American Journal of Case Reports

ISSN 1941-5923
© Am J Case Rep, 2015; 16: 886-892
DOI: 10.12659/AJCR.894974

Received: 2015.06.11
Accepted: 2015.09.16
Published: 2015.12.18

Is Mixed Apnea Associated with Non-Rapid Eye Movement Sleep a Reversible Compensatory Sign of Heart Failure?

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 Lyudmila Korostovtseva
BCDEF 2 Yulia Sazonova
CDE 3 Nadezhda Zvartau
BDF 4 Andrew Semenov
BF 1 Viktoriya Nepran
BCE 1 Mikhail Bochkarev
BEG 2 German Nikolaev
BCE 5 Lyubov Mitrofanova
DEG 1 Yurii Sviryaev
BEG 6 Mikhail Gordeev
EG 3 Aleksandra Konradi

1 Somnology Group, Hypertension Research Department, Almazov Federal North-West Medical Research Centre, St. Petersburg, Russian Federation
2 Cardiothoracic Surgery Research Department, Almazov Federal North-West Medical Research Centre, St. Petersburg, Russian Federation
3 Hypertension Research Department, Almazov Federal North-West Medical Research Centre, St. Petersburg, Russian Federation
4 Department of Cardiology #2, Almazov Federal North-West Medical Research Centre, St. Petersburg, Russian Federation
5 Pathologic Laboratory, Almazov Federal North-West Medical Research Centre, St. Petersburg, Russian Federation
6 Surgery Department, Almazov Federal North-West Medical Research Centre, St. Petersburg, Russian Federation

Corresponding Author: Lyudmila Korostovtseva, e-mail: korostovtseva@almazovcentre.ru, lyudmila_korosto@mail.ru
Conflict of interest: None declared
Source of support: The work was supported by the grant of the Russian Humanitarian Scientific Grant, project № 14-06-00219

Patient: Male, 24
Final Diagnosis: Dilated cardiomyopathy
Symptoms: Biventricular heart failure • sleep apnea
Medication: —
Clinical Procedure: Heart transplantation
Specialty: Cardiology

Objective: Unusual or unexpected effect of treatment

Background: Sleep-disordered breathing is common in heart failure (HF), and prolonged circulation time and diminished pulmonary volume are considered the main possible causes of sleep apnea in these patients. However, the impact and interrelation between sleep apnea and HF development are unclear. We report the case of a patient with complete elimination of non-rapid-eye-movement (NREM) sleep-associated mixed apnea in HF after heart transplantation.

Case Report: After unsuccessful 12-month conventional treatment with abrupt exacerbation of biventricular HF IV class (according to New York Heart Association Functional Classification), a 26-year-old man was admitted to the hospital. Based on a comprehensive examination including endomyocardial biopsy, dilated cardiomyopathy was diagnosed. Heart transplantation was considered the only possible treatment strategy. Polysomnography showed severe NREM sleep-associated mixed sleep apnea [apnea-hypopnea index 43/h, in rapid eye movement (REM) sleep 3.7/h, in NREM sleep 56.4/h, mean SatO2 93.9%], and periodic breathing. One-month post-transplantation polysomnography did not show sleep-disordered breathing (apnea-hypopnea index 1.0/h; in REM sleep – 2.8/h, in NREM sleep 0.5/h, mean SatO2 97.5%). The patient was discharged from the hospital in improved condition.

Conclusions: NREM sleep-associated mixed apnea occurring in severe systolic HF due to dilated cardiomyopathy might be reversible in case of successful HF treatment. We suggest that mixed sleep apnea strongly associated with NREM sleep occurs in HF, when the brain centers regulating ventilation are intact, and successful HF compensation might be highly effective regarding sleep-breathing disorders without non-invasive ventilation. This is important to know, especially with regard to the recently published data of potentially unfavorable effects of adaptive servoventilation in systolic HF, and the lack of other treatment options.

