TINNITUS, BIOMARKERS AND QUALITY OF LIFE IN AN OLDER POPULATION

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*Equal contributions

“In life there is nothing to fear, but to understand.”

(Marie Curie)

“What we know is a drop; What we do not know is an ocean.”

(Isaac Newton)

Sumário

O acufeno é um sintoma referente à perceção de um som nos ouvidos ou na cabeça, sem que exista um estímulo acústico externo correspondente. Está presente em diferentes patologias (otológicas ou não) e tem um impacto importante na qualidade de vida da pessoa afetada. Atualmente, o seu diagnóstico e monitorização são baseados em medidas subjetivas audiométricas e psicométricas, sendo que não existem métodos objetivos para a identificação do acufeno. Além disso, os mecanismos fisiopatológicos subjacentes ao acufeno subjectivo permanecem desconhecidos.

O objetivo da presente tese é estudar os mecanismos subjacentes ao acufeno subjectivo e a sua relação com a surdez, visto que a surdez é a co-morbilidade mais frequentemente associada ao acufeno. Pretende-se também avaliar a contribuição dos fatores genéticos, audiológicos e imunológicos na etiologia do acufeno.

Para isso, foram realizadas revisões sistemáticas (RS) sobre esta temática de forma a conhecer o estado de arte, primeiramente em relação à forma como os pacientes e os familiares percecionam o acufeno e também sobre os ensaios clínicos existentes acerca da eficácia do tratamento do acufeno. Ambas as RS contribuíram para a identificação de um conjunto de domínios relacionados com o acufeno, usado pelo COMIT'ID (*Core outcome measures in tinnitus international Delphi*), num método de consensos Delphi, baseado na Internet, com o objetivo de identificar um 'Core Outcome Set' (ou seja definir quais as queixas relacionadas com o acufeno que são imprescindíveis para a sua avaliação) recomendado para ensaios clínicos de eficácia terapêutica para o acufeno assim como para o seu diagnóstico. Estas recomendações são específicas para as três modalidades terapêuticas principais: sonora, psicológica e farmacológica uma vez que cada modalidade tem fundamentos específicos e por isso visam avaliar diferentes aspetos do acufeno.

Com o objetivo de contribuir para a padronização da avaliação e do tratamento clínico do acufeno, foi constituída a TINNET, uma rede europeia para a investigação científica do acufeno. Considerando o objetivo do presente estudo e a hipótese de integrar esta rede europeia, foram desenvolvidas um conjunto de atividades que em muito contribuíram para o conhecimento sobre o acufenos. Entre as diferentes atividades realizadas com o apoio da TINNET destaca-se a realização de uma revisão sistemática sobre as 'guidelines' clínicas existentes para o diagnóstico e tratamento do acufeno. Esta revisão foi uma das bases que conduziu ao desenvolvimento das 'guidelines' europeias multidisciplinares para o acufeno: diagnóstico, avaliação e tratamento. Estas 'guidelines' foram apresentadas na conferência final do TINNET e estão atualmente em fase de disseminação.

Outro foco de interesse da presente tese foi a realização de trabalhos de revisão sobre o acufeno somatosensorial (nomeadamente sobre a fisiopatologia, diagnóstico e

tratamento), bem como a participação num grupo de consenso internacional sobre o diagnóstico deste subtipo do acufeno, de forma a contribuir para uma melhor compreensão deste subtipo do acufeno. Também estas atividades contribuíram para o desenvolvimento de competências cientificas essenciais ao desenvolvimento do presente estudo, dado que permitiram uma melhor compreensão deste subtipo do acufeno, demonstrando-se a heterogeneidade e diversidade do acufeno.

De forma a alcançar os objetivos deste estudo de doutoramento, recrutaram-se 114 voluntários da população portuguesa com idade dos 55 aos 75 anos. Os indivíduos desta amostra permitiam a realização de diferentes estudos nomeadamente os laboratoriais, tendo a analise dos resultados envolvido a amostra dividida em quatro grupos consoante a presença/ausência do acufeno e de surdez. Dos resultados desta tese fazem parte quatro artigos originais que e incluem uma caracterização demográfica, aspetos relevantes a nível psicológico e de qualidade de vida, marcadores audiológicos do acufeno, perfil imunológico da população e biomarcadores da presbiacusia e do acufeno.

Os resultados obtidos sugerem a perda auditiva como fator de risco para o desenvolvimento do acufeno e as queixas a nível psicológico como fator de risco para o acufeno mais grave e consequentemente associado a menor qualidade de vida nos pacientes com este sintoma.

A nível da caracterização dos marcadores audiológicos, verificou-se que a presença de antecedentes de exposição ao ruído e a perda auditiva aumentam a probabilidade de desenvolver acufeno. Também, os participantes com um início abrupto do acufeno e que apresentam um efeito negativo ou '*rebound*' na inibição residual têm maior probabilidade de desenvolver acufeno grave ou catastrófico. Encontrou-se nos Potenciais Evocados Auditivos, uma redução da amplitude na onda I em pacientes com acufeno, bem como valores maiores no 'Ratio de amplitude das ondas V e I de ambos ouvidos' estando associados a maiores probabilidades de desenvolver acufeno severo ou catastrófico.

O perfil inflamatório da nossa população mostra diferenças significativas entre o grupo com e sem acufeno quando comparados para a IL10. Quanto à relação entre os parâmetros imunológicos e a acufenometria, verificou-se uma correlação entre o aumento da IL1α e acufeno tonal, bem como entre o aumento da IL2 e a inibição residual do acufeno. Foi também encontrada uma correlação negativa para a IL10 e a duração do acufeno e para o HSP70 e a intensidade do acufeno. Estes resultados são muito originais e suscitam a necessidade de estudos futuros que permitam esclarecer os mecanismos subjacentes às correlações encontradas.

Em relação aos biomarcadores, foi efetuada uma revisão sistemática com a finalidade de sintetizar evidências para a existência e utilidade clínica dos

biomarcadores para o desenvolvimento ou gravidade do acufeno. Foi também realizado um estudo acerca do papel do *GRM7* e do *NAT2* na nossa amostra. Os resultados apontam para uma maior prevalência do alelo T no gene *GRM7* (60,3% T/T e 33,3% A/T). Os participantes com um genótipo T/T parecem ter um maior risco para o desenvolvimento de ARHL e 33% apresentam menor risco para o desenvolvimento do acufeno, em comparação com indivíduos com A/A e genótipo A/T. Em relação ao fenótipo *NAT2*, o acetilador lento (53%) foi o mais comum seguido pelo intermediário acetilador (35,9%). Os nossos resultados sugerem que o genótipo A/T de *GRM7* e o fenótipo acetilador lento de *NAT2* como potenciais biomarcadores da severidade do acufeno.

Os resultados obtidos são originais e no seu conjunto são muito interessantes, apontando para a necessidade de estudos futuros em larga escala de forma a aprofundar as conclusões aqui obtidas. Por outro lado, os estudos translacionais poderão ser a chave para esclarecer os dilemas da fisiopatologia do acufeno.

Summary

Tinnitus is a symptom involving the perception of sound in the ears or head, without a corresponding external acoustic stimulus. It is related to many different conditions and has a major impact on quality of life of the affected person. Currently, its diagnosis and monitoring are based on subjective audiometric and psychometric measures. There are no objective methods for tinnitus identification. In addition, the pathophysiological mechanisms underlying tinnitus remains unknown.

The purpose of this thesis was to study the mechanisms underlying tinnitus and their relationship to hearing loss, being that hearing loss is the comorbidity most frequently associated with tinnitus. It also aimed to evaluate the contribution of genetic, audiological and immunological factors to the etiology of tinnitus.

For this purpose, systematic reviews (SR) were performed, in order to account the state of art, the perspectives of the patient and their relatives, and previous clinical trials of tinnitus treatments. SRs contributed to the identification of a pool of tinnitus-related complaint domains used by COMIT'ID (Core outcome measures in tinnitus international Delphi) in a 3-round internet-based Delphi survey to identifying core outcome sets (COS), i.e., which complaints related to tinnitus are essential for evaluation in clinical trials. These recommendations are specific to the three main therapeutic modalities: sound, psychological, and pharmacological.

In order to contribute to the standardization of tinnitus clinical evaluation and treatment, TINNET, a European network for scientific tinnitus research, was created. Among the different activities carried out in were a systematic review of existing national clinical practice guidelines for the diagnosis and treatment of tinnitus. This review contributed to the development of a multidisciplinary European guideline for tinnitus: diagnosis, evaluation and treatment. This guideline was presented at TINNET final meeting and it is being disseminated widely.

Another aim of the present thesis was to review work on somatosensory tinnitus (pathophysiology, diagnosis, treatment and the participation in an international Delphi consensus group on the diagnosis of this subtype of tinnitus), to contribute to a better understanding of this subtype of tinnitus.

In order to achieve the objectives of this PhD study, 114 participants aged 55 to 75 years were recruited from the Portuguese population. Participants were divided into four groups according to the presence/absence of tinnitus and hearing loss. The completion of the study protocol gave rise to four original research articles, including a demographic characterization, relevant psychological and quality of life aspects comparing the studied population and the published literature, audiologic markers of

tinnitus, and immunological profile of population and biomarkers of presbycusis and tinnitus.

The results point to hearing loss as a risk factor for the development of tinnitus and psychological complaints as a risk factor for more severe tinnitus and consequently less quality of life in patients with this symptom.

In characterizing audiological markers, the presence of previous noise exposure and the hearing loss increased the probability of developing tinnitus. Also, participants with an abrupt onset of tinnitus and who had a negative effect or rebound on residual inhibition were more likely to develop severe or catastrophic tinnitus. For the population with tinnitus, a reduction in amplitude of auditory evoked potentials wave I and a higher values in the 'Ratio of Waves V/I for both ears' were associated with a greater probability of developing severe or catastrophic tinnitus.

The inflammatory profile of the study population showed significant differences in IL10 levels between the group with and without tinnitus. IL1α was significantly higher in patients with tonal tinnitus, while IL2 was higher in participants who reported negative or rebound effect on residual inhibition of tinnitus. A negative correlation was also found between IL10 and tinnitus duration, and between HSP70 and tinnitus intensity.

Biomarkers were explored in this thesis. A systematic review was performed to synthesize evidence for the existence and clinical usefulness of biomarkers. *GRM7* and *NAT2* were evaluated in the thesis population. The results indicate a higher prevalence of the T allele in the *GRM7* gene (60.3% T/T and 33.3% A/T). Participants with a T/T genotype appeared to be at a higher risk for ARHL development, and 33% have a lower risk of developing tinnitus compared to participants with A/A and A/T genotype. Regarding the *NAT2* phenotype, the slow acetylator (53%) was most common, followed by the intermediate acetylator (35.9%). These results suggest that the AT allele of *GRM7* and the slow acetylating phenotype of *Nat2* are potential biomarkers of tinnitus severity.

The results in this thesis are very interesting and original, showing us the need for future research in larger samples, and employing rigorous methodological design in order to control for confounding variables. On the other hand, translational studies may be the key to clarifying the pathophysiologic dilemmas of tinnitus.

Dedication

To my son with endless Love

Acknowledgment and motivation

Tinnitus has been the area of my special interest since at least two decades of my professional life. So, the opportunity of developing PhD studies in this area gave me the exceptional opportunity to increase knowledge regarding tinnitus pathophysiology, management and treatment.

The chance of working with other colleagues of different professional grounds sharing the same interest on tinnitus in TINNET, a European research network, was a unique occasion for improvement of research skills and learn to see different angles of the same problem, team working and also create friendships.

Those were also hardworking years, trying to reconcile research and clinical activity was sometimes difficult. But, the passion for learning, improving research skills and having more insight about scientific critical thinking, as well knowledge about tinnitus and related comorbidities has driven me through the whole process of PhD.

Nevertheless, the development of the studies related to this thesis were only possible with the structured team work of several professionals with whom I had the honor to work with, and this is the moment to express them my profound and sincere appreciation for their contributions.

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List of abbreviations

ABR	Auditory brainstem response
AC	Auditory Cortex
ACE	Angiotensin-Converting Enzyme
ADD1	Alpha-Adducin
AEPs	Auditory-evoked potentials
AMPA	Alfa-amine propionic acid
ANX	Anxiety
ARHL	Age related hearing loss
ASSIA	Applied Social Sciences Index and Abstracts
AVCN	Antero-ventral Cochlear Nucleus
BAER	Brainstem auditory evoked response
BDNF	Brain-derived neurotrophic factor
BF	Basal forebrain
BIAP	Bureau International d'Audiophonologie
BioISI	Biosystems & Integrative Sciences Institute
BOLD	Blood oxygenation level-dependent
BP	Bodily Pain
BZR	Benzodiazepine receptor distribution
C	Control Group
CANS	Central Auditory Nervous System
CBC	Complete blood count
CBT	Cognitive Behavioral Therapy
CDSR	Cochrane Database of Systematic Reviews
CENTRAL	Cochrane Central Register of Controlled Trials
CINAHL	Cumulative Index to Nursing and Allied Health Literature
CIs	Cochlear implants
CN	Cochlear nuclei
CNS	Central Nervous System
COMET	Core Outcome Measures in Effectiveness Trials
COMiT	Core Outcome Measures in Tinnitus
COMIT'ID	Core outcome measures in tinnitus international Delphi
CONSORT	Consolidated Standards of Reporting Trials
COS	Core outcome set
COST	Cooperation in Science and Technology
COX	Cyclooxygenase
COX-2	Cyclooxygenase type-2
CR	Chromium
cRP	C - reactive Protein
CSOL	Complex Superior Olivary Lateral
CSOM	Complex Superior Olivary Medium
CT	Computer tomography
Cu	Copper
dACC	Dorsal anterior cingulate cortex
DCN	Dorsal Cochlear Nucleus
DEP	Depression
DHEA	Dehydroepiandrosterone
DPOAEs	Distortion product otoacoustic emissions
DSA	Digital subtraction angiography
DSM	American Diagnostic and Statistical of Mental Disorders
EBSCO Host	Elton Bryson Stephens COmpany
EEG	Electroencephalography
EMBASE	Excerpta Medica dataBASE
ENT	Ear nose and throat

EPOC	Effective Practice and Organization of Care;
Fe	Iron
fMRI	Functional magnetic resonance imaging
FR	Free radical
FTQ	Fear of Tinnitus Questionnaire
GABA	Gamma-Aminobutyric Acid
GDNF	Glial cell-derived neurotrophic factor
GGA	Geranylgeranylacetone
GHP	General Health Perceptions
GP	General practitioner
GPNs	Global perceptual networks
GPX	Glutathione peroxidase
GRM7	Metabotropic glutamate receptor subtype 7
GST	Glutathione S-transferase
GSH-PX	Plasma glutathione peroxidase
GSI	General Severity Scale
GWAS	Genome-wide association study
HADS	Hospital Anxiety and Depression Scale
HC	Homocysteine
HF_PTA	High frequency pure-tone average
HG	Heschl's gyrus
HHL	Hidden hearing loss
HL	Hearing Loss
HOS	Hostility
HPA	Hypothalamic-pituitary-adrenal
HPLC	High performance liquid chromatography
HR-QoL	Health-related quality of life questionnaire
HSP-70	Heat shock protein 70
IHC	Inner Hair Cells
IAC	Internal auditory channel
IC	Inferior colliculus
ICF	International Classification of Functioning, Disability and Health
ICTRP	International Clinical Trials Registry Platform;
IFN- γ	Interferon-gamma
IL1α	Interleukin-1 alfa
IL1 β	Interleukin-1 beta
IL-1b	Interleukin-1b
IL-2	Interleukin-2
IL-6	Interleukin-6
IL-10	Interleukin-10
IPC	Inferior parietal cortex
I-S	Interpersonal sensitivity
ISRCTN	International Standard Randomized Controlled Trial Number registry
ISSNHL	Idiopathic Sensorineural Sudden Hearing Loss
K+	Potassium
LDL	Loudness discomfort level
MCS	Mental Component Summary scale
MDA	Malonaldehyde
MEG	Magnetoencephalography
MeSH	Medical subject headings
MFT	Myofascial trigger
MGB	Medial geniculate body
MH	Mental Health
MMA	Methylmalonic Acid
MML	Minimum masking level
Mn	Manganese
MOS SF-36	Medical Outcomes Study Short Form Health Survey

MPO	Myeloperoxidase
MPV	Mean Platelet Volume
MRA	Magnetic Resonance Angiography
MRI	Magnetic resonance imaging
Na+	Sodium
NIHL	Noise-induced hearing loss
NIPT	Nutritional intervention program to Tinnitus
NLR	Neutrophil-to-Lymphocyte Ratio
NMDA	N-methyl-**D**-aspartate
NMDAR	N-methyl D-aspartate receptor
NOx	Nitrite plus nitrate orofacial movements
OAE	Otoacustic emission
O-C	Obsessive–compulsive
OR	Odds Ratio
OFM	Orofacial movements
OHC	Outer Hair Cells
PAR	Paranoid ideation
PC	Platelet count
PCS	Physical Component Summary scale
PDW	Platelet distribution width
PET	Positron emission tomography
PF	Physical Functioning
PFL	Paraflocculus lobe of the cerebellum
PHC	Parahippocampal cortex
PHOB	Phobic anxiety
PICOS	Patient, Intervention, Comparison, Outcome, Setting
PREC	Precuneus
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-analyses;
PROSPERO	International Prospective Register of Systematic Revisions
PSDI	Positive Symptom Distress Index
PST	Positive Symptom Total
PSY	Psychoticism
PsychINFO	Database of abstracts of literature in the field of psychology, produced by the American Psychological Association
PTA	Pue Tone Average
PubMed	Database maintained by the United States National Library of Medicine at the National Institutes of Health.
PVCN	Postero-ventral Cochlear Nucleus
QoL	Quality of life
RAAS	Renin-angiotensin-aldosterone system
RE	Role-Emotional;
RI	Residual inhibition
ROS	Reactive oxygen species
RP	Role-Physical
rTMS	repetitive Transcranial Magnetic Stimulation
S	Specific (lemniscal) auditory thalamus
SC	Standard Care
SF	Social Functioning
SG/G/IG	Supragranular/granular/infragranular neuronal layers
SLC12A2	Solute carrier family 12, member 2
SLC6A4	Serotonin transporter gene polymorphism
SNHL	Sensorineural hearing loss
SNP	Single Nucleotide Polymorphism
SOC	Superior Olivary Complex
SOD	Superoxide Dismutase
SOM	Somatization
SOPs	Standard operation procedures

SPECT	Single-photon emission computed tomography
SPM	Statistical parametric mapping
SR	Systematic Review
SRT	Speech recognition threshold
ST	Somatosensory tinnitus/somatic tinnitus
STG	Superior temporal gyrus
STMQ	Self-efficacy for Tinnitus Management Questionnaire
SV	Sedimentation Velocity
TCDS	Transcranial direct stimulation
T-Cog	Tinnitus Cognitions Scale
TEOAE	Transient evoked optoacoustic emission
TEQ	Tinnitus effects Questionnaire
T-FAS FTQ	Tinnitus Fear-Avoidance Cognitions and Behaviors Scale
TFI	Tinnitus Functional Index
TGF-β	Transforming growth factor
Th1	Type 1- derived cytokine
THI	Tinnitus Handicap Inventory
THQ	Tinnitus Handicap Questionnaire
TINNET	TINnitus research NETwork
TMD	Temporomandibular joint disorders
TMJ	Temporomandibular joint
TMS	Transcranial magnetic stimulation
TMT	Tinnitus management therapy
TNF-α	Tumor necrosis factor alpha
TP	Tinnitus Patients
TPFQ	Tinnitus Primary Function Questionnaire
TQ	Tinnitus Questionnaire
TRI	Tinnitus Research Initiative
TRQ	Tinnitus Reaction Questionnaire
TRN	Thalamic reticular nucleus
TRT	Tinnitus retraining therapy
TSI	Tinnitus Severity Index
TSST	Trier Social Stress Test
UCL	Unconfortable listening level
VAS	Visual analog scale
VBM	Voxel-based morphometry
VIT	Vitally
vl/vmPFC	Ventrolateral/ventromedial prefrontal cortex
VNTR	Variable number tandem repeats
VS	Vestibular Schwannoma
vWF:Ag	von Willebrand factor antigen
WG	Working Groups
WHO	World Health Organization
WHOQOL-BREF	World Health Organisation Quality of Life (brief version)
Zn	Zinc

1. Introduction

Tinnitus derives from the latin word *tinnire* (to ring). Subjective tinnitus is a symptom involving the perception of a sound without an external source, which is only heard by the affected person. Since ancient times, we can find records reporting the condition and its treatment. For example, an Egyptian medical document originated in 2500 BC called the Ebers Papyrus refers to the 'bewitched ear' and recommends intra-aural infusions as a treatment (Heller, 2003; Sandlin & Olsson, 2000).

Tinnitus can be a symptom of various diseases, is described in a variety of ways (e.g., buzzing, ringing, roaring) and can be a single sound or combination of different sounds (Coles, Vernon, & Moller, 1995; Stouffer & Tyler, 1990). It can also be perceived in one ear, both ears, or in the head, as a constant sound or fluctuating in intensity (loudness) or pitch. The sound level can vary from barely noticeable to very disturbing, and this perception also varies among individuals and within an individual over time. Tinnitus most commonly occurs bilaterally (Andersson et al., 2005).

Due to our progressively aging population it is estimated that in 2050 there will be 2 billion people older than 65. Results from the most recent World Health Organization (WHO) Global Burden of Diseases (2015) reports hearing loss as the fourth leading cause of years lived with disability. Given the strong link between hearing loss and tinnitus, we can assume that tinnitus follows this growing trend. It is estimated that one in ten people has tinnitus, and so the global burden of tinnitus is very high (Bhatt, Lin & Bhattacharyya, 2016).

Tinnitus has a variety of etiological factors and may be associated with other diseases. It often accompanies hearing loss or hyperacusis, but neither is necessary for its presence (Eggermont, 2013, 2015; Eggermont & Roberts, 2004). There are two broad categories of tinnitus; objective and subjective. Tinnitus can be objective when it is audible by others, but these account for less than 1% of all cases. In the majority of cases tinnitus is subjective and only heard by the affected person. The prevalence of tinnitus in adult population is around 10% to 15% (Henry, Dennis & Schechter, 2005) and rises to 59 to 86% whenever there is associated hearing loss (Spoendlin, 1987). In 20% of people tinnitus has a significant impact on their quality of life, with repercussions for sleep, concentration, emotional stability, and social activities (Davis & Refaie, 2000).

The majority of tinnitus cases are associated to hearing loss (Roberts 2010), which is considered to be the major risk factor for the development of tinnitus (Chung, Gannom, &Mason, 1984; Sindhusake et al., 2003, 2004). Some previous studies with audiological markers, such as the high frequency thresholds, allow us to perceive differences when tinnitus is accompanied by hearing loss, and when it is not. Also,

tinnitus psychoacoustic assessment allows us to draw a different picture from individuals with hearing loss and individuals without hearing loss. For example, it was found that tinnitus pitch is higher among individuals without hearing loss, whereas the opposite is true for regarding to loudness (Prestes & Gil, 2009).

The heterogeneity of tinnitus causes a substantial problem in its classification, which has hampered both basic and clinical research. A major challenge for the field is to identify the underlying causes of tinnitus for developing specific treatments that address the distinct manifestations (Noreña, 2015). Although much research is underway, the precise pathophysiology of tinnitus remains unclear.

For this purpose, TINNET a pan-European multidisciplinary network (COST action) has been gathering efforts to standardize the methodology for assessment, diagnosis and treatment of tinnitus. This initiative comprised five working groups with different objectives in order to facilitate the standards for clinical assessment and outcome measurement, by large-scale multi centric data assessment and by data management in a quality-controlled database. Moreover, it has the main goal of better understanding the underlying tinnitus mechanisms in order to achieve better treatments.

Although there are multiple management options for tinnitus, the majority lack high quality scientific evidence to support claims of benefit. Perhaps of all therapeutic options, Cognitive Behavioral Therapy (CBT) delivered by a qualified clinical psychologist has the most support for its effectiveness in reducing tinnitus symptom severity (Cima et al., 2012; Hesser, Weise, Westin & Andersson, 2011; Martinez Devesa, Waddell, Perera & Theodoulou, 2007).

The present thesis aims to contribute with systematic reviews of the literature in order to increase knowledge regarding clinically relevant tinnitus subtyping, the tinnitus management standardization, the patient's perspective and the underlying pathophysiological tinnitus mechanisms, as well exploring the contribution of audiological, immunological and genetic factors to tinnitus etiology in an older population. This study has revealed new and extremely interesting results regarding tinnitus etiology and factors associated with more severe grades of tinnitus, also pointing us to future research studies.

2. State of art

2.1. Ear Anatomy

2.1.1. Summary of ear anatomy and physiology of hearing

The ear is a mechano-receptor organ that acts as a link between the outer environment and the nervous system, referring to complex auditory functions. Humans can hear frquencies from 20 to 20.000Hz. The ear is composed by three primary parts: the outer ear, middle ear and inner ear. Each section is comprised of structures that play distinct roles in the process of converting sound waves into electric signals that go into the brain. The external ear collects sound waves from the external environment and funnels them toward the tympanic membrane. The middle ear ossicles transmit the sound waves to the inner ear (Figure 2.1-1).

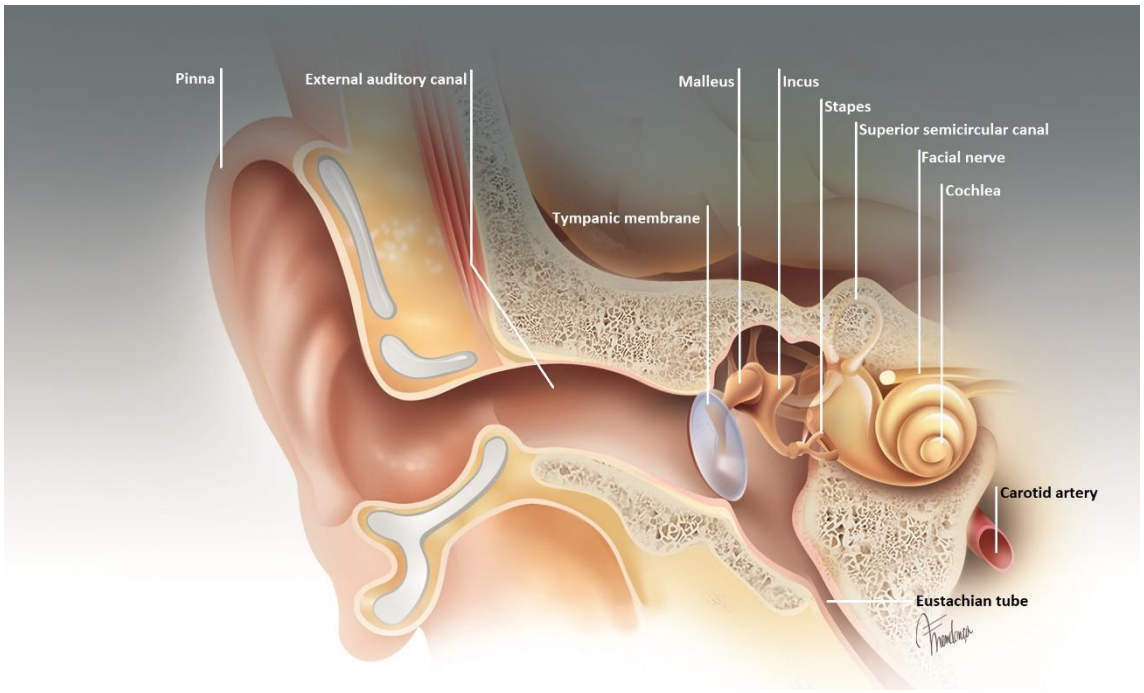

Figure 2.1-1 - Schematic view of the structures of the ear.
(Illustration provided by Fernando Vilhena de Mendonça, MD)

The **outer ear and middle ear** collect, amplify, and conduct sound waves to the inner ear, where the auditory receptors are to be stimulated.

