

# Prémio Thomé Villar/Boehringer Ingelheim 2004\*

## Thomé Villar/Boehringer Ingelheim Award 2004\*

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### Participação das vias aéreas centrais na doença vibroacústica

### *Involvement of central airways in vibroacoustic disease patients*

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#### Resumo

Nos últimos vinte cinco anos constatou-se que o aparelho respiratório constitui um alvo do ruído de baixa frequência (RFB < 500Hz, incluindo infra-sons). Denomina-se doença vibroacústica (VAD) a patologia sistémica causada pela exposição excessiva a RBF. Em ratos Wistar expostos a RBF, as alterações morfológicas observadas nos tecidos das vias aéreas centrais explicam, parcialmente, os sintomas apresentados pelos doentes com a VAD. Apesar disso, muitas perguntas permanecem por responder. Recentemente, voluntários com a doença vibroacústica submeteram-se a exames broncos-

#### Abstract

Introduction. Vibroacoustic disease (VAD) is the whole-body pathology caused by excessive exposure to LFN. For the past 25 years, it has been known that low frequency noise (LFN, < 500 Hz, including infrasound) targets the respiratory system. In LFN-exposed rodents, the morphological changes of respiratory tract tissue partially explained some respiratory symptoms reported by VAD patients. However, many questions remain unanswered. Recently, some volunteer VAD patients underwent bronchoscopy in order to ascertain possible damage that could be associated with

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cópicos para esclarecer e, se possível, demonstrar possíveis lesões das vias aéreas centrais que estariam associadas com as queixas respiratórias. Treze doentes voluntários, exaustivamente esclarecidos sobre este exame, submeteram-se a broncoscopias, durante as quais se colheram fragmentos para exame histológico e ultra-estrutural. Em todos os indivíduos se observaram lesões de tipo vascular na submucosa próximas das bifurcações das vias aéreas. Estas lesões caracterizavam-se por numerosos pequenos vasos com paredes espessadas, mergulhados em feixes de colagénio e algumas fibras de elastina. Em cinco indivíduos as lesões evidenciavam degenerescência e ruptura do colagénio. Histologicamente, na mucosa normal observaram-se alterações ciliares, hiperplasia da membrana basal e espessamento dos vasos sanguíneos. Em nenhum caso se observaram infiltrados celulares de natureza inflamatória. O estudo da ultra-estrutura revelou numerosos axonemas (de dois a oito) contidos numa membrana ciliar comum, algumas vesículas emanando dos cílios, imagens de apoptose com reforço do citosqueleto celular e das ligações intercelulares. Não se observaram diferenças entre fumadores e não fumadores. Estes dados estão de acordo com o que já fora observado nas vias aéreas centrais de oito doentes com carcinoma pavimento-celular do pulmão e também com as imagens observadas em ratos expostos a RBF. Em broncoscopias efectuadas em doentes sem a doença vibroacústica, não é normal observar-se este tipo de lesões vasculares. Assim, estas lesões vasculares das vias aéreas centrais podem ser específicas da doença vibroacústica.

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**Palavras-chave:** Ruído de baixa frequência, doença vibroacústica, lesões vasculares, displasia, doença auto-imune, doença do colagénio, estrutura ciliar, apoptose, mecano-transdução.

their respiratory complaints. Methods. Fourteen fully-informed and volunteer VAD patients were submitted to bronchoscopy, and biopsies were removed for analysis. Results. All patients exhibited small submucosal vascular-like lesions near the spurs, consisting of increased collagen and elastin fibres. Histology disclosed ciliary abnormalities, basal membrane hyperplasia, and thickening of vessel walls. In five patients, collagen bundles appeared degenerative and disrupted. No inflammatory process was ever identified, and no differences were seen between smokers and non-smokers. Discussion. Data is in accordance with what was observed in LFN-exposed animal models and also in 8 VAD patients who developed lung tumours. Collagen disruption and degeneration was also observed in electron microscopy images of the respiratory tract of LFN-exposed rodents. Thickened blood and lymphatic vessel walls have been consistently seen in images of VAD patients and of LFN-exposed rodents. During bronchoscopy performed by other reasons, this sort of structural aspects is not frequently seen. Taken together, it is strongly suggested that these findings could be VAD-specific.