MeSH Keywords: Cardiomyopathy, Dilated • Heart Failure • Heart Transplantation • Sleep Apnea Syndromes • Noninvasive Ventilation

Full-text PDF: http://www.amjcaserep.com/abstract/index/idArt/894974

1537 4 5 18

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Background

Sleep-disordered breathing is known to be associated with cardiovascular diseases and complications: it is an independent risk factor for hypertension, and it also increases risk of coronary heart disease, heart rhythm disorders, and cerebrovascular events [1–5]. However, the data regarding a relation between sleep-disordered breathing and heart failure (HF) are more disputable. On the one hand, there is an evident association between HF and abnormal breathing patterns, and in particular, with Cheyne-Stokes and periodic breathing, which are considered central types of apnea. These central breathing patterns are assumed to be linked to poor prognosis, as well as more severe and terminal states [6–9]. Recent evidence has shown a beneficial effect of the successful treatment of sleep-disordered breathing on cardiac function. On the other hand, there are singular observations of sleep apnea attenuation in patients with improved HF [6–8]. Recently preliminary results of a randomized, controlled trial warn about possible unfavorable prognostic effects of sleep apnea treatment in HF. Thus, the association of sleep-disordered breathing with prognosis and HF treatment remains controversial. We present a case of complete elimination of non-rapid eye movement (NREM) sleep-associated mixed sleep apnea after heart transplantation, with discussion of possible mechanisms and treatment options.

Case Report

A male 26-year-old man was admitted to the hospital with progressive worsening of biventricular HF IV class (according to New York Heart Association Functional Classification). In the past, at age 24 years, dilated cardiomyopathy was diagnosed after acute viral myocarditis. A year later the patient had a respiratory infection, leading to HF worsening. Despite the multicomponent conventional treatment (Table 1), at 1-year follow-up biventricular dysfunction had progressed significantly.

On admission the physical examination demonstrated the following: weight – 68.7 kg, height – 177 cm; body mass index – 21.93 kg/m^2 (normal), skin was normal in appearance, texture and temperature, and mucous membranes were moist and pale-pink, and icteric sclera. No lymphadenopathy or thyromegaly were found. There was lower-limb edema up to the proximal part of the shins. Cardiac system: sinus rhythm, 97 beats per minute, blood pressure – 90/60 mmHg in sitting position, 80/50 mmHg while standing. At auscultation S1, S2 were muffled, and systolic murmur was auscultated on the apex. Chest was symmetric, moderate both-sided bubbling rales were auscultated, and there was a right-sided dullness of the sound. The abdomen was symmetrical without distention, soft and non-tender; bowel sounds were normal in quality and intensity in all areas. Hepatomegaly (+2 cm) was found, but there was no splenomegaly. Other vital signs were normal.

Table 1. Medication treatment at different time points.

Type of medication	Before admission to the hospital	At the hospital and intensive care unit	At discharge (after heart transplantation)
Diuretics	Loop diuretics Spironolactone	Combined diuretic therapy: Loop diuretics Aldosterone antagonists Acetazolamide intermittently	–
Inotropics	–	Combination of dobutamine and adrenaline	–
Anticoagulants	–	Anticoagulants (enoxaparin), later changed to warfarin	Warfarin was discontinued 1 year after pulmonary thromboembolism
Other cardiovascular medications	Angiotensin converting enzyme inhibitors Beta-blockers	Beta-blockers Angiotensin converting enzyme inhibitors Amiodarone	–
Other	Immune globulin	–	Tacrolimus Mycophenolate mofetil Methylprednisolone Gastroprotectors Valganciclovir Calcium-vitamin D

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Table 2. Laboratory analyses at baseline and after heart transplantation.