The **outer ear** consists of the pinna and the external auditory canal and has the function of collecting and transmitting the sound to the tympanic membrane (Figure 2.1-2). It also protects from parasitic sounds.

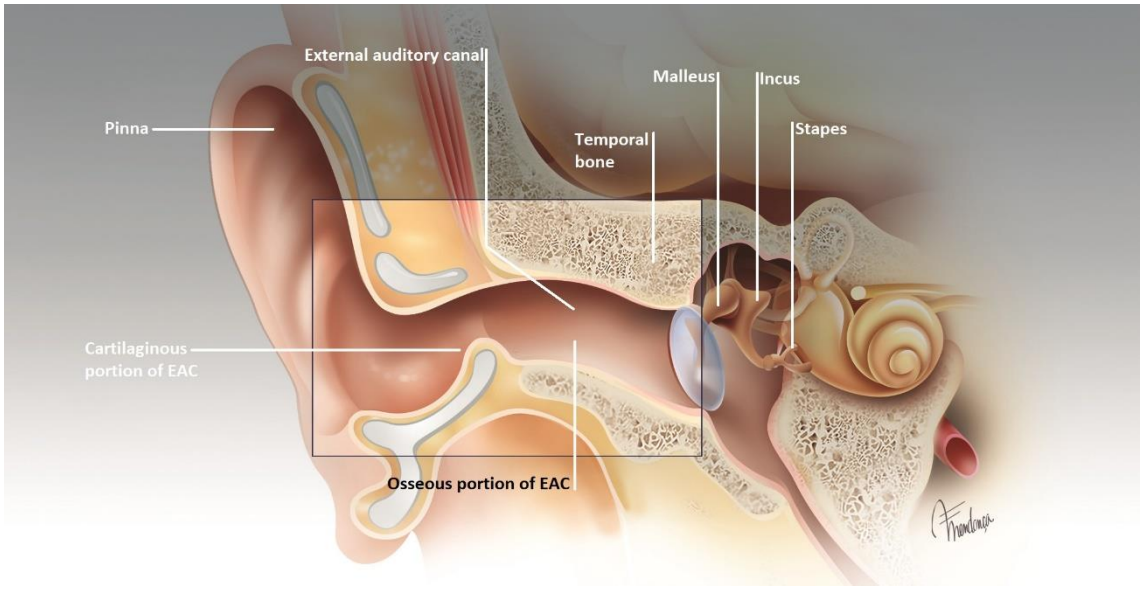

Figure 2.1-2 - Schematic view of the structure of the outer ear.
Legend: EAC, External Auditory Canal. (Illustration provided by Fernando Vilhena de Mendonça, MD)

The pinna, which part protrudes from the side of the skull, attached to the temporal bone, captures sound and channels to the ear canal. The pinna is shaped like a cone that allows amplifying the sound, differently depending on its origin (Figure 2.1-3) (Geisler, 1988).

The most amplified sounds come from about 45 ° (forwards and backwards) of the ear in the horizontal plane and about 60 ° above the plane of the ear. Less-amplified sounds originate on the opposite side of the head through the mascara effect (Rosowski, 2012). Anatomically the auricle or pinna is constructed as an organ for "catch" incoming sound waves and then funnel them down the external auditory canal. The main structures are the *tragus* and *anti-tragus*, *helix* and *anthelix* (Figure 2.1-3) (Weber, Deschler, & Sokol, 2006).

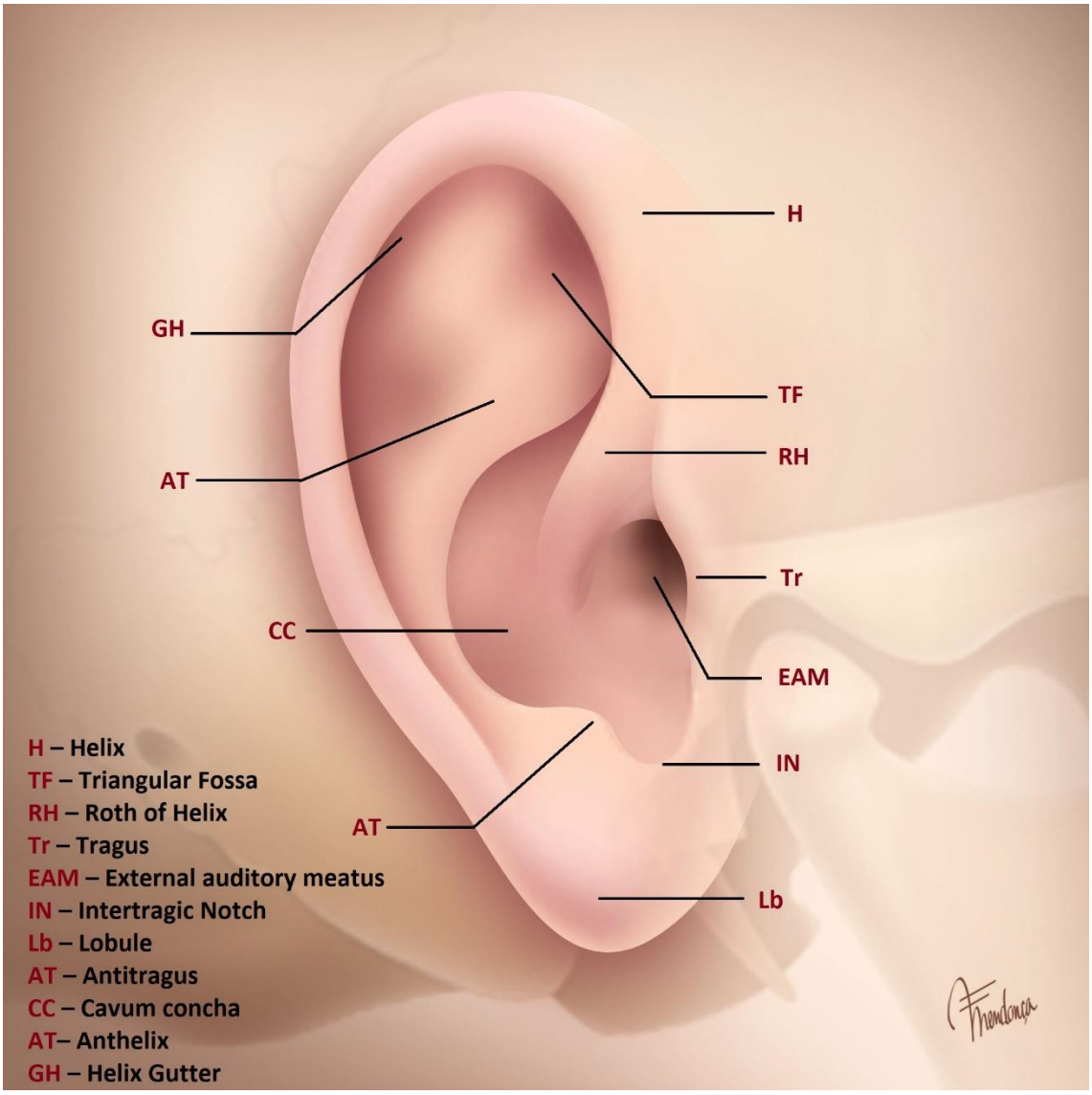

Figure 2.1-3 - Schematic view of the anatomy of the pinna.
(Illustration provided by Fernando Vilhena de Mendonça, MD)

The shape of outer the ear allows a gain of approximately 20dB for sounds from 2kHz to 4kHz. The tympanic membrane separates the outer and middle ear (Alberti, 2001) (Figure 2.1-4). It is a thin (0.1 mm), cone-shaped membrane that has three layers. The external layer derived from the ectoderm is a stratified *squamous epithelium*. The internal layer comes from endoderm and is a *cuboidal mucosal epithelium*. The intermediate layer comes from *mesenchyme and*, is the fibrous layer (Figure 2.1-5). It is divided into the *pars flaccida* and the *pars tensa*. The *pars tensa* has a central fibrous layer (lamina propria), while the *pars flaccida* is slightly thinner (without the intermediate layer). The three layers are important in maintaining the strength of the tympanic membrane as well as in aiding the proper vibration with different frequency sounds (Lalwani, 2007). The tympanic membrane acts as a mirror of the interior of the

middle ear, and knowledge of this structure is fundamental to understand the multiple dysfunctions that affect the middle ear (Paço, 2003). According to Paço (2003), the tympanic membrane can be divided topographically into six quadrants, of which four are referred to as the *pars tensa* (postero-superior, postero-inferior, antero-superior and antero-inferior) and two are referred to as the *pars flaccida* (Figure 2.1.4).

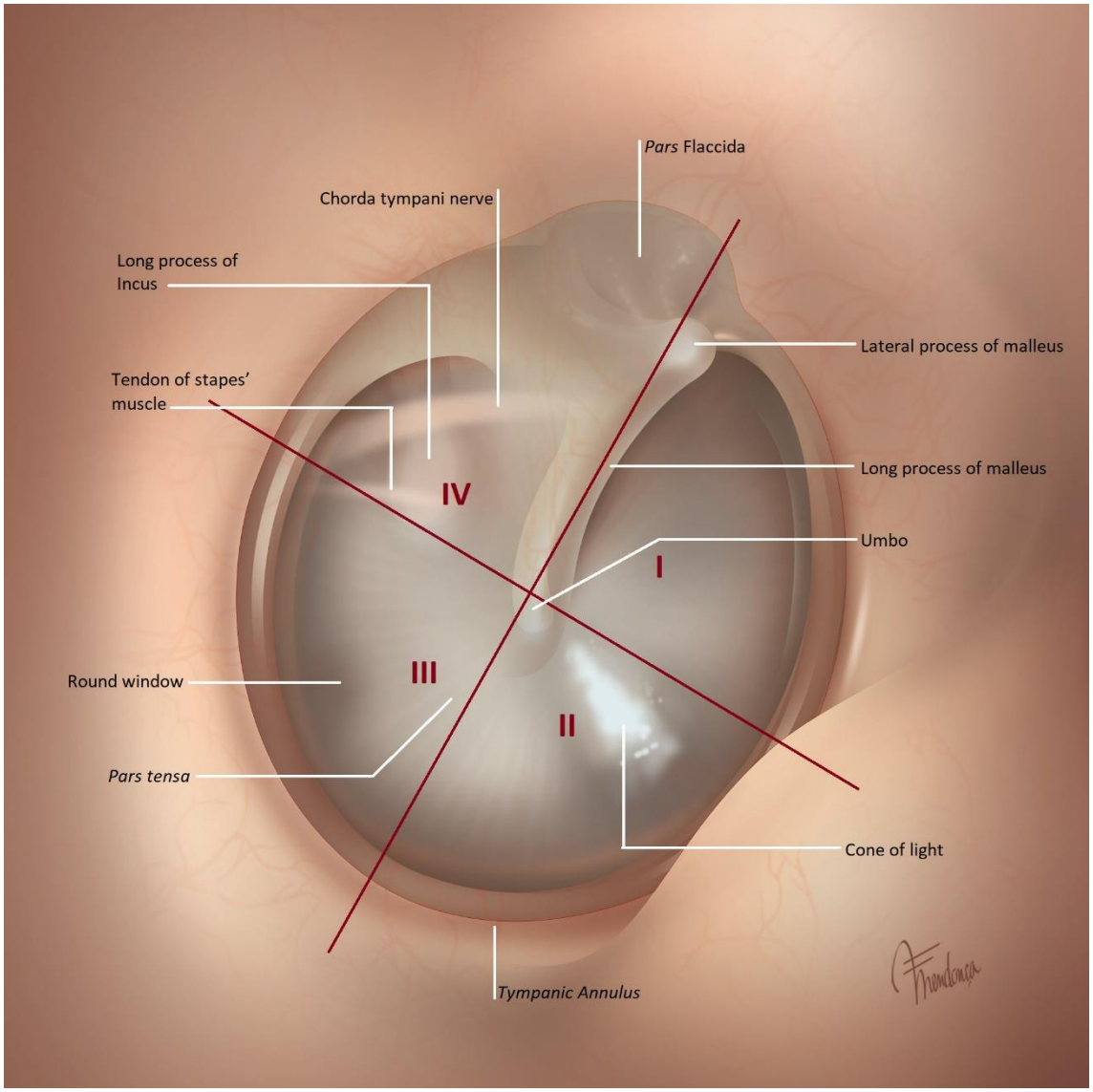

Figure 2.1-4 Anatomy of the tympanic membrane.
(Illustration provided by Fernando Vilhena de Mendonça, MD)

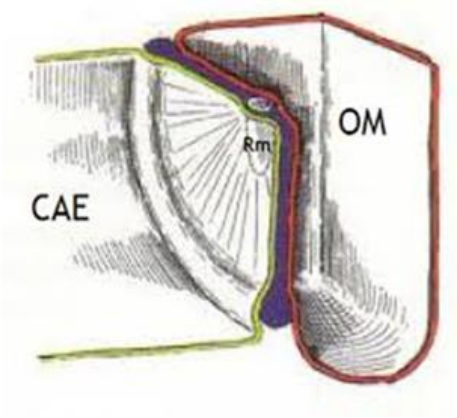

Figure 2.1-5 - The layers of tympanic membrane.
Legend: CAE external auditory canal, OM middle ear. (Lalwani, 2007)

The **middle ear** comprises the tympanic cavity, mastoid air cells, and Eustachian (auditory) tube. The air filled space, also known as the tympanic cavity, is slightly concave and suspended by a bony ring. Posteriorly this includes the mastoid cells that communicate to tympanic cavity through the *aditus ad antrum*. It is connected to the back of the nose by a 35-45 mm long thin tube called the auditory tube (necessary for equalization of pressure between exterior and middle ear and drainage of mucous produced in the middle ear) (Moller, 2006). The auditory tube has an osseous portion (posterior 1/3) that opens into the tympanic cavity and a fibro-cartilaginous portion that opens into the *nasopharynx*. There are three important muscles for its function - *tensor veli palatini*, *levator veli palatini* and *salpingopharyngeus* (Correa & Gómez, 2007).

Sound is conducted from the tympanic membrane to the inner ear by three bones that constitutes the ossicular chain – the *malleus*, *incus* and *stapes* (Figure 2.1-6). The ossicular chain is attached to one side of the tympanic membrane by the *malleus, which* inserts into the vestibular window of the inner ear by the footplate of the stapes. When a sound wave hits the tympanic membrane, it propagates through the ossicular chain and into the vestibular window of the inner ear. There are two muscles in the middle ear, namely the *stapedius* and *tensor tympani*, which insert into the stapes and malleus, respectively. The middle ear amplifies sound via two mechanisms: The proportion of tympanic membrane and stapes *platinum* surface is 14:1 and the lever effect (*malleus* and *incus* assured by the malleus and incus ligaments respectively anterior and posterior) is 18.3:1 which provides a gain of 20-35dB. The ossicular chain maximizes transference of sounds from 1-10kHz (Erminy, Skanavi, Van Den Abbeele, Avan, & Bonfils, 1995). The stapes arch reflex causes contraction of *stapedius* when the ear is exposed to sounds louder than 70dB, raising rigidity to ossicular chain and protecting the inner hear from noise induced damage.

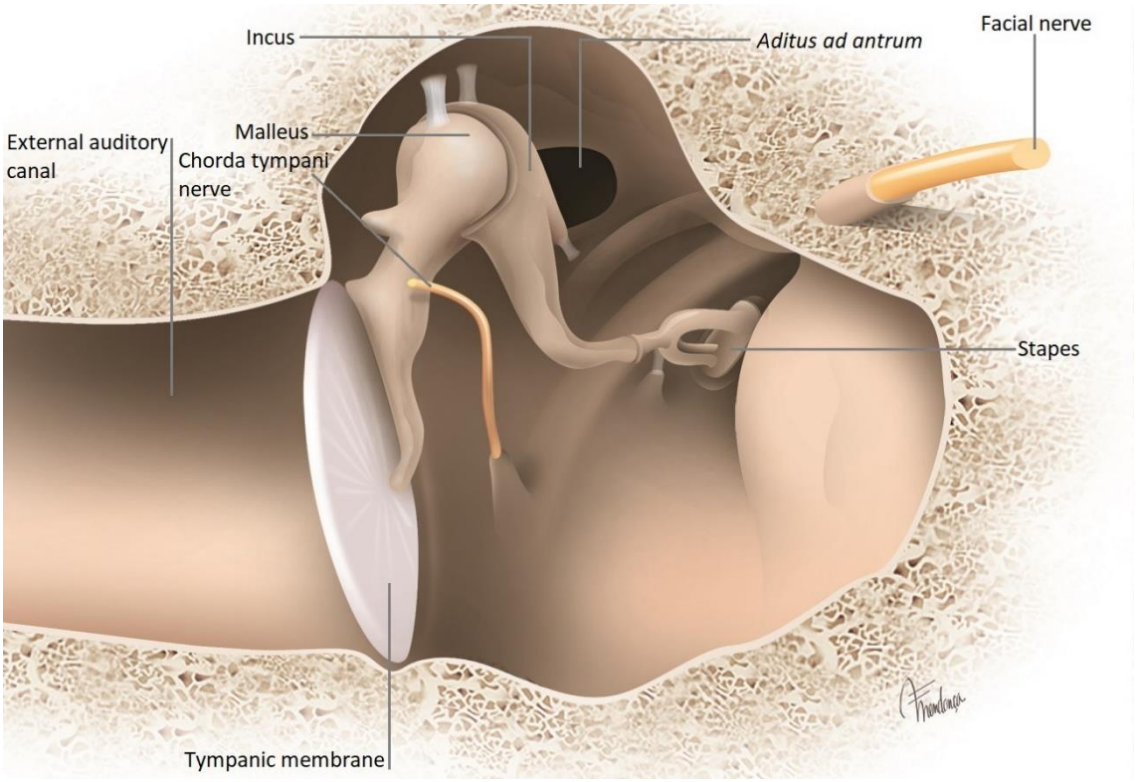

Figure 2.1-6 - Schematic view of the structures of the middle ear ossicles.
(Illustration provided by Fernando Vilhena de Mendonça, MD)

The **inner ear** contains a group of interconnected, fluid-filled chambers. The snail-shaped chamber, called the cochlea, is an approximately 30-mm-long coiled tube, of 2 and 3/4 turns along an osseous axis called *modiolus*, located within the petrous bone and divided by membranes into three chambers (Figure 2.1-7).

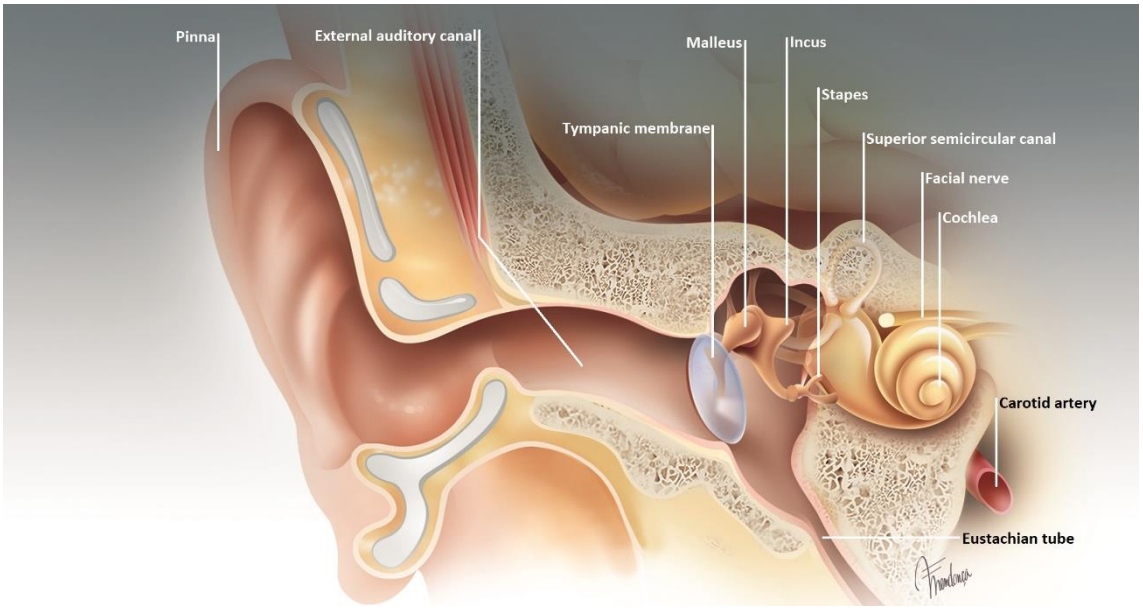

Figure 2.1-7 - Schematic view of the structures of Inner Ear.
(Illustration provided by Fernando Vilhena de Mendonça, MD)

The cochlea is one of the smallest organs of the human body (Figure 2.1-8). Its small size is important because if it was larger the necessary mechanical force to vibrate its structures would have to be higher and humans would only be able to hear very loud sounds. The cochlea acts as active and passive sound filter.

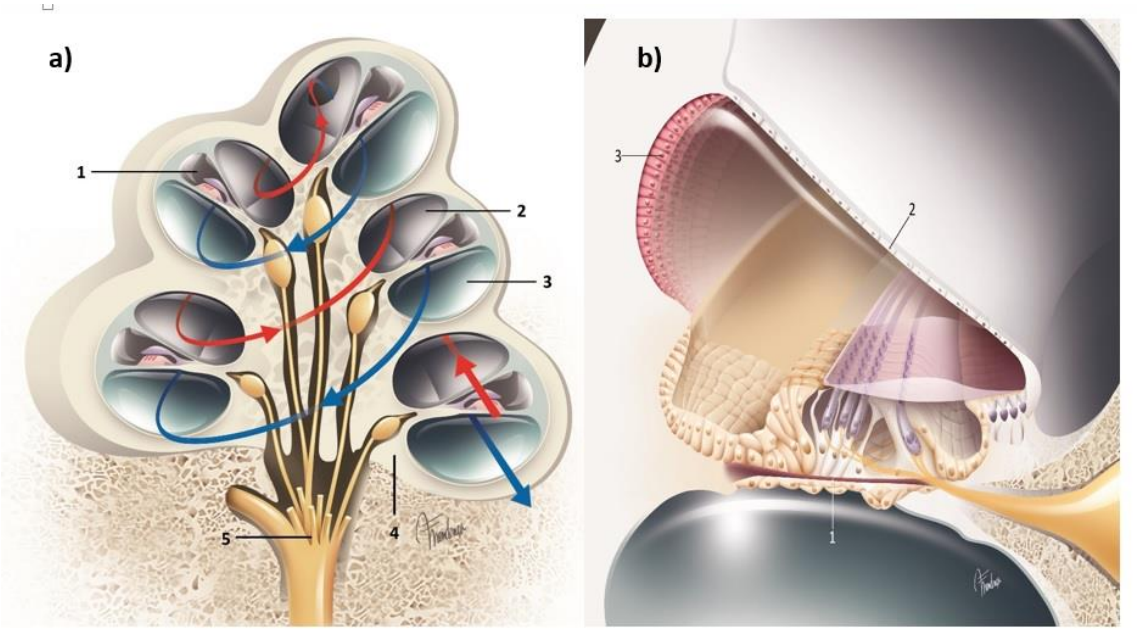

Figure 2.1-8 - Cochlea. a) Structures of cochlear channel; b) Cross-section of a single cochlear turn.
Legend: a) *1 – the scala media; 2 – the scala vestibuli; 3 – the scala tympani; 4 – the spiral ganglion neurons; 5 – Auditory nerve VIII; b) 1 – Reticular Lamina; 2 –scala vestibule ; 3 – Lateral wall (stria vascularis).* (Illustration provided by Fernando Vilhena de Mendonça, MD).

The three fluid-filled chambers of the innear ear are: the *scala vestibuli*, the *scala tympani*, and the *scala media* (Figure 2.1-8). The *scala media* or cochlear, located in the center, is separated from the *scala vestibuli* (superiorly) by vestibular membrane and from the *scala tympani* (inferiorly) by the basilar membrane (Moller, 2006). Sound vibrations from the bones of the middle ear are transferred to the fluids of the cochlea through the vestibular window to the *scala vestibuli*. Near the apical termination of the bony labyrinth there is an opening called the helicotrema. This allows communication between the *scala vestibuli* and *scala tympani* that are filled by a fluid rich in Sodium (Na^+) called *perilymph*, which is similar to cephaloraquidian fluid. The *Scala tympani* ends at the round (tympanic) window, and serves as an escapement of the sound wave. The *scala vestibuli* receives the sound wave from the vestibular window (connection to ossicular chain). The *scala media* narrows towards the apex of the cochlea ending just short of the apical termination of the bony labyrinth. It is filled with a fluid rich in Potassium (K^+) called endolymph, similar to intracellular fluid, possesses a potential of +80mV. The basilar membrane has the ability to separated sounds according to their frequency (tonotopy spectrum). It becomes larger and less tense near the apex and so,

more sensitive to higher frequencies at the basal area where the cochlea has lower mass and higher rigidity. It has higher mass and lower rigidity at the apex where it is more sensitive to lower frequencies- This process is called passive tonotopy (Figure 2.1-9). The sound vibration propagates through *cochlear scala* and is maximal in the area corresponding to the sound frequency.

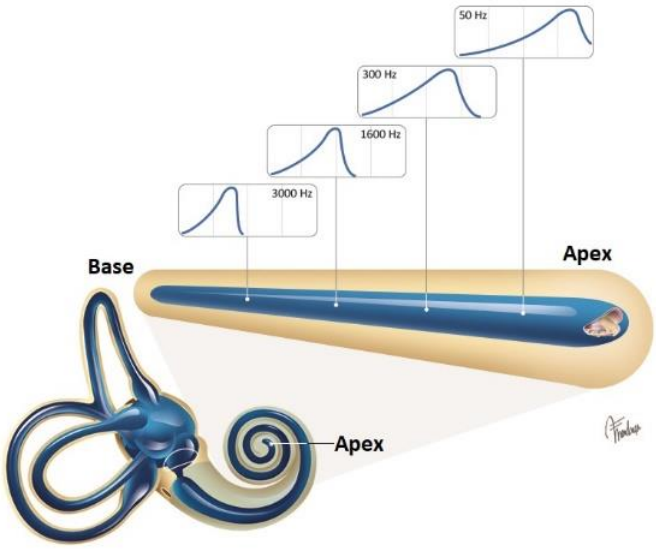

Figure 2.1-9 - Travelling wave.
(Illustration provided by Fernando Vilhena de Mendonça, MD).

