathy in patients with acute infective endocarditis and we can say that patients with infective endocarditis have a septic SIRS too. Thus we could change the title to: „SCMP: Evidence for a reduced force generating capacity of human atrial myocardium in patients with acute infective endocarditis and the need of urgent/ emergent surgery caused by beginning septic and cardiogen (acute valve insufficiency) shock" to underline the accompanied SIRS, but this would be too long, wouldn`t it? We explain it better in the methods.
Cit p.6: SCMP may be induced by IE as demonstrated by animal-studies showing that the vegetation-associated host (bacteria) protease activity not only leads to proteolytic tissue damage, as evidenced by the release of extracellular matrix fragments such as fibrinogen, but also induces inflammation and myocardial cell damage [7]. In this respect, it is essential to note that SCMP can not only be caused by IE but by any infective focus in the organism that gives rise to systemic endotoxemia [8].
4. All patients with infective endocarditis (group E) showed preoperatively positive blood cultures and needed urgent or emergent surgery caused by theier unstable hemodynamics caused by acute valve insufficiency or/ and septic shock / SIRS.
5. Please have a look at my citation:
Denk K, et al. Evidence for a negative inotropic effect of obesity in human myocardium?. Eur J Cardiothorac Surg 2009;36(2):300-5.
"For that reason an experimental set-up was selected, which warrants exactly defined nutritive conditions for atrial myocardial tissue. Trabeculae from the right auricle were used, which are neither affected by elevated systemic blood pressure nor coronary artery stenosis. The present study was designed to analyze, based on a large number of patients, whether or not there is a relationship between body weight and atrial myocardial contractility using the experimental model of isolated human right atrial myocardium. "
6. The protocol was described in the methods.
Cit. P.9: Electrical stimulation was applied in square wave at a frequency of 60 beats per minute. During electrical stimulation, the trabeculae clamped into the apparatus were pre-streched, during continuous force measurement, from slack to optimal length, the latter being defined as the length at which maximum force output was achieved. At optimal length, the diameter of the fibre was measured under the microscope. The respective resting force and isometric force were recorded.
References are:
Denk K, et al. Evidence for a negative inotropic effect of obesity in human myocardium?. Eur J Cardiothorac Surg 2009;36(2):300-5.
"The trabeculae were mounted horizontally in a perfusion fixed at one end to micro tweezers closed by a micro ring and at the other end to an isometric force transducer as previously described [Vahl CF, Bonz A, Timek T, Hagl S. Intracellular calcium transient of working human myocardium of seven patients transplanted for congestive heart failure. Circ Res 1994;74(5):952—8.]. The preparations were continuously perfused with 37.8°C oxygenated Krebs—Henseleit solution (composition: in mM: NaCl 119.0, $NaHCO_3$ 25.0, 141 KCl 4.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.3, glucose 11.0) for at least 15 min. Then electrical stimulation (frequency: 1 Hz, amplitude: 10% above threshold, duration: 5 ms, mode: square wave) was carried out while the preparation was slowly stretched to optimal length. Optimal length is defined as the muscle length associated with the maximum active tension. The tension recorded at optimal length without electrical stimulation was measured (passive resting tension). The solution was gassed with a mixture containing 95% O_2 and 5% CO_2. Measurements were started as soon as steady state conditions of passive resting tension and active tension were observed. Muscle length, muscle diameter, passive resting tension and active tension were measured (Güth scientific instruments, Heidelberg, Germany)."
7. Referring to same paragraph in 6 (not in 5) above: Yes, it is only an assumption that the impaired systolic function is caused by a reduced number of contractile units.