Parameter		Baseline, on admission	One month after heart transplantation	Reference values
Clinical blood analysis	Hemoglobin, g/l	96.0	161.4	130–160
	Red blood cells, 10^{12}/l	3.17	4.87	4.00–5.00
	Hematocrit, %	28.3	46.1	40.0–48.0
	Platelets, 10^9/l	142	292	180–320
	White blood cells, 10^{12}/l	9.0	6.9	4.0–9.0
Biochemistry	C-reactive protein, mg/l	70.55	0.57	0.00–5.00
	Total protein, g/l	57.00	65.00	64.00–83.00
	Albumin, g/l	34.0	42.00	35.00–50.00
	Creatinine, mcmol/l	112	75	64–111
	eGFR, ml/min/1.73 m^2	73.15	109	>60
	Alaninaminotransferase, IU/l	20.0	14.0	0.0–55.0
	Aspartataminotransferase, IU/l	47.0	14.0	5.0–34.0
	N-terminal pro-brain natriuretic peptide, pg/ml	2071.00	–	0.00–125.00
	Myoglobin, ng/ml	204.2		
	Glucose, mmol/l	6.5	4.55	3.89–5.83
Blood gases and acid-base balance (arterial)	pH	7.36	–	7.35–7.45
	pCO2, mmHg	31	–	32–48
	sO2,%	98	–	95–99
	HCO3-(P), mmol/l	19	–	21–28
	BE, mmol/l	–5.5	–	–2.5–2.5

Blood tests are summarized in Table 2, revealing moderate normochromal anemia and mild hypoproteinemia; N-terminal pro-brain natriuretic peptide was significantly elevated.

During hospitalization, based on a comprehensive examination, familial (genetic), endocrine, ischemic, and other potential causes of dilated cardiomyopathy were excluded. The patient had no history of smoking, alcohol and/or drug abuse, confirmed by negative test results.

Electrocardiography showed sinus rhythm, 99 beat per minute, and complete right-bundle branch block**.** Baseline echocardiography (on admission) showed extremely low ejection fraction, significant enlargement of all cardiac chambers, eccentric left ventricular hypertrophy, and significant tricuspid and mitral insufficiency (Table 3). Hepatomegaly, ascites, and right-sided hydrothorax were verified by ultrasound.

Right heart catheterization showed decreased cardiac index (2.1 l/min/m^2) and stroke volume (40 ml/beat), increased pulmonary artery pressure (43/33/21 mmHg), transpulmonary gradient (12 mmHg), and pulmonary vascular resistance (2.7 Wood). Right ventricular endomyocardial biopsy did not verify acute myocarditis, but post-myocarditis changes were found (Table 4).

Taken into account all the data, dilated cardiomyopathy was confirmed. While in intensive care, episodes of ventricular tachycardia (heart rate – 213 bpm) and pulmonary embolism occurred. Despite high-dosage inotropic support and intensive treatment (Table 1), biventricular cardiac failure progressed and multiorgan failure manifested.

Within a complex examination, due to the witnessed apneas, full polysomnography was performed. It showed severe mixed sleep apnea (apnea-hypopnea index – 43/h, mean O_2 saturation – 93.9%) and periodic breathing [10]. A strong association between sleep-disordered breathing and NREM sleep was found: in rapid eye movement (REM) sleep apnea-hypopnea index – 3.7/h, in NREM sleep – 56.4/h (Figure 1A).

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Table 3. Echocardiography parameters at baseline and after heart transplantation.