The spiral organ (of Corti), located along the basilar membrane, contains the sensory cells (hair cells) (Moller, 2006) (Figure 2.1-10). These tiny sensors (hair cells) are mechanoreceptors that convert the sound vibrations of the basilar membrane into electrical impulses that are transmitted along the auditory nerve to the brain through the auditory pathway – this mechanism is called transduction. The Inner Hair Cells (IHC) are the principal auditory receptors. They constitute the most internal row along the spiral organ (of Corti) (approximately 3500 cells). Their function is to transform the mechanical sound stimulus transmitted from the outer and middle ear into and electrical message to be send to auditory nervous centers (in temporal lobe) through the auditory pathway (Abbas & Miller, 1993).

IHCs are sensory cells that transform the hydromechanical, vibratory energy of cochlear liquids into bioelectric energy. About 80% of the time the transducer channels are closed and only open in the excitatory phase of the stimulus. The process of transduction begins when the energy is sufficiently intense, and the stimulation leads to the depolarization of IHCs, which leads to the release of neurotransmitters, causing one or more afferent fibers of the auditory nerve to fire (Monteiro & Trigueiros, 2018).

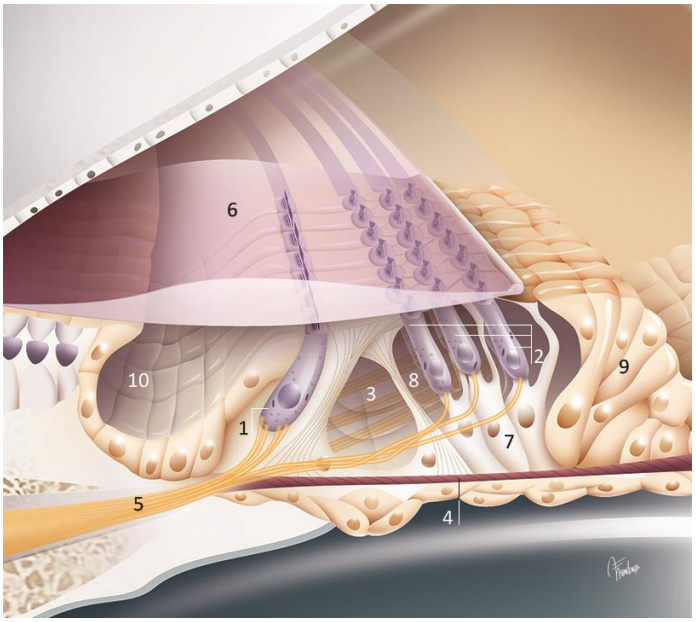

Figure 2.1-10 - Schematic of the organ of Corti.
Legend: 1-Inner hair cell (IHC); 2-Outer hair cells (OHC); 3-Tunnel of Corti; 4-Basilar membrane; 5-Habenula perforate; 6-Tectorial membrane; 7-Deiters' cells; 8-Nuel's space; 9-Hensen's cells; 10-Inner spiral sulcus. (Illustration provided by Fernando Vilhena de Mendonça, MD).

The three outer rows along the spiral organ (of Corti) corresponds to the Outer Hair Cells (OHC), nearly 12000 cells (Figure 2.1-10). Surrounded by supporting cells – Hensen and Claudius cells also have a role in the cycle of K^+ and Glutamate (Monteiro & Trigueiros, 2018).

OHCs are pressurized and have a central core without a cytoskeleton which gives them strength, flexibility and electromotility according to the stimulus, providing a refinement in frequential sensitivity and selectivity. Hence OHC are called the cochlea amplifiers. OHCs length can vary from 12µm in the basal region to more than 90µm in the apex of the cochlea (Brownell, Spector, Raphael, & Popel, 2001).

Because only the apical part of ciliated cells are in contact with endolymph cochlea found a process to transport K^+ from or to cells without ATP consumption, this electrochemical gradient is achieved through the *stria vascularis*, located at external wall of *scala media* and lying in the basilar membrane. It is a highly vascularized and metabolically active organ (Figure 2.1-8b).

Within the cochlea, vibratory energy results in an interaction called the traveling wave, which is a wave of fluid vibration along the basilar membrane along the cochlear coil. Wave energy is placed at a particular site of the basilar membrane, depending on the frequency of vibration. Through the depolarization of cells, the action of mechanical vibratory energy is used more effectively in the transduction of bioelectric energy. This gradient acts like a battery whose energy permits hearing. Ciliated cells have synapses

at their basal pole. The delivery of neurotransmitters to the synaptic cleft (transforming electrical energy in chemical) is regulated by alterations in cellular membrane Differentiated vibration of the basilar membrane and tectorial membrane causes stereocilia flection. The OHCs respond to stimulation when their stereocilia are flexed in an external direction. When the stereocilia are flexed in the excitatory direction (towards the top), the links between tops are stretched, which increases the likelihood of calcium channel opening. Calcium plays a determinant role in the intracellular balance, being that it maintains cellular homeostasis through the mechanotransducing channels (Figure 2.1-11). The energy of acoustic stimulus induces the movement of the basilar membrane synchronized with the deflection of the stereocilia that triggers transducer currents. This allows the entrance of K+ into the OHCs and leads to their depolarization. The resulting action potentials produce a motor response caused by the properties of the *prestin* protein. This vibratory energy is returned to the basilar membrane (Monteiro & Trigueiros, 2018).

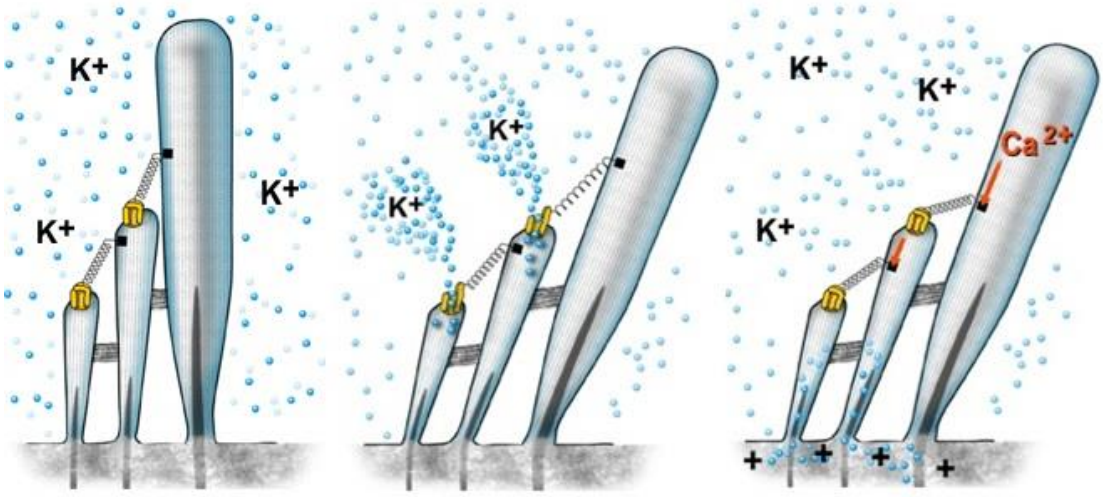

Figure 2.1-9 - Transduction channels: opening and adaptation to potassium levels. (http://www.cochlea.eu/po/)

The middle portion of OHCs is where electrical energy is converted into mechanical – electromotility. If electrical energy resulting from sound vibration coincides with the natural frequency of that cochlear portion then the magnitude of vibration augments. Otherwise it decreases. Accordingly, there is a release of neurotransmissor in the inferior pole of the cell.

The OHCs produce a sound that can be detected through a microphone in the outer ear – Otoacoustic emissions (OAE). This is the demonstration of the active cochlea mechanisms that are exclusive of OHCs. No other cells in the human body have this electromotility, a biological form of piezoelectricity.

Hearing occurs by air conduction or bone conduction. In air conduction sound reaches the inner ear by propagating in the air reaching tympanic membrane through the external auditory channel. Movement of the tympanic membrane is transmitted to the ossicular chain that propagates to the cochlea through the oval window connected to stapes footplate. The structures of middle ear serves as an impedance-matching mechanism, improving the efficiency of energy transfer from the air to the fluid-filled inner ear. Hearing by bone conduction happens when the sound source is in physical contact with the head, causing vibration of the bones of the skull that generate a travel wave in the cochlea's basilar membrane (Brownell et al., 2001; Dallos & Fakler, 2002).

The nervous fibers that come from IHCs converge to form the spiral ganglion. From here originates the cochlear part of VIII cranial nerve, the anterior portion of which goes to the internal auditory channel (IAC).

2.1.2. Central Auditory Nervous System (CANS)

The cochlear nerve contains a total of 30,000 afferent nerve fibers (Spoendlin, 1987). The cell bodies of these fibers are found in the spiral ganglion, the cochlear part (auditory) of VIII cranial nerve. The anterior portion goes to the IAC and enters the brain at the spinal bulb (Bonaldi, Lago, Crema, Fukuda, & Smith, 2004; Ruah, 2002)

At low frequencies individual auditory nerve fibers can respond more or less synchronously with the stimulating tone. At higher frequencies phase-locking occurs so that neurons can alternate in response to particular phases of the sound wave cycle. Three aspects encode the intensity of the sound wave cycle: 1) the amount of neural activity in individual neurons, 2) the number of neurons that are active, and 3) the specific neurons that are activated.

The central auditory pathways are composed of various structures, beginning in the auditory nerve, followed by the cochlear nuclei, the superior olivary complex, the lateral lemniscus, the lower colliculus, the middle geniculate body, terminating in the auditory cortex (Figure 2.1-12 and 2.1-13). The auditory nerve divides in two pathways. The ascending/afferent auditory pathway (afferent fibers (myelinated bipolar type I neurons) carries sound information to the brain mostly come from IHCs, and the descending/efferent auditory pathway (efferent fibers, non-myelinated bipolar type II neurons) carries information back from the brain to the OHCs (Malmierca & Hackett, 2010).

- Ascending or afferent auditory pathway, is a fast pathway, which begins in the cochlear nucleus – **first integrator centre**, are composed by three subnucleus: Dorsal Cochlear Nucleus (DCN), the postero-ventral Cochlear Nucleus (PVCN) and the antero-ventral Cochlear Nucleus (AVCN) (Malmierca & Hackett, 2010; Marinho, 2011; Moller, 2006; Phillips, 2007), where the central auditory system

begins its course to the auditory cortex (Aquino, Chandra, Haines, & Micco, 2002; Ruah, 2002) located at spinal bulb, the fibers keep the cochlear frequencial selectivity. From here some projections go to ascendant reticular pathway – **non-primary auditory pathway**, the main function of these pathways, also connected to wake and motivation centers as well as to vegetative and hormonal systems, is to select the type of sensory message to be treated first. This pathway is inactive during sleep (Cochlea, 2013).

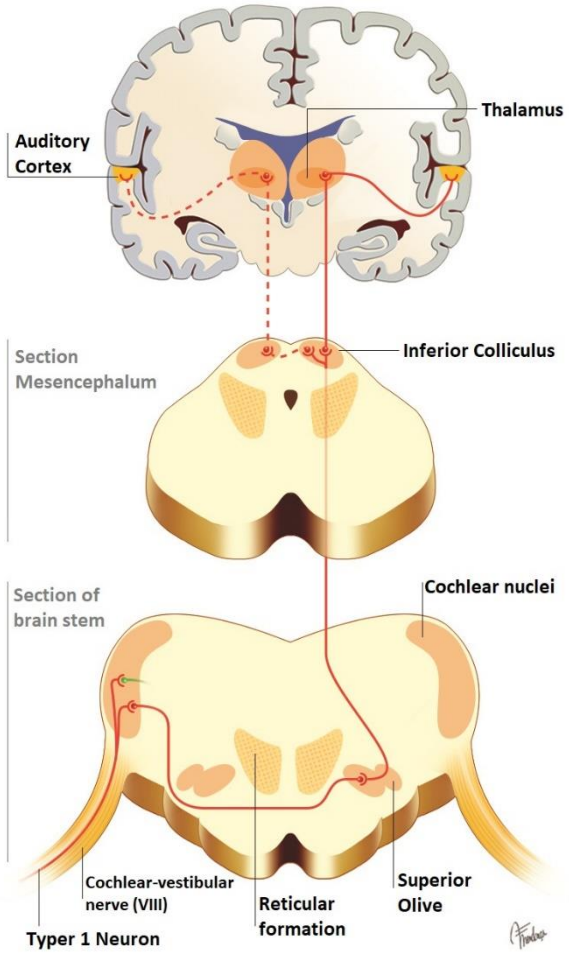

Figure 2.1-10 - Primary auditory pathways.
(Illustration provided by Fernando Vilhena de Mendonça, MD).

- **Second integrator centre** is Superior Olivary Complex (SOC) also located at spinal bulb, majority of fibers cross to the opposite side, SOC is divided in three nucleuses: the Complex Superior Olivary lateral (CSOL), the Complex Superior Olivary medium (CSOM) and the medial nucleus of do trapezoid body (Martínez & Nieto, 2003; Musiek & Baran, 1986; Neijenhuis, 2003).
- The **third integrator centre** is Inferior Colliculus, located at midbrain. Together with SOC have important role in the localization of sound and integration of sounds with a complex temporal pattern, also receives fibers from the Cochlear

Nucleus.

- The **fourth integrator centre** before cortex is medial geniculated body located in thalamus, here starts the integration of information. Can be subdivided in three regions: the ventral projects to the primary auditory region of temporal lobe, the middle projects to the other temporal lobe regions and the dorsal projects afferent to cerebellar associative areas (Seikel, King, & Drumright, 2009).

Primary auditory cortex is located in the temporal lobe, hidden by the lateral sulcus incisures. Auditory message arrives here largely decoded by the previous nucleus, is memorized, and possibly integrated in a motor response like vocalization. The primary auditory cortex has an important role in phoneme discrimination, and is also involved in temporal and spectral discrimination (Bellis, 2003). Posteriorly, angular girus, represents the Wernicke region, responsible for linguistic stimuli recognition and speech understanding (Specht, 2014).

The descending or efferent auditory pathway extends from the auditory cortex to the hair cells. The majority of the bodies of the efferent fibers are located in the SOC of the brainstem.

The anatomical description and role of this pathway is still a matter of debate, however it is agreed that it begins at the auditory cortex and associative areas. It is divided in two segments. The **rostral segment**, involves auditory cortex, associative secondary areas, medial geniculated body, inferior colliculus and the lateral lemniscus. It is possible that this segment has a regulator role of afferent pathway, namely cochlea, the auditory nerve, and the inferior nucleus of the brainstem (Baran, Brooke Shinn, & Musiek, 2006). The **caudal segment** comprises the SOC (lateral and middle), cochlear nucleus, and ends at the cochlea (IHCs and mainly at OHCs). This segment is also called medial olivo-cochlear bundle. In the efferent pathway the main neurotransmitter is acetylcholine but dopamine, dynorphins and encephalin are also present. It is considered that the physic-acoustic model closest to reality should be based on oscillators tuned in frequencies (OHCs) that would be regulated through the medial olivo-cochlear bundle.

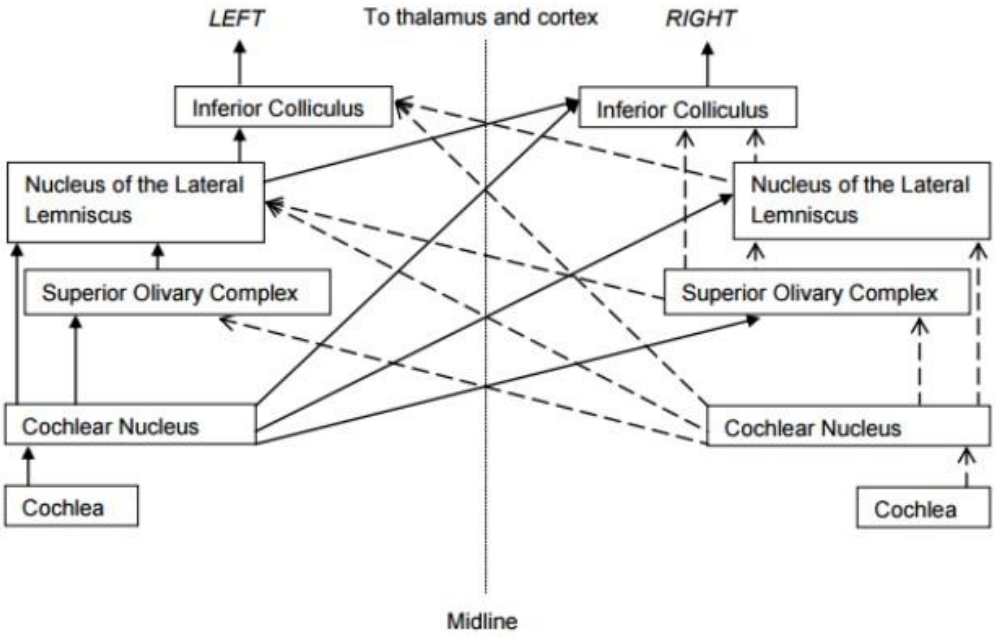

Figure 2.1-11 - Main nuclei involved in binaural hearing.
(Porter, 2012)

2.1.3. The close correlation between hearing and cognition

As far as hearing is concerned we do not hear using a single part of our brain. Instead hearing stimuli 'switch on' several brain areas. Even a simple word has the capacity to activate not only the auditory cortex, but also several other areas where it is 'understood' or semantically or cognitively connected. Evidence demonstrates a very close association between hearing and cognition. Recent studies on people aged 50 to 79 included tests to evaluate hearing capacity, central auditory processing and cognitive skills. The most predictive factor of speech discrimination in noisy environment was central processing of sound, followed by cognitive skills (such as working memory and short-term memory), and by life experience such as socio-economic status. Hearing sensitivity evaluated by tonal audiometry was the 'weakest' contributor to performance of this task (Figure 2.1-14).

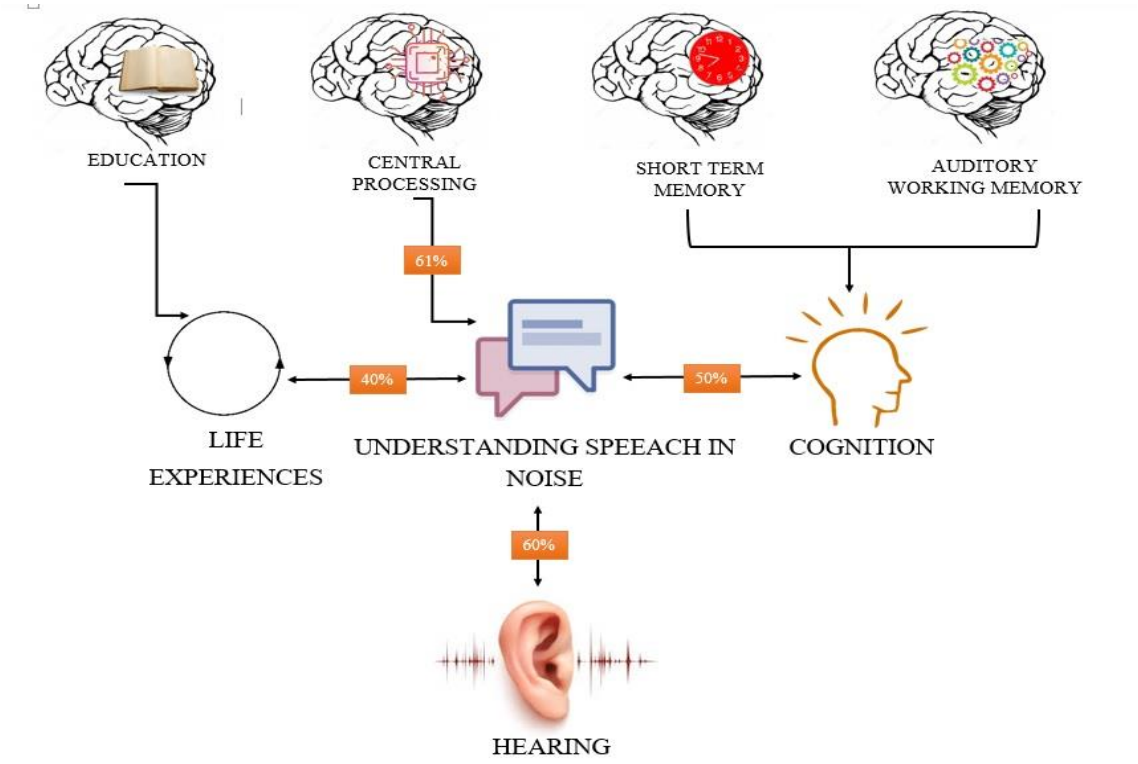

Figure 2.1-12 - Relationship between cognition, life experience and economic state with hearing. (Anderson et al., 2013)

An auditory stimulus activates the entire cerebral cortex and the cognitive processes influence 'how' we hear (Anderson, White-Schwoch, Parbery-Clark, & Kraus, 2013). In a complex situation for hearing, the central processing of sound information is the most predictive factor for a better speech comprehension, followed by cognitive skills – short-term memory and auditory memory – and by life experience such as education (Figure 2.1-14). This demonstrates a complex interaction of factors influencing hearing in noise and the importance of cognitive skills, lifestyle and central auditory processing in the diagnosis and treatment of patients with difficulties hearing in noise (Anderson et al., 2013).

Studies using magnetic resonance imaging showed that hearing loss is correlated with reduced volume of the primary auditory cortex (Chang et al., 2004; Eckert et al., 2013). Furthermore, a longitudinal study revealed that hearing deficiency is accompanied by overall cerebral atrophy most evident in the superior, median and inferior gyri critical areas for auditory and cognitive processing (Lin et al., 2014). Along with diminished volume there is also a reduced neuronal activity in those areas and other sub-cortical regions. Moreover, there is also evidence that the brain tries to compensate by activating collateral circuits, requiring greater expenditure of mental resources (Peelle, Troiani, Grossman, & Wingfield, 2011). The deviation of attention-

related resources to listening tasks explains the residual attention for the remaining cognitive activities. For example, people with hearing impairment that prevents them from having a conversation, present a 24% higher probability of declined cognitive skills such as concentration, memory and planning capacity (Lin et al., 2013).

At the same time the brain will change and experience distress. One of the reasons for these alterations is social isolation that is associated with diminished psychological well-being and impaired self-esteem that results in a poorer lifestyle and a diminished quality life. This takes a bi-directional vicious circle, in which on the one hand, hearing loss involves structural and functional changes to the brain and on the other hand cognition declines correlated with ageing facilitates the onset of hearing loss. There are common mechanisms, e. g. atherosclerosis, that contribute both for hearing loss and cognitive decline (Figure 2.1-15).

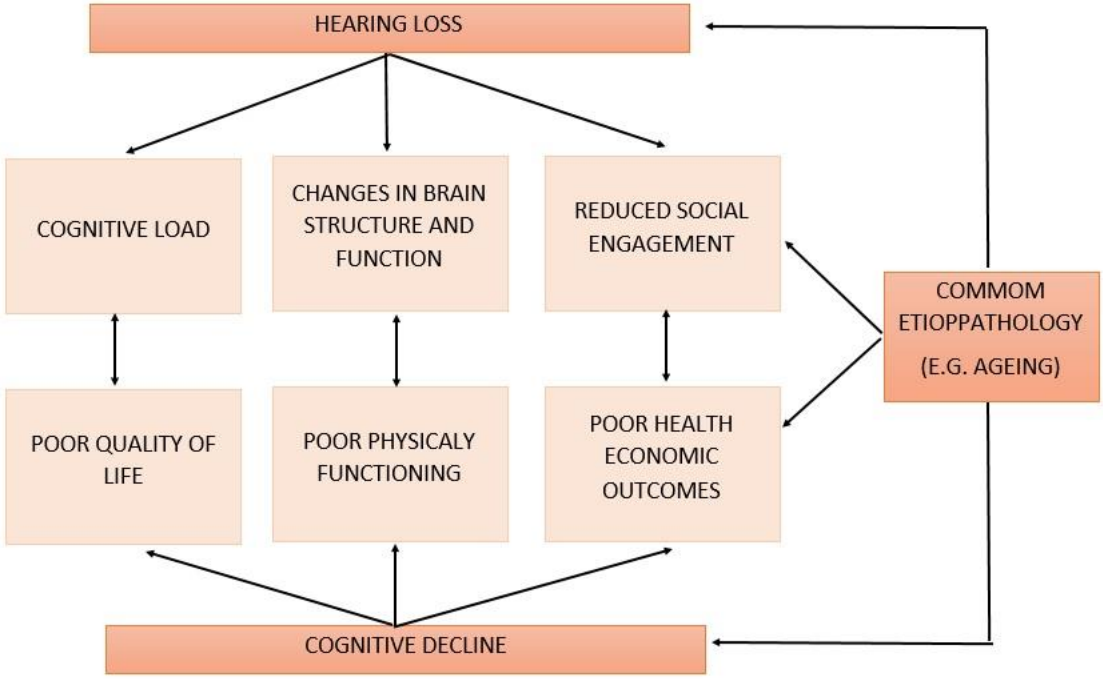

Figure 2.1-13 - Vicious circle between hearing loss and cognitive decline.
(Lin et al., 2013)

2.1.4. Hearing Loss

Hearing loss is extremely common and the degrees of hearing loss can vary from a nearly undetectable degree of disability to a profound loss of ability to function in society. Although it is a prevalent condition, it is most evident in older adults, many of whom require hearing rehabilitation (e.g. hearing aids, bone integrated hearing aids, middle ear hearing implantable devices or cochlear implants). Hearing loss can result from disorders of the auricle, external auditory canal, middle ear, inner ear, or central

auditory pathways. In general, lesions in the auricle, external auditory canal, or middle ear cause conductive hearing loss.

In mammals, ciliated cells do not regenerate if are destroyed after birth (Ruben, 1967). Therefore, the loss of ciliated cells results in irreversible hearing loss (Abbas & Miller, 1993; Riva, Donadieu, Magnan, & Laville, 2007).

In terms of audiometry findings, there is a general guideline for interpreting degrees of hearing loss. Levels should be categorized somewhat more stringently for children (Table 2.1-1).

Table 2.1-1 - Guidelines for interpreting hearing loss according to Bureau International d'Audiophonologie (BIAP).