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**Key-words:** Low frequency noise, vibroacoustic disease, vascular lesions, displasia, auto-immune disorders, collagen disease, ciliar structure, apoptosis, mecano-transduction.

## Introduction

Excessive exposure to low frequency noise (LFN) ( $\leq 500$  Hz, including infrasound) can lead to the development of vibroacoustic disease (VAD)<sup>1,2</sup>. VAD is a systemic pathology with an insidious and silent progression, characterized by the abnormal proliferation of extra-cellular matrices, namely collagen and elastin. Pericardial thickening in the absence of an inflammatory process and with no diastole is VAD specific<sup>3</sup>, and can be assessed through echocardiography<sup>4</sup>. Other diagnostic tests such as brainstem auditory evoked potentials<sup>5</sup>, P300 wave<sup>6</sup> and the PCO<sub>2</sub> respiratory drive test are available,<sup>7,8</sup>. However, to date, no medical diagnostic test has been found to readily assess pathognomonic signs.

Respiratory pathology in VAD was not immediately acknowledged because the first VAD study group consisted of aircraft technicians who are often exposed to airborne agents that could justify their respiratory complaints. These included (in both smokers and non-smokers alike) non-productive cough, repeated oropharynx infections, bronchitis and unexplained hoarseness<sup>2</sup>. Most of these situations, however, were not responsive to the typical therapeutics. Several cases of pleural effusion of unknown aetiology also developed among these workers. Unexplained lung fibrosis was a 1987 autopsy finding in a deceased VAD patient<sup>9</sup>.

All these circumstances prompted the 1892 investigations of the respiratory tract of LFN-exposed rats<sup>10-18</sup>. Here, significant morphological changes of respiratory epithelia such as abnormal amounts of collagen, thickened blood vessel walls, and

damaged cilia, were observed through electron microscopy. Pleura investigations in these rats showed that in addition to the morphological changes, mesothelial cells lost their phagocytic ability<sup>15,17</sup>. Mesothelial changes in the rat pleura are of the same morphological type as those observed in the respiratory epithelial cells: de-differentiation, reinforcement of cytoskeletal structures, and abnormally high frequency of apoptotic processes. Increased collagen in tracheal subepithelial cells, in lung parenchyma, and in pleural structures disclosed foci of foamy-like degeneration (Figs. 1 and 2).

Noise-induced respiratory pathology have also been studied by other authors over the past 4 decades<sup>19-24</sup>. Results show that acoustic phenomena have a direct effect on respiratory system structures, whether the exposure is long-term or short-term. In 2003, this team was contacted by a couple from Dublin, Ireland, suspected of suffering from VAD due to excessive in-home exposure to LFN caused by Dublin buses. Upon their trip to Lisbon, a VAD diagnosis was confirmed, and the case proceeded through the Irish courts with success for the plaintiffs. As in previous VAD patients, non-productive cough, unexplained hoarseness and frequent, atypical oropharynx infections were reported by the 54 year-old female, a mild smoker. She promptly agreed to undergo a bronchoscopic examination in order to better characterize the nature of her complaints and, possibly, to provide the Irish courts with additional data favourable to their case.

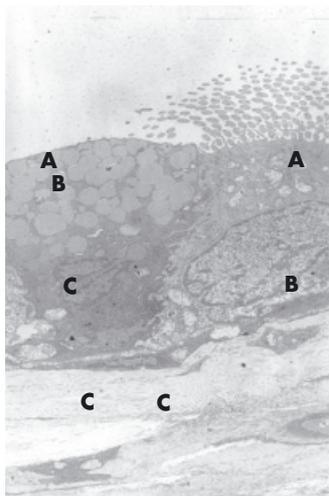
Fiberbronchoscopy was performed. Pink lesions were identified in small submu-

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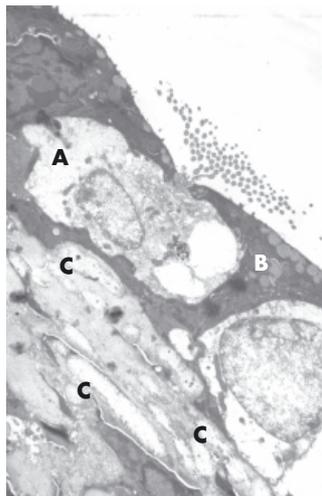
*VAD is a systemic pathology with an insidious and silent progression, characterized by the abnormal proliferation of extra-cellular matrices, namely collagen and elastin*

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*Acoustic phenomena have a direct effect on respiratory system structures, whether the exposure is long-term or short-term*



**Fig. 1** – Transmission electron microscopy of control rat respiratory epithelia. Ciliated cell (A) next to a serous cell (B), over a normal subepithelial layer of collagen. Numerous ciliary vesicles are patent. (x5600).