Parameter		Baseline, on admission	After heart transplantation
Left ventricle	Interventricular septum	8 mm	12 mm
	Posterior wall	10 mm	10 mm
	Relative wall thickness	0.24	0.44
	Myocardial mass index	181 kg/m^2	123 kg/m^2
	End-diastolic dimension	74 mm	50 mm
	End-systolic dimension	68 mm	31 mm
	End-diastolic volume	285 ml	131 ml
	End-systolic volume	240 ml	49 ml
	Stroke volume	45 ml	82 ml
	Ejection fraction	**16%**	**62%**
	Local contractility	Diffuse hypokinesia	No regional abnormalities
Right ventricle	Dimension at parasternal axis	39 mm	30 mm
	4-chamber dimension	43 mm	39 mm
	Anterior wall	3 mm	5 mm
	TAPSE	16 mm	13 mm
	TAVS	10 cm/sec	10 cm/sec
Left atrium	Dimension	53 mm	52 mm
	Volume index	69 ml/m^2	54 ml/m^2
Right atrium	Dimensions	63×59 mm	37×50 mm
Aorta	At sinus level	31 mm	37 mm
	Ascending	23 mm	34 mm
Pulmonary artery	Diameter	25 mm	25 mm
	Estimated pulmonary systolic pressure	50 mmHg	29 mmHg
Aortal valve		Normal cusps, Vmax 0.6 m/sec, dPmax 1.4 mmHg, no insufficiency	Normal cusps, Vmax 1.22 m/sec, dPmax 6.0 mmHg, no insufficiency
Mitral valve		Normal cusps, Ve 1.03 m/sec, Va 0.36 m/sec, Ve/Va 2.86, Tdec 91 m/sec, E/Em 15, **regurgitation II degree**	Normal cusps, Ve 0.98 m/sec, Va 0.43 m/sec, Ve/Va 2.27, Tdec 131 m/sec, E/Em 5.5, **no insufficiency**
Tricuspid valve		Normal cusps, Vmax 0.69 m/sec, dPmax 1.68 mmHg, **regurgitation III–IV degree**	Normal cusps, Vmax 0.89 m/sec, dPmax 3.18 mmHg, **no insufficiency**
Pulmonary valve		Vmax 0.58 m/sec, dPmax 1.35 mmHg, no insufficiency	Vmax 1.11 m/sec, dPmax 4.92 mmHg, no insufficiency

In light of unsuccessful conventional therapy and HF progression despite intensive care, orthotopic heart transplantation was considered the only possible treatment strategy. The operation and postoperative period were successful, and ejection fraction was 53% and 62% at early and 1-month post-surgery follow-up (Table 2), respectively. No signs of HF were present and immunosuppression therapy was started (Table 1).

Repeated endomyocardial biopsies did not show acute cellular rejection (Table 4, Figures 2–4). Based on pathology data, primary dilated cardiomyopathy as a result of lymphocyte myocarditis was verified (Figures 2–5).

The repeated polysomnography (1 month after the operation) did not show sleep-disordered breathing (apnea-hypopnea

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Table 4. Summary of the pathomorphology examination.

Right ventricular endomyocardial biopsy	Post-transplantation examination of the heart: macrostructure	Post-transplantation examination of the heart: histology
Histology: – Stroma edema – Local fibrosis – Aggressive cellular infiltration without evident cardiomyocyte necrosis – Moderate dystrophy of muscle fibers – Endotheliosis **Immunohistochemistry:** 26 CD3+/mm, 26 CD45+/mm, 17 CD68+/mm, HLA-DR+++, Th17+, perforin +/–	– Globe-shaped heart – Weight – 410.8 G – Dilation of all heart chambers – Interventricular septum – 11 mm – Left ventricle dimension – 12 mm – Right ventricle dimension – 3 mm – Tricuspid valve – 10 cm – Mitral valve – 10 cm – Left atrium – 7 cm – Aorta – 7 cm – Myocardium is pink – No cardiosclerosis	– Focal lipomatosis of interventricular septum at the border with posterior wall of left ventricle (Figure 2) – Isolation and dystrophy of muscle fibers of right ventricle and interventricular septum (Figure 3) – Few foci of aggressive lymphocyte infiltration and cardiomyocyte necrosis (lymphocyte count > 20/mm) of right and left ventricles and interventricular septum (Figures 4, 5)