Hearing Threshold	Interpretation
The average tone loss is below 20 dB.	Normal or subnormal hearing. Mild tone disorder with no social consequences.
Average tone loss between 21 and 40 dB.	Mild hearing loss. Speech is perceived if the voice is normal, difficulties arise if the voice is low-pitched or distant from the subject. Most of the daily life noises are perceived.
- 1st degree: average tone loss between 41 and 55 dB. - 2nd degree: average tone loss between 56 and 70 dB.	Moderate hearing loss. Speech is perceived if the voice is loud. The subject understands better what is being said if he can see his/her interlocutor. Some daily life noises are still perceived.
- 1st degree: average tone loss between 71 and 80 dB. - 2nd degree: average tone loss between 81 and 90 dB.	Severe hearing loss. Speech is perceived if the voice is loud and close to the ear. Loud noises are perceived.
- 1st degree: average tone loss between 91 and 100 dB. - 2nd degree: average tone loss between 101 and 110 dB. - 3rd degree: average tone loss between 111 and 119 dB.	Very severe hearing loss. Speech is not perceived. Only very loud noises are perceived.

2.1.4.1. Type and classification of Hearing Loss

There are three types of hearing loss according to the tonal audiometry pattern: Conductive hearing loss, Sensorineural hearing loss (SNHL), and mixed hearing loss due to a combination of conductive and SNHL (Figure 2.1-16).

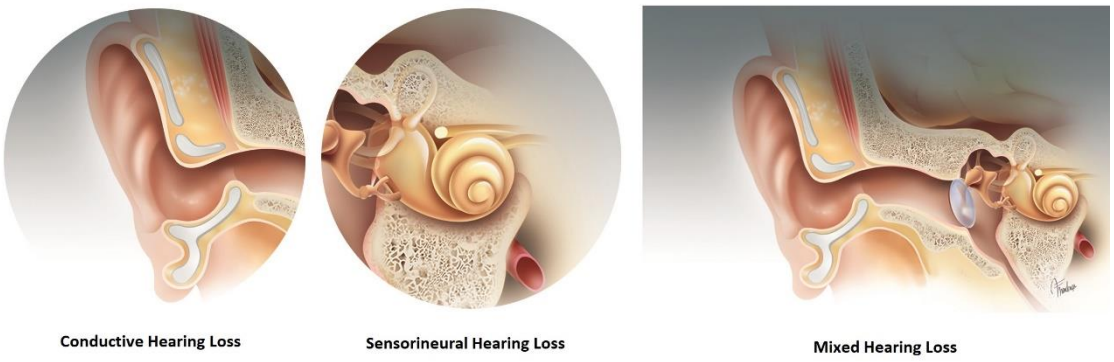

Figure 2.1-14 - The three different types of hearing loss.
(Illustration provided by Fernando Vilhena de Mendonça, MD).

Conductive hearing loss is usually due to a disease or condition that occurs in the ear canal or the middle ear. The signal transmission from the outer ear/middle ear to the inner ear decreases independently of the sound pressure level of the stimulus. Conductive hearing loss is diagnosed by audiometry, when there is an air-bone gap above 10 dB, indicating that the transmission of sound between the outer ear/middle ear to inner ear does not function optimally over the entire frequency range (Gelfand, 2009). This may be due to problems such as outer ear obstruction due to wax, or outer or middle ear infection.

Mixed hearing loss invovles air-bone gaps with the bone conduction thresholds outside of the normal range. For example, impairment of ossicular chain transmission such as in otosclerosis, usually begins with stapedo-vestibular fixation causing a conductive hearing loss, with a later progression of disease and cochlear involvement leading to a mixed or sensorineural hearing loss. Other causes are transverse or longitudinal temporal bone fractures, head trauma, chronic otitis media, and middle ear tumors. Some inner ear malformations such as large vestibular aqueduct, lateral semicircular canal dysplasia and a bulbous lateral end of internal auditory canal can also be the cause of mixed hearing loss.

SNHL appears as an increase in both bone and airway conduction threshold, determined by an audiometry reading that indicates no gap between the air and bone thresholds, i.e., the air-bone conduction is equal to the bone conduction. This result means that the signal transmission from the outer ear/middle ear to the inner ear functions well, but some other obstacle prevents the sounds from being perceived by the brain. Usually the lesions involve the cochlea and/or auditory nerve and may affect sensory receptor (hair) cells, auditory neurons, and/or any of the many structures and processes that enable them to be activated and to function properly. The resulting impairment of auditory functioning is called a SNHL (Gelfand, 2009). After any

aggression first damaged cells of the cochlea are supporting (Deiters) cells, next are OHCs to be damaged, and posteriorly the other cochlear structures start degenerating: IHCs and cells of auditory nerve (Ling et al., 2005). Once hair cells die they do not regenerate resulting in irreversible hearing loss. Damage of OHCs blocks the amplifier mechanism but the passive tonotopic mechanism persists. Damage of the IHCs reduces global cochlear function because they are responsible for the detection of acoustic signal and transmission to the brain through VIII cranial nerve.

Diseases or damage to hair cells cause a reduction in the sensory function. SNHL is the most common type of hearing impairment, and can sometimes be due to damage to the central pathways, termed central hearing loss.

Tinnitus patients frequently have hearing loss but not every patient with hearing loss has tinnitus, furthermore some tinnitus patients do not have hearing loss.

2.1.4.2. More frequent causes of SNHL

Broadly speaking there are two categories of SNHL, genetics and non-genetic (Table 2.1-2). Among non-genetic causes are the damage to the hair cells caused by intense noise, viral infections, fractures of the temporal bone, meningitis, and ageing. Also, certain types of medication – such as ototoxic drugs (eg, salicylates, quinine, and the synthetic analogs of quinine), aminoglycoside antibiotics, loop diuretics (eg, furosemide and ethacrynic acid), and cancer chemotherapeutic agents (eg, cisplatin) – can produce SMHL. Medical advances in vaccines and antibiotic therapy led to a significantly decline in the infectious and teratogenic intrauterine causes. Nowadays genetic causes are responsible for more than half of childhood hearing impairments. Nearly one-third are syndromic (hearing loss is associated with anomalies in other organ systems) and two-thirds are non-syndromic (when hearing loss is the only clinical abnormality). Between 70 to 80% of non-syndromic NSHL is inherited in an autosomal recessive manner, the remaining 15-20% is autosomal dominant. Less than 5% is X-linked or maternally inherited via the mitochondria. Much progress has been made in the identification of responsible genes. In general, hearing loss associated with dominant genes has its onset in adolescence or adulthood and varies in severity, while the hearing loss associated with recessive inheritance is congenital and profound and almost always related to cochlear defects (Petit, 1996).

Noise-induced hearing loss (NIHL) is currently a growing problem worldwide. Intense noise exposure (due to military service, some professions or leisure activities) sometimes leads to a temporary threshold shift in the cochlea but can also lead to permanent hearing loss that may be accompanied by other auditory disorders, such as tinnitus and hyperacusis (Axelsson & Sandh, 1985). Exposure to sounds greater than 85 dB for prolonged periods of time can result in high-frequency, symmetric, hearing loss

that is typically maximal at 4kHz. Continued acoustic trauma may lead to irreversible hearing loss including at lower frequencies. Noise exposure can cause changes throughout the entire auditory pathway and an imbalance of the excitatory and inhibitory transmitter systems (Dong et al., 2009; Milbrandt et al., 2000). Hyperacusis is a generalized reduced sound tolerance. People with hyperacusis report an unusual intolerance to ordinary environment sounds that are not bothersome to other people (Baguely, 2003; Vernon, 1987).

Table 2.1-2 - Etiology of SNHL.

Category	Example
Development and hereditary	
Syndromic	Alport syndrome, Usher syndrome
Non-syndromic	Larger vestibular aqueduct syndrome
Infectious	Otitis media, viral, syphilis
Pharmacologic toxicity	Aminoglycosides, loop diuretics, anti-neoplastics
Trauma	Head injury, noise-induced, barotrauma
Neurologic disorders	Multiple sclerosis
Vascular and hematologic disorders	Migraine, cryoglobulinemia, sickle cell
Immune disorders	Polyarteritis nodosa, HIV, Coghan sind
Bone disorders	Paget's disease
Neoplasms	Vestibular schwannoma
Unknown etiology	Presbycusis, Ménière's disease

Hearing loss induced by ototoxic drugs can be temporary or permanent. Drugs such as aminoglycosides (amikacin, streptomycin, gentamicin, kanamycin, neomycin, tobramycin), macrolides (azithromycin, clarithromycin, erythromycin), chloramphenicol, tetracyclines (minocycline), metronidazole, vancomycin, tuberculostatics (capreomycin), anti-phungals (amphotericin B), anti-virals (didanosyne, ribavirin, zidovudine), non-steroidal anti-inflammatories (acetylsalicylic acid - Especially doses > 2.7 g per day; mefenamic acid, indomethacin, naproxen, piroxicam), quinine and derivates, anticonceptional drugs, loop diuretics (sulfonamides), analgesic (hydromorphone), anesthesic (nitric oxide), antidepressants (mianserina), antipsychotics (chlorpromazine), anti-epileptics (valproic acid, carbamazepine, gabapentin), anti-neoplastics and others: deferoxamine, methadone, sildenafil (Rybak & Whitworth, 2005) are associated with a risk of high-frequency hearing loss and loss of

outer hair cells in the cochlea. It is interesting to note that some of this drugs such as quinine has a long period of wash-out from the inner ear that can last until one year.

Sudden hearing loss can be defined as the loss of 30 dB in three consecutive frequencies with less than 72 hours of evolution (Fetterman, Saunders, & Luxford, 1996; Wilson, Byl, & Laird, 1980).

Idiopathic Sensorineural Sudden Hearing Loss (ISSNHL) is described as a symptom rather than a disease (Hallberg, 1956) and can be accompanied by tinnitus and/or vertigo. There are different theories regarding ISSNHL; some researchers state that it can be a result of local autoimmune processes that affect the cochlea (Campbell & Klemens, 2000), while others claim that this disease may be a consequence of infection or a vascular disorder (Hultcrantz et al., 1994; Kellerhals, 1972; Wilson, Veltri, Laird, & Sprinkle, 1983). There are different types of treatments, including corticosteroids, hyperbaric oxygen that are based on the immune theory (Kanzaki, Taiji, & Ogawa, 1988; Nosrati-Zarenoe & Hultcrantz, 2012; Russolo & Bianchi, 1997).

Cochlear otosclerosis is an uncommon disease and usually presents in young patients. This disease often occurs between 30 and 50 years of age and is known to worsen during pregnancy. The stiffening of osseous bone causes a mixed hearing loss (sensorineural and conductive hearing loss) that is occasionally combined with tinnitus (Hayashi et al., 2006; Youssef, Chandrasekhar, Rosen, & Lee, 1998).

Retrocochlear lesions are located beyond the cochlea on the vestibulocochlear nerve or in one of the auditory areas of the Central Nervous System (CNS). Retrocochlear hearing loss, or neural hearing loss, is the result of damage to structures beyond the cochlea or neural systems occurring at the level of the auditory nerve or the auditory pathway, which causes degeneration of the hearing nerves. Alternatively, retrocochlear hearing loss can result from the inability of the hearing nerves themselves to convey neurochemical information through the central auditory pathways (Moore, 2008). A rather common cause of retrocochlear hearing loss is the growth of a benign tumor (vestibular schwannoma) that presses on the auditory nerve. The types of retrocochlear hearing loss are divided into two groups: central and vestibular nerve diseases (Moore, 2008). Auditory diseases (central) are the disorders of hearing or auditory perception resulting from diseases of the central auditory pathways or auditory associated cortical areas, such as cortical deafness. Above the level of the pons, bilateral lesions are usually required to produce auditory dysfunction. Vestibulocochlear nerve diseases are the diseases that damage the vestibular and/or cochlea nerves (vestibular schwannoma).

By far presbycusis or age-related hearing loss (ARHL) is the leading global cause of hearing loss in adults. It is most commonly characterized by symmetric, high-frequency hearing loss, and difficulties understanding conversation in loud environments (Figure 2.1-17). It is a complex process, not entirely clarified, involving oxidative stress, cellular apoptosis, and mitochondrial DNA deletions that conduct to a deficient protein synthesis and/or accumulation insoluble pigments. Other relevant contributive factors are genetic predisposition, smoking, diet, noise exposure and socio-economic factors (Nelson & Hinojosa, 2006; Seidman et al., 2004).

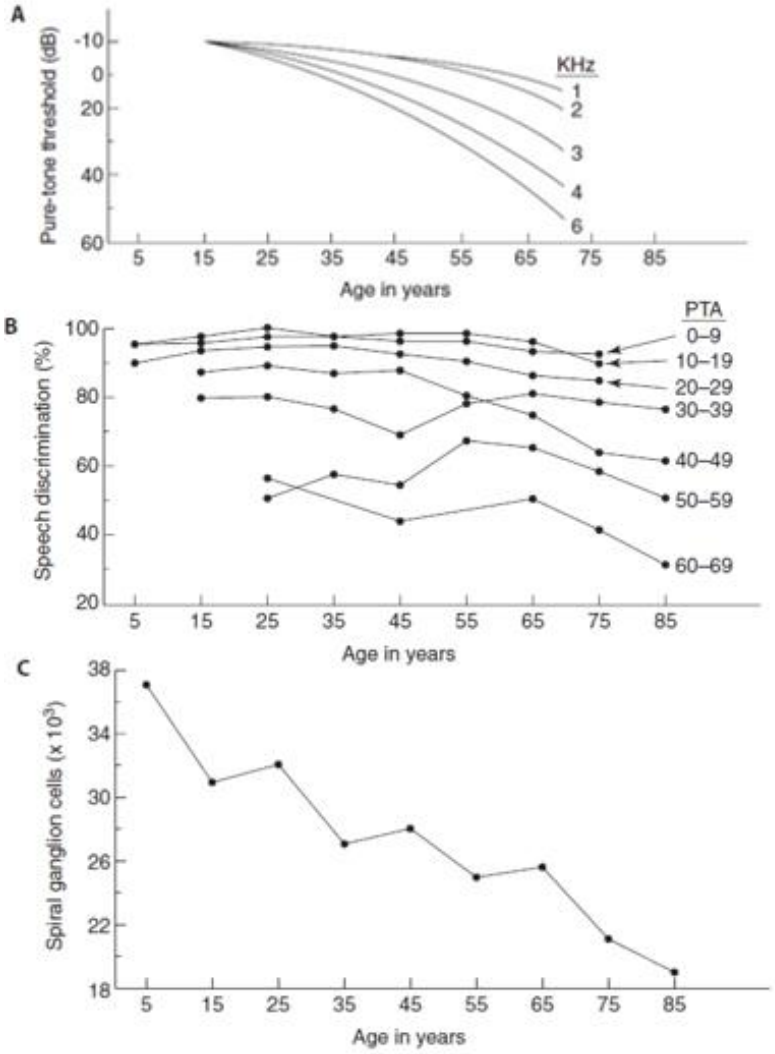

Figure 2.1-15 – Age related hearing loss alterations.
Legend: A) Hearing level as a function of age. Pure-tone hearing level increases with age, and higher frequencies are affected more than the lower frequencies. (B) Speech discrimination as a function of age. For a given pure-tone hearing loss, the speech discrimination as a function of age. For a given pure-tone hearing loss, the speech discrimination decreases with aging. (C) Total ganglion cell population versus age. There is progressive loss of cochlear neurons as a function of aging. (Lalwani, 2007).

ARHL classification (initially proposed by Schucknecht, later modified by Nelson and Hinojosa, 2003) are based on audiometric and histopathologic findings (Nelson & Hinojosa, 2006) (Figure 2.1-18):

Sensory presbycusis – is the most common, the symmetrical abrupt downward slope of the audiogram begins above the speech frequencies (4kHz), therefore speech discrimination is often preserved. At histology there is a progressive epithelial atrophy with loss of sensory hair cells and corresponding neurons.

Neural presbycusis – audiometry corresponds to a gentler downward slope, usually speech discrimination is severely affected. Histopathology shows 1st neuron degeneracy that begins at the basilar region of the cochlea.

Strial presbycusis (metabolic) – begins early in life, slow progression, it has a hereditary component, and is more common in women. Audiometry has a flat pattern, with a preserved speech discrimination. Histopathology corresponds to atrophy of the stria vascularis (with big intracellular vacuoles, cystic structures and/or basophilic deposits).

Cochlear presbycusis (conductive / mechanical) – Audiometry is pan-tonal or in a gradual downward slope with poor speech discrimination. Histopathology reveals thickening and secondary stiffening of the basilar membrane of the cochlea with changes in the spiral ligament, in particular a loss of type IV fibrocytes adjacent to the basilar membrane. The mechano-electric mechanism is compromised.

In 25% of the cases:

Mixed presbycusis – Mixture of the above.

Indeterminate - None of the above.

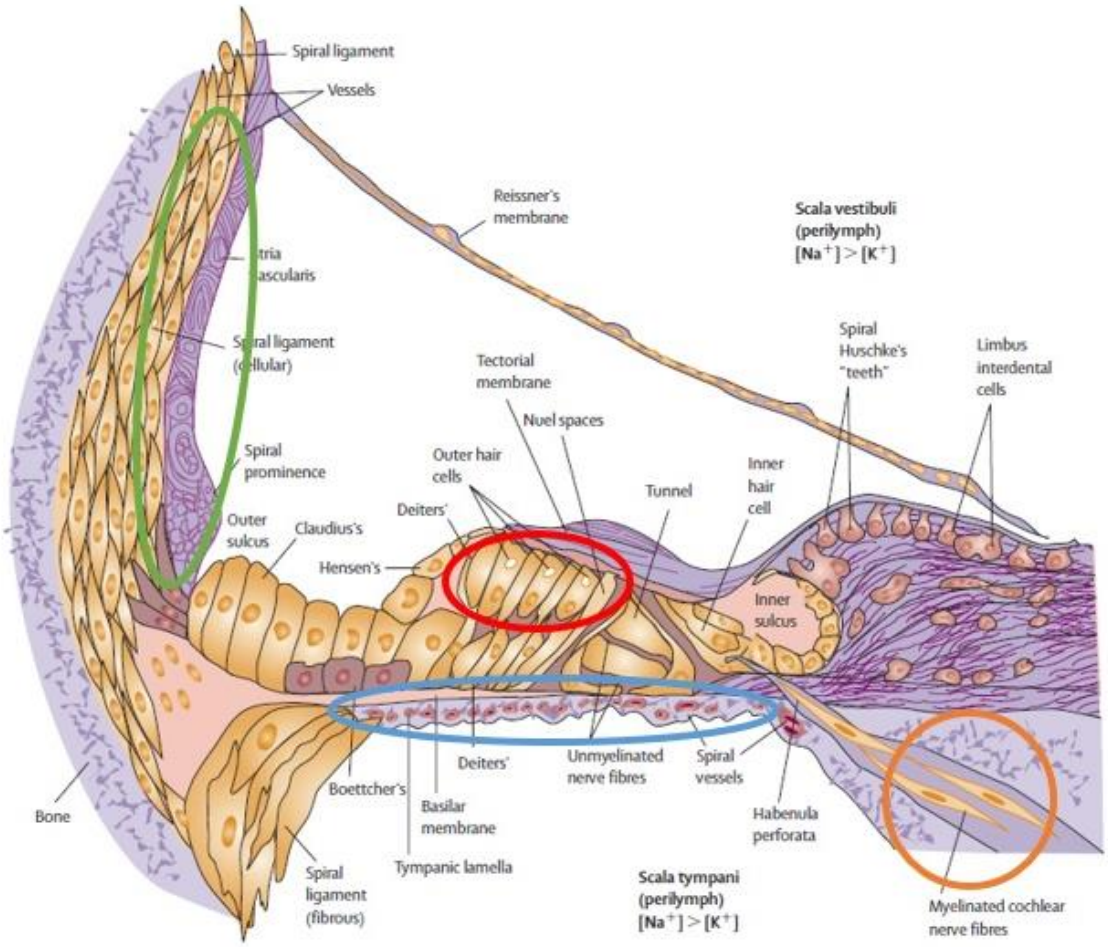

Figure 2.1-16 - Different structures involved in the types of presbycusis.
Legend: Red circle – sensory presbycusis; Orange circle – Neural presbycusis; Green circle – Strial presbycusis; Blue circle – Mechanical presbycusis.
(https://www.sobiologia.com.br/conteudos/FisiologiaAnimal/sentido6.php)

It is generally accepted that one of the contributors for the mechanisms leading to ARHL is the natural ageing of the organism where occurs a progressive state of hypoperfusion of cochlear tissues leading to ischemia, and the production of free radicals which are very toxic and harmful to auditory epithelium. This process induces mitochondrial mutations and a state of bioenergetic inefficiency (Terao et al., 2018).

A genetic predisposition of ARHL has the implication that this entity may be treatable or preventable. There are studies describing an association between ARHL and genes involved in hereditary hearing loss (e. g. *GJB2* e *KCNQ4*); or genes related to oxidative metabolism (e. g. *NAT2, GSTM1, GSTT1);* other studies involve *GRHL2* and *GRM7* and also concerning mitochondrial DNA, the 4977bp deletion and the U and K haplogroups.

Ageing involves a progressive loss of sensorial epithelium in the inner ear. This process begins at the age of forty (Seidman et al., 2004). Seidman and colleagues found an average loss of 2000 cochlear neurons for each decade of life (Seidman et al., 2004). There are also losses at central auditory pathways, with a 50% reduction of the total number of cochlear dorsal and ventral nucleus neurons at the age of 80.

There has been recent research concerning *Heat shock proteins* (HSP).HSP-70. is phylogenetically very ancient and present in bacteria, and in humans is genetically codified in at least 17 variants. In the nervous and immune systems HSP-70 has both intra and extracellular roles with many paracrine effects (Giffard, Macario & Macario, 2013). It works like an alarm signaling activating cytokine chains such as MYK88/IRAK/NFkß. HSPs are present in many tissues: liver, muscles, neurons etc, (Pujol & Puel, 1999). In the immune system HSP-70 can have an immunosuppressive or immune stimulating effect depending on the cell type involved (Giffard et al., 2013; Pujol & Puel, 1999).

Several studies have shown that in response to an insult the Shock transcription factor (HSF1) is activated, which induces several HSPs. This activation is reduced in the process of ageing and consequently the cellular protection is also reduced (Lobo, García-Berrocal, Trinidad, Verdaguer, & Ramírez-Camacho, 2013; May, 2013).

2.1.4.3. Relationship with tinnitus

Hearing loss is considered a risk factor for tinnitus development (Chung et al., 1984; Sindhusake et al., 2003, 2004). Although tinnitus can be triggered by a variety of causes, the majority of cases are associated with hearing loss (Roberts et al., 2010). Usually, tinnitus patients with normal standard audiograms have some hearing loss at frequencies above 8 kHz (Roberts, Moffat, & Bosnyak, 2006). However, not all patients with hearing loss develop tinnitus, as demonstrated by the higher prevalence of hearing loss compared to tinnitus (Lockwood, Salvi, & Burkard 2002). Although it is unclear which factors of hearing loss contribute to the occurrence of tinnitus it is hypothesized that interventions that prevent hearing loss may also prevent tinnitus (Roberts et al., 2010).

2.2. Pathophysiology of subjective tinnitus: triggers and maintenance

Submitted to Frontiers in Neuroscience

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Abstract

Tinnitus is the conscious perception of a sound without a corresponding external acoustic stimulus, usually described as a *phantom* perception. One of the major challenges for tinnitus research is to understand the pathophysiological mechanisms triggering and maintaining the symptoms. Our objective was to synthesize the published literature in order to provide a comprehensive update on theoretical and experimental advances and to identify further research and clinical directions. We performed literature searches in three electronic databases, complemented by scanning reference lists from relevant reviews in our included records, citation searching of the included articles using Web of Science, and manual searching of the last six months of principal otology journals. One-hundred and thirty-two records were included in the review and the information related to peripheral and central mechanisms of tinnitus pathophysiology was collected in order to update on theories and models. A narrative synthesis examined the main themes arising from this information. Tinnitus pathophysiology is complex and multifactorial, involving the auditory and non-auditory systems. Recent theories assume the necessary involvement of extra-auditory brain regions for tinnitus to reach consciousness. Tinnitus engages multiple active dynamic and overlapping networks. We conclude that advancing knowledge concerning the origin and maintenance of specific tinnitus subtypes origin and maintenance mechanisms is of paramount importance for identifying adequate treatment.

Keywords: Tinnitus; Auditory system; Tinnitus pathophysiology; Central tinnitus; Peripheral tinnitus; Causes of tinnitus; Tinnitus maintenance

Introduction

Tinnitus is a prevalent symptom associated with various conditions and diseases; both otological and non-otological (Baguley et al., 2013). It affects over 70 million people in Europe and more than 50 million people in the United States (Heller, 2003; Henry et al., 2005; Baguley et al., 2013). The heterogeneity of tinnitus causes a substantial problem in its classification, which has hampered both basic and clinical research. A major challenge for the field is to identify the underlying causes of tinnitus for developing specific treatments that address the distinct manifestations of tinnitus (Noreña, 2015). Although much research is underway, the precise pathophysiology of tinnitus remains unclear.

Tinnitus can be classified according to various criteria including causes, comorbidities, symptoms characteristics, and psychological burden. The most common form of tinnitus is described as the conscious perception of a phantom sound or noise perceived in the ear(s) or head in absence of a known external or internal stimulus (Schlee et al., 2014) and this is often associated with a hearing loss. Tinnitus has been further classified according to its initial triggers as a *primary tinnitus,* which is either associated with sensorineural hearing loss (SNHL) or is idiopathic (or unknown cause), and a *secondary tinnitus,* which is related to other causes such as an organic origin (Tunkel et al., 2014). Somatic or somatosensory tinnitus is a subtype of subjective tinnitus, and the patients's tinnitus perception its cause by the alteration in somatosensory afference from the cervical spine or temporomandibular area (Michiels et al., 2018). Another causal classification strategy is based on the origin of tinnitus in relation to the site of impairment in the auditory pathway, and splits tinnitus into peripheral and central types (Henry et al., 2014). Tinnitus duration is also a common symptom classification since this can distinguish patients where tinnitus is maintained over the longer term after its initial onset. *Acute tinnitus* has been defined as an onset within the past 6 months, whereas *chronic tinnitus* refers to symptoms lasting 6 months or longer (Tunkel et al., 2014). However, the precise temporal boundary from acute to chronic is not standardized, since other authors report the transition from acute to chronic tinnitus anywhere between 3 and 12 months (Hall et al., 2011; Rabau et al., 2015). Another symptom classification is based on a description of the tinnitus sound such as whether it is continuous or intermittent, pulsatile or non-pulsatile. Questions about duration and symptom characteristics are often asked in case history questionnaires (e.g. Tinnitus Sample Case History Questionnaire, Schecklmann et al., 2015).

