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Carotid body resection for sympathetic modulation in systolic heart failure – results from first-in-man study

Running title: Carotid body resection in heart failure

Department of Cardiology, Centre for Heart Disease, 4th Military Hospital, Wroclaw, Poland

Piotr Niewinski[1], Dariusz Janczak[2], Artur Rucinski[2], Stanislaw Tubek[1,3], Zoar J. Engelman[4], Paweł Piesiak[5], Przemyslaw Jazwiec[6], Waldemar Banasiak[1], Marat Fudim[7], Paul A. Sobotka[4,8], Shahrokh Javaheri[9,10], Emma C.J. Hart[11], Julian F.R. Paton[11], Piotr Ponikowski[1,3]

[1] Department of Cardiology, Centre for Heart Disease, 4th Military Hospital, Wroclaw, Poland; [2] Department of Vascular Surgery, 4th Military Hospital, Wroclaw, Poland; [3] Department of Heart Diseases, Faculty of Health Sciences, Wroclaw Medical University, Wroclaw, Poland; [4] Cibiem Inc., Los Altos, CA, USA; [5] Department of Pulmonology and Lung Cancer, Medical University, Wroclaw, Poland; [6] Department of Radiology and Diagnostics Imaging, 4th Military Hospital, Wroclaw, Poland; [7] Department of Cardiology, Duke University School of Medicine, Durham, NC, USA; [8] The Ohio State University, Columbus, OH, USA; [9] Bethesda North Hospital, Cincinnati, OH, USA; [10] University of Cincinnati, Cincinnati, OH, USA; [11] School of Physiology and Pharmacology, Clinical Research & Imaging Centre, University of Bristol, Bristol, UK.

Corresponding Author:

Piotr Niewinski, MD, PhD

Cardiology Department, Centre for Heart Diseases, Military Hospital

Ul. Weigla 5, 50-981, Wroclaw, Poland

Telephone: +48261660237, Email: pnsky@wp.pl

ABSTRACT

Aims

Augmented reflex responses from peripheral chemoreceptors, which are mainly localized in the carotid bodies (CB), characterize patients with systolic heart failure (HF) and contribute to adrenergic hyperactivation. We investigated whether surgical resection of CB in these patients can be performed safely to decrease sympathetic tone.

Methods and results

We studied 10 male patients with systolic HF (age: 59±3 years, left ventricular ejection fraction: 27±7%) who underwent unilateral right-sided CB resection (4 patients) or bilateral CB resection (6 patients). Primary endpoints of the study were changes in muscle sympathetic nerve activity (MSNA) and peripheral chemosensitivity measured as ventilatory response to hypoxia from baseline to 1 month post-CB resection. Safety analysis included analysis of arterial blood gas and oxygenation at night through 2 months post-procedure and adverse events assessed up to 12-months.

At 1-month visit, CB resection was associated with a significant decrease in both MSNA (86.6±3.1 vs. 79.7±4.2 bursts/100 beats, p=0.03) and in peripheral chemosensitivity (1.35±0.19 vs. 0.41±0.17 L/min/SpO_2, p=0.005). It also resulted in improved exercise tolerance. Amongst some patients with bilateral CB resection there was a trend towards worsening of oxygen saturation at night, which in one case required therapy with non-invasive ventilation.

Conclusion

We present first-in-man evidence that CB resection in patients with systolic HF is associated with decrease in sympathetic activity. Bilateral procedure may carry a risk of worsening oxygenation at night. CB modulation constitutes an interesting research avenue, but careful consideration of the balance between safety and efficacy is necessary before further clinical trials.