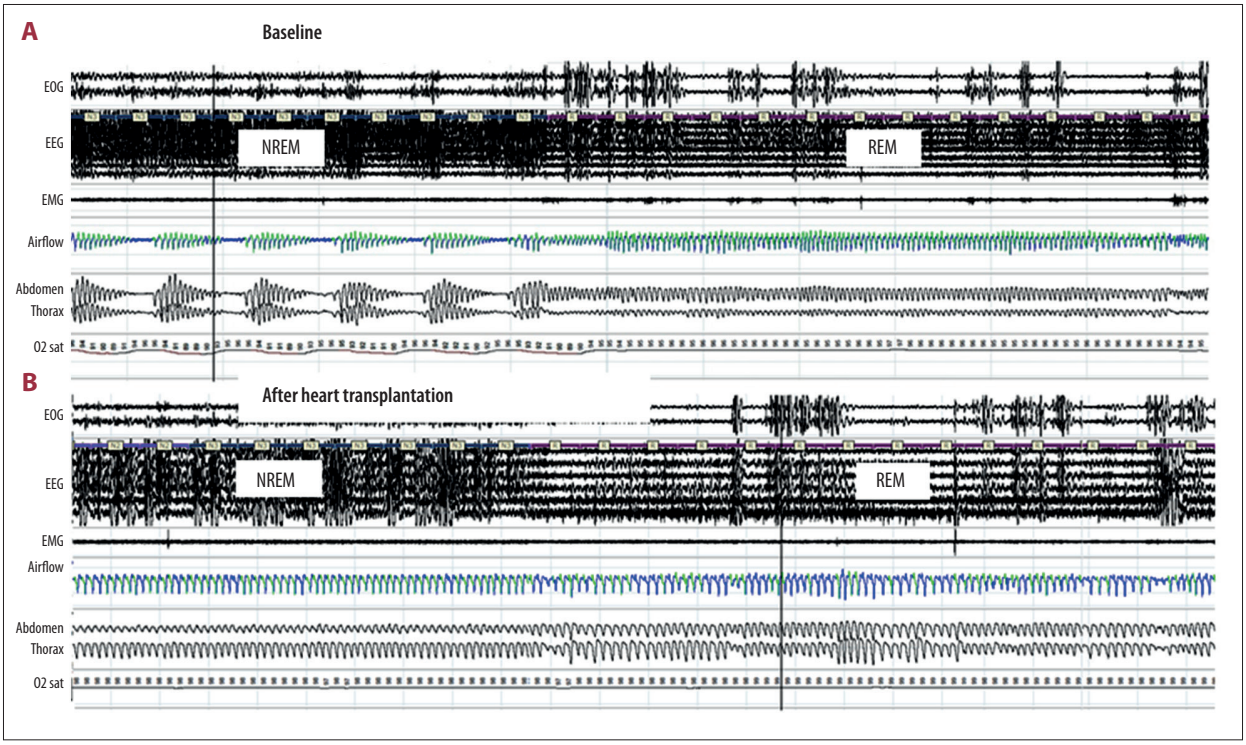

Figure 1. Polysomnography at baseline (**A**) and 1 month after heart transplantation (**B**). The figure shows a 12-minute epoch of the polysomnography recording. The traces downward (at both **A and B** sections of the figure) are the following: electrooculogram, electroencephalogram, electromyogram, nasal airflow, abdomen and thorax excursions, oxygen saturation. At baseline (**A**) sleep-disordered breathing (mixed apneas and hypopneas mainly of central origin) was registered almost exclusively during non-rapid eye movement sleep (NREM) (total apnea-hypopnea index – 43/h). After heart transplantation (**B**) there was no pathological sleep-disordered breathing (total apnea-hypopnea index – 1.0/h of sleep).

index – 1.0/h of sleep; in REM sleep – 2.8/h, in NREM sleep – 0.5/h, mean O_2 saturation – 97.5%) (Figure 1B).

The patient was discharged from the hospital in improved condition (the on-going therapy is presented in Table 1).