**Fig. 2** – Transmission electron microscopy of LFN-exposed rat respiratory epithelia (2438 hrs of total exposure). Brush cell (A) with 2 vacuoles and surrounded by serous cells, both de-differentiated. Scarce ciliary vesicles are present. Underneath is a layer of hyperplastic collagen with multiple foci of "foamy" (C) degenerated bundles. (x5600).

cosal vascular-like lesions, located distally in both tracheal and bronchial trees, and uniformly distributed bilaterally near the spurs. Informal calculations indicated that the diameter of these lesions was not over 3mm. No biopsy was initially taken from the pink areas. Given the possibility that these areas could be of a vascular nature, biopsy could lead to bleeding with possibly serious consequences. Histological studies of the non-pink areas disclosed abnormal amounts of collagen, damaged cilia and thickened blood vessel walls.

These observations confirmed previous findings in LFN-exposed rats, as well as in 3 other VAD patients who underwent bronchoscopic biopsies for other purposes

– neoplasm screening. In these 3 other individuals, biopsies of the pink areas were never performed due to the suspicion of their vascular nature.

The results were submitted to the Hospital Ethics Committee to obtain authorization to perform a biopsy in the pink areas, with the necessary precautions. Authorization was obtained. The Irish female volunteered for a second biopsy, fully-informed of the procedure and the risks, while additional medical staff was present, as well as thoracic surgeons. To date, 13 bronchoscopic examinations have been performed on volunteer VAD patients with biopsy of the pink areas.

The goal of this report is to describe the histological results of bronchoscopic biopsies with relevance to the pink areas identified in VAD patients.

## Methods

Thirteen patients volunteered for this study, as described in Table I. All had been previously diagnosed with VAD and exhibited pericardial and cardiac valve thickening, as assessed through echocardiography. Brainstem auditory evoked potentials and the P300 wave disclosed the usual changes in VAD patients, and all had impaired respiratory drive with simultaneous normal pulmonary function tests. They performed routine lab tests, C-Reactive Protein and serum antinuclear antibodies (ANA).

The procedures were conducted under general anesthesia, with jet ventilation system (Sanders-Venturi valve) and through rigid bronchoscope (Efer-Dumon, France). After identification of the lesion, it was punctured with a Wang needle

## PARTICIPAÇÃO DAS VIAS AÉREAS CENTRAIS NA DOENÇA VIBROACÚSTICA

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Table I – Description of study population

Case # (Male/Female)	Age	Exposure type	Smoker
1 – Male	48	Aircraft technician	Mild
2 – Male	37	Merchant marine	No
3 – Female	56	Nurse (helicopter)	No
4 – Female	36	Flight attendant	No
5 – Male	61	Combat pilot	No
6 – Female	54	Home-maker	Mild
7 – Female	59	Home-maker	No
8 – Male	52	Aircraft technician	No
9 – Male	59	Aircraft technician	Mild
10 – Male	59	Helicopter pilot	Moderate
11 – Male	54	Aircraft pilot	No
12 – Female	39	Flight attendant	No
13 – Female	40	Flight attendant	No

which produced a slight and self limited haemorrhage. Biopsies were obtained with standard bronchofiberscope flexible forceps (Olympus, Japan). For safety reasons only small volumes of biopsy material were removed.

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, chromotrop aniline blue, and PAS.

Specimens for electron microscopy were placed in a solution of 3% gluteraldehyde in 0.1 M phosphate buffer, pH 7.2 and

then washed with several changes of 5% sucrose in 0.1 M phosphate buffer, pH 7.2, for ultrastructural studies.