Key words: heart failure, carotid body, sympathetic modulation

INTRODUCTION

Augmented activation of peripheral chemoreceptors (PChR) is a common feature in patients with systolic heart failure (HF)[1,2] associated with lower exercise capacity, greater arrhythmic burden, attenuated left ventricle function and higher levels of natriuretic peptides.[1-3] An exaggerated ventilatory reflex response to PChR activation is an independent predictor of poor long-term prognosis in the HF population.[4] Recent data from animal models of HF suggest that overactive peripheral chemoreceptors localized in the carotid bodies (CB) may be responsible for disease progression.[5,6]

Both animal and clinical studies confirm a relationship between PChR afferent discharge and the level of sympathetic activity. Blockade of PChR with hyperoxia in humans[7,8] or resection of CBs in animals[5,6] result in a significant decrease in sympathetic nerve traffic. Hyperactivity of the sympathetic nervous system is well defined in patients with HF, where it remains the priority target of both pharmacotherapy and device based interventions.[9] Thus, this has led to the novel concept that reducing afferent signalling of the CB may have therapeutic benefit in patients with systolic HF.[10]

Transient inhibition of PChR with dopamine or oxygen has been shown to acutely improve exercise capacity.[11,12] However, whether chronic desensitization of PChR translates into sustained improvements in exercise tolerance and hence better quality of life in the HF population is unknown.

Historically, the CBs have been surgically resected as a palliative therapy in patients with respiratory disorders in order to reduce or eliminate the respiratory drive of PChR.[10,13] Moreover, based on a vast number of published cases, both unilateral and bilateral surgical CB resection have been shown to be safe in the short and long term perspective.[10] However, CB resection itself carries some surgical risks related specifically to the operating field that include: inadvertent vessel and nerve damage, risk of central nervous system embolism and persistent headache. Nonetheless, such complications have been described as rare with an incidence rate of less than 3%.[10]

Based on these premises, we tested the hypothesis that surgical resection of CB in patients with systolic HF and augmented peripheral chemosensitivity could be safely performed and would result in a decrease in sympathetic tone and reduction in peripheral chemosensitivity. We previously presented a case report[14] and hemodynamic data obtained from the same cohort of patients.[15]

METHODS

Study population

The study was performed in two stages. First, a group of 4 patients underwent unilateral CB removal (CBu). After reviewing all the cases in the context of safety, a second group of 6 patients was selected to undergo bilateral CB removal (CBb). Decision to move into bilateral procedures was based on the notion that more meaningful clinical changes may be obtainable with virtually complete elimination of PChR activity. This decision was made following interim safety and efficacy analysis of the data from unilateral group.

Inclusion criteria for entering both stages were identical and included: stable HF symptoms in NYHA II-III class for at least 4 weeks prior to inclusion; left ventricular ejection fraction (LVEF) $\leq$ 45%; optimized HF treatment; presence of both CB in computer cervical angiotomography; and augmented peripheral chemosensitivity determined as >0.6 L/min/%SpO_2 (cut-off based on previous findings from our lab and other reports).[1-3] Exclusion criteria were as follows: acute coronary syndrome; coronary revascularization; HF related hospitalization; clinically significant infection; surgery under general anesthesia within 3 months prior to inclusion; history of stroke; transient ischemic attack or clinically significant chronic neurological disorder; heart transplant; hemodialysis or peritoneal dialysis; obstructive carotid atherosclerotic disease with >50% stenosis; severe sleep apnea and chronic obstructive pulmonary disease (COPD) stage III and IV. We decided to include one patient with a history of recent ventricular arrhythmia (registered and treated by implantable cardioverter-defibrillator) as previous studies have

showed improvement in the burden of ventricular arrhythmia following autonomic modulation (e.g. renal nerve ablation).[16,17] None of the patients reported hospital admission due to HF decompensation within 1 year preceding the study, and 7 patients reported history of HF hospitalization in the earlier period. We did not include NYHA IV patients as in this group the unstable course of the disease (together with the open design of the study) would complicate the evaluation of the effects of CB denervation.

The local Ethics Committee of Wroclaw Medical University approved the study protocol based on encouraging results of risk/benefit analysis taking into account low rates of the complications from >15000 published cases of CB resection in humans and potential clinical benefit demonstrated in the animal models of heart failure and previous studies with CHF patients.[5-8,10-12] The study protocol was identical for already published case report[14] and for CBb patients presented in the hemodynamic context elsewhere.[15] All subjects gave written informed consent before the study entry. The study was performed in accordance with the latest review of the Helsinki Declaration. The protocols are registered with Clinical Trials for unilateral and bilateral procedures (NCT01653821 and NCT01782677).