Discussion

In this reported case, severe sleep apnea of the evident central origin was observed during NREM sleep in a patient with severe systolic HF; it completely disappeared after successful treatment of HF and restoration of cardiac systolic function.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

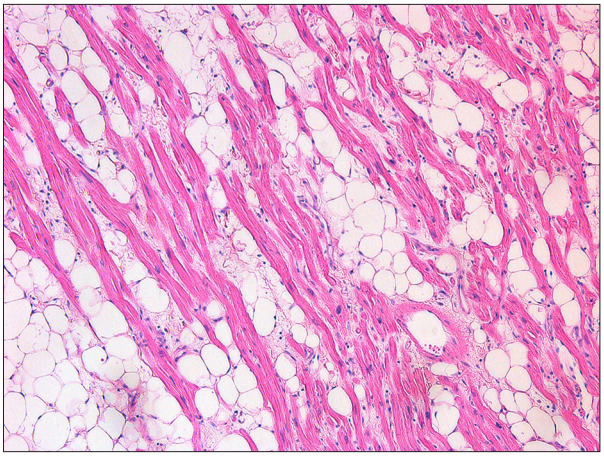

Figure 2. Lipomatosis of the myocardium (post-transplantation exam) ×100, hematoxylin-eosin stain.

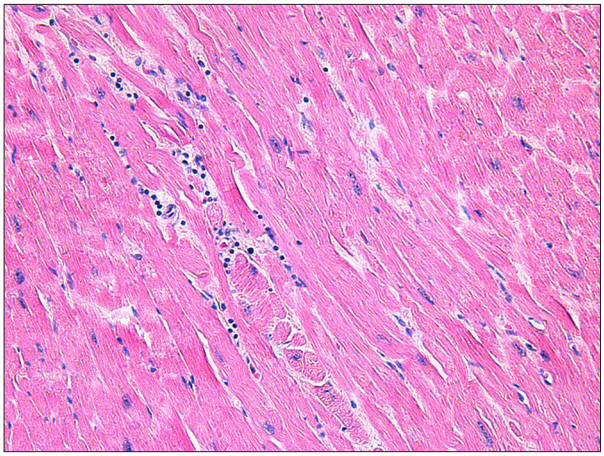

Figure 4. Focal lymphocyte infiltration of left ventricle (post-transplantation exam) ×200, hematoxylin-eosin stain.

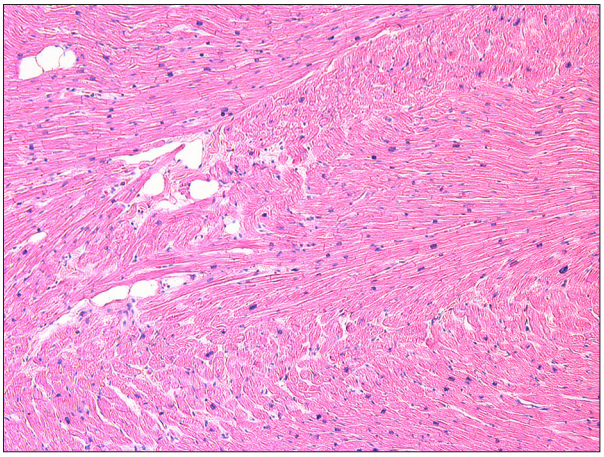

Figure 3. Isolation and dystrophy of muscle fibers of right ventricle and interventricular septum (post-transplantation exam) ×100, hematoxylin-eosin stain.

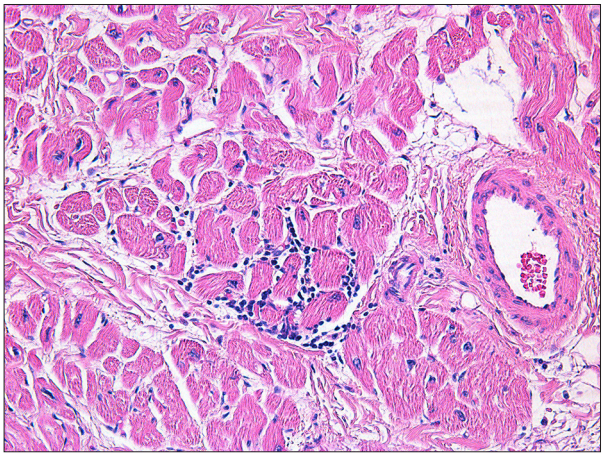

Figure 5. Focal lymphocyte infiltration at the peripheral region of atrioventricular node (post-transplantation exam) ×200, hematoxylin-eosin stain.