Another classification system takes account of the functional and psychological impacts caused by tinnitus, and this is particularly important for those with chronic bothersome tinnitus. A number of questionnaires have been designed to assess self-reported impacts and examples include the Tinnitus Handicap Inventory (THI, Newman

et al., 1996), Tinnitus Questionnaire (TQ, Hallam et al., 1988), Tinnitus Functional Index (TFI, Meikle et al., 2012), and Tinnitus Primary Function Questionnaire (TPFQ, Tyler et al., 2014). The correlation between total scores of THI and TQ is 0.641 (P<0.0001), indicating that they assess a similar tinnitus-related construct. Of note, the German version of the TQ (Hiller and Goebel, 1992), frequently used in the German-speaking countries, is a modified version of the original TQ developed in the UK. Burden can be represented by a score on a continuous scale, by narrative description on a categorical scale, or by a dichotomous distinction such as between "*compensated*" or "*decompensated"* tinnitus as measured by the German version of the TQ.

Whether or not any of these classification strategies are informative with respect to the pathophysiology of tinnitus remains controversial. Concerning its origin, there is a minimum consensus that tinnitus is related to aberrant neural activity at certain levels of the auditory system (Jastreboff, 1990). "Peripheral tinnitus" refers to the auditory perception that results from aberrant neural activity at the cochlear level and transmitted through the auditory pathways (Jastreboff, 1990; Guitton et al., 2003; Puel and Guitton, 2007). "Central tinnitus" refers to the auditory perception that is generated in auditory brain centers by the aberrant neural activity and is sustained by that aberrant neural activity (Eggermont, 2005; Kaltenbach, 2006; Eggermont, 2007; Kaltenbach, 2007; Mulders and Robertson, 2009). The auditory centers perform an important role because they are involved in the generation of the tinnitus-related activity (Liberman and Dodds, 1984b;a; Heinz and Young, 2004; Noreña, 2015). Despite this distinction, "peripheral tinnitus" and "central tinnitus" are not completely independent forms (Noreña, 2011). This article uses systematic review methodology to identify the latest knowledge regarding the different pathophysiological mechanisms that trigger and maintain tinnitus symptoms.

Identifying and selecting appropriate literature sources

Eligible information sources were review articles and original research articles reporting basic science, exploratory and investigational studies. We included animal and human studies investigating tinnitus pathophysiology, but we did not include studies where the primary focus was an associated condition (such as Ménière's disease, otosclerosis, vestibular schwannoma, chronic otitis media, tumor, autoimmune diseases, neurodegenerative or demyelinating disease, or cases of ototoxicity) with tinnitus as an incidental observation. Other exclusion criteria were articles not written in English language, and records relating solely to objective or somatosensory tinnitus.

Initial literature searches were conducted in October 2017 using three literature search platforms: PubMed, Medline and Web of Science and the search terms "pathophysiology" and "subjective chronic tinnitus". The initial search was complemented by scanning reference lists from relevant reviews in our included

records, citation searching of the included primary scientific articles using Web of Science. Additionally, in May 2018, we performed an update by manually searching key otology journals.

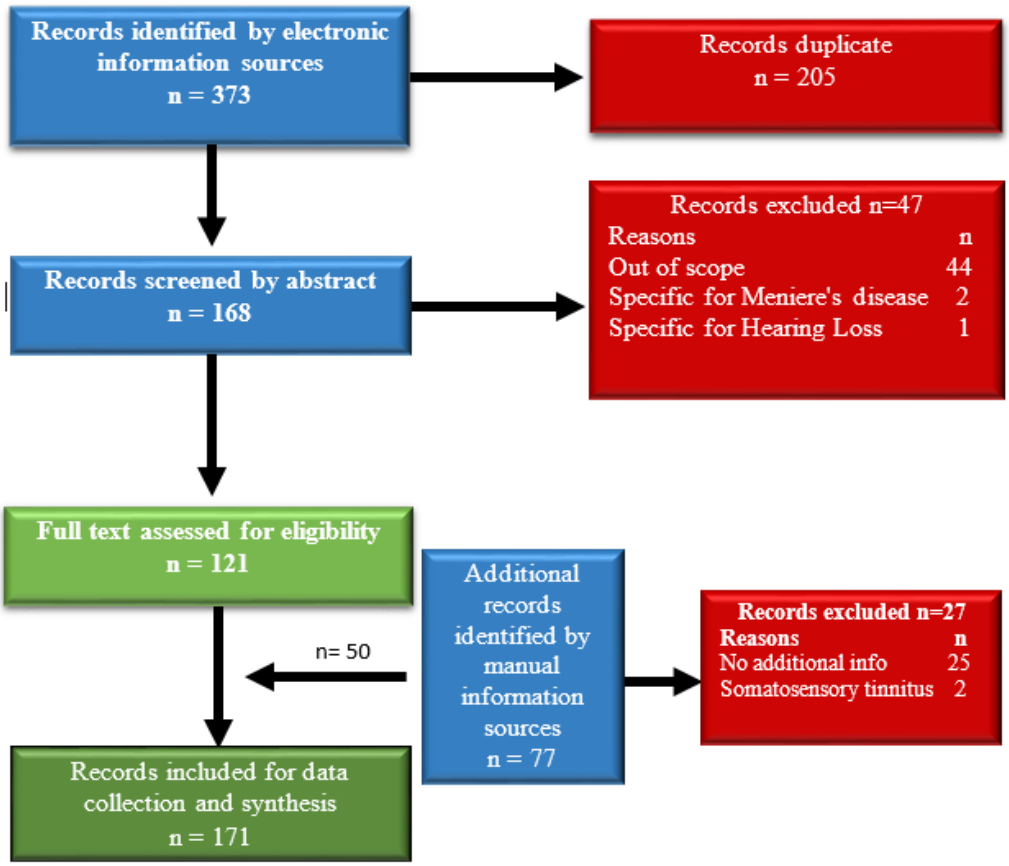

Figure 2.2-1 - Flowchart of the literature search and selection process.

The initial search retrieved 373 records. After duplicates had been removed, 168 records remained for abstract screening. From those, 47 were excluded as not related to the topic of the review or not meeting the inclusion criteria. The remaining 121 full texts were screened again for eligibility (Figure 2.2-1). Fifty additional records were identified from the manual searching to give a total of 171 records. At least two authors independently, from review team, reviewed all records, and in case of disagreement, a third opinion was obtained. Information extraction and synthesis focused on tinnitus pathophysiology.

Population characteristics indicating pathophysiology

A study in Italy performed by Martines and collaborators estimated that in 30% of cases, tinnitus had an undetermined etiology (Martines et al., 2010a;Martines et al., 2010b;c). It is well established that tinnitus often accompanies noise-induced hearing loss and presbycusis. According to Davis and Rafaie, approximately 90% of people with tinnitus in the UK have some form of hearing loss (Davis and Rafaie, 2000). Large-scale population studies have identified other risk factors such as vascular disease,

hypertension, diabetes, autoimmune disorders, head injury, and degenerative neural disorders (Rojas et al., 2003;Sindhusake et al., 2004).

Comparing animal and human neurophysiological studies

Some of the major advantages of the animal model as a way to investigate the pathophysiology of tinnitus are the ability to i) control the etiology via controlled experimental manipulation of the noise environment or ototoxic drug exposure, ii) to randomly assign animals to experimental or control groups, increasing the power of statistical testing, and iii) to apply a wide range of experimental tools (from molecular to behavioral). Nevertheless, some disadvantages of using animals for tinnitus research exist, the main one being the lack of a standardized animal model of tinnitus. These fundamental challenges give rise to concerns about the reliability and interpretation of results (Lobarinas et al., 2013; Brozoski and Bauer, 2016). Noise exposure in the animal model is often traumatic and acute, unlike the more common human experience of moderate and prolonged noise exposure, while exposure to highly concentrated ototoxic agents such as salicylate are rare in humans. An unresolved issue is the distinction between acute and chronic tinnitus in animal models, mainly due to different experimental paradigms and different species used. An agreed classification of what constitutes acute versus chronic tinnitus in the animal model is of special importance for future studies regarding the progression from acute to chronic forms, especially since this could provide the basis for seeking objective markers of its natural history. The majority of research done with help of animal models points to noise-induced hearing loss and tinnitus as an adequate model for the development of chronic tinnitus (Bauer and Brozoski, 2001;Turner and Larsen, 2016). The report of Pace and collaborators focuses on a novel experimental paradigm and makes distinction between the salicylate-induced tinnitus (tinnitus duration 5 days) and noise-induced tinnitus (tinnitus duration 7 weeks) (Pace et al., 2016). An attempt to define such criteria has already been made using clinical studies (Leaver et al., 2016a). Based on the obtained findings, species-specific criteria could be expected to emerge in animal models of tinnitus.

The pioneering and widely applied salicylate model (Jastreboff et al., 1988) induces tinnitus both by direct central effects on the auditory system and by induction of peripheral hearing loss (Eggermont, 2015). For a detailed review on animal models of tinnitus, the reader could refer to Brozoski and Bauer (2016). Questions about altered neural spontaneous firing rates in the auditory pathway, abnormal neural synchrony and changes in tonotopic representation have been obtained from animal studies at the level of individual neurons and neuronal assemblies, and in human studies at a much more macroscopic population level (Adjamian et al., 2009; Eggermont, 2015). The main problem here is the translation of research from subcellular neuronal events found in animal models to the brain activity patterns observed in people with tinnitus. The differences in measurement technique bring important caveats for drawing analogies

between animal and human findings. For example, the assumption that the interpretation of coupling between local neural activity and the responses monitored using blood oxygenation level-dependent functional magnetic resonance imaging (BOLD-fMRI) are still unclear (Adjamian et al., 2009).

One of the overall impressions about the neurophysiological results obtained from animal models of tinnitus is that they typically consider tinnitus as the consequence of an acute peripheral lesion associated with severe hearing loss. In contrast, human neuroimaging studies tend to emphasize the role of auditory thalamus and auditory cortex in the chronification and maintenance of tinnitus (Eggermont, 2015;Brozoski and Bauer, 2016).

Sites of tinnitus generation

A fundamental question in tinnitus pathophysiology concerns the neural component that generates tinnitus (Henry et al., 2005). Zenner initially postulated that tinnitus could originate in any relevant anatomical structure; from the ear throughout the central auditory pathways (Zenner, 1998). Initial speculations favored a cochlear origin since tinnitus can be perceived in the ears and also due to the fact that there is a strong association between the frequency of psychoacoustic identified tinnitus and the audiometric profile of hearing thresholds (Sereda et al., 2011). These opinions were contradicted by the fact surgical section of the auditory nerve does not eliminate tinnitus in every case, which favors the hypothesis about the central rather than peripheral origin of tinnitus (House and Brackmann, 1981).

Nowadays, it is well established that many forms of tinnitus reflect a complex interaction between peripheral and central mechanisms within the auditory pathway (Noreña and Farley, 2013). Usually two or more triggers (e.g. noise exposure, hearing loss, emotional distress, and somatosensory factors) are necessary to elicit a noticeable tinnitus (Shore et al., 2007). Tinnitus can be seen as a pathology of neural plasticity with a molecular and a systemic component. The molecular component has a cochlear component related to the initiation phase of tinnitus; while the systemic component has a central aspect associated to the long-term maintenance of tinnitus (Satar et al., 2003; Guitton, 2012; Noreña and Farley, 2013; Noreña, 2015; Sedley et al., 2015). It has been suggested that peripheral tinnitus may originate from the dysfunction of cochlear outer hair cells and the consequent changes in endocochlear potential, leading to increased spontaneous cochlear activity. This suggestion provides a possible explanation of different causes behind cochlear tinnitus, including tinnitus induced by an acute noise exposure (Noreña, 2015). Meanwhile, central tinnitus is mediated by the neuronal activity in the auditory centers. A good illustration is the chronic tinnitus induced by a noise trauma in the absence of changes in cochlear activity following the trauma (Liberman and Dodds, 1984b;a; Heinz and Young, 2004; Noreña, 2015). Although central

mechanisms are important for explaining the generation of tinnitus-related activity, much of these mechanisms appear to be triggered by a reduction of cochlear activity. However, damage to cochlear tissues is not necessary to produce central changes related to tinnitus, since a conductive hearing loss can also induce tinnitus (Ayache et al., 2003; Midani et al., 2006; Schaette et al., 2012).

Based on the above assumptions, Noreña proposed three distinct subtypes of tinnitus: cochlear tinnitus, peripheral-dependent central tinnitus and peripheral-independent central tinnitus (Noreña, 2015). Cochlear tinnitus refers to a tinnitus generated by aberrant activity in the inner ear, which is propagated through the cochlear nerve and the central auditory pathway. This activity may lead to an auditory perception, depending on the firing neuronal rates and top-down modulation, (Noreña and Farley, 2013;McKenna et al., 2014; Noreña, 2015). Peripheral-dependent central tinnitus refers to a tinnitus associated with cochlear spontaneous activity, while peripheral independent central tinnitus refers to a tinnitus that is independent from cochlear spontaneous activity (Noreña, 2011;2015).

Cellular mechanisms

Cochlear damage may include loss of Outer Hair Cell (OHC) electromotility, loss of synapses between Inner Hair Cells (IHCs) and spiral ganglion neurons (synaptopathy), damage to the stereociliar bundle, death of OHCs or IHCs, or rupture of the basilar membrane. All of these processes can be seen in rodents by means of histology, but are not easily measureable in humans due to difficulty in access to tissue. These mechanisms lead to a decrease in neuronal output from the cochlea to the brain and they could account for the potential generation of compensation mechanisms in the brain (Chen and Fechter, 2003).

Position of the tectorial membrane

Change in the position of the tectorial membrane may be a pathophysiological trigger for acute tinnitus following an intense noise exposure. It is well established that after noise trauma, the rootlets of stereocilia are altered leading to stiffness and contributing to acute increase in cochlear spontaneous activity (Liberman and Dodds, 1984b;a;1987). The prolonged depolarization of IHCs can occur through any condition that changes the relative position of the tectorial membrane. This may originate after an increased pressure in the scala media, tectorial membrane detachment, degeneration of OHCs or stereocilia (LePage, 1989). In some cases, there might exist areas of damaged OHCs but intact IHCs, and so the tectorial membrane can touch the IHCs stereocilia, consequently causing their depolarization (Baguley, 2002).

Outer Hair Cells (OHC)

Another pathophysiological trigger for acute tinnitus concerns damage to the stereocilia of OHCs, again often following an intense noise exposure. High noise levels damage first the OHCs and then the IHCs (Nicolas-Puel et al., 2006). The initiation of pathological process starts at the stereocilia of OHCs, with two fundamental processes damaged by the noise: intracellular calcium levels and biochemical changes of their structural proteins. Eggermont suggested that increased intracellular calcium could be the pathological substrate of peripheral tinnitus, by increasing the neurotransmitter release of the cells and subsequent activity of afferent fibers (Eggermont, 2000).

Inner Hair Cells (IHC) and the cochlearNMDA receptors

The *N*-methyl-**D**-aspartate (NMDA) receptor has been found to play an essential role in noise-induced tinnitus. In a behavioral animal model, pharmacological interventions that antagonize the NMDA receptors prevent tinnitus (Guitton et al., 2003). These NMDA receptors appear to predominate on the modiolar side of IHCs (Pujol et al., 1992), with a higher percentage of lateral olivocochlear efferent fibers that seem to terminate on low-SR high threshold fibers (Liberman, 1980). It seems that an increase in glutamate levels derived from IHCs, activates the NMDA receptors that release excessive Ca2+ in the dendrites of the spiral ganglion neurons. This causes an over-excitation of NMDA-receptors and consequently a calcium influx during the damage. This process may contribute to hearing loss, neural presbycusis and tinnitus via the aberrant excitation of the auditory nerve (Sanchez et al., 2015). Underlying the over-excitation, there is an increase in adenosine triphosphate (ATP) which consequently increases the reactive oxygen species in the synapses between IHCs and spiral ganglion neurons (Sahley et al., 2013). An increase in levels of Ca^{2+} in the NMDA receptors can trigger a successive metabolic events such as production of reactive oxygen or hydrogen species or even death of spiral ganglion neurons (Parsons and Raymond, 2014). It is likely that the blockade of NMDA-receptor activation prevents the loss of IHC ribbons after noise damage (Bing et al., 2015). Therefore, concerning the lower auditory pathway, the NMDA receptor plays a role in numerous functions such as neuronal plasticity, synapse modifications, temporal processing and onset of disease (Sanchez et al., 2015).

Increase of the endocochlear potential

The endocochlear potential is a prerequisite for auditory signal transduction. It is maintained by keeping high concentrations of K+ in the endolymph and is strongly associated with cochlear spontaneous activity (Sewell, 1984; Mittal et al., 2017). An increase in the endocochlear potential can depolarize IHCs, which triggers a sequence of events that includes opening the voltage-gated Ca2+ channels, an intracellular influx of Ca2+ and fusion of the synaptic ribbon to plasmatic membrane. This culminates in glutamate release and depolarization of cochlear fibers (Hudspeth, 1985;Moser et al.,

2006). OHCs can regulate the endocochlear potential, through their mechano-electrical transduction channels. In other words, the opening of these channels depends on steriociliar bundle deflection. This process seems to be induced by acute noise trauma that reduces the opening probability of this channels, consequently increasing the endocochlear potential (Patuzzi, 2002).

Biochemical changes seem to be most relevant to the acute phase of tinnitus. The heat-shock protein group (stress proteins), interacts with structural proteins of hair cells, giving them support and protecting them from further damage. Any disturbance that causes a deficient heat-shock protein system response can lead to incurring tinnitus to the person exposed to loud noise (Dechesne et al., 1992).

Cochlear synaptopathy

Although the majority of people with tinnitus have a clinically measurable hearing loss, a good number do not. According to different series more than 60% of people with normal hearing (based on tonal audiometry) have tinnitus (Tucker DA et al., 2005; Heller & Bergman, 1953). Animal data suggest that the permanent loss of synapses between the IHCs and the cochlear nerve fibers occurs because external factors such as noise exposure or aging (Kujawa and Liberman, 2009;Sergeyenko et al., 2013;Kujawa and Liberman, 2015). This condition is popularly called 'hidden hearing loss' (HHL) (Schaette and McAlpine, 2011), since it is not possible to diagnose through conventional tonal audiometry using quiet sounds. In the ear, noise overexposure causes a rapid excessive release of the neurotransmitter glutamate from electron-dense ribbon synapses in the IHC. This excitotoxic insult induces the swelling of the dendrites, which causes an important level of hearing loss at a particular frequency due to a partial disconnection among the IHCs and the afferent neurons (Pujol et al., 1993). The ear possesses a remarkable healing capacity that allows these neuronal terminals to regrow towards the sensory cells and reestablish functional connections restoring hearing (Pujol and Puel, 1999), as people experience after noise exposure (e.g., concerts) and have their hearing thresholds recovering and their tinnitus disappearing after some time. However, in some cases, even if the terminals have grown back, the reconnection can be incomplete and synaptic coupling remains incomplete due to either a decrease in the number of ribbons (Ruttiger et al., 2013) or a decrease in the number of paired pre-and post-synaptic entities (Kujawa and Liberman, 2009). The damage seems to selectively affect low spontaneous rate of the cochlear neurons responsible for high thresholds and coding moderate-to-high sound intensities (Furman et al., 2013). Recently, Wan and Corfas (2017) reported another mechanism underlying HHL. The authors found that transient Schwann cells loss results in permanent disruption of the cochlear heminodal and consequently in permanent auditory deficits characteristic of HHL. Interestingly, these auditory deficits are not related to the synaptic loss, but with the affection of the

first heminodes at the auditory nerve peripheral terminal. This study provides new insights on the mechanisms, causes and long-term consequences underlying HHL.

The extent to which cochlear synaptopathy contributes to tinnitus in animals and in humans is still uncertain. Schaette and McAlpine first demonstrated the reduced amplitude of wave 1 in the auditory brainstem response (ABR) in the subjects with tinnitus but with normal audiogram, when compared to controls (Schaette and McAlpine, 2011). An appealing interpretation of these findings is that they are evidence for reduced cochlear nerve output as a direct result of cochlear synaptopathy. However, there are some important caveats to data interpretation. First, the match between tinnitus and control groups was not 100% regarding the high frequency sensitivity, yet wave 1 ABR amplitude is known to be predominantly raised by responses to high-frequency tones (Don and Eggermont, 1978). Second, this finding has not withstood replication (Gilles et al., 2016;Guest et al., 2017). Methodological differences might underlie the lack of replication, but another plausible explanation is that tinnitus in young audiometrically normal adults is not related to cochlear synaptopathy but may reflect other effects of the exposure to noise (Guest et al., 2017). Clear directions for further research are to improve the sensitivity of non-invasive electrophysiological measures of cochlear synaptopathy in humans, and to examine the broader neurophysiological impacts of noise exposure.

Mechanisms involved in maintenence of tinnitus

The link between hearing loss and tinnitus is well substantiated. For example, patients with conductive hearing loss (e.g. otosclerosis) frequently report having tinnitus and these symptoms are usually abolished after surgery (Gersdorff et al., 2000; Ayache et al., 2003; Sobrinho et al., 2004). Ear plugging is a way to induce a temporary hearing loss in otherwise normally hearing people. Participants who wear a silicone earplug for 7 days develop tinnitus symptoms, which disappear after removing the earplug (Schaette et al., 2012). Implantable and non-implantable hearing devices improve tinnitus in 50% of treated patients and eliminating it in 20% of cases (Schaette, 2013), likely by partially restoring cochlear output. More specifically, published data confirm a strong association between high-pitched tinnitus and high-frequency SNHL, suggesting again that hearing loss is a main cause of tinnitus (Noreña et al., 2002; Martines et al., 2010a; Martines et al., 2010b;c; Sereda et al., 2011). Many theories suggest that the underlying cause of tinnitus may be associated with damage to the sensory cochlear epithelium (Henry et al., 2005), and if acute then this can be assessed in the patient by asking about the temporal association between noise exposure events, abrupt changes in hearing and tinnitus onset or exacerbation. In a review, Zhao and collaborators found that specific insults to the peripheral auditory system (e.g. cochlear ablation, selective IHC or OHC loss, and mixed or incomplete IHC and OHC injuries) can all reduce cochlear output (Zhao et al., 2016). The edge theory of tinnitus proposes having cochlear

disturbance inducing tinnitus and caused by the shift of OHCs in the organ of Corti from the apical side towards the lesion in a high-frequency basal side (Nuttal AL, 2004). In almost all types of peripheral insults, OHCs are more damaged than IHCs. Combined with the edge theory, this provides the foundation of the Discordant Theory, which predicts that tinnitus is associated with a disinhibition of neurons in the dorsal cochlear nucleus (DCN), due for example to DCN receiving excitation from IHC and not from damaged OHC and consequently leading to increasing spontaneous activity in the central auditory system (Levine, 1999;Jastreboff and Hazell, 2004).

Reduced cochlear output through hearing loss likely triggers a cascade of neuromodulatory events ultimately causing hyperactivity in central auditory circuits (central gain). This process has been proposed to contribute to tinnitus. It seems to be associated with neuronal hyperactivity and could likely be a common consequence of various kinds of cochlear damage (Parra and Pearlmutter, 2007). It could also explain individual cases of tinnitus without hearing loss, since there can be up to 30% damage to the OHCs before hearing loss is detectable using pure tone audiometry (Chen and Fechter, 2003).

Hearing loss decreases the input to the central auditory system. This may in turn modify the gain of central neurons, resulting in increased spontaneous activity. The functional aberrations resulting from either model (tonotopic over-representation, enhanced synchronicity, or elevated spontaneous firing rates) may underlie the induction of tinnitus (Adjamian et al., 2014) (Figure 2.2-2).

The sensation of pain and phantom limb perception is often used as an analogy to the pathophysiology of tinnitus. Damage in the cochlea (e.g. hair cell loss or synaptic damages) leads to a frequency-specific decrease in output from the cochlear nerve. An upregulation of activity in the central auditory pathway is a compensatory effort to counteract the lack of signals in the particular frequency area. This effort increases the gain, falsely leading to the perception of a non-existing sound and possibly accompanying hyperacusis (Auerbach et al., 2014). In addition to the auditory pathway, tinnitus shares non-auditory networks, similar to these know in chronic pain (perception, salience, distress, memory). Such networks, may maintain, in absence of the initial “tinnitus-initiator” (De Ridder et al., 2011a;De Ridder et al., 2014;Rauschecker et al., 2015). De Ridder and others consider phantom pain and phantom sound to share basic underlying mechanisms. The model assumes sensory differentiation resulting in cortical activity within the primary and secondary auditory cortices. This activity becomes a conscious percept upon connection to a larger brain networks located in the frontal and parietal areas of cortex, such as “self-awareness” and “salience network”. The latter network intersects with the central autonomic control system and affects the limbic-auditory and somatosensory interaction indispensable for consciously maintaining the phantom perception (Figure 2.2-2 and 2.2-3). This perception may

associate with distress, simultaneously co-activating non-specific distress networks located in the anterior cingulate cortex, anterior insula and amygdala. At the same time, it is proposed that memory mechanisms may reinforce and maintain the awareness of the phantom percept (De Ridder et al., 2011a).