Study endpoints

The primary outcome measures were defined as changes in: a. muscle sympathetic nerve activity (MSNA) measured by burst frequency normalized to heart rate and b. peripheral chemosensitivity measured as ventilatory response to acute hypoxia at 1 month after CB resection. Secondary outcome measures included: changes in MSNA and peripheral chemosensitivity at 2 months after CB resection; changes in exercise tolerance and serum levels of natriuretic peptides at 1 and 2 months after CB resection; change in left ventricular function at 2 months after CB resection. Safety assessment comprised: analysis of procedural safety, arterial blood gas sampling, nocturnal oxygenation evaluated through 2 months post procedure and surveillance for adverse events for up to 12 months after CB resection. The study was designed as proof-of-the-concept, safety and feasibility trial, hence we decided for relatively short follow-up periods for primary and secondary end-points. However, we also briefly report clinical data available at 6 months following CB resection.

Procedures

Peripheral chemosensitivity was measured using the standard transient hypoxia test employing brief administrations of nitrogen gas as described in more detail elsewhere.[2] Direct assessment of muscle sympathetic nerve activity (MSNA) was achieved with multi-unit microneurography. Briefly, a tungsten microelectrode was inserted directly into the peroneal nerve posterior to the fibular head. The nerve signal was amplified and filtered (Neuro Amp Ex, ADInstruments, Sydney, Australia), which allowed for calculation of burst incidence per 100 heart beats as a measure of global sympathetic tone.[18] MSNA at baseline was performed in all subjects, but in one case was not repeated after CB resection due to the request of the patient. Despite not being a pre-specified end-point, we decided to include heart rate variability (HRV) into our analysis. It was assessed using spectral method. Low frequency (LF) (0.04 to 0.15 Hz) and high frequency (HF) (0.15 to 0.40 Hz) spectral bands were computed at rest in the supine position. LF/HF ratio was used as an indirect measure of the sympathetic–parasympathetic balance. Such analysis was precluded in 3 patients due to frequent supraventricular arrhythmia or atrial fibrillation. Baroreflex control of MSNA was assessed with baroreflex threshold method[19] using simultaneous MSNA and blood pressure recordings (Nexfin, BMEYE B.V., Amsterdam, The Netherlands).

Exercise capacity was assessed using treadmill spiroergometric test (modified Bruce protocol; Ultima, Medgraphics, St. Paul, MN, USA) and included calculation of peak oxygen consumption (peakVO_2), slope relating minute ventilation to carbon dioxide production (VE/VCO_2 slope), total exercise time and respiratory exchange ratio (RER).

Study design is presented in details in Table S1 in the Supplementary Appendix.

Carotid body resection

Carotid body resections were performed by a team of experienced vascular surgeons under local anaesthesia (2% lidocaine). Before surgery, the exact location of CB and surrounding anatomy was assessed using cervical CT angiography as described previously.[20] CB resection followed the method described earlier by Winter[13] which consists of a lateral approach to the carotid bifurcation with optional mobilization of external carotid artery. Care was exercised to avoid damage to the vasculature and adjacent structures including: hypoglossal nerve, vagus nerve, thyroid artery, facial nerve and carotid

sinus baroreceptors. All unilateral procedures were performed on the right side for consistency. In every case surgeon was able to macroscopically identify CB and resect it within the small block of adjusting tissues. Sample containing CB was then put into formaldehyde, stained using hematoxilin-eosin method, sectioned and analysed for the presence of CB by experienced pathologist. CB was defined as easily discernible structure consisting of multiple glomoids containing chemosensitive cells (see Figure 1). Blood pressure, heart rate, respiratory rate, electrocardiogram and oxygen saturation were monitored continuously during surgery and for 24 hours after the procedure. In the group of patients receiving CBb resections these were staggered in time (2-3 days apart). Patients were discharged from the hospital on the third day following the second procedure.

