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Vibroacoustic Disease and Respiratory Pathology III – Tracheal and Bronchial Lesions

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Abstract [638] **BACKGROUND.** Wistar rats exposed to low frequency noise (≤500 Hz, including infrasound) (LFN) have been used as experimental models for vibroacoustic disease (VAD) since 1992. The respiratory tract has been extensively studied through light microscopy, and scanning and transmission electron microscopy. LFN-induced tracheal lesions are abundant: loss of cilia, fusion of brush cell microvilli, deformation of secretory cells, and epithelial metaplasia. In VAD patients, respiratory complaints appear in the first 4 years of occupational exposure to LFN (in smokers and non-smokers alike). Thus, it became pertinent to offer a bronchoscopy examination to volunteer VAD patients, in order to investigate the type of lesions present, and whether they could be responsible for some of the respiratory pathology observed in these patients. **METHODS.** Four fully-informed and volunteer VAD patients (2 males-1 mild smoker, 2 females-1 mild smoker) received a bronchoscopy, and biopsies were removed for analysis. **RESULTS.** In all patients, small submucosal vascular-like lesions were observed in the vicinity of the spurs, consisting of increased collagen and elastin fibers. In all cases, histology disclosed cilliary abnormalities, basal membrane hyperplasia, and also thickening of vessel walls. No inflammatory process was ever identified, and no differences were seen between smokers and non-smokers. **DISCUSSION.** These data are in accordance with what has been observed in LFN-exposed animal models and also in 8 VAD patients who developed lung tumours. Moreover, this type of imaging is not frequent in the bronchoscopies given for other reasons. Taken together, it is strongly suggested that these findings could be LFN-specific.

1 INTRODUCTION

Vibroacoustic disease (VAD) is a systemic pathology caused by long-term (years) exposure to low frequency noise (<500 Hz, including infrasound) (LFN), and characterized by the abnormal growth of the extra-cellular matrices (1-3).

Respiratory complaints appear within the first 4 years of exposure, in smokers and non-smokers alike (1). Standard pulmonary function tests are usually normal in VAD patients, with the exception of focal fibrosis, as seen through high resolution CT scan (4), and a compromised neurological control of breathing, as measured by the PCO2 index obtained with the CO2-rebreathing technique (5). Non-productive cough and oropharynx infections are frequent complaints that, initially, seem reversible. In younger workers, these symptoms disappear during vacation and, in retired VAD patients, respiratory symptoms subside and often evolve to asymptomatic situations (1-3). In LFN-

exposed rat models, 48 hours of continuous exposure showed reversibility of LFN-induced tracheal lesions (6,7). On the other hand, studies of LFN-exposed rats while *in utero* demonstrated that severe cellular damage still existed after one-year of post-birth silence (6,8).
In this report, we investigate tracheal biopsy material taken from 4 VAD patients who volunteered for a bronchoscopy, only one of whom is still being exposed to LFN.

2 METHODS

2.1 Study Population

All patients volunteered to receive a tracheal bronchoscopy. This study was approved by Hospital Scientific Ethics Committees. All 4 cases have been diagnosed with Stage III VAD. (See Table 1.)
Case 1 has been a volunteer member of the VAD (aircraft technician) study population since 1980. He discloses a very thickened pericardium (hallmark of VAD (9)) and very large delays in nerve conduction times for his age group, also common in VAD patients (10).
Case 2 has been a volunteer VAD patient since 1988, when he suffered a cerebral vascular event that left him aphasic. He also discloses the characteristic thickening of cardiac structures, as seen through echocardiography. He was a combat helicopter pilot while an officer in the Portuguese Air Force, grounded since 1979, and retired since 1991.
Case 3 is an airline flight attendant, on sick leave since 2001. She has filed for disability retirement, and contacted our team in 2002. Along with the usual cardiac structure thickening and neurophysiological abnormalities, this patient also discloses severe balance disturbances, as seen in other VAD patients (11).
Lastly, Case 4, contacted our team from abroad, and is exposed to environmental LFN in the home, in the form idling bus engines, approximately 825 hours /month. Buses are located approximately 16 meters from the windows of her home. She has been residing in these conditions since 1999. In May 2001 she suffered an epileptic seizure and, in 2003, she travelled to Portugal and was diagnosed with VAD; she discloses very thickened pericardium and thickened cardiac valves, moderate neurophysiological abnormalities, and brain MRI revealed lesions typically seen in other VAD patients (12).

Case No.	1	2	3	4
Gender	M	M	F	F
Smoker	Mild	No	No	Mild
Age	48	61	36	54
Occupation	Aircraft Technician Retired, (14 yrs)	Air Force Colonel Retired (13 yrs), Combat Pilot until 1979	Airline Crewmember, On sick leave (3 yrs)	Homemaker Still exposed to environmental LFN

Table 1: *Summary of VAD patient information.*

2.2 Bronchoscopy

The procedures were conducted under general anaesthesia, with the jet ventilation system (Sanders-Venturi valve) and through rigid bronchoscopy (Efer-Dumon, France). After identification of the lesion, it was punctured with a Wang needle which produced a slight and self limited hemorrhage. Biopsies were obtained with standard flexible forceps of the bronchofiberscope (Olympus, Japan). For safety reasons, only small volumes of biopsy material were removed.