For TEM, samples were fixed at room temperature in an aldehyde mixture consisting of 4% paraformaldehyde, 1.25% glutaraldehyde, and 10mM CaCl<sub>2</sub> in 0.05 M cacodylate buffer, and pH 7.2. Specimens were washed in buffer, and postfixed in a ferricyanide-reduced osmium solution made up of 1% potassium ferricyanide and 1% osmium tetroxide in distilled water, dehydrated through a graded ethanol series, and embedded in Epon. The samples were sectioned in an ultramicrotome (LKB, Sweden) and the thin sections stained with uranyl acetate and lead citrate. Preparations were then examined with electron microscopy (JEOL 100C, Japan).

### Results

In all patients, bronchoscopy observation disclosed small submucosal vascular-like lesions located distally in both tracheal and bronchial trees, and uniformly distributed bilaterally near the spurs (Fig. 3). Biopsies were performed on the abnormal mucosa (pink areas) and apparently normal mucosa (outside the pink areas). Pink areas did not bleed as would be expected from typical vascular lesions. No other abnormalities were observed.

Light microscopy of the non-pink areas disclosed irregular cilia distribution, with some areas with large groupings, while others had scarce damaged cilia. Dysplastic foci were identified as well as basal hiperplasia (Fig. 4). The basal membrane was thickened with an abnormal amount of collagen, and some blood vessels with

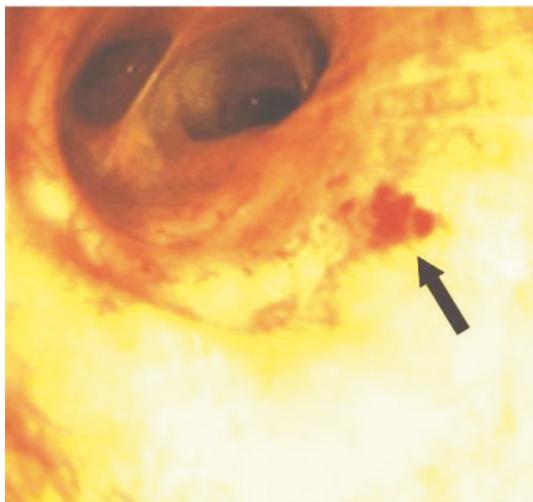


Fig. 3 – Patient #5: Pink area lesion (arrow) as seen through bronchoscopy.

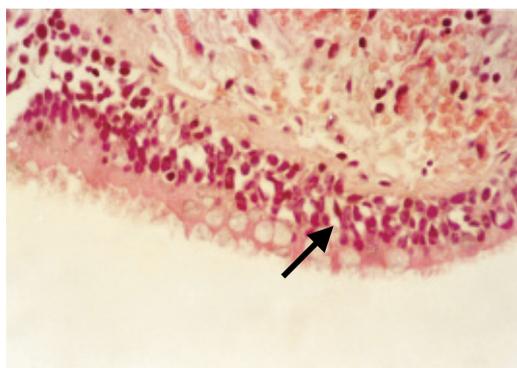


Fig. 4 – Patient #7: Light microscopy. Tracheal “normal” epithelium of the female VAD patient, non-smoker. Basal hyperplasia and partial destruction of cilia are visible, with subepithelial loose tissue. (x 200) (H&E)

thickened walls were visible. In the 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 abnor-

mal neo-vascularization, disclosing very thickened small blood vessel walls with scarce lumen. Lymphatic vessel walls were also thickened. This intense neo-vascularization was embedded within the collagen bundles. The amount of elastic fibres was also increased when compared with the non-pink areas. No inflammatory cellularity nor processes were observed, nor was retraction of neighbouring structures observed in the vicinity of collagen fibres (Figs. 5-8).