Data and statistical analysis

Statistica 10 (StatSoft Inc., Tulsa,OK, USA), LabChart Pro (ADInstruments) and MATLAB (MathWorks, Natick, MA,USA) were used to analyse the data. Variables were expressed as the mean and standard error of the mean (SEM) for repeated measurements before and after CB resection. In cases where repeated data were not available (e.g. death, refusal), a baseline value for that particular variable was not taken into account. Thus in some statistical comparisons less than 10 participants were taken into account (e.g. for statistical analysis of MSNA 9 cases were analysed at 1 month and 7 cases were analysed at 2 months). T-test for dependent samples and Wilcoxon matched pairs test were performed depending on the type of distribution (analysed with Shapiro-Wilk test) to assess differences in measured parameters after CB resection compared to baseline. A p value < 0.05 was considered statistically significant. Due to small sample size statistical analysis was performed only in combined CBu and CBb groups.

RESULTS

Study participants

Studied patients presented with moderate systolic HF as evidenced by decreased peakVO_2 (mean – 16.2 ml/kg/min), compromised left ventricular systolic function (mean left ventricular ejection fraction [LVEF] – 27.1%) and elevated N-terminal prohormone of brain natriuretic peptide (NT-proBNP; mean – 2337 pg/mL). They received optimal treatment – all were treated with combination of angiotensin converting enzyme inhibitor / angiotensin receptor blocker with a beta-blocker (carvedilol at mean daily dose of 43.75 ± 13.5 mg) and 9 (90%) had either implantable cardioverter-defibrillator or cardiac resynchronisation device implanted. Of importance, all patients demonstrated elevated MSNA ranging from 71 to 98 bursts/100 beats (in healthy volunteers of similar age MSNA was reported as 64 ± 4 bursts/100 beats)[18] and presented with moderate risk of death within 3 years as per MAGICC score[21] (mean: 27.6 ± 14.2 %, range: 12.2 - 55.6 %). Baseline clinical characteristics of studied patients are shown in Table 1.

Carotid body resection

Results of both CBu and CBb are presented in a case-by-case fashion in Table 2, S2, S3 and S4. Tables S5 and S6 show results of CB resection according to group participation (CBu vs. CBb). A case report from one patient receiving CBu and data on hemodynamic response to hypoxia following CBb have been published elsewhere.[14,15] Histologic examination confirmed completeness of surgical CB resection in all patients (Figure 1).

Primary end-points

Muscle sympathetic nerve activity at 1 month post-resection

Nine patients underwent MSNA assessment at 1 month visit. Compared to baseline there was a significant decrease in bursts frequency normalized to heart rate (86.6±3.1 vs. 79.7±4.2 bursts/100min, p=0.03) (Figure 2).

Peripheral chemosensitivity at 1 month post-resection

Carotid body resection (CBu + CBb) resulted in a significant decrease in peripheral chemosensitivity at 1 month visit compared to baseline (1.35±0.19 vs. 0.41±0.17 L/min/SpO_2, p=0.005). Peripheral

chemosensitivity was virtually eliminated in all patients receiving CBb (range 0.04-0.35 L/min/SpO_2), but did not reach levels regarded as normal (<0.6 L/min/SpO_2) in two of the four patients with CBu (Table 2).

Secondary end-points

Muscle sympathetic nerve activity at 2 months post-resection

Seven patients underwent MSNA assessment at 2-month visit: 2 from CBu group and 5 from CBb group. There was a significant decrease in number of bursts normalized to heart rate compared to baseline (85.6±3.9 vs. 75.4±3.0 bursts/100 beats, p=0.008) (Figure 2). A numerical reduction in bursts/100 beats was seen in all patients studied at 2 months post-resection.

Peripheral chemosensitivity at 2 months post-resection

Carotid body resection (CBu + CBb) resulted in a significant decrease in peripheral chemosensitivity at the 2 month visit compared to baseline (1.40±0.22 vs. 0.41±0.14 L/min/SpO2, p=0.006). Numerically PChS was reduced in all patients with CBb (almost complete elimination relative to baseline) and in 3 of 4 patients with CBu (approximately halves of the pre-procedural values) (Figure 3 and Table 2).