2.3 Light Microscopy

Specimens for light microscopy were fixed in 10% buffered formalin, sectioned and prepared for histological observation using standard methods. The sections were stained with hematoxylin-eosin, Masson trichrome solution, chronotrop aniline blue, and PAS.

3 RESULTS

In all patients, bronchoscopy imaging revealed small submucosal vascular-like lesions located distally in both tracheal and bronchial trees, uniformly distributed bilaterally near the spurs. These lesions were pink in color. Informal calculations indicated that maximal diameter of these lesions was approximately 3mm, and many were dot-like. Biopsies were performed on the abnormal mucosa (pink dots) and apparently normal mucosa (outside the pink dots). Pink areas did not bleed as would be expected from typical vascular lesions. No other abnormalities were observed. Case 1 presented the largest number of these pink lesions. Light microscopy of the non-pink areas disclosed irregular cilia distribution, with some areas containing large groupings, while others had scarce cilia. The basal membrane was thickened with abnormal amounts of collagen, and some blood vessels with thickened walls were visible.

In the abnormal (pink) areas, the number of ciliated cells and the cilia were also abnormally distributed. The basal membrane appeared even thicker than in the non-pink areas, with very large amounts of collagen. The entire basal membrane featured an abnormal neo-vascularization, disclosing very thickened blood vessel walls with scarce blood. This intense neo-vascularization was embedded in the collagen bundles that were not continuous, and exhibited ruptures. The amount of elastic fibers was also increased when compared with the non-pink areas.

No inflammatory cellularity nor processes were observed.

4 DISCUSSION

Thickening of blood vessel walls and abnormal growth of collagen was not a surprising observation since this feature has been consistently observed in LFN-exposed humans and animals:

a) in the 1987 autopsy of a VAD patient, fibrosa thickening of blood vessel intima was identified in all observed vessels. Interstitial and focal lung fibrosis was observed in both lungs (13);
b) in LFN-exposed Wistar rats, focal and interstitial fibrosis can be found along the entire respiratory epithelia. Collagen bundles appear foamy. Blood vessel intimas are very thickened with collagen. Alveoli walls are also thickened (6,8,14);
c) in electron microscopy studies of VAD-patient pericardial fragments, pericardial thickening (as seen through echocardiography), has been shown to be caused by an intense proliferation of collagen and other extra-cellular matrix components. Sandwiched within these thickened layers is a newly-formed layer of loose tissue, that includes thickened blood vessels (15-18);
d) in the lung and pleural biopsy of an aircraft technician, diagnosed with VAD (since deceased with a squamous-cell carcinoma of the lung, located in the upper right lobe), vessel walls were thickened, and focal fibrosis was a prominent feature. Thickening of the pleura, due to intense collagen proliferation, was remarkable (19); and
e) in tracheal biopsy material taken from the above patient as well as from a female military parachutist (with >2000 logged hours in military helicopters), basal membrane hyperplasia was identified (20).

This study discloses the first cases where collagen disruption was identified in humans, although foamy, degenerative foci of collagen has already been identified in LFN-exposed animal models

(6,8). Abnormal growth of the extra-cellular matrices has been a consistent and characteristic feature of LFN-exposed humans, and is believed to be intimately related to the specific biomechanical response of living tissue to LFN-induced vibration, on the tissue and cellular structures. This is the object of independent studies (21-23).
Despite the differences in the a) time exposure patterns, and b) types of LFN-rich acoustical environments of these patients, these lesions appeared in all, although case 1 was the most severe. The fact that these pink dots never appear to exceed about 3 mm in diameter is reminiscent of the results obtained in a experiment conducted in 1969, by Ponomarkov *et al* (24). Here, dogs were exposed to 1.5-2 hours of about 120-130 dB. Under the pleura, autopsy results revealed 3mm diameter hemorrhages, which increased in number - but not in size – with increasing noise exposure time.
It is important to emphasize that even though the retired VAD patients (Cases 1-3) are no longer working in LFN-rich environments that produced the onset of their pathology, as residents of urban and suburban communities, they are still exposed to environmental LFN. LFN exposure is not restricted to the aeronautical industry and military settings. Unfortunately, as Case 4 clearly attests, excessive exposure to environmental noise can also lead to the development of VAD. Cars, public transportation, and high volume roadways are all significant sources of LFN to which Cases 1-3 are still exposed. It should be noted that for a period of time post-retirement, Case 1 took up a job as vendor, which required that he spend very large amounts of time travelling in his car, a known LFN-rich environment (25). Additionally, Case 1 has the greatest amount of cumulative exposure time to LFN. Studies in immunohistochemistry and electron microscopy are ongoing.

5 SUMMARY

The first bronchoscopies performed on 4 volunteer VAD patients demonstrate, once again, that LFN exposure induces the abnormal growth of collagen and thickening of blood vessels. These lesions may by LFN-specific.

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