In five patients (cases 1-4, 13), some collagen fibres disclosed images of degeneration and disruption. These five cases also tested positive for anti-nuclear antibodies. No inflammatory cellularity was identified. In TEM, the most noticeable feature of gross changes in ciliary morphology are the multiple ciliary axonemes surrounded by a common membrane (Fig. 9). The cell life-cycle seems to be greatly accelerated given the frequency of apoptotic images in all epithelial cell ultramicrographs. All

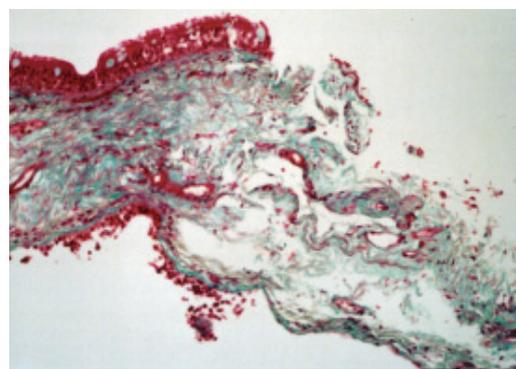
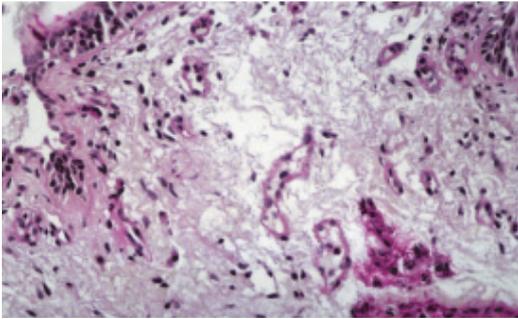


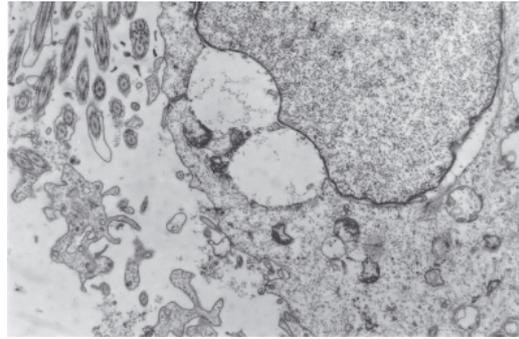
Fig. 5 – Patient #4: Ciliary changes are visible with subepithelial fibrosis, as well as vascular hyperplasia within subepithelial loose tissue. A longitudinal cut of a lymphatic vessel (arrow) shows thickened walls (x100) (Masson trichrome solution staining).

## PARTICIPAÇÃO DAS VIAS AÉREAS CENTRAIS NA DOENÇA VIBROACÚSTICA

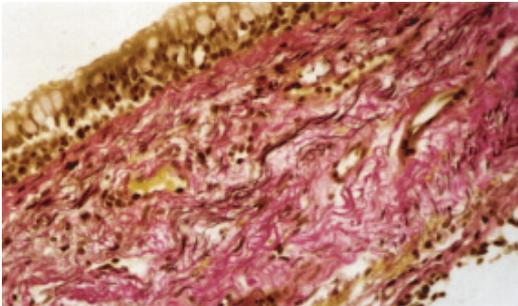
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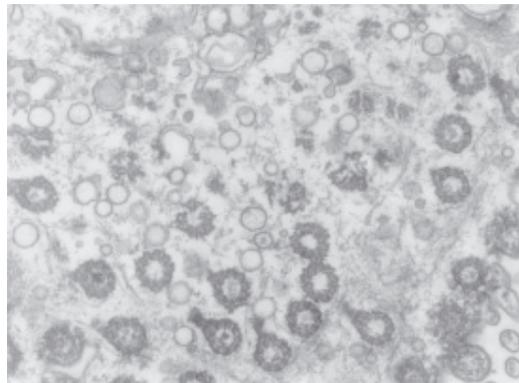
**Fig. 6** – Patient #4: Subepithelial loose tissue. Deep abnormal collagen proliferation with neo-vascular formations. (x200) (Masson trichrome solution staining).



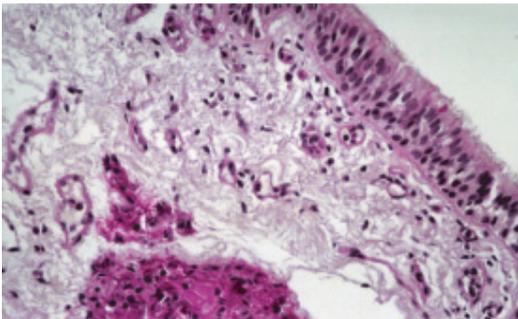
**Fig. 9** – Patient #7: TEM. Tracheal epithelium of non-smoker female VAD patient. An apoptotic cell and abnormal cilia with more than one axoneme per membrane. Two ciliary vesicles are visible. (x16000).