Exercise tolerance

All patients (CBu + CBb) exhibited significant prolongation of exercise time compared to baseline at 1 month (561±58 vs. 624±57 s, p=0.003) and 2 months (567±70 vs. 648±68 s, p=0,008). Peak oxygen consumption did not differ significantly at 1 month (16.2±1.0 vs. 17.1±1.1 ml/min/kg, p=0.27) or 2 months (16.6±1.0 vs. 18.3±1.7 ml/kg/min, p=0.17). The VE/VCO_2 slope decreased at 2 months relative to baseline (36.4±3.0 vs. 32.8±3.2, p=0.03), although this did not reach significance at 1 month compared to baseline (37.5±2.5 vs. 35.3±2.8, p=0.15) (Table 2 and Table S4).

Quality of life

The quality of life measured using KCCQ (clinical summary score) showed significant improvement (increase) at 1 month visit compared to baseline (65.4±5.5 vs. 76.1±3.6, p=0.005). However, the score at 2 month visit did not differ significantly from the baseline (63.6±5.4 vs. 69.4±4.3, p=0.12). Fatigue and

shortness of breath scores (based on raw data extracted from KCCQ) showed improvement at every time point following CB resection. However, these changes reached statistical significance only for fatigue score at 1 and 2 months visits. Details are given in Table S7 in the Supplementary Appendix.

Natriuretic peptides

There were no significant changes in NT-proBNP serum concentration at 1 month (2337±719 vs. 2596±834 pg/ml, p=0.24) or at the 2 month visits (2536±895 vs 2652±1042 pg/ml, p=0,89) compared to baseline.

Left ventricular function

Left ventricle ejection fraction did not change significantly at 2 months relative to baseline (28.5±2.6 vs. 29.6±2.9 %, p=0,39). Similarly, there was no significant difference in left ventricle end-diastolic diameter (70.0±5.4 vs. 71.5±5.2 mm, p=0.13).

Heart rate variability

Ratio of LF/HF did not change at 1 month (1.00±0.13 vs. 0.73±0.12, p=0.24) when compared to baseline. However, there was a significant decrease in LF/HF ratio at 2 months visit (1.04±0.15 vs. 0.48±0.1, p=0.028). At 2 months LF/HF ratio was found to be reduced in all patients with available data (n=6) when compared to baseline.

Safety assessment

Periprocedural safety

There were no acute changes in blood pressure, heart rate, oxygen saturation (SpO_2), respiratory rate and electrocardiogram immediately after either bilateral or unilateral CB resection(s) and during 24 hours of post-procedural continuous monitoring.

There was one inadvertent arterial puncture during the surgery which was promptly repaired by the operating surgeon without any long term consequences. In two patients, we noted ipsilateral damage

to the facial nerve which tended to resolve without intervention within the follow-up period. One participant complained of headache and jaw numbness which gradually improved during the follow-up.

Oxygenation at night

One patient from the CBb group with pre-existing moderate obstructive sleep apnoea exhibited significant prolongation and deepening of apnoeic episodes and the minimal oxygen saturation (SpO_2) value decreased from 79% at baseline to 49% at 2 months. This was successfully addressed with the prescription of an adaptive servoventilation device. In two other patients with CBb we noted less pronounced changes in minimal SpO_2 at night (from 82% at baseline to 72% at 2 months and from 83% at baseline to 73% at 2 months) without the need for clinical intervention. In one patient from the CBu group we found a reduction in minimal SpO_2 level at night from 88% to 78%. There were no significant changes in minimal SpO_2 levels at night in the remaining patients. See Table S8 for detailed results.

Arterial blood gas analysis

There were no significant changes in the oxygen arterial partial pressure (PaO_2) at 1 month (74.5±1.9 vs. 73.9±2.8 mmHg, p=0.96) or 2 months post-resection relative to baseline (75.8±2.2 vs. 75.0±3.7 mmHg, p=0.78). The lowest PaO_2 level noted at either 1 or 2 month visits was 57 mmHg.

Concomitantly, we observed an increase in carbon dioxide arterial partial pressure ($PaCO_2$) in 5 patients at 1 month (all from CBb group) and in 5 patients at 2 months (one with CBu and 4 with CBb) compared to baseline. These changes reached statistical significance at 2 months after CB resection (36.9±0.9 vs. 39.5±1.0 mmHg , p=0.07 at 1 month and 37.5±0.8 vs. 40.3±1.3, p=0.03 at 2 months). The highest measured $PaCO_2$ level at either 1 or 2 month visits was 44 mmHg (Table S2).