The prevalence of sleep apnea in systolic HF is 50% and higher, and, according to some authors, it is associated with worse outcomes [6]. However, the data on the effects of HF treatment, including heart transplantation, on sleep apnea are controversial, and sleep-stage-dependency of sleep-disordered breathing has not received much research attention.

The pathophysiology of sleep apnea in HF includes combinations of different mechanisms. Hypoxia and hypocapnia (due to high respiration rate), prolonged circulation time, and diminished pulmonary volume are considered possible causes, although they are not themselves pathogenetic mechanisms [6]. Recently, White and Bradley suggested a novel theory of sleep apnea development in states associated with fluid-retention (such as HF) [11], giving some new insights into the classical concept of central breathing patterns in HF. According to this hypothesis, posture-dependent fluid shift (from legs to upper body) due to gravity can augment venous return to the thorax and heart, increasing pulmonary capillary wedge pressure, pulmonary congestion, and interstitial pulmonary edema. These factors lead to hyperventilation, thus decreasing PCO_2 below the apnea threshold [6,9,11] causing cessation of breathing, which in turn results in CO_2 accumulation, chemoreceptor activation, and respiration resumption. The impaired chemosensitivity observed in HF causes higher ventilation response and more abrupt fluctuations [6]. In our patient, pulmonary thromboembolism further decreased the ventilation volume, worsening hypoxia and hypercapnia and leading to hyperventilation, thus contributing sleep apnea onset.

There is an evidence of sleep-stage dependent differences of ventilator control. Ventilation during sleep, especially in NREM-sleep, is thought to be dependent on chemoreceptor stimulation associated with arterial PCO_2 level, and apneic threshold plays a pivotal role. This was confirmed in animal models, and is assumed to be a possible mechanism of sleep apnea

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

in HF [12,13]. In contrast, in REM sleep, ventilator responses to the changes in PO_2 and PCO_2 are less prominent, which can lead to a more stable breathing pattern during this sleep phase, as observed in our patient [6,14,15].

The prognostic role and possible treatment of central and mixed sleep apnea in HF need further clarification. There is rather contradictory evidence of the effects of oxygen and there are few medication options (including acetazolamide, and theophyllines) for sleep-disordered breathing [6]. Positive airway pressure therapy, especially adaptive pressure support ventilation, seemed promising, although low compliance is common in HF patients. However, preliminary results of the SERVE-HF study demonstrating higher cardiovascular mortality rate in patients with systolic HF who used the devices compared to non-users (10% *vs.* 7.5%, respectively), prompted discussion about the possible role of central breathing patterns in systolic HF being somehow protective [16]. There are reports showing improvement or complete elimination of sleep-disordered breathing following successful treatment of HF, including medications, biventricular assist devices, and heart transplantation [6,17,18], although the data on sleep-stage association of sleep-breathing disorders is lacking. Our case report confirms the possibility of the complete elimination of NREM-associated mixed sleep apnea after successful treatment of severe systolic HF by heart transplantation.

Conclusions and Implications for Clinical Practice

Our clinical case demonstrates that NREM sleep-associated mixed sleep apnea occurring in patients with severe systolic HF due to dilated cardiomyopathy is reversible in case of successful elimination of HF. We suggest that it might be a marker of HF and the possible mechanisms of sleep apnea elimination include improvement of systolic heart function, decrease in circulation time, and beneficial changes in neurohumoral regulation, which play pivotal roles in ventilation control, especially during NREM sleep.

Thus, we strongly suspect that NREM sleep-associated mixed sleep apnea occurs at initial stages of HF, when the brain centers regulating ventilation are intact, and that compensation of HF might be highly effective in NREM sleep-associated mixed sleep apnea without non-invasive ventilation. This is important to know, especially with the regard to the recently published data of potentially unfavorable effects of adaptive pressure support servoventilation in systolic HF, and the lack of other treatment options.