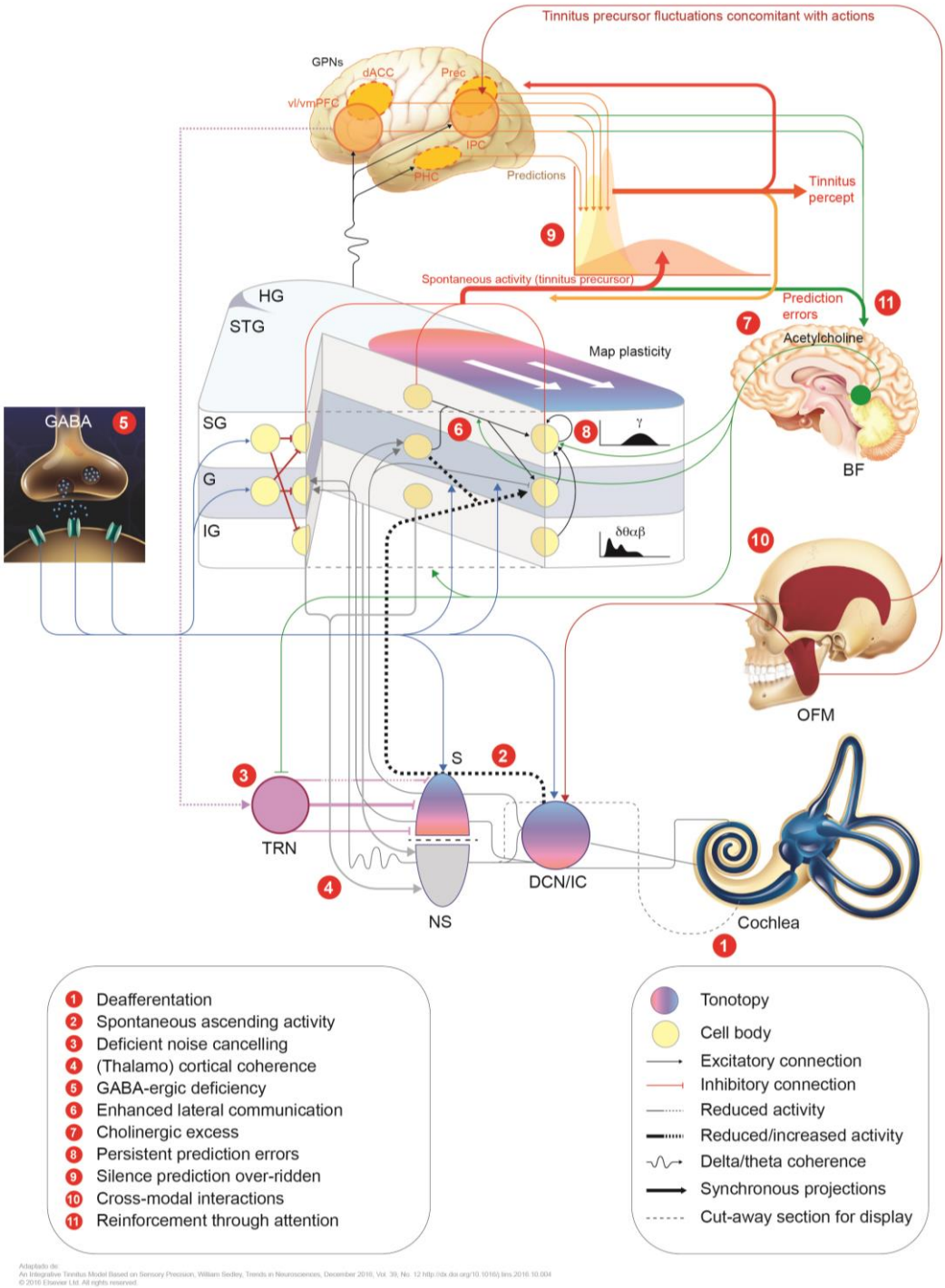

Figure 2.2-2 - Potential mechanisms involved in tinnitus pathophysiology.
Legend: GPNs – global perceptual networks; vl/vmPFC – ventrolateral/ventromedial prefrontal cortex; dACC – dorsal anterior cingulate cortex; Prec. – precuneus; IPC – inferior parietal cortex; PHC – parahippocampal cortex; HG – Heschl's gyrus; STG – superior temporal gyrus; SG/G/IG, supragranular/granular/infragranular neuronal layers; BF – basal forebrain; OFM – orofacial movements; S – specific (lemniscal) auditory thalamus; TRN – thalamic reticular nucleus; NS – non-specific auditory thalamus; ; DCN – dorsal cochlear nucleus; IC – inferior colliculus.

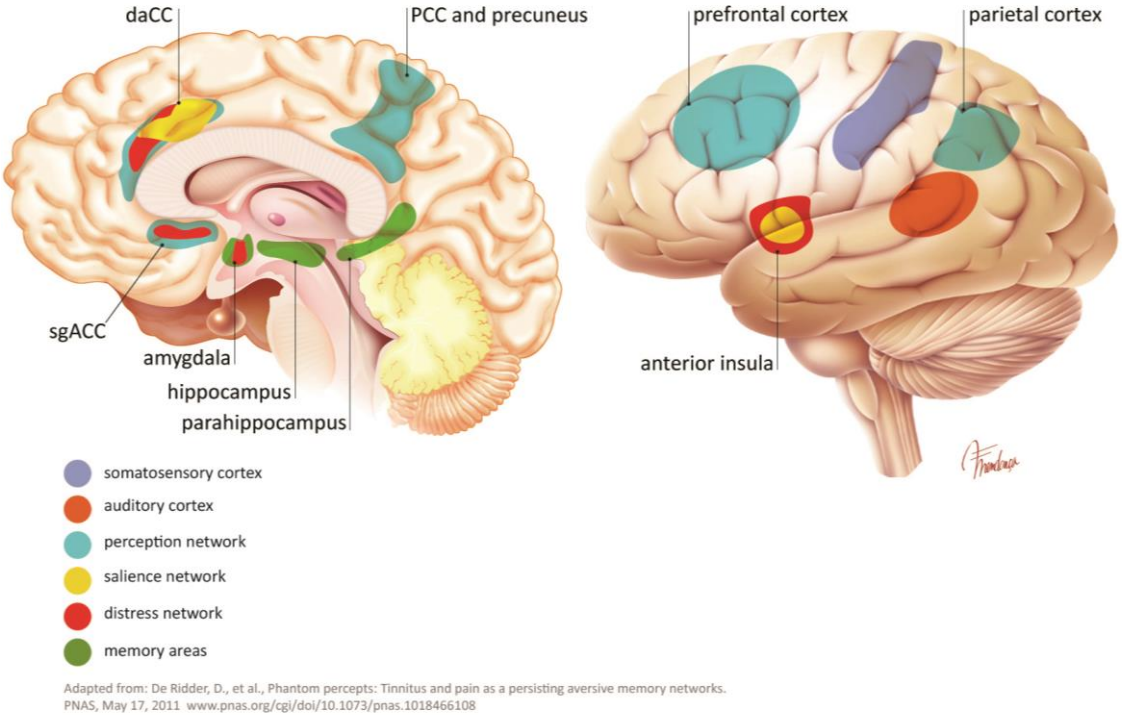

Figure 2.2-3 - Some extra auditory regions involved in tinnitus pathophysiology.

Central mechanisms

The compensation mechanism occurring in the central nervous system during tinnitus is called “homeostatic plasticity”. This is a phenomenon whereby auditory neurons in the brain adapt their synaptic connections in attempt to maintain a neuronal network similar to the one before the peripheral damage occurred. Neuronal correlates of tinnitus have been proposed as neuronal hyperactivity in the posteroventral cochlear nucleus (PVCN), the inferior colliculus (IC), DCN, and the paraflocculus lobe of the cerebellum (PFL) (Cacace et al., 2014). Specifically, it has been suggested the presence of elevated responses to sound in subcortical areas, in particular in the IC, as a common effect among individuals with tinnitus and normal thresholds (Melcher et al., 2009). A large body of data supports the view that DCN is the induction site of tinnitus, which then spreads to higher areas (Brozoski et al., 2012;Dehmel et al., 2012;Wu et al., 2015). Animal studies show an increased activity in fusiform neurons of the dorsal cochlear nucleus during noise-induced tinnitus (Brozoski et al., 2002). Being the site of convergence of different somatosensory pathways (trigeminal nucleus and dorsal somatosensory pathway), cholinergic and serotonergic systems, it has been proposed that the DCN is an important site of maladaptive auditory-somatosensory plasticity (Wu et al., 2015). Supporting the importance of the DCN in tinnitus generation is the identification of a role of the Kv7.2/3 channel, which shows decreased activity in the DCN after noise-induced tinnitus. However, a specific drug compound that modulates Kv channels (Kv3.1) has been found not to alleviate subjective tinnitus in humans (https://clinicaltrials.gov/ct2/show/NCT02315508?term=QUIET-1&rank=1).

Another hypothesis views tinnitus as a product of neuronal hyperactivity in particular regions of the central auditory system such as cochlear nucleus, IC and thalamus (see (Dong et al., 2010;Middleton et al., 2011;Vogler et al., 2011;Manzoor et al., 2013;Kalappa et al., 2014). There is no consensus about cannabinoids, which activate the CB1 receptors and which may have an effect on exacerbation or worsening tinnitus. However, there seems to be an effect of cannabinoids and their receptors that exist in the cochlear nucleus, where they are functional and may have an effect in tinnitus. The function of CB1 receptors in the circuitry of the DCN suggests that could increase rather than inhibit it, neuronal excitation which if causing tinnitus might exacerbate rather than relief it (Smith and Zheng, 2016).

There are two main, partially compatible theories on the role of medial olivocochlear bundle in tinnitus onset. The first theory emphasizes the role of decreased neural efferent input to the cochlear amplifier which, in this way, increases its spontaneous activity and induces a chain reaction of neuroplastic changes in the afferent auditory relays up to the auditory cortex. The second theory focuses on the brainstem as the place of integration of efferent neuronal drive and afferent tinnitus-related stimuli (Riga et al., 2015). Considering that some studies could not confirm the role of medial olivocochlear bundle in tinnitus, this finding is still controversial (Riga et al., 2015).

The auditory cortex also shows evidence of frequency-dependent reorganization, although in people with tinnitus but without measurable hearing loss, tonotopic map reorganization is not essential (Langers et al., 2012). Comparing cortical hubs that involve multiple brain regions in people with tinnitus and in the healthy controls through electrophysiological measurement demonstrates fundamental differences between the groups (Muhlnickel et al., 1998;Schlee et al., 2009). Mapping the cortical hubs has demonstrated essential differences in the global networks, mainly hyperactivity in the gamma frequency range within the temporal cortex associated with tinnitus (Schlee et al., 2009). According to this view, the global network may influence the auditory cortex in a top-down process and regulate the degree of tinnitus-related distress. Those alterations seem to be associated with conscious tinnitus perception (Schlee et al., 2008). In particular, the activity and connectivity patterns detected in the posterior cingulate cortex and the precuneus region, associate with a distressing tinnitus (Maudoux et al., 2012). When cochlear damage causes a reduction of electric signals at a given frequency, neurons within the primary auditory cortex responsive to these frequencies start responding to adjacent frequencies, as exemplified by the broadening of the frequency tuning in this region (Engineer et al., 2011;Yang et al., 2011). Aberrant neuronal oscillations have also been observed in the alpha and gamma frequency range within the frontal cortex (Muller et al., 2013). These results are in agreement with the work of Weisz and collaborators, who were the first group to use the EEG oscillation to study tinnitus (Weisz et al., 2005). That first study revealed the dissimilarities of power

spectra between a group of people with tinnitus and hearing loss and a matched group of control subjects. Over the years, other results provided mixed support for this finding (Weisz et al., 2007;Moazami-Goudarzi et al., 2010;De Ridder et al., 2011b;Adamchic et al., 2012;Adjamian et al., 2012;Adamchic et al., 2014), and there is not yet any clear agreement in the field. For example, recently, Pierzycki and collaborators (Pierzycki et al., 2016) found no evidence that resting state whole-scalp EEG reflects any tinnitus-related percept or symptom severity and so should not be assumed as a biomarker for tinnitus. Moreover, the correlation between perception of tinnitus and the frequency band power in EEG and magnetoencephalography (MEG) remains unclear.

Overall, it is now rather well established that most of the nuclei in the auditory pathway can be affected during tinnitus. These compensatory mechanisms seem to be related to the loss of GABAergic inhibition and decreased activity of specific potassium channels (Kv7.2/3) (Yang et al., 2011;Li et al., 2013). However, whether the changes seen in central gain are directly related to tinnitus or instead more related to hyperacusis is still a matter of discussion (Knipper et al., 2013;Auerbach et al., 2014).

Non-auditory neuronal networks involved in tinnitus

Recent work in rodents (with fMRI) and humans (intracranial recordings) strongly support the involvement of emotional/cognitive relays of the brain such as temporal, parietal, sensorimotor, and limbic cortex in the pathophysiology of tinnitus (Frank et al.,2011; Vanneste and De Ridder, 2011). Neuronal emotional networks which influence peripheral and central circuits during tinnitus, involve most likely central regions implicated in a normal emotional behavior and in mood altered disorders. Such regions comprise the medial prefrontal cortex and ventro-medial parts of the basal ganglia (also known as limbic frontostriatal network) (Lowry et al., 2004;Cheung and Larson, 2010). In addition, they include dorsal prefrontal regions, the medial and caudolateral orbital cortex (medial prefrontal network), insula, posterior thalamus, anterior cingulate, posterior cingulate, amygdala (Shulman, 1995;Mirz et al., 2000), parahippocampus, hippocampus (Lockwood et al., 1998;Landgrebe et al., 2009), and the subcallosal region (Muhlau et al., 2006;Leaver et al., 2011) including the nucleus accumbens (Jastreboff, 1990;Drevets et al., 2008). The precise functional role of the numerous extra-auditory structures is difficult to establish because some of them participate in the generation or in the chronification of tinnitus, some in psychological reactions to the tinnitus, some are associated with hearing loss and others with hyperacusis (Leaver et al., 2016a;Leaver et al., 2016b) (Figures 2.2-2 and 2.2-3). It is highly plausible that there is no coherent model for the involvement of extra-auditory structures in chronic tinnitus but rather that the patterns are highly dependent on the individual tinnitus profile. A tight interaction between limbic non-auditory and auditory pathways and the presence of both anatomical and functional abnormalities has been confirmed by different neuroimaging techniques (stimulus evoked BOLD fMRI, diffusion MRI, resting-state fMRI

and PET) (Leaver et al., 2016b). On the other hand, other groups have not been able to determine significant differences in the connectivity of auditory network between control and tinnitus groups (Davies et al., 2014). One of the important observations is that the involvement of the extra-auditory brain areas traces the evolution of acute tinnitus to its chronic form (Leaver et al., 2016b). Because a relationship between the psychoacoustic tinnitus characteristic, the degree of tinnitus distress and underlying neural patterns of activity is not scientifically confirmed, there is an urgent need for systematic studies to address these questions further (Leaver et al., 2016b).

The frontostratial circuits appear to have a central role in the development and maintenance of both tinnitus and chronic pain (Rauschecker et al., 2015). Two structures are essential in this process: the ventromedial prefrontal cortex and the nucleus accumbens. Both of them play a role in evaluating the relevance and emotional significance of sensory stimuli and in managing of the information flow via descending pathways. The damage in frontostratial areas could explain tinnitus pathophysiology and provide new insights for the therapeutic design or prevention of tinnitus and chronic pain (Rauschecker et al., 2015). The tinnitus percept seems to be mediated by a somatotopic map and the corresponding somatic memory. Furthermore, somatic memories depend on somatotopic maps and their active use in the specialized cortical areas (Eggermont and Kral, 2016). The genetically defined somatic memories and the somatotopic maps are shaped by experience during early development, and are independent of auditory input (Bonham et al., 2004;Pienkowski and Harrison, 2005;Eggermont and Moore, 2012). Corroborating this observation, it was noted that the individuals born without limb(s) are free of phantom limb and phantom limb pain phenomena. These observations reinforce the relationship between tinnitus and the phantom limb that occurs as references to sensory surface maps (Eggermont and Kral, 2016).

The medial geniculate body (MGB) within the thalamus has been suggested to gate the perception of sound on its way to the auditory cortex and to limbic system (Caspary and Llano, 2017). The key component in the pathology of the tinnitus network strongly implicates MGB and its ascending inputs from the brainstem, thalamic reticular nucleus and, limbic structures, as well as descending inputs from the auditory and nonauditory cortices (Shinonaga et al., 1994;Bajo et al., 1995;Lee and Winer, 2008a;c;b;Rauschecker et al., 2010;Leaver et al., 2011). In addition, a functional model of tinnitus suggests that in the affected individuals, tinnitus-related distress correlates with abnormal functions in limbic and thalamocortical circuits (Winer et al., 1999;Rauschecker et al., 2010;Leaver et al., 2011). Concerning the role of MGB, opposing hypotheses offered GABA-related explanations. The first one assumes tinnitus-related up regulation of GABAergic inhibition whereas the second one assumes tinnitus-related suppression of GABAergic inhibition. GABA mediates fast synaptic inhibition and a persistent tonic inhibition (Caspary and Llano, 2017).

One study has evaluated the cortical benzodiazepine receptor distribution in patients with tinnitus, using venous blood samples after radiolabeling with 123I-iomazenil, radiochemical purity, single-photon emission computed tomography (SPECT) and MRI. A comparison of participants with severe chronic tinnitus and controls revealed a significant trend toward bilaterally reduced benzodiazepine receptor density in the frontal lobes ($p < .001$) and a reduction in the cerebellum ($p = .045$) (Daftary et al., 2004).

An MRI study, involving people with hearing loss affected or not by tinnitus, demonstrated increased gray matter in the temporal and limbic areas, and decreased gray matter in frontal and occipital areas when compared to a control group. In detail, analyses of all cortical areas of the tinnitus participants demonstrated an increase of gray matter in cerebellum and subcortical auditory nuclei with the most significant effect in the left primary auditory cortex when compared to controls and those with hearing loss only. On the other hand, people with hearing loss had decreased gray matter in frontal areas and increases in limbic areas, compared to controls. These findings imply a particular role for the left primary auditory cortex and other non-auditory brain structures in tinnitus development (Boyen et al., 2013). Another study, with a similar design, using diffusion tensor imaging and voxel-based morphometry (VBM), found both gray and white matter changes in the auditory cortex of people with hearing loss but without tinnitus, compared to people with tinnitus and controls. Thus, the authors concluded that hearing loss rather than tinnitus was associated with the observed changes (Husain et al., 2011). A large-scale study examining VBM and surface-based morphometry changes in brain anatomy from 128 participants with tinnitus and hearing loss, tinnitus with clinically normal hearing, and non-tinnitus controls with clinically normal hearing managed to replicate some of the morphological differences that had been reported in previous studies, but found other differences that contradicted previous results (Allan et al., 2016). The variability of morphometry results obtained by different teams and by different analysis methods is confusing. It perhaps indicates the need for greater standardization in study design, and in analysis techniques, as well as more precise subtyping of the condition.

Theories and models of tinnitus pathophysiology

A recent report supports the notion that tinnitus is not associated with increased metabolic activity in localized auditory regions (Geven et al., 2014), but rather with neural synchrony between different cortical networks (Noreña and Farley, 2013;Sedley et al., 2015), including the thalamus (Eggermont, 2013;Husain and Schmidt, 2014). Oscillatory activity within large neuronal ensembles is one method for measuring neural synchrony in the human brain. The power of the oscillatory activity can be separated into different frequency bands, the premise being that these reflect different functional processes. A steep audiometric edge between regions of normal and impaired hearing may be sufficient to disrupt the normal pattern of neural synchrony in tonotopically

organized regions of the central auditory system. De Ridder et al. (2015) have observed that oscillatory activity in the gamma frequency band usually appears bilaterally in tinnitus patients and they have proposed this to be the substrate of tinnitus. However, the evidence only partially supports this model because there are a number of methodological issues that complicate the attribution of findings to the tinnitus versus the hearing loss (Adjamian et al., 2012). With respect to this edge region, some studies have found tinnitus-related changes in the magnitude of the oscillatory power in delta/theta frequency bands (1-4/4-8 Hz), as well as in alpha (8-12 Hz) and gamma (>30Hz) frequency bands (Eggermont and Tass, 2015). These authors observed tinnitus-related low-frequency delta oscillation that are hypothesized to originate from the thalamus low frequency bursting (Sedley et al., 2015). The delta activity extended beyond auditory cortex to the temporal, parietal, sensorimotor and limbic cortices. The diffuse distribution of activity was too extensive to be consistent with the putative "edge effect" theory. Rather, delta frequency band activity has been found to interact with alpha, beta and gamma frequency band activities in specialized brain regions such as parahippocampal and inferior parietal regions. And this has been proposed as a neurophysiological correlate of the network-based interactions between tinnitus perception and memory processes. In line with further development of the synchronicity model, Schlee and others investigated the correlation between chronic tinnitus and cortical activity in the alpha frequency range (Schlee et al., 2014). The authors confirmed the reduction of alpha power and auditory alpha variability in the tinnitus brain. According to their conclusions, changes in alpha power reflect the enhanced and reduced excitability of engaged neuronal networks (Schlee et al., 2014).

Overall, these results suggest a role for neural synchrony both for establishing pathological activity within the auditory cortex and for recruiting extra-auditory networks in tinnitus. However, the precise details of these mechanisms warrant further attention.

De Ridder and collaborators proposed a pathophysiological framework that involves deafferentation and the concept of dysfunctional noise-canceling tinnitus can be defined as a result of activity of and connectivity between multiple, parallel subnetworks, (De Ridder et al., 2014) (Figure 2.2-2). Such subnetworks could be responsible for encoding specific tinnitus characteristics. The model includes the brain areas directly associated with the perception of tinnitus and the areas that are essential for the affected person to perceive tinnitus as an external sound source. The inferior parietal-ventrolateral prefrontal cortex-auditory cortex participate in retrieving a sound from memory and in the auditory awareness. The core tinnitus subnetwork can be defined as simultaneously activated brain areas necessary to perceive tinnitus, devoid of its affective components. Because of communication within the core tinnitus subnetwork, the affected brain areas undergo adaptation process, possibly mediated by neuronal synchrony (De Ridder et al., 2014).

Recently, Sedley and collaborators proposed another framework to explain tinnitus pathophysiology from the ear to the cortex (Sedley et al., 2016). That model assumes a so-called predictive coding model, in which spontaneous activity of the auditory subcortex involves 'tinnitus precursor', which is normally ignored against the prevailing percept of 'silence' (Figure 2.2-2). This model explains the simple and unitary content of tinnitus. The sensory precision tinnitus model comprises causes of spontaneous sensory input and their graded processing in a predictive coding framework.

The broader framework is equally applicable to other conditions similar to tinnitus, such as chronic nociceptive pain. Nevertheless, some types of pain such as central post-stroke pain, cannot be explained by this framework (Klit et al., 2009).

There are a number of psychological models of tinnitus. The neurophysiological model (Jastreboff, 1990) proposes that fear is a conditioned responses that is responsible for generating a bothersome tinnitus (Jastreboff and Hazell, 1993). The neurophysiological model draws on behavioral psychology and has the following stages: (1) generation of the tinnitus-initiating signal in the peripheric auditory system; (2) detection of the neuronal activity induced by tinnitus; (3) perceptional evaluation of tinnitus. Husain proposed a neuropsychological model that includes the regions and connections involved in mediating chronic tinnitus (Husain, 2016). The brain regions identified incorporate the neuropsychological (Jastreboff, 1990; Kaltenbach, 2006; Eggermont and Roberts, 2012) and psychological (e.g. Sweetow, 1986; Hallam et al., 1988) components of tinnitus. This model differs from those already existing in that it uses MRI evidence to explain habituation to tinnitus. The model predicts a key role of the amygdala in a severe, non-habituated tinnitus. The frontal cortex becomes more engaged in subjects with mild, habituated tinnitus, and this may facilitate bypassing the emotional processing from the amygdala and the use of alternate limbic pathways involving the insula and parahippocampus gyrus (Husain, 2016).

Tinnitus models that are influenced by the cognitive psychology movement include the cognitive behavioral model (McKenna et al., 2014) and fear-avoidance model (Cima, 2018). Both of these seek to explain the causes and chronicity of tinnitus-related distress from a cognitive perspective, and both offer an integrative approach that could shed insights on higher-order pathological processes of tinnitus-related distress.

Conclusions

Significant advances in understanding the molecular, cellular and system-level mechanisms of tinnitus have been made in the last decade. Although tinnitus may be induced by a peripheral insult, the tinnitus generators are found mainly centrally, in and around the primary auditory cortex as well as in many non-auditory higher-order processing centers. Reduced input to the auditory nerve shifts the balance of central

excitation and inhibition, and this may lead to hyperactivity, increased bursting activity and increased synchrony. This view is consistent with the multifactorial nature of tinnitus, which involves auditory, attentional, memory and emotional systems (Kaltenbach, 2011).

The current view on tinnitus therefore is that it is a symptom encompassing a distributed network across the peripheral and central auditory system. Many studies would indicate that the restoration of cochlear output to the brain should also abolish tinnitus. Preliminary evidence reporting benefit from hearing aids and cochlear implants for tinnitus support this view.

Recent novel findings may open perspectives for new therapeutic approaches on molecular level (e.g. intracochlear application of NMDA antagonists, modulation of microtubule associated proteins molecular pathway, GABA modulation); on a systemic level (behavioral strategies, transcranial magnetic stimulation); “hybrid” solutions that would involve synergistic action of pharmacotherapy and Vagal Nerve Stimulation (Bojic et al., 2017) and lastly the intracochlear pharmacological interventions supported by a nonspecific, mostly anxiolytic pharmacotherapy (Guitton, 2012). Factors that determine the phase of tinnitus pathophysiological evolution (initiation or maintenance), the level (molecular or systemic), the mechanism (neurotransmission or neuromodulation) (Guitton, 2012) will in the future determine the therapeutic approach. The therapy of tinnitus will have to be strictly individualized, with an assessment protocol that would define tinnitus in the sense of the phase (chronicity), level of lesion (peripheral or central) and whenever possible – the mechanism of tinnitus maintenance. This approach in tinnitus evaluation will engage specific multidisciplinary teams whose collaboration will have as a center the subjective wellness and improvement of tinnitus patients.

2.3. Tinnitus in adults, a health problem, implications for the patients and clinicians

2.3.1. Symptoms, causes and global burden

Tinnitus is a symptom that comprises the perception of a sound in the ears or head, without a corresponding external acoustic stimulus. The percept can be a simple sound like hissing, sizzling, or ringing, or a complex composite of sounds, or multiple separate sounds (Baguley, McFerran, & Hall, 2013).

Tinnitus can cause significant emotional reactions to it and negatively impact the quality of life of the affected person. Clinicians talk about habituation to tinnitus so that over time symptom severity tends to diminish (Cima, Crombez, & Vlaeyen, 2011a). Indeed, a systematic review that pooled outcomes for 'no-intervention' controls across a number of randomized controlled trials also confirmed a small but consistent reduction in symptom severity (Phillips, McFerran, Hall, & Hoare, 2018). Those individuals who do remain disturbed by their tinnitus over the longer term may be those with some additional comorbidity such as generalized anxiety, depression, hyperacusis, headache, and vertigo, which may make the individual more prone to focus attention on their tinnitus symptoms (Cima, Vlaeyen, Maes, Joore, & Anteunis, 2011b; Dobie, 2003; Holgers, Zöger, & Svedlund, 2005; Kennedy, Wilson, & Stephens, 2004; Møller, 2003).

Tinnitus can also be categorized according to its duration as acute (duration inferior to 6 months) or chronic (if tinnitus lasts longer than six months) (Tunkel et al., 2014). It is believed that chronic tinnitus may be related to somatosensory manifestations (Kennedy et al., 2004) and related to a decrease in alfa neuronal activity in the temporal areas of the brain (Schlee, Herrmann, Pryss, Reichert, & Langguth, 2014).

There are two broad categories of tinnitus; objective and subjective (can not be heard by others). Objective tinnitus can be audible by others and, account for less than 1% of all cases, mainly originates from vascular or muscular problems in the vicinity of the ear or in the head and neck (Folmer, Martin, & Shi, 2004; Sismanis, 2003). Tinnitus can be pulsatile and if it is synchronous with the heart beat then a vascular pathology might be suspected. In particular, when there is a unilateral pulsatile tinnitus that is synchronous with the heart beat then there is an 80% higher probability that the origin is a vascular loop adjacent to the VIIIth cranial nerve (Chadha & Weiner, 2008).