Serious adverse events

Two deaths occurred during 12-month follow-up period.

One patient from the CBb group with a history of ventricular arrhythmias and adequate ICD interventions died at night 55 days after CB resection due to multiple episodes of ventricular tachycardia and ventricular fibrillation.

One patient from the CBu group with severe dilated cardiomyopathy died 8 months after the study intervention. This event occurred suddenly during the day-time and its cause remains unclear. Post mortem examination was not carried out. Additional details on two deaths are provided in Table S9 in the Supplementary Appendix.

There was also one hospitalization due to decompensated heart failure which took place 11 months after CBb. Patient`s ability to recognize symptoms of worsening heart failure was not distorted by the lack of hypoxic sensing.

Blood pressure, heart rate and arrhythmic burden

There were no significant changes in mean office blood pressure either 1 or 2 months post-CB resection (82.5±3.7 vs. 85.3±3.2 mmHg, p=0.19 and 85.2±4.1 vs. 84.6±3.0 mmHg, p=0.91, respectively) (Table S3). We did not observe any signs of baroreceptor dysfunction following CB resection such as fluctuation in clinical blood pressure measurements or orthostatic hypotension as reported by study participants. Data on baroreflex control of MSNA are provided in Table S10 in the Supplementary Appendix.

Similarly, we found no significant change in mean 24 hours heart rate at 2 months post-CB removal compared to baseline (81.6±2.1 vs. 81.0±2.5 min^{-1}, p=0.82) (Table S3).

Eight patients had Holter ECG recording repeated 2 months after CB resection. Overall there was no change in supraventricular/ventricular arrhythmia burden.

Clinical data at 6 months following CB resection

We found the following significant changes at 6 month visit relative to baseline: (1) decrease in peripheral chemosensitivity (p=0.009), (2) decrease in LF/HF ratio (p=0.04), (3) increase in exercise time (p=0.03), (4) increase in PaCO2 (p=0.049), (5) increase in serum creatinine (p=0.046), (6) decrease in office HR (p=0.046) - see Table S11 for details. Described changes in serum creatinine and office HR were not seen at 1 month and 2 months visits.

DISCUSSION

To our knowledge, this is the first human study showing decrease in sympathetic tone following CB resection. We studied patients with moderate HF who demonstrated augmented peripheral chemosensitivity and elevated MSNA (direct and reproducible measurement of efferent sympathetic traffic) despite optimal pharmacological and device therapy. This is of particular importance as exaggerated sympathetic tone and high activity of peripheral chemoreceptors are related to increased mortality in systolic HF.[4,22,23] By blocking PChR with transient hyperoxia Despas et al. showed decrease in muscle sympathetic nerve activity in HF patients.[8] Similar results were obtained from patients with hypertension.[24] Recent data from rat and rabbit model of systolic HF further support this concept. Del Rio et al. found that bilateral CB denervation led to significant decrease in various indices of sympathetic outflow that translated into improved barosensitivity, augmented left ventricle function, reduced cardiac arrhythmias and better survival.[5] Similar results were obtained by Marcus et al.[6] It has been established that afferent discharge from PChR impinges on neurons in the nucleus of tractus solitarii that relay to activate sympathetic neurones in various regions of central nervous system (e.g. paraventricular nucleus, rostral ventrolateral medulla oblongata).[25] This in turn results in augmentation of sympathetic post-ganglionic activity.[26] Thus, bilateral resection of CB resulting in eradication of afferent signalling to these structures is most likely responsible for the reduction in MSNA seen in the current study. Another possible explanation involves a decrease in the central coupling between respiratory and sympathetic neural networks[27] related to diminished ventilatory response to hypoxia after bilateral CB resection – this however was not directly assessed in our study. While numerically decline in MSNA might seem small (10 bursts/100 beats at 2 months), it must be noted that it equates to approximately 45% of the excess sympathetic activity related to HF state (when compared to healthy volunteers of similar age).[18]