Cit. p-13: In group E, the maximal force amplitude was significantly lower than in group C. This suggests (a) a reduction of contractile units contributing to force generation per square millimeter or (b) a reduced force output per contractile unit. A reduced number of contractile units per square millimeter may be the result of myocardial edema or destruction of contractile units. Destruction of contractile units in this respect may be functional, i.e. after a history of overstretching, or morphologic, i.e. as a function of insufficient supply (infarction). Clinically, these findings translate into an impairment of the systolic function.
We indicated the missing histologic data in our Limitations.
Cit p. 15: The study protocol was very simple and focused on mechanical performance. Additional histopathological data of the preparations is not available.
8. In paragraph 5 we state the diastolic dysfunction, in paragraph 4 the systolic. And in summary in paragraph 6 we state that our data suggest a severe impairment of systolic and diastolic function. The therapeutic approach with Milrinon and Dobutamin as well with levosimendan agrees with our therapeutic recommendation of higher heart rate and lower preload and atrial filling pressure. We did not recommend intravasal volume and/or arterenol, what is the gold standard in sepsis therapy.
Cit. p.16: the need for a high heart rate, a low preload and low atrial filling pressures. Unfortunately, however, this deviates from the current therapeutic approach to hemodynamics in septic patients.
9. With contribution of our study it is clarified that the infective endocarditis (with the bacterial host in the endocard and the inflammatory reaction there) ends in a SCMP that we could proof with our electromechanical measurements.
Cit. p.16: The present paper represents an initial contribution to describing the effects of presence of SCMP in the atrial myocardium of patients with IE. Regardless of whether myocardial edema, a poor blood supply or other factors are involved in the generation of SCMP, our data show clearly that the problem of IE is not automatically solved as soon as the valve is repaired or replaced. IE is a systemic disease, and cardiac surgery represents only one factor within the comprehensive treatment approach to this particular entity. In this respect, we are convinced that improving hemodynamics is per se an important factor in the treatment of SCMP.
10. We have no histopathological proof for fibrotic remodeling in our group E patients. But as we showed in the literature, post mortem our assumption is proved [28]. cit. p.14: It is well possible that the quantity of sarcomeres in a muscle stripe of standardized muscle thickness could be reduced due to edema / swelling of myocytes or swelling of the extracellular structure. Edema could therefore be a reason for a significantly lower active force in SCMP. Of note, histopathological post mortem observations of patients who died of septic shock showed interstitial fibrosis in 100% and interstitial edema 90% [29].
It is no contrariety that the SCMP can be reversible. There are other studies that state that the fibrotic cardiac remodeling after aortic valve stenosis can also be reversible after correction of the stenosis (by valve replacement). [Wallenborn J1 et al: Prognostic significance of serial high-sensitivity troponin I measurements following acute cardiac decompensation-correlation with longer-term clinical outcomes and reverse remodelling. Int J Cardiol. 2017 Jan 5. pii: S0167-5273(17)30040-2.]
Chest 1999 showed patients with septic shock (plus positive blood culture) who recovered in a few days with their systolic function, - but over half oft he group died!.
We are not suggesting that the systolic dysfunction results from the preload defect.
Cit. p.13: The calculated resting force/ active force ratio was significantly higher in group E than in group C, which indicates that in SCMP an elevated preload (resting force) is required to achieve a given active force amplitude. On the muscle preparation level, this means that there seems to be a diastolic dysfunction.
We also described a lot of other investigators` work about the intrinsic induced SCMP:
Cit.p.15: We assume that any infectious agent initially causes inflammation with circulating cytokines induced by endotoxemia. Sepsis and its hemodynamic changes including a reduced afterload caused by vascular nitric oxide (NO) result in the release of cytokines such as TNF alpha and interleukins [29]. As it is also well known, TNF-alpha in its turn stimulates macrophages to produce NO [30]. Elevated nitric oxide and increased reactive oxygen species (ROS) lead firstly to myocardial hibernation and reduced contractility and secondly, after 7-10 days [21], to fibrotic remodeling by tipping the balance between ROS and antioxidant enzymes.
11. We had to correct our references: “Elevated nitric oxid and increased ROS lead firstly to myocardial hibernation and reduced contractility [30, this is the correct reference] and secondly to fibrotic remodeling [32-34].
12. We changed our recommendation in an assumption of therapeutic approach, but further histopathological studies have to be observed. We also changed our conclusion to a convincingly proof of myocardial depression – no longer based on the mechanism of this myocardial depression that we did not oberserve.
13. This is the first in vitro study in human myocardium asking for septic induced myocardial depression. We will underline this.
Furthermore we would like to mention while we are at it that this study has won a price for the best poster presentation on our annual cardiothoracic meeting some years ago.

Reviewer 2:

See 1. above (Reviewer 1). We deleted our recommendation because this was not proved, right.