**Fig. 7** – Patient #4: Ciliary changes are visible, as well as fibrosis and numerous elastic fibres. Some collagen bundles disclose degeneration and disruptions (x200) (chromotrop aniline blue staining).



**Fig. 10** – Patient #7: TEM - Tracheal epithelium of non-smoker female VAD patient. Ciliated cell under apoptosis. In this tangential cut, the cell death process is seen to reach the basal bodies. (x20000).



**Fig. 8** – Patient #4: Histological cut showing ciliary changes with subepithelial loose tissue. Deep fibrosis with neo-vascular proliferation. (x200) (Masson trichrome solution staining).

cell structures seem to be under the same process of death (Figs. 9-11), in contrast with a marked reinforcement of the cytoskeleton and intercellular junctions.

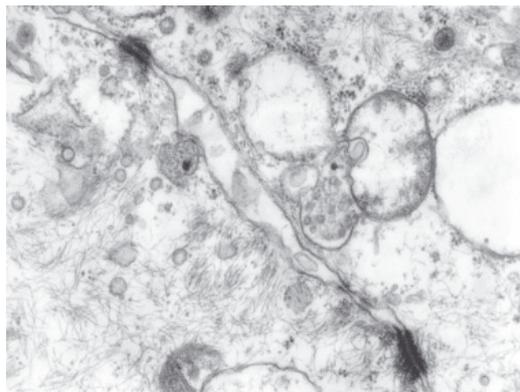


Fig. 11 – Patient #9: TEM - Tracheal epithelium of a male VAD patient, smoker. Intercellular junctions of two apoptotic epithelial cells with impressive desmosomes and a marked reinforcement of the cytoskeleton. (x40000).

## Discussion

Thirteen subjects may seem to be a small number of cases. Bronchoscopic examinations are very invasive procedures, and even though all aspects are explained to volunteer patients before the examination, it naturally frightens many individuals. Nevertheless, this is already an important number considering that all (100%) have disclosed the same type of lesions, even though they have distinct types of LFN exposure – occupational and environmental, with different frequency distributions. Additionally, the vascular component is the same in all cases observed – thickened blood vessel walls – as has already been seen in autopsy<sup>9</sup>, in LFN-exposed rats<sup>10-14</sup>, as well as in pericardial fragments of VAD patients<sup>25,26</sup>. Lymphatic vessel abnormalities were also similar to those seen in LFN-exposed rats<sup>27</sup>, as were those observed in blood vessels<sup>28</sup>. Electron microscopy studies of biopsy material are still underway. In an inflammatory process, the growth

of collagen is not well organized, and does not include embedded neo-vascularization, as is seen in these patients. In the formation of scar tissue, there is retraction of the neighbouring structures that is not observed here. This does not mean that the inflammatory cascade is not present; however the classical inflammatory response, with collagen production as an end result, is not exhibited by VAD patients. The inflammatory cascade within the scope of LFN-exposure is the focus of an independent and ongoing project.

In cases 1-4 and 13, collagen degenerative processes were observed. Although none of these five patients have been diagnosed with collagen diseases, namely systemic lupus erythematosus, other VAD patients have indeed been diagnosed with this form of collagen disease, and other auto-immune disorders<sup>29,30</sup>. The airway pink lesions described herein have also been observed in systemic lupus erythematosus<sup>31</sup>. The involvement of the immune system in VAD patients has long been recognized. Previous studies have demonstrated that lupus-prone mice have an earlier onset and increased mortality when exposed to LFN<sup>32</sup>, while circulating CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes suffer modulations with LFN exposure in aircraft technicians<sup>33</sup>. Collagen plays a key role in maintaining structural integrity of biological tissue, allowing movement without rupture. The different arrangements of collagen fibres, relative angular organization and interlacing of elastic fibres provide tissues with the ability to sustain movement without rupture, to maintain functionality despite (mechanical) disturbances. Production of

**Collagen plays a key role in maintaining structural integrity of biological tissue, allowing movement without rupture**

collagen, therefore, can be elicited by biological structural requirements.