In the majority of cases, tinnitus is subjective because it is audible only by the person affected. Subjective tinnitus can be associated with a variety of medical conditions, both otological and non-otological (Martines et al., 2010a; Martines, Bentivegna, Martines, Sciacca, & Martinciglio, 2010b, 2010c). For example, a low

frequency tinnitus is most typical of Ménière's disease (Havia, Kentala, & Pyykkö, 2002). However, nearly 30% of all tinnitus cases are considered to be idiopathic (Martines et al., 2010a,b,c). Tinnitus etiology can be multifactorial and complex, and given it is often of gradual onset, even careful medical history taking cannot always identify a cause.

Epidemiological case control studies confirm hearing loss and ageing (Hoffman & Reed, 2004), noise exposure (Nondahl et al., 2002; Shargorodsky, Curhan, & Farwell, 2010; Sindhusake et al., 2003) exposure to ototoxic medication (Cianfrone et al., 2011; Seligmann, Podoshin, Ben-David, Fradis, & Goldsher, 1996) and depression (Zeman, Koller, Langguth, & Landgrebe, 2004) as some of the most common risk factors. Nearly 40% of people with tinnitus also report some degree of sound intolerance (hyperacusis) (Anari, Axelsson, Eliasson, & Magnusson, 1999), but whether there is a pathophysiological link between these two conditions remains unknown. Other known associations include sleeping problems (Alster, Shemesh, Ornan, & Attias, 1993; Crönlein, Langguth, Geisler, & Hajak, 2007; Fioretti, Fusetti, & Eibenstein, 2013), head or neck injury (Kreuzer, Landgrebe, Schecklmann, Staudinger, & Langguth, 2012), emotional exhaustion (Hébert, Canlon, & Hasson, 2012), anxiety, irritation and frustration (Zeman et al., 2014). For some authors tinnitus is regarded as the result of chronic stress and related immune response (Szczepek & Mazurek, 2017).

With respect to hearing loss, associations are more than simply having both conditions. When a hearing loss is present, the tinnitus frequency usually matches the region of greatest threshold losses, especially in the case of down-sloping SNHLprofiles (Schecklmann et al., 2012). Nevertheless, not everyone with a hearing loss has tinnitus, and some people with tinnitus have a normal clinical audiometric profile.

It is widely posited that if prognostic indicators and clinically meaningful subtypes or profiles of tinnitus could be identified then this would be an effective approach to reducing the variable responsiveness to the different management options currently available. However, pathophysiological mechanisms underlying tinnitus are so far elusive and so, without this basic biological understanding and the identification of tinnitus biomarkers, these goals have not yet been achieved.

It is estimated that one in ten people have tinnitus, and so the global burden of tinnitus is very high (Bhatt, Lin, & Bhattacharyya, 2016). Information about the prevalence of tinnitus is rather patchy. A recent systematic review of adults (aged 18 years or more) found that global prevalence ranged from 1.5 to 42.7% (McCormack, Edmondson-Jones, Somerset, & Hall, 2016). But many of these studies defined tinnitus in slightly different ways and so this may explain the wide range. For those population-based studies which used the same tinnitus definition, prevalence rate was somewhat narrower (11.9 to 30.3%). Examining the study information indicated that prevalence increased with age, and was also slightly higher for men than for women, but this may be explained by occupational rather than biological differences between the sexes.

A large American cross-sectional study found that 21.4 million people have tinnitus, and of those 36% experience constant tinnitus, 15% experienced tinnitus at least once every day and 49% at least once a week (Bhatt et al., 2016). Concerning tinnitus symptom severity, 7.2% described their condition as a 'big or very big problem', 20.2% as a 'moderate problem', and 72.6% as 'not a bothersome problem' (Bhatt et al., 2016). The Tinnitus Functional Index (Meikle et al., 2012) is an instrument that can be used as a diagnostic tool to grade overall symptom severity and this has five category levels from 'very big problem' to 'not a problem'.

The majority of individuals with tinnitus do not report major discomfort, and learn or finding strategies quickly to live with their tinnitus, ignore it or become habituated to it (Cima et al., 2011a). When tinnitus becomes uncomfortable this is mainly due because the presence of other factors or comorbidities interfering with the daily life of the patient, directing excessive attention to the tinnitus, interfering with sleep, disturbing concentration and work (Cima et al., 2011b; Dobie, 2003). The characteristics of the personality of the person, as well as the effectiveness of their coping strategies, allow us to understand how they will react to the presence of tinnitus, and how they will deal with the emotional aspects that underlie them (Vallianatou, Christodoulou, Nestoros, & Helidonis, 2001). The particularity of the psychological aspects related to the presence of tinnitus may contribute to the variability of the reactions described by individuals with tinnitus (Cima et al., 2011b). This is reflected in a higher prevalence of somatic disorders, depression, anxiety, phobias, psychoticism and hostility in people who report tinnitus (Oliveira & Trigueiros, 2002).

Results from the most recent World Health Organization (WHO) Global Burden of Diseases (2015) reports hearing loss as the fourth leading cause of years lived with disability. Given the strong links between hearing loss and tinnitus, then tinnitus surely follows this trend. It has been recognized that the economic burden to society is high compared to some other chronic conditions. For example, a large prospective Swedish cohort study found that sickness absence at work due to tinnitus and hearing loss increased the risk of future claims for disability pension by three times compared to non-audiological diagnoses (Friberg, Jansson, Mittendorfer-Rutz, Rosenhall, & Alexanderson, 2012). Furthermore, tinnitus is the most common complaint in modern warfare. For example, the annual disability compensation paid by US department of Veteran's Affairs for tinnitus and hearing loss exceeds $2 billion USD (Yankaskas, 2011).

2.3.2. Diagnosis

Several studies compare tinnitus to chronic pain, some common complaints are present in both of them, such as sleep disturbances, depression and/or anxiety, high levels of hypochondria and obsessive-compulsive disturbances, coping strategies poorly adjusted, reflecting difficulties in controlling the tinnitus percept and daily life activities.

Among patients suffering from chronic pain tinnitus is a prevalent complaint, although usually not disturbing (Cima et al., 2011b).

Tinnitus can be a symptom of many diseases, the generalized lack of knowledge regarding those impairs standardization of diagnosis assessment (Figure 2.3-1). It is important to highlight that the sooner a diagnosis is reached the better the prognosis for treatment effectiveness (Herraiz, 2008). Nevertheless, the advised therapy should be chosen according to the tinnitus impact on patient's life (Grewal, Spielmann, Jones, & Hussain, 2014). To inform a patient that there is "nothing can be done" regarding his problem or that they have to "learn to live with it", is inappropriate and risks making tinnitus an even greater problem for the patient (Newman et al., 2011).

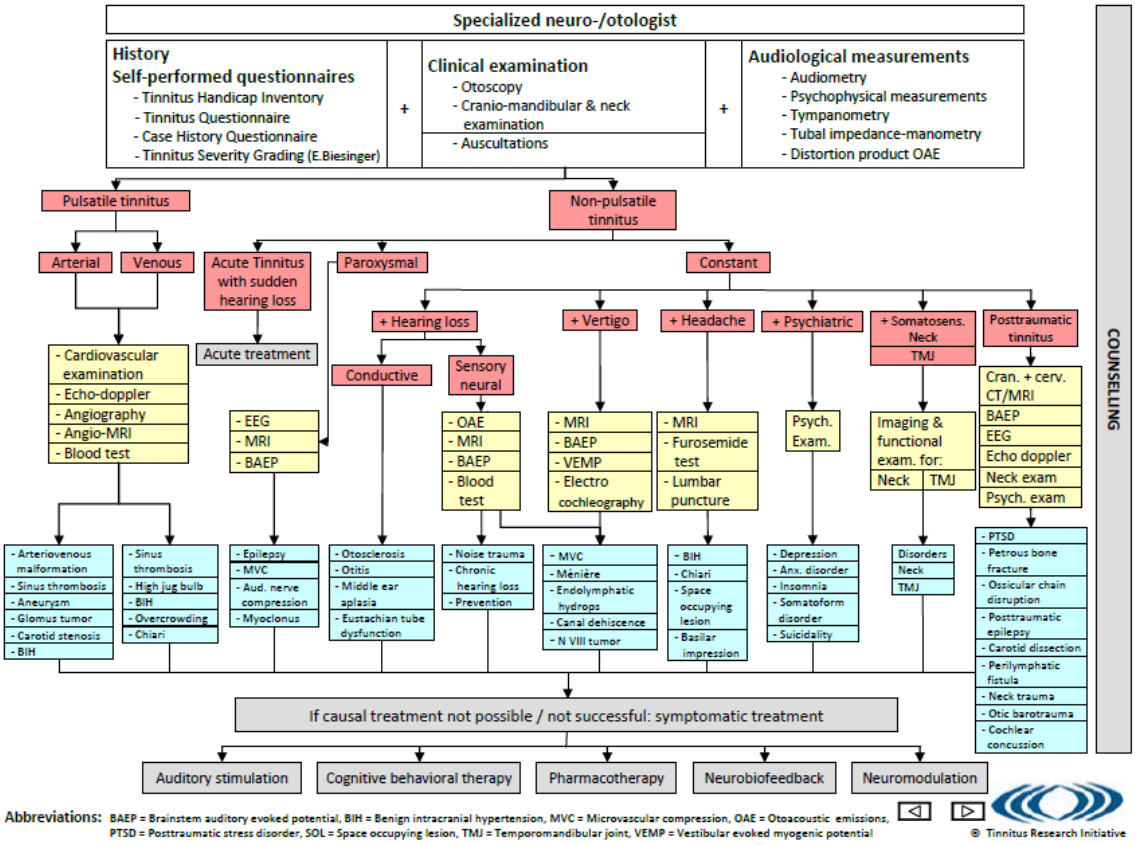

Figure 2.3-1 - Diagnosis and treatment of tinnitus.
(TRI Tinnitus Clinic Network (2009) – "TRI Flowchart for Patient Management". *Tinnitus Research Initiative*).

2.3.2.1. Clinical history and Questionnaires

Because there is not an objective and strict method for tinnitus evaluation we have to rely on patient's collaboration, their description and information (Baguley et al., 2013).The methodology applied to tinnitus diagnosis involves a detailed anamnesis enquiring about associated or triggering factors and comorbidities. It is important to ask questions about the patient's lifestyle regarding noise exposure, at work or for leisure.

It is also important to evaluate other symptoms that may be related to tinnitus such as hearing loss, difficulties in speech discrimination, dizziness, hyperacusis, headaches, sleeping disturbances, etc. Paying attention to the patient, trying to better understand their problem, is not only important for an accurate diagnosis, but may also facilitate counselling (Langguth, Kreuzer, Kleinjung, & De Ridder, 2013). A complete ENT observation and head and neck auscultation should always be performed in cases of pulsatile tinnitus. Tinnitus intensity modulation according to certain neck or temporomandibular joint movements should also be evaluated. To evaluate the impact of tinnitus on patient's life it is recommended to use questionnaires (e.g. Tinnitus Handicap Inventory, Tinnitus Functional Index), although they may retain some bias inherent to the formulation of the questions (Møller, 2016). There are no studies establishing correlations between these questionnaires and tinnitus intensity or discomfort (Hiller & Goebel, 2006), so there is a strong emotional component to the clinical evaluation (Jastreboff, 1990; Li, Gu, & Zeng, 2015).

2.3.2.2. Audiological evaluation

In addition to standard tonal audiometry, high frequencies audiometry, and speech audiometry, audiological evaluation includes the tympanogram. Tympanometry is important to discard tympanic membrane or ossicular chain alterations, or auditory tube dysfunctions, that may originate be responsible for tinnitus (Ambrosetti & Del Bo, 2011). Stapedius reflex evaluation, in cases of intermittent or pulsatile tinnitus, may be indicative of dysfunctions, particularly of the stapedius muscle (Levine, 2013).

Psychoacoustic measurements inform us about the tinnitus type of sound, duration, loudness, pitch, minimum masking level (Van de Heyning, Gilles, Rabau, & Van Rompaey, 2015) and residual inhibition (Ambrosetti & Del Bo, 2011). This allows the patient to identify among the offered sounds, which corresponds to their tinnitus (Langguth et al., 2013). However, this type of measurement lacks objectivity and consistency, especially in cases where tinnitus involves complex sounds, is changeable in time, or is fluctuating (Ambrosetti & Del Bo, 2011; Baguley et al., 2013). It is also important to evaluate the presence of hyperacusis, or generalized oversensitivity for sounds, characterized by loudness discomfort levels below normal (Ambrosetti & Del Bo, 2011).

2.3.2.3. Otoneurolgic and neurophisiologic assessment

Other exams that are usually included in tinnitus diagnosis include auditory brainstem evoked response (ABR) and OAE. Through the evaluation of the latencies and peaks of several waves, ABR verifys the integrity of central auditory pathways, providing information about the location of pathology in the cochlea or retro cochlear. If the result is normal it is possible to assure the patient that there no tumors of the auditory

pathways. This is useful both for diagnosis and therapy because it is common that tinnitus patients, at the initial phase of the complaints, are worried about the possibility of having a tumor. Reassuring the patient regarding this fear will reduce levels of anxiety (Vallianatou et al., 2001). The distortion products of OAE evaluate the functioning of the ciliated OHC, and understand determines if are there any lesions at specific cochlear areas This evaluation is particularly important for patients without hearing loss detected by tonal audiometry, according to the theory of cochlea's ciliated cells discordant functioning (Ambrosetti & Del Bo, 2011).

Imaging techniques are useful in some clinical cases such as tumoral, inflammatory or infectious pathology. These can be essential for certain specific therapies in identification of underlying cause. Human imaging studies have given important contributions for the identification of brain centres involved in tinnitus pathogenesis (Adjamian, Sereda, & Hall, 2009) (figure 2.3-2).

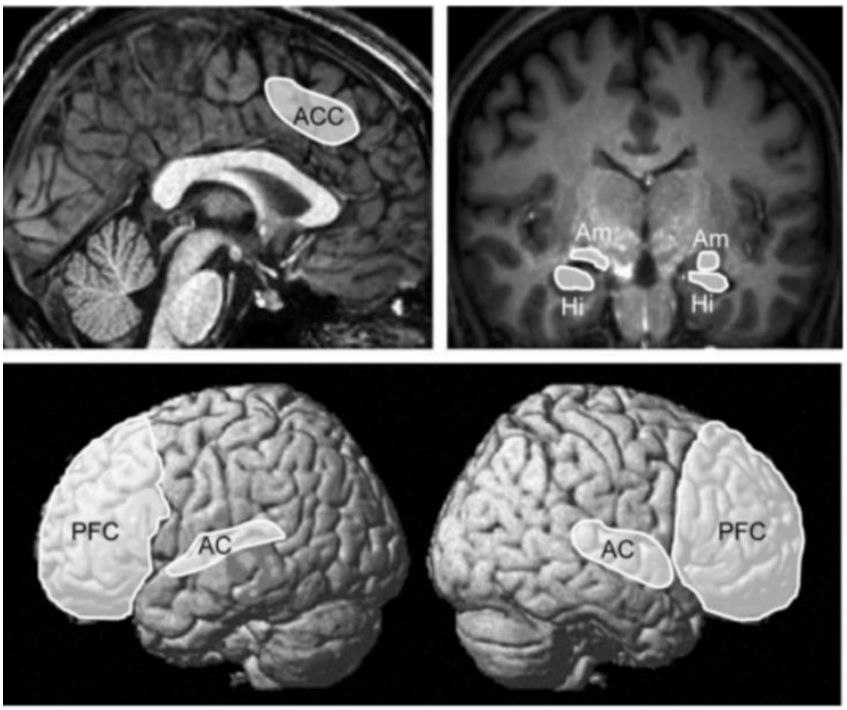

Figure 2.3-2 - Principal structures of the limbic system involved in the pathophysiology of tinnitus, identified through imaging.
(Adjamian, Sereda, & Hall, 2009).

2.3.2.4. Difficulties in tinnitus diagnosis

There are several strategies for tinnitus management among multidisciplinary teams of health professionals (Hall, Szczepek, Kennedy, & Haider, 2015). For this reason, there is often a lack of communication between and within the health professional teams. The inexistence of standardization has hampered the development of large scale randomized clinical trials and scientific evidence (Møller, 2007). In fact, the absence of

standardized methodologies for tinnitus diagnosis and treatment prevents meta-analysis that are necessary for the interpretation of large databases (Ward, Vella, Hoare, & Hall, 2015), and would be very helpful in the realization of more effective tinnitus clinical interventions. The COMiT (Core Outcome Measures in Tinnitus) initiative (a subgroup of experts from TINNET WG5 dedicated to the definition of Core Outcomes Set (COS) for tinnitus) published two systematic reviews for the identification of clinical outcomes domains, one from the patient perspective (Hall et al., 2016) and the other from existing randomized clinical trials on treatment effectiveness for tinnitus (DOI 10.1186/s13063-016-1399-9). Furthermore, and in accordance to COMET (Core Outcome Measures in Effectiveness Trials) Initiative recommendations (Kirkham et al., 2016), three internet-based Delphi rounds for the definition of core outcome sets (COS), the minimum reporting standard for outcomes to be assessed and reported in all clinical trials, in accordance to at final face-to-face meetings for sound-, psychology-, and pharmacology-based interventions to manage chronic subjective tinnitus in adults, were conducted (Hall et al., 2015). Fuller, Haider et al. (2017) published a systematic review of existing tinnitus guidelines. Reviewing five guidelines for the management and treatment of subjective tinnitus they discriminate fundamental parameters: clinical history, patient's observation, audiological evaluation, evaluation of how bothersome is tinnitus using a validated questionnaire or visual analogue scale, referral to mental health in cases of elevated tinnitus-related distress, and performance of imaging studies in selected cases.

2.3.3. Treatment

The shortage of scientific knowledge regarding tinnitus pathophysiology is one of the main obstacles for the achievement of more effective tinnitus therapies (Lee et al., 2014). There is no cure for subjective tinnitus and most of the existing therapeutic options are focused on the management of tinnitus collateral effects without truly reducing tinnitus perception (Cima, Andersson, Schmidt, & Henry, 2014; Hall et al., 2011; Tyler, 2012). Example of treatment include therapies for temporomandibular joint pathology, miofascial trigger points, dental treatments, chiropractors, muscle relaxation, electric stimulation, some pharmacotherapies for anxiety or depression, surgery and CBT (train, habituation and counselling). Nevertheless, and besides the apparently high diversity of therapeutic options, there is scarce scientific evidence of their effectiveness, which may exacerbate symptoms of distress and depression in tinnitus patients (Tyler, 2012). At the same time, 2110 dollars per chronic tinnitus patient is spente annually in healthcare management, unfortunately in most of the cases without therapeutic success (Goldstein et al., 2015).

2.3.3.1. Drugs therapy

There are no drugs approved by any regulatory agency of medical drugs as specific for tinnitus, which would probably be the patient's first and 'easy' choice. Nevertheless, it is predictable that such drugs under research would be based on antagonists of NMDA receptors (e. g. dopamine, GABA and encephalines) applied in the tympanic cavity (through the tympanic window) in order to stabilize synaptic activity and reduce excitotoxicity at primary auditory neurons (Puel, 1995; Ruel et al., 2001).

Notably, some medical drugs can alleviate some symptoms associated to tinnitus such as dizziness, depression, anxiety and sleep disturbances (Hall et al., 2011).

2.3.3.2. Acoustic therapy e Auditory stimulation

Acoustic therapy, initially proposed by Vernon in 1976, allows the patient to have tinnitus control, promotes habituation and distraction, neutralizes the threat and fear related to the presence of an unknown sound, and may contribute to reorganization of CANS through neural plasticity (Newman et al., 2011). Equipment includes electroacoustic hearing aids (amplification of environmental sounds masks the tinnitus), noise generators (initially white noise and more recently noise bands in frequencies that promote a more effective masking, adjusted by audiologists using some filters), or systems combining hearing aids to noise generator (when it is necessary to have both hearing rehabilitation and tinnitus treatment). The option is defined by the existence of and type of hearing loss (Del-Bo, Baracca, Forti, & Norena, 2011). Electroacoustic hearing aids have the advantage of improving speech recognition that may be disturbed by tinnitus percept. The most recent option includes the open-fit systems that are more comfortable and reduce the sense of ear occlusion. This allows better amplification of the more highe pitched sounds than other types of electroacoustic hearing aids (Del-Bo et al., 2011). Moreover, bilateral equipment fitting represents a significant improvement compared to unilateral fitting, and electroacoustic hearing aids using frequential transposition systems present better results (Levine, 2013).

As part of a more recent therapy, the Neuromonics Tinnitus Therapy invovles acoustic stimulation is obtained through an instrument similar to MP3, using soft music (baroque type or new age) adapted to individual tinnitus frequency spectra, in order to compensate for hearing loss in those frequencies areas and reduce the emotional component. In preliminary studies better results were reported for Neuromonics than stimulation with narrow band sounds (Baguley et al., 2013; Langguth et al., 2013; Newman et al., 2011).

2.3.3.3. Tinnitus Retraining Therapy

Tinnitus retraining therapy (TRT) is based in the neurophysiological model of tinnitus. Treatment is based on the process of tinnitus habituation, through CNS plasticity, and aims to reduce the impact of tinnitus on daily life, and promote habituation through reduction of tinnitus intrusiveness (Jastreboff & Jastreboff, 2000; Sáez-Jiménez & Herráiz-Puchol, 2006). Treatment involves a series of counselling sessions (directive counseling) after patients initial evaluation. The aim of these counselling sessions is to eliminate or reduce anxiety or fear reactions. Sound generators, hearing aids or background noise are also used to provide the auditory systems with constant neutral signs to decrease the strength of tinnitus signal and decreases the gain within the auditory pathways (Jastreboff, 2011; Langguth, 2015; Hesse, 2016; Shin & Lee, 2016). Based on a medical evaluation of tinnitus, patients are placed into one of five general categories that guide the treatment recommended (Table 2.3-1) (Jastreboff & Jastreboff, 2000).

Table 2.3-1 - TRT-Categories of Tinnitus and Hyperacusis patients.

Category	Impact on life	Tinnitus	Subjective hearing loss	Hyperacusis	Prolonged sound induced exacerbation	Treatment
0	Low	Present	Not present	Not present	Not present	Abbreviated version of counselling.
1	High	Present	Not relevant	Not present	Not present	Full counselling and sound therapy with sound generators
2	High	Present	Significant presence	Not present	Not present	Full counselling (with the stressing matters) and sound therapy with combination instruments
3	High	Not relevant	Not relevant	Present	Not present	Full counselling with stress issues related to hyperacusis and sound therapy using sounds generators and hearing aids
4	High	Not relevant	Not relevant	Present	Present	Full counselling, sound therapy with sound generators set at the threshold.

There is direct involvement of the patient in these sessions, explaining and demystifying the tinnitus, often using illustrative support material (Jastreboff & Hazell, 2004; Sáez-Jiménez & Herráiz-Puchol, 2006). However, this therapy shows better long-

term results and is poorly studied (Grewal et al., 2014). A randomized controlled trial comparing TRT to Standard Care (SC) for chronic tinnitus concluded that adults with moderate to severe tinnitus and hearing loss amenable to amplification, benefited from either TRT or SC treatment when combined with hearing aid use. TRT benefit may exceed that of SC. The global improvement in tinnitus severity that accrued over an 18-month period appeared to be robust and clinically significant (Bauer, Berry, & Brozoski, 2017). TRT is also used to treat hyperacusis, through sound desensitization (Jastreboff and Jastreboff, 2000; Vernon and Press, 1998). Hyperacusis is a common symptom accompanying tinnitus, and results from enhanced functional gain at auditory pathway (Bartnik & Skarżyńsky, 2005).

2.3.3.4. Surgical treatment

There may also be surgical options for tinnitus. An example of this is the case of stapedectomy or stapedotomy in patients with otosclerosis which may improve moderate or severe subjective tinnitus (Chang & Cheung, 2014). In cases of vascular-nervous compression, microvascular decompression in the initial stages of tinnitus up to the fourth year may be beneficial (De Ridder et al., 2010). Gritsenko, Caldwell, Shaparin, Vydyanathan, & Kosharskyy, (2014) describe a clinical case of a 65-year-old patient with left cervical pain and homolateral tinnitus who referred with ablation of the medial (C2-C3) branches of the dorsal branch on the side where the patient felt tinnitus. Cochlear implants may be indicated for tinnitus with severe or profound SNHL, especially for older patients (Kim et al., 2013). Brainstem auditory implants, especially for individuals who have had both VIII cranial nerves destroyed by trauma or bilateral schwannomas, have also been showed to reduce the intensity of tinnitus (Kaltenbach, 2006). However, the use of these devices is not free of drawbacks, inherent to their economic value, surgical risk and possible loss of any residual function in the affected ear (Kim et al., 2013). Because tinnitus is often associated with hearing loss at high frequencies, middle ear implants can also be an alternative as they achieve greater acoustic gains directly in the cochlea in this frequency region, promoting relief in the perception of tinnitus (Biesinger & Mazzoli, 2011). In cases of unilateral hearing loss bone-anchored-hearing-aids can be a useful solution. On the other hand, Zenner and colleagues (2016) reviewed the literature on these devices, and found poor evidence of their success in the treatment of chronic tinnitus due to inconsistent methodologies of studies and sample sizes.

The use of low-intensity laser therapy, with commercially available equipment, which has one hundredth the power of lasers used in surgery, has been used to accelerate the recovery of peripheral nerve lesions, soft tissue lesions and to reduce inflammatory processes and pain (Baguley et al., 2013; Kleinjung, 2011). However, so far

the results of the clinical investigation does support the use of laser therapy, although this therapy has been used for about 20 years in patients with tinnitus (Kleinjung, 2011).

2.3.3.5. Physiotherapy Treatments

In this therapeutic modality we can consider therapies for the treatment of diseases of the temporo-mandibular joint, myofascial trigger points, dental treatments, chiropractic and muscle relaxation of the head and neck chains. Many of these options may be effective in controlling somatosensory tinnitus, especially if it is of mild-moderate intensity.

There is intense debate about the efficacy of cervical spine therapy, which is defined by Michiels and colleagues (2015) but contested by Bhatt and colleagues (2015). It is expected that new scientific studies can deepen the relevance of this form of treatment for chronic subjective tinnitus.

Functional dental therapy was also recommended for the treatment of chronic tinnitus by Buergers and colleagues (2014).

2.3.3.6. Psychological treatment and Cognitive Behavioral Therapy

More recently, the first tests were designed to treat tinnitus through virtual reality immersion (3D auditory and visual environments), with a success rate equivalent to cognitive therapies. In this case, virtual reality seems to regulate neural plasticity (Malinvaud et al., 2016)

Patients with decompensated chronic tinnitus, particularly subjective tinnitus, present a high discomfort and very high comorbidities with psychological or psychiatric alterations. Hence their evaluation and immediate interventional response should be considered, including by a mental health professional (D'Amelio, Archonti, Falkai, Plinkert, & Delb, 2004).

The main purpose of psychological therapy in tinnitus is to reduce the negative impact on the individual's life by acting on the negative thoughts or cognitive distortions associated with to tinnitus (Searchfield, Magnusson, Shakes, Biesinger, & Kong, 2011). The therapist and patient work together in a way to identify these cognitive distortions for the patient to construct a more logical and rational meaning, reducing the occurrence and level of discomfort associated with them, in a counseling process.