Reviewer 3:

1. The abstract is shorter than 350 words and structured now.

2. We want every paragraph in the introduction not to be shortened.

3. Cardiac malfunction really occurred in these patients, because we proved it in comparison to a control group without septic component and without positive blood cultures but undergoing same anaesthesia for cardiac surgery as the patients with endocarditis. Perhaps in vivo patients with SCMP have some compensatory mechanism to keep in stable hemodynamics, but as our measurements could show: there is a real depression of cardiac function under in vitro conditions in patients with infective endocarditis compared to a very similar control group also with valve insufficiency but not caused by infective endocarditis and undergoing same anaesthesia.

4. Anti-edema therapy is deleted as a recommendation.

5. The skewness of resting force is 0.347, of force amplitude ist 0.487 and of active force 0.219. Thus the values are normally distributed and the T-test may be used.

Reviewers' Comments to 1st Revision

Reviewer 1: Kwabena Frimpong-Boateng

Feb 28, 2017

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	N/A

Custom Review Questions	**Response**
Is the subject area appropriate for you?	5 - High/Yes
Does the title clearly reflect the paper's content?	4
Does the abstract clearly reflect the paper's content?	5 - High/Yes
Do the keywords clearly reflect the paper's content?	5 - High/Yes
Does the introduction present the problem clearly?	4
Are the results/conclusions justified?	4
How comprehensive and up-to-date is the subject matter presented?	4
How adequate is the data presentation?	4
Are units and terminology used correctly?	4
Is the number of cases adequate?	4
Are the experimental methods/clinical studies adequate?	4
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	3
Please rate the practical significance.	3
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	4
Please rate the appropriateness of the figures and tables.	3
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	No: the manuscript may be accepted for publication in its revised form

Comments to Authors:

The authors have answered the queries satisfactorily and the manuscript may be accepted for publication in its revised form.

Reviewer 3: anonymous

Feb 20, 2017

Reviewer Recommendation Term:	Revise with Major Modifications
Overall Reviewer Manuscript Rating:	81

Custom Review Questions	**Response**
Is the subject area appropriate for you?	4
Does the title clearly reflect the paper's content?	1 - Low/No
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	4
Are the results/conclusions justified?	4
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	4
Are units and terminology used correctly?	4
Is the number of cases adequate?	5 - High/Yes
Are the experimental methods/clinical studies adequate?	4
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	1 - Low/No
Please rate the practical significance.	3
Please rate the accuracy of methods.	2
Please rate the statistical evaluation and quality control.	4
Please rate the appropriateness of the figures and tables.	4
Please rate the appropriateness of the references.	5 - High/Yes
Please evaluate the writing style and use of language.	3
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
Still, I´m not convinced that the authors really took atrial material from patients with septic cardiomyopathy. With their new comments in the manuscript, the authors even increase my doubts: They write that patients were in cardiogenic shock due to severe valve insufficiencies. This is something completely different than septic cardiomyopathy! The authors should look up their data again to see which patients were in septioc and which were in cardiogenic shock. Having done this, all patients undergoing surgery due to cardiogenic shock should be taken out of the analysis! The reason for this step is that the decribed changes in atrial function are probably NOT due to sepsis, but due to cardiac failure!

Authors' Response to Reviewer Comments

Mar 07, 2017

Reviewer 2:
Thank you for your important comment. We have included this aspect in our discussion and limitations. Unfortunately, IE is a highly complex disease in the course of which a variety of factors including valve insufficiency and sepsis exert their respective influences on hemodynamics. These facts cannot be easily in- or excluded in a human setup.
To differentiate the "septic cardiac depressant effect" in addition to insufficient valve dependent cardiac failure we compared our "septic" collective to human patients only showing valve insufficiency.

Reviewers' Comments to 2nd Revision

Reviewer 3: anonymous

Mar 13, 2017

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	85

Custom Review Questions	**Response**
Is the subject area appropriate for you?	4
Does the title clearly reflect the paper's content?	4
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	4
Are the results/conclusions justified?	2
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	4
Are units and terminology used correctly?	4
Is the number of cases adequate?	3
Are the experimental methods/clinical studies adequate?	2
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	3
Please rate the practical significance.	2
Please rate the accuracy of methods.	2
Please rate the statistical evaluation and quality control.	4
Please rate the appropriateness of the figures and tables.	3
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
The paper can now be published as it is.