Vibratory stress, such as that induced by LFN exposure, can threaten the structural integrity of almost any type of biological (and non-biological) material. In aircraft fuselage, for example, vibratory stress can give rise to micro-fractures that, in turn, can jeopardize flight safety. In biological tissue, LFN-induced vibratory stress, as potentially related to pathological evolutions, is an underdeveloped scientific field. In biological tissue, vibratory stress can play a key role in the maintenance of the tissue's structural integrity. However, unlike aircraft, a biological organism can mount a response associated with assuring that structural integrity is not lost or, in other words, that functionality is maintained – at least at a required minimum for immediate and/or long-term survival of the organism.

Maintenance of structural integrity can be achieved by structural reinforcement. In layman's term: if it's shaking, tie it down. The possibility of rupture along tissue surfaces can elicit a biological response that counteracts by reinforcing the surface. This structural reinforcement can be achieved through the production of collagen – a strong, tensile material. If enough elastic fibres are simultaneously produced, it is possible for the tissue surface to maintain its functionality while assimilating abnormal vibratory stress.

Considering that respiratory airways can be thought of as flexible hoses and that LFN exposure translates into vibratory stress, it is not difficult to speculate on a reason for the existence of these respiratory tract lesions. Indeed, they can be

thought of as the equivalent of micro-fractures that appear along aircraft fuselage as a result of excessive vibratory stress. Following this line of reasoning, the biological response of increased collagen and elastic fibres is not surprising if maintaining structural integrity is an issue. Biointegrity concepts, continuous tension with zero torque, may be the guiding pathways for this occurrence<sup>34-39</sup>. Mechanotransduction signalling is an important pathway for cell signalling. It is achieved through the biopolymers that compose both the cytoskeleton and the extra-cellular matrix. Given the morphological changes seen in FLN noise exposed humans and animals, it is pertinent and logical to view VAD as a mechanotransduction disease.

However, disruptions in the collagen bundles, giving them their “foamy” appearance, and reproducing previous results in LFN-exposed rats, strongly suggests that functionality is *not* being fully maintained.

**Vesicles.** Ciliary vesicles cannot be ignored given their recurring presence in both non-exposed (Fig. 1) and exposed (Fig. 2) rats and humans (Fig 9). No reference to this feature was found in the literature except for one micrograph<sup>40</sup> It is therefore unknown if they are a product of exocytosis (coated vesicles?) or something else. Regardless of their contents or biochemical role, their physical presence alone as microspheres spewed into the respiratory fluid can have important consequences on drainage processes. The number of vesicles is decreased in the exposed specimens, which suggests that their function might be compromised. This

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***Vibratory stress, such as that induced by LFN exposure, can threaten the structural integrity of almost any type of biological (and non-biological) material***

would not be entirely surprising since other functional capabilities such as the phagocytic ability of the pleural mesothelial cells<sup>18</sup> are known to be lost due to LFN exposure.

**Low Frequency Noise.** LFN targets the respiratory epithelia. This has been suspected for decades. In 1987, Svirgovi et al. investigated the effects of infrasound on the pulmonary ultra structure of white mice<sup>22</sup>. Noise was characterized as 2, 4, 8, or 16 Hz, at 90-140 dB. The mice were exposed for 3 hr daily, for 1, 5, 10, 15, 24 and 40 days. After 3 h of exposure, "point, mosaic-type" hemorrhages were identified over the entire lung surface. These hemorrhages were observed beneath the pleura for exposures of 2-4 Hz at 92-100 dB, and large bleeding spots were identified in lung exposed to 8-16 Hz, at 120-140 dB. After 10-15 d of exposure, parts of the lung tissue are filled with blood and the walls between alveoli are swollen and thick. Dramatic morphological changes of alveolar, cellular, and blood vessel structures are described after exposures of 24-40 days, at 8-16 Hz and 120 dB. These included deformation of nuclei and concentration of chromatin under the nuclear membrane in type I and II pneumocytes, and disruption and deformation of alveolar sacs, destruction of alveolar walls, and burst capillaries and larger vessels.