Acknowledgements

The authors would like to thank Professor Eugene Shlyakhto, MD, PhD, Academician of Russian Academy of Sciences, Director of Federal Almazov North-West Medical Research Centre (St. Petersburg, Russia) for general support. We also would like to thank the patient and his family for the consent to publish these data.

References:

1. Lavie P, Hever P, Peled R et al: Mortality in sleep apnea patients: a multivariate analysis of risk factors. Sleep, 1995; 18: 149–57
2. Logan AG, Perlikowski SM, Mente A et al: High prevalence of unrecognized sleep apnoea in drug-resistant hypertension. J Hypertens, 2001; 19: 2271–77
3. Parati G, Lombardi C, Hedner J et al: Position paper on the management of patients with obstructive sleep apnea and hypertension. J Hypertens, 2012; 30: 633–46
4. Peker Y, Kraiczi H, Hedner J et al: An independent association between obstructive sleep apnea and coronary artery disease. Eur Respir J, 1999; 14: 179–84
5. Young T, Finn L, Peppard PE et al: Sleep disordered breathing and mortality: eighteen-year follow-up of the Wisconsin Sleep Cohort. Sleep, 2008; 31: 1071–78
6. Javaheri S. Heart failure. In: Kryger MH, Roth T, Dement WC (eds.), Principles and Practice of Sleep Medicine. Canada, 2011; 1400–15
7. Calvin AD, Somers VK, Van Der Walt C et al: Relation of natriuretic peptide concentrations to central sleep apnea in patients with heart failure. Chest, 2011; 140: 1517–23
8. Olson LJ, Somers VK: Treating central sleep apnea in heart failure: Outcomes revisited. Circulation. 2007; 115: 3140–42
9. Lieber C, Mohsenin V: Cheyne-Stokes respiration in congestive heart failure. Yale J Biol Med, 1992; 65: 39–50
10. AASM Manual for the Scoring of Sleep and Associated Events, version 2.0. USA; 2013
11. White LH, Bradley TD, Logan AG: Pathogenesis of obstructive sleep apnoea in hypertensive patients: role of fluid retention and nocturnal rostral fluid shift. J Hum Hypertens [Internet]. J Hum Hypertens, 2015; 29(6): 342–50
12. Dempsey JA, Skatrud JB: A sleep-induced apneic threshold and its consequences. Am Rev Respir Dis, 1986; 133: 1163–70
13. Xi L, Smith CA, Saupe KW et al: Effects of rapid-eye-movement sleep on the apneic threshold in dogs. J Appl Physiol, 1993; 75: 1129–39
14. Conwell W, Patel B, Doeing D et al: Prevalence, clinical features, and CPAP adherence in REM-related sleep-disordered breathing: a cross-sectional analysis of a large clinical population. Sleep Breath, 2012; 16: 519–26
15. Campos-Rodríguez F, Fernández-Palacín A, Reyes-Núñez N, Reina-González A: Clinical and polysomnographic features of rapid-eye-movement-specific sleep-disordered breathing. Arch Bronconeumol, 2009; 45: 330–34
16. ResMed Provides Update on Phase IV SERVE-HF Study of Adaptive Servo-Ventilation (ASV) Therapy In Central Sleep Apnea and Chronic Heart Failure [Internet]. [cited 2015 May 22]. Available from: *http://www.resmed.com/us/en/consumer/newsandinformation/news-releases/2015/resmed-provides-update-on-phase-iv-serve-hf-study-of-adaptive-servo-ventilation-therapy.html*
17. Vermes E, Fonkoua H, Kirsch M et al: Resolution of sleep-disordered breathing with a biventricular assist device and recurrence after heart transplantation. J Clin Sleep Med, 2009; 5: 248–50
18. Mansfield DR: The effect of successful heart transplant treatment of heart failure on central sleep apnea. Chest, 2003; 124: 1675–81

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License