CBT based on methods of psychology, are defended as therapeutic options referred to by several authors and recently, strong scientific evidence (Cima et al., 2014). The CBT approach intends to reduce patient’s emotions regarding his tinnitus, improving

daily life. Because this therapy does not focus on tinnitus pathophysiology it does not reduce the intensity of tinnitus (Grewal et al., 2014), but it is highly recommended to control tinnitus related distress and anxiety (Cima et al., 2014). This effect persists long term (Goebel, Kahl, Arnold, & Fichter, 2006). CBT is based in cognitive restructuring and behavioral modification, including mechanisms of physichopsycho-education, relaxation, mindfulness training and control of attention techniques (Langguth et al., 2013). Because this modality has many dropouts, an internet-based model of self-help CBT was created. It is known that many tinnitus patients perform internet searches in attempts to get information. It has been found a significant improvement at 3 months follow-up of this self-help modality (Andersson & Kaldo, 2005).

Although reported since the 1980's, 'mindfulness-based therapies' have only recently been actively applied to tinnitus treatment, especially for chronic tinnitus (Ludwig & Kabat-Zinn, 2008). It is one of the sub-categories of behavioral therapies that intends to manage negative feelings, stress and anxiety in tinnitus patients. In parallel, acceptance and commitment therapy has already given the first steps, always in connection with other behavioral therapies. Both reveal a strong potential in chronic tinnitus treatment (Cima et al., 2014), nevertheless there are still no comparative studies and standardized methodologies for a systematic evaluation of these therapies. In fact, there are still no systematic comparative studies among several therapies of psychological counselling (Zenner et al., 2016). There is an urgent need for standardization of assessment methodology to allow progression of studies (Grewal et al., 2014). The same is said for acoustic therapies (with sound or music) (Zenner et al., 2016).

2.3.3.7. Transcranial Magnetic Stimulation

One of the oldest tinnitus therapies is based on electrical stimulation. Besides the persisting droughts regarding its efficiency it has been used regularly for tinnitus suppression since the 1960's (Lee et al., 2014). This method seems to present higher success rate for low frequency type of tinnitus and when there is moderate hearing loss (Lee et al., 2014). Repetitive transcranianal magnetic stimulation has been a therapeutically option for chronic tinnitus (Figure 2.3-3), regardless of tinnitus location or psychological patient's status (Rossi et al., 2007). This is less painful than electrical transcranianal stimulation (Kleinjung, Steffens, Londero, & Langguth, 2007). However, the information regarding long-term results is scarce (Lehner et al., 2015). The placebo effect of this therapy still confuses the researchers (Rossi et al., 2007). On the other hand, recent studies failed to present benefic results for subjective chronic tinnitus patients. Hence most recent tinnitus guidelines recommend against this treatment modality (Tunkel et al., 2014).

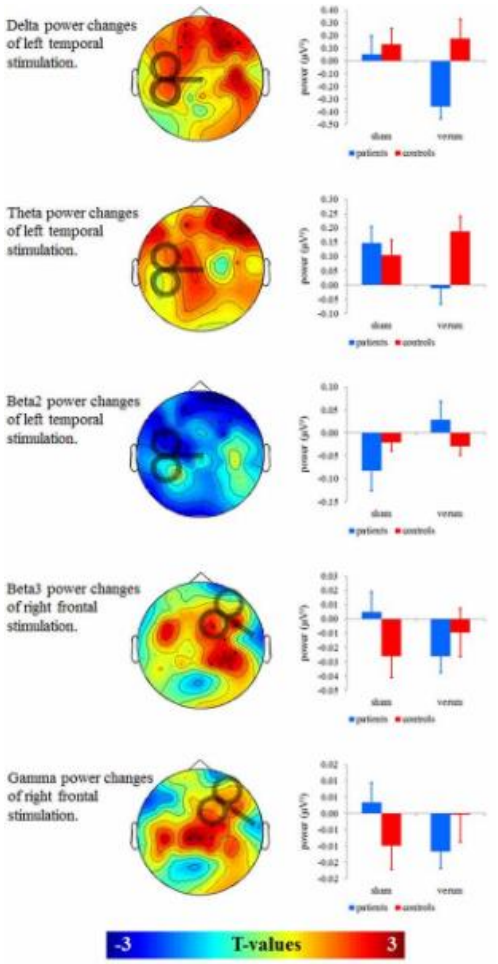

Figure 2.3-3 - Transcranial Magnetic Stimulation in a patient with chronic tinnitus compared to a control group.
(Schecklmann et al., 2015)

Final Considerations

It is common to use distraction strategies and techniques of attention control in these patients, as well as relaxation training, in order to reduce the excessive attention that patient pays to tinnitus and help him to focus and concentrate in other activities. This includes strategies of sound enrichment complemented by other therapeutic procedures (Greimel & Kröner-Herwig, 2011).

Fuller and collaborators presented in their systematic review, six proposals of shared therapies from the analyzed tinnitus guidelines (Fuller, Haider et al., 2017). Overall, patients should have access to the information regarding available therapeutic options; the use of hearing aids can improve tinnitus in patients with hearing loss and cognitive behavioral therapies are useful. Moreover, there is still controversy regarding the efficacy of TRT. It is agreed that drugs are ineffective for tinnitus, except for the treatment of accompanying comorbidities such as anxiety or depression. In summary,

treatment goals should be realistic in order to prevent feelings of disappointment and depression in patients, whose own coping skills regarding tinnitus certainly influence the treatment outcome (Møller, 2016).

Due to the huge diversity of tinnitus etiologies it is important to appropriately perform clinical meaningful tinnitus subtyping, in order to identify potential treatable causes and obtain the appropriate tinnitus management for each patient. In order to perform this task multidisciplinary guidelines for tinnitus diagnosis and treatment are essential.

The assessment of the individual with tinnitus and therapy strategies should be multidisciplinary to ensure coverage of all dimensions of the patient. Moreover, therapeutic strategies should be tailored to the individual, after proper information and with respect for patient choice and individual needs.

3. Objectives

3.1.General Objective

This study aims to evaluate the contribution of inflammatory, immunological or genetic factors in the etiology of tinnitus in patients with or without hearing loss.

3.2. Specific Objective

- To evaluate the existence of differences in the tonal and vocal audiogram in the four subgroups (according to having or not tinnitus and hearing loss).
- To determine by auditory brainstem responses distinctive factors in the subgroups.
- To determine by distortion product otoacoustic emissions distinctive factors in the subgroups.
- To evaluate the quality of life of these subgroups (using MOS SF-36, and in tinnitus groups THI).
- To assess the susceptibility to anxiety and/or depression (BSI).
- To evaluate in the peripheral blood parameters of inflammation and immunity (HSP-70, IL1-alfa, IL1-beta, IL2, IL-6, IL10, TNFalfa, IFNgamma e TGFbeta) in the subgroups.
- To determine the correlation between phenotype and genotype (*NAT2* and *GRM7*).

4. Methods

4.1. Ethic Commission and National Committee for Personal Data Protection

The study was drafted in accordance with the Declaration of Helsinki for the Protection of Human Subjects, one of the principles of medical research and on the evolution of Europe, the World Health Organization and the Community Involves beings, as well as the constant DL 43/04 of 19 August, DR I Series.

The study protocol was approved by the Ethics Committees of the Hospital Cuf Infante Santo and Faculty of Medical Sciences of the New University of Lisbon (number:

65/2014/CEFCM) (Appendix I and II). All participants signed the Informed Consent (Appendix III).

The study protocol was approved by the National Committee for Personal Data Protection (number: CNPP 1637/2016) (Apendix IV).

4.2.Study methodology

Transversal cohort study to analyze the etiological factors of tinnitus in a population aged between 55 and 75 years, with or without hearing loss.

4.2.1. Study population and sample size calculation

The population was composed of individuals between the ages of 55 and 75 years. Participants were recruited at the ENT consultation of the Hospital Infante Santo CUF in the period defined for data collection.

Due to the complexity of sample size calculation in the presence of several primary variables and scarce information regarding the variables (genetic and inflammatory parameters) it was decided to perform the calculation using a pilot sample involved in the study. Therefore, the sample size calculation was based on information about IL1-alfa, IL1-beta, IL2, IL-6, IL10, TNFalfa, IFNgamma regarding 40 participants, to compare groups with tinnitus (n=20) and without tinnitus (n=20). For a power of 80% and a significance level of 95% where obtained very high sample sizes (58 to 1240 in each group) not being possible to reach it because of time and financial restrictions related to the present PhD study.

The organization of subjects in the sample (n = 114 individuals) was performed according to the audiological data being integrated in four separated groups:

- Subgroup I – composed by individuals without hearing loss and without tinnitus (control group);
- Subgroup II – composed by individuals without hearing loss and with tinnitus;
- Subgroup III – composed by individuals with hearing loss and without tinnitus;
- Subgroup IV – composed by individuals with hearing loss and with tinnitus.

These subgroups allowed the comparison of the presence of tinnitus (subgroup II + subgroup IV) with its absence (subgroup I + subgroup III), as well the presence of hearing loss (subgroup III + subgroup IV) with its absence (subgroup I + subgroup II).

4.2.2. Inclusion/Exclusion criteria

We considered in the present study all individuals between the ages of 55 and 75 years, from Hospital Cuf Infante Santo ENT consultation, where it is expected a proportional distribution between sexes. Included volunteers agreed to participate, gave written informed consent, and performed all the required tests. Individuals with or without hearing loss, with or without tinnitus, who did not have any of the exclusion factors were included. For the purposes of inclusion hearing loss was defined as bilateral sensorineural deafness in down slope audiometric pattern, above 1000 Hz with poor speech discrimination (Speech Recognition Threshold (SRT) > 40 dB SPL and 100% discrimination to 60 dB or worse).

The primary evaluation variable was tinnitus, and the tinnitus participants were evaluated by psychoacoustic measurements with an expected tinnitus frequency between 2kHz and 16kHz and at any level of sound intensity.

Exclusion factors included inability to understand and sign the informed consent due to a significant cognitive impairment, or uncompensated medical disorder or a serious psychiatric disorder that requires urgent medical evaluation. Also, individuals over 55 years of age who presented possible factors that may overlap with the study variables were excluded, i.e. Ménière's disease, chronic otitis media, otosclerosis, tinnitus from diseases of the outer ear (e. g. occlusive exostosis, outer otitis), history of massive ototoxic drugs use, massive noise exposure, a history of previous malignancy with chemotherapy, history of autoimmune disorders and neurodegenerative and demyelinating diseases. Individuals who did not attend all clinical evaluation visits were be excluded from the sample.

4.2.3. Outcomes variables

The primary outcome evaluation variable was tinnitus. This study aimed to evaluate the contribution of audiological, inflammatory, or genetic factors (secondary outcome variables) to the etiology of tinnitus in patients with or without hearing loss

4.2.4. Study planning and Institutions involved

The participants were recruited at ENT consultations at the Hospital Cuf Infante Santo.

In the first visit – *Screening* (S), eligibility was assessed. Before being included in the study all participants were given explanation about what the study involved and the purpose of the study before signing the Informed Consent.

Baseline (V0) – this visit took place a few days after screening according to participant availability. It involved a detailed clinical history, and characterization of

hearing loss and tinnitus (if present: date of beginning of the complaint, if was sudden, insidious or gradual) tinnitus characterization (confirm the type (pitch) and number of sound perceptions – tonal, noise, pulsatile or other; if loudness is steady or has fluctuations; if the location is in one ear, both ears or in the head; if the timing is intermittent or continuous), associated factors, and trigger factors. Previous intake of ototoxic drugs was recorded (Appendix V). In this visit a complete ENT observation (microscopic otoscopy, anterior rhinoscopy, oropharynx and head and neck inspection, with stethoscope auscultation in cases of pulsatile tinnitus was performed).

Also, in this visit participants were asked to complete the *Tinnitus Handicap Inventory* (*THI*) – Portuguese validate version (only the tinnitus subgroups) (Oliveira & Meneses, 2008) (Appendix VI), the *Brief Symptom Inventory* (*BSY*) - Portuguese validate version (CANAVARRO, 1999) (Appendix VII), and the Portuguese validated version Medical Outcomes Study Short Form Health Survey (MOS SF-36) (Ferreira, 2000), (Appendix VIII).

In the same visit tonal audiometry standard and high frequency, speech audiometry, auditory brainstem responses and distortion product otoacoustic emissions (all subgroups) and psychoacoustic measurements (only the tinnitus subgrups) were performed.

On the same day all participants had peripheral blood sample collection to evaluate parameters of inflammation and immunity (HSP-70, IL1-alfa, IL1-beta, IL2, IL-6, IL10, TNFalfa, IFNgamma e TGFbeta) and genetic analysis.

Participants attended a final visit, at the end of the study, in which they were informed about the obtained results, and given final recommendations regarding their complaints and preventive measures.

This study was realized at **Hospital Cuf Infante Santo**, in the ENT departament, in collaboration with the **Immunology** and **Biostatistics departments of Nova Medical School** and also **BioISI** – Biosystems & Integrative Sciences Institute Deafness group from **Science Faculty of Lisbon University.** The genetic parameters were obtained from the collaboration with their study 'Age-related hearing loss: genetic susceptibility and social impact'.

5. Tinnitus as a health problem

5.1. Tinnitus in the patient's perspective

In our study we have tried to increase knowledge concerning tinnitus while a health problem and from the patient's perspective and their significant others (Haider, Fackrell, Kennedy, & Hall, 2016). With this purpose we have performed a systematic review that was also included as the initial phase roadmap of the TINNET outcomes measures group of TINNET. This work allowed to detail more insights regarding the different dimensions of tinnitus symptom and its repercussions on the patient's daily life. With a similar objective another study performed a retrospective qualitative analysis anonymized clinical data from patients who attended a UK tinnitus treatment centre between 1989 and 2014. Content analysis was used to code and collate the responses of 678 patients to the clinical interview question 'Why is tinnitus a problem?' into categories of problems (domains).

The obtained results allowed to define a pool of domains that was the basis for developing COS for early-phase clinical trials of sound-, psychology-, and pharmacology-based interventions to manage chronic subjective tinnitus in adults: the COMIT'ID study using a Delphi process and face-to-face meetings to establish consensus (Hall, Smith, Heffernan & Fackrell, 2018b; Smith et al., 2018).

5.1.1. Why is Tinnitus a Problem? A Qualitative Analysis of Problems Reported by Tinnitus Patients

Submitted to Trends in Hearing

Emily J. Watts, Kathryn Fackrell, Sandra Smith, Jacqueline Sheldrake, Haúla Haider, Derek J. Hoare

Abstract

Objectives

Tinnitus is a prevalent complaint and people with bothersome tinnitus can report any number of associated problems. Yet to date only a few studies, with different populations and relatively modest sample sizes, have qualitatively evaluated what those problems are. Our primary objective was to determine domains of tinnitus problem according to a large clinical dataset.

Design

This was a retrospective analysis of anonymised clinical data from patients who attended a UK tinnitus treatment centre between 1989 and 2014. Content analysis was used to code and collate the responses of 678 patients to the clinical interview question 'Why is tinnitus a problem?' into categories of problems (domains).

Results

We identified 18 distinct domains of tinnitus-associated problems. Reduced quality of life, tinnitus-related fear, and constant awareness were notably common problems.

Conclusion

Clinicians need to be mindful of the numerous problem domains that might affect their tinnitus patients. Current questionnaires, as well as being measures of severity, are useful clinical tools for identifying problem domains that need further discussion, and possibly measurement with additional questionnaires. The domains identified in this work should inform clinical assessment and the development of future clinical tinnitus questionnaire.

Keywords: Psycho-social/Emotional; Behavioral Measures; Adult or General Hearing Screening

Introduction

Often described as a ringing, whistling or buzzing sound, tinnitus is a complex and diverse symptom, defined as the perception of a sound that has no external source (McFadden, 1982). For some, the experience of tinnitus goes beyond the 'phantom' sensation of sound. It can cause problems such as insomnia, difficulty concentrating, and poor psychological well-being, ultimately decreasing symptom-specific health-related quality of life (Langguth & Landgrebe, 2011; Nondahl et al., 2011; Pierce et al., 2012; Stevens, Walker, Boyer, & Gallagher, 2007; R. S. Tyler & Baker, 1983; Hall et al., 2018). The impact of tinnitus on a person can range from mildly problematic to completely debilitating with significant social and economic consequences (Andersson, 2002; Stockdale et al., 2017). Of the 10% of the general population who experience chronic tinnitus (Landgrebe et al., 2012; McFerran & Phillips, 2007) 20% experience 'clinically significant tinnitus' and seek relief from their symptoms (Henry, Jastreboff, Jastreboff, Schechter, & Fausti, 2003). However, management of tinnitus can be complex, requiring an individualised and often multifaceted approach to reduce symptoms and associated co-morbidities (Decot, 2005; Department of Health, 2009; Hoare, Gander, Collins, Smith, & Hall, 2012).

Psychoacoustic estimates of tinnitus provide little information on its impact and the associated problem symptoms (Andersson, 2002; Jakes, Hallam, Chambers, & Hinchcliffe, 1985). Typically, clinicians and researchers alike rely on multi-attribute self-report questionnaires to measure tinnitus severity and identify appropriate management pathways. For example, tinnitus questionnaire items can ask about particular difficulties with concentration, sleep, coping, and emotional wellbeing.

Negative consequences or limitations of tinnitus can be categorised into 'domains' that are theoretically similar or contribute to a specific aspect of tinnitus distress or annoyance, and many tinnitus questionnaires provide measures, to varying degrees, of different problem domains associated with tinnitus. Patient interviews, to assess the effects of treatment for example, can be structured around what are considered important domains (Andersson & Edvinsson, 2008). To belong to the same domain, consequences or limitations would have to produce a sufficiently similar effect on the patient such that questionnaire items could logically be combined to measure a specific problem caused by tinnitus. There is however, no universal agreement on what these domains are, how many domains of tinnitus problem there are, or how these domains should be assessed (Baguley & Andersson, 2003; Hall et al., 2018). For example, the Tinnitus Handicap Questionnaire (THQ; Kuk, Tyler, Russell, & Jordan ,1990) assesses handicap in relation to psychological and auditory problems, whilst the Tinnitus Handicap Inventory (THI; Newman, Jacobson, & Spitzer, 1996) probes problems with function, emotion, and catastrophizing. One of the more recent multi-attribute questionnaires to be developed is the Tinnitus Functional Index, (TFI, Meikle et al.,

2012). The TFI was specifically developed to cover multiple distinct problem domains (intrusiveness, sense of control, cognition, sleep, auditory, relaxation, quality of life and emotional impact of tinnitus), to measure tinnitus severity, and to be a responsive outcome measure (Meikle et al., 2012). More recent still, the Tinnitus Primary Function Questionnaire (TPFQ, Tyler et al., 2014) was developed to measure *"the primary ways tinnitus impacts on a person's life"* (p.261) with domains covering problems with sleep, hearing, concentration, and emotions.

Whilst many of questionnaires drew heavily on previous questionnaires when selecting items or potential domains to include, the true starting point to developing any questionnaire is to identify and understand what the problems that need to be measured are. This is something that can only be answered by people who experience tinnitus and might include not only problems that arise because of tinnitus, but also problems that patients confuse with or ascribe to their tinnitus (e.g. consider hearing difficulties or cognitive problems as tinnitus problems when they are more likely due to an unacknowledged hearing loss). Tyler & Baker (1983) surveyed 72 members of a Nottingham-based tinnitus self-help association asking why tinnitus was a problem to them. Respondents had an average age of 61 years (SD = 13.1), 66% were women, 34% were men, and the mean age at onset of tinnitus was 51.9 years (range = 9-73). On average, respondents reported 4.6 difficulties due to tinnitus (range = 1-13) with fewer difficulties being reported by those who had experienced tinnitus for a longer time. The 31 problems reported were grouped into four main domains: (1) 'Effects on hearing' including problems understanding speech and television, listening to the radio, appreciation of music, use of the telephone, localisation of sounds, and listening to environmental sounds; (2) 'Effects on lifestyle' including problems getting to sleep, persistence of tinnitus, worsening upon waking, requiring/avoiding noisy situations, and conversely requiring/avoiding quiet situations, withdrawing from/avoiding friends, family problems, interference with work, difficulty learning to drive, and explaining tinnitus to others; (3) 'Effects on general health' including dependence on drugs, pain and headaches, giddiness/imbalance, general ill health, ineffectiveness of drugs, tiredness, and ineffectiveness of tinnitus masker; (4) 'Emotional problems' including despair, frustration and depression, annoyance, irritation and inability to relax, difficulty concentrating, confusion, insecurity, fear and worry, and consideration of suicide. The most commonly reported problems in that study were 'getting to sleep', and the 'persistence of tinnitus'. Tyler & Baker acknowledge that there may have been some bias in their dataset towards patients who suffer more severely as all respondents were members of a tinnitus self-help group. The same survey was subsequently used by Sanchez & Stephens (1997, 2000) to assess why tinnitus was a problem amongst a population of 436 tinnitus clinic patients (mean age of 57 years, range 14-92 years, 51% women, 49% men), at baseline and at follow-up (1.5 to 5 years later). In this study, all respondents were patients attending a tinnitus clinic for the first time. Duration of tinnitus ranged from 1 month to 70 years, and 394 (90.1%) had some degree of hearing

loss. They reported, on average, 3.78 distinct problems (range = 1-12). Younger patients (those under 50 years) reported more problems. Thirty distinct problems were reported, many of which were common to Tyler & Baker (1983), but Sanchez & Stephens (1997, 2000) determined there were five problem domains; sleep, auditory, health, situational, and psychological problems. The most common problems were 'hearing difficulties' and 'sleep difficulties'. More recently, Manchaiah et al. (2018) took a deductive approach to quantify tinnitus-related problems in a population of 240 tinnitus research volunteers (Mean age of 57 years, 57% men, 43% women), using the International Classification of Functioning, Disability and Health (ICF) framework (World Health Organization, 2001). Most but not all reported problems could be classified according to the framework. The most commonly reported problems were coded as 'emotional functions' and 'sleep functions'.

Therefore, to date there have been three studies evaluating problem domains associated with tinnitus, in different populations, and with relatively modest sample sizes. Here we performed a retrospective analysis of anonymised clinical data from 678 patients who attended a tinnitus treatment centre in the UK. The primary aim was to identify the domains of tinnitus problem according to this large patient population.

Materials and Methods

This study was a retrospective analysis of anonymised data that had been routinely collected from patients attending the Tinnitus and Hyperacusis Centre (London, UK) between 1989 and 2014. Data use and analysis complies with the governance procedures of the data controller (JS).

Data collection

The Tinnitus and Hyperacusis Initial Interview Form (Jastreboff & Jastreboff, 1999) was completed by an audiologist (JS) during the first consultation to assess each patient's suitability for Tinnitus Retraining Therapy (TRT). The interview includes questions on tinnitus laterality, constancy, percentage awareness and annoyance, and the degree of severity, annoyance and effect on life experienced over the last month (using a 0-10 rating scales) and a single question asking patients to say "Why is Tinnitus a Problem?" in one sentence. For this question, the audiologist recorded the exact wording of patients' responses. For example one patient responded with "sleep disturbance is a problem, apprehension and waking sleeping". The same questions were asked about sound tolerance and hearing loss, if indicated. A further question used a 0-10 rating scales to determine the degree to which each complaint (tinnitus, sound level tolerance, and hearing loss) is a life problem. For the present study, we were primarily interested in the patients' recorded responses (free-text) to the single question: *"Why is Tinnitus a Problem?"*

Participants

The responses from 678 patients to the question *"Why is Tinnitus a problem?'* were analysed.

Content analysis of free-text data from responses to "Why is Tinnitus a Problem?"

Free text responses were analysed using a conventional content analysis approach, i.e. information was collated directly from patients' responses without imposing pre-existing categories or theories (Hsieh & Shannon, 2005). Hence the goal of content analysis here was to provide knowledge and understanding of the phenomenon under study, i.e. why tinnitus is a problem, through the subjective interpretation of text data using a systematic process of coding and identifying themes in the data. Patient responses given to the question *"Why is tinnitus a problem?"* were in general short, such as "can't control it" or "cannot work". To avoid any misinterpretation of meaning that could occur due to a lack of context, we coded responses using only what was written rather than what was implied (Elo & Kyngäs, 2008).

Content analysis was conducted by EW (all data), KF, SS, and DH (each analysed one third of responses, allocated using a random number generator: https://www.random.org/) First, authors independently analysed their assigned dataset. This involved data familiarisation (reading and rereading) and the extraction of any meaningful initial units (problem codes) from each response. Meaningful units constituted parts of a sentence, a whole sentence, or a passage of text that pertains to the same topic, and had to contain enough information to allow meaningful interpretation with respect to the research question. Two authors assessing the same dataset met to examine and discuss their independently extracted problem codes. Each author presented their interpretations of the data, rotating who presented first in each meeting to ensure that no one author led the identification of problem codes. Any disagreements regarding these codes were discussed until consensus was reached or the other authors were consulted to reach a majority decision. To ensure consistency of coding across all pairs of authors, one author (EW) was involved in coding the entire dataset. Finally, the extracted 994 problem codes were reviewed and categorised by four authors (EW, KF, SS, DH) into domains that were considered representative of the themes emerging from the problem codes. This was an iterative process involving (i) data familiarisation of all problem codes involved all authors reading all codes; (ii) identification of potential conceptual labels (domains) based on data familiarisation. Any of the four authors could suggest a domain that they believed was representative of the data, but the domain was only included if all four authors agreed that it reflected the content of the problem codes; (iii) allocation of each problem code to a relevant domain, continuing until all codes were allocated to a domain (Elo & Kyngäs, 2008;

Graneheim & Lundman, 2004). These initial domains were then refined by all authors, checking for commonality or overlap between the content of the codes that were allocated to different domains, whether codes should be reallocated to a different domain, whether domains should be merged together, or whether there was more than one domain emerging from the group of problem codes allocated to the same domain. For example, the problem codes grouped under initial domains described as 'Distraction' and 'Concentration', were sufficiently similar to combine and form a single domain, subsequently described as 'Inability to Concentrate'. Initial domains described as 'Anger', 'Frustration' and 'Stress' were combined to form an 'Emotional Reaction to Tinnitus' domain. This iterative process continued until every problem code and domain was deemed valid, with each code only being allocated to one domain (Elo & Kyngäs, 2008).

In a final validation step, audiologist members of the British Society of Audiology tinnitus and hyperacusis special interest group (who were not authors or otherwise involved in the project) checked that the 994 extracted problem codes and domain grouping captured the essence of the raw data and represented the core themes from the data. The original raw data responses, the extracted problems codes and associated domains, and codes (25) removed for being ambiguous were examined and discussed. Each domain was discussed in turn with further refinements made through an iterative process and the domains were finalised. One major revision to the domains