In 1969, Ponomarkov et al. explored the effects of wide-band noise at 105-155 dB (no frequency distribution information was provided) on dogs<sup>20</sup>. After 1.5-2 h of exposure, the animals were sacrificed. Autopsy results revealed hemorrhages up to 3 mm in diameter in the lungs of the

animals exposed to about 126 dB, located beneath the pleura in the form of convex vesicles. They were most common in the costal surface of the upper lobe of the right lung. The authors claim that as the dB level of the noise increased, the number of the hemorrhages increased, but they never exceeded 3mm in diameter. Microscopic analysis of the hemorrhaged section of lung tissue revealed ruptured capillaries and larger vessels. Microscopic analysis of the emphysematous areas showed focal enlargement of the alveoli, stretching of the connective-tissue structures of the alveolar walls, and compression of lung tissue. They conclude their report by giving a possible explanation for this pathology: "The lungs, as a system open to the external environment, are subjected to the greatest influence of changes in pressure in this medium. Depending on the magnitude of the pressure differential created by sound in the lungs during its transformation, and of the threshold of resistance of the lung tissue to pressure changes, the severity of the lung lesions gradually increases."

The effects of LFN on human populations have been partially studied in the past and confirm that this acoustic stressor is a potential agent of respiratory illness. An interesting study was conducted in the mid-60's<sup>19</sup>. Unfortunately the number of subjects involved (N=5, all military personnel) renders them statistically non-significant. However, the acoustic simulation facilities used were unique: they allowed simultaneous subjective and objective stress responses during exposure to a well-defined acoustic environment, which provided detailed information on

**Dramatic morphological changes of alveolar, cellular, and blood vessel structures are described after exposures of 24-40 days, at 8-16 Hz and 120 dB**

amplitude (95-140 dB), frequency (spikes at 30-100 Hz), and exposure time (25 sec-5 min). Subjective complaints involving the respiratory tract are abundant and included coughing, gagging sensations, chest wall and throat pressure, and pain on swallowing.

LFN is not currently legislated, nor is it assessed during routine noise evaluations, hence this agent of disease is ubiquitous in modern society with the potential consequences if imolies for Public Health.

That LFN exposure induces severe lesions of the respiratory system is a fact. The evidence gathered with animal models clearly shows that the respiratory epithelia are not, at all, impervious to acoustic phenomena. Despite the immune depression of VAD patients, many of their immediate and long-term respiratory illnesses are certainly due to the direct impact of this agent of disease. The involvement of the CNS in LFN-induced respiratory pathology is still understudied and cannot be excluded.

Unfortunately, noise-exposed workers are only required to undergo an audiogram in order to evaluate hearing loss, particular at the 4000 Hz notch. VAD patients normally have loss at the 250-500 Hz range, which, if observed, is normally overlooked as a curiosity. In fact, the most common auditory complaint of VAD patients is that “they hear too much, can’t stand any type of sound, not even music or television”.

**Commentary.** Regarding LFN as a mutagenic and genotoxic agent raises unsettling concerns<sup>41-43</sup>. Not only because LFN is rampant in our society, but also because the cause of many malignancies are being

erroneously imputed to other causes. For example, given the information provided here, what is the validity of studies concerning the respiratory system where the acoustic environments of the study population are unknown? Many animal laboratories are kept in basements, where hot/cool air conditioning systems, as well as electromechanical laboratory equipment, are powerful sources of LFN. Likewise, many human subjects have an extensive history of LFN exposure that must be taken into account if their respiratory tract is under study or monitoring.

The fact that LFN is not legislated, not evaluated during routine noise assessment procedures, and that LFN-exposed workers are not afforded any protection against this agent of disease is a very worrisome state of affairs. In fact, a large part of the medical community still believes that acoustic phenomena only impacts the auditory system, producing hearing loss, or via the auditory system, producing annoyance. It is painfully clear that a new attitude toward noise-exposed people is urgently warranted.

### Conclusions

Despite the small number of subjects (n=13), all have disclosed the same type of lesions even though their LFN exposures are distinct. This type of lesion is considered to be pathognomonic of VAD but, given the invasive nature of bronchoscopic examinations, the search for this sign should be reserved for critical diagnosis, namely for forensic purposes. The results herein further substantiate that VAD is in fact a mecano-transduction disease.

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*A large part of the medical community still believes that acoustic phenomena only impacts the auditory system*

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