In the present study, we also describe an improvement in exercise capacity. This manifested as longer exercise times in all subjects (CBu and CBb). At the same time, we noted a decrease in the VE/VCO_2 slope in all CBb patients 2 month post-operatively reflecting enhanced efficiency of ventilation during exercise – possibly as a result of attenuation in hypercapnic ventilatory response. Experimental

studies involving acute PChR blockade showed similar results. Chua et al. using hyperoxia to block PChR in HF subjects described prolongation of exercise time.[12] Furthermore, Notarius et al. reported improved exercise time in HF patients following caffeine administration[28] (known chemoreflex attenuator[29]). In another study employing dopamine to inhibit PChR in healthy volunteers a decrease in VE/VCO_2 slope was also reported.[11] The beneficial effect of CB resection on exercise capacity might be related to the attenuation of sympathetic constraint towards skeletal muscle arterioles as described in HF animal model.[30] On the other hand, it could be hypothesised that exercise improvements might be related to the subjective perception of reduced dyspnoea, which has been demonstrated after CB resection for other respiratory disorders[31] and following oxygen administration in HF.[32] Also, because of the lack of the sham-control group we cannot rule-out the placebo effect in relation to the improvements seen in exercise capacity.

There was no significant change in NT-proBNP levels following CB intervention. While on the one hand it could point towards futility of described intervention, on the other hand it suggests that decrease in sympathetic activity and improvement in exercise time may well be independent of myocardial performance.[33]

During available follow-up we observed no significant changes in left ventricle function and morphology. This observation is consistent with the lack of improvement in NT-proBNP levels. Thus, it could be speculated that augmented MSNA seen commonly in HF patients has predominantly secondary role and its involvement in further progression of the disease is overrated – at least in a case of long-standing and advanced systolic HF.

As expected from previous observations carried out on individuals after CB removal[34] we observed an increase in $PaCO_2$ levels, which was more notable in the CBb group. This effect might have been mediated by a reduction in resting ventilation caused by a loss of excitatory input from the CB to the central chemoreceptors.[35] Yet, this was not directly evaluated in this study. Similar effect together with reduced chemoreflex CO_2 sensitivity was observed in CHF rabbits following CB ablation.[6] While being relatively small (of approximately 3-4 mmHg) the rise in $PaCO_2$ could be of some clinical significance. As demonstrated by Lorenzi-Filho et al.[36] even such degree of increase in $PaCO_2$ may alleviate central

sleep apnoeas and hypopnoeas. Because our population of CBb patients consisted of individuals with obstructive pattern of sleep disordered breathing this effect could not be seen.

In all subjects 1 month after CB resection, quality of life measured with KCCQ improved. It could be related to less dyspnoea perception and better exercise tolerance after CB removal. In fact, our analysis of raw data obtained with KCCQ showed degree of improvement in both fatigue and shortness of breath scores. However, a short lived "placebo" effect related to the surgical procedure is a possibility supported by less obvious improvements in quality of life seen at 2 and 6 months. At the same time, the latter could reflect the ongoing adaptation to diminished ventilatory drive. Whether the observed improvements in quality of life are related to reduced subjective sensation of dyspnoea or to primarily enhanced exercise capacity cannot be conclusively derived from our findings.

In some patients CB resection resulted in the reduction of minimal level of SpO_2 at night. This was mostly seen in CBb group and had serious clinical consequences in one patient who required intervention with non-invasive ventilation. Such effect is most likely related to the elimination of PaO_2 sensing which is mainly provided by the chemoreceptors in the CBs. Central sensors predominantly detecting changes in PaCO2 are delayed in the relation to the CBs, therefore resulting in longer apnoeas and hypopnoeas leading to deeper desaturations after bilateral CB removal. Conversely, unilateral CB removal has less significant effect on sleep disordered breathing likely because of the preservation of hypoxic sensing by the contralateral CB. Exacerbated apneas / hypopneas in CBb group may be also related to reduced central CO_2 chemosensitivity as discussed above.[6]

Despite the mitigation of 45% of sympathetic excess in HF patients included in our study we did not see a significant impact on blood pressure. This can be explained by low baseline blood pressure due to low cardiac output and up-titrated pharmacotherapy. We also did not see a meaningful change in HR at 2 months. However at 6 months we noted small (6 bpm) but statistically significant decrease in office HR measurements. Changes in HR might have been blunted by the presence of atrial arrhythmia in 3 out of 10 study participants.

Authors cannot rule out the relation between CB resection and two deaths that occurred during follow-up. However, such relationship seems rather unlikely. The first event occurred in a patient with a history of previous malignant ventricular arrhythmias. The second adverse event took place late (8

months) after unilateral surgery in a patient with severe dilated cardiomyopathy, in whom safety analysis after CB resection did not show significant disturbances in blood gas analysis, oxygenation at night and blood pressure. The cause of death remains unknown (see also Table S9 for details).

Currently tested devices used for neuromodulation in HF (baroreceptor stimulation and vagal nerve stimulation) may be associated with poorly tolerated side effects occurring during the therapy which may limit the efficacy of such modalities.[37,38] On the contrary, CB denervation is free from the ongoing sensation related to continuous electrical stimulation. However, as seen in this study, CB denervation is a definitive procedure with the possibility of side effects related to the operative field. To avoid the risks related to the surgical approach a transvascular method of CB ablation might be an interesting option.

Moving forward into future clinical trials requires careful consideration of potential safety issues and should rather focus on unilateral procedures (which showed some positive efficacy signals in current study, see Tables S5, S6) with exclusion of patients with pre-existing obstructive sleep disordered breathing. Also, it is not clear at which stage of the natural course of systolic HF sympathetic modulation by means of CB resection should be tested.

Our study is not without limitations. Because of the surgical nature of the CB removal we did not include a control group with sham procedures or a control group of "matched" HF patients receiving standard treatment. Nonetheless, we believe that relatively short follow-up period makes changes related to the progression of HF less likely. We present MSNA data only for 1 month and 2 months visits, which is consistent with predefined study endpoints. However, long-standing reduction in sympathetic tone is confirmed by heart rate variability analysis available for 6 months follow-up. We did not include female participants which could influence some of the results (e.g. oxygenation at night due to lower prevalence of sleep disordered breathing in women). Finally, we acknowledge missing data (two drop-outs) related to death and patient`s wish to terminate study participation due to personal reasons and subjectively demanding study protocol.

In conclusion, CB resection constitutes an interesting novel approach to sympathetic modulation in HF. Our first-in-man data purport a decrease in MSNA and improvement in some indices of exercise capacity with concomitant reduction in peripheral chemosensitivity. Bilateral CB resection may result in

clinical worsening of nocturnal hypoxemia, while unilateral CB resection appears to have little effect on oxygenation at night. Careful consideration of the balance between safety issues related to invasiveness and definitiveness of the procedure and somewhat limited clinical efficacy is necessary before further clinical trials.

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CONFLICT OF INTEREST

PP has received consultancy contract and research grant for institution from CIBIEM, Inc.

PN and ST have received research support from CIBIEM, Inc.

ZJE is full time employee of CIBIEM, Inc. and has patents related to carotid body modulation.

MF has stock options for CIBIEM, Inc., patents related to carotid body modulation and has received consultancy fees from Axon Therapies Inc.

PS has stock options for CIBIEM, Inc.

Other authors have no conflict of interest.

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FIGURE LEGENDS

Figure 1

Example of microscopic examination of resected tissues documenting completeness of carotid body removal (H+E stain, magnification x20).

Figure 2

Changes in muscle sympathetic nerve activity (MSNA) after carotid body resection. Panel A depicts changes in bursts frequency normalized to heart rate. Panels B and C show an example of raw MSNA recording (patient #09) before (B) and 1 month after (C) bilateral carotid body resection illustrating significant decrease in the number of bursts/100 beats (reduction from 98 to 86 bursts/100 beats); * $p<0.05$ comparing to baseline.

Figure 3

Changes in peripheral chemosensitivity 2 months after carotid body resection. Values of peripheral chemosensitivity are showed only for patients with complete data at baseline and at 2 months visit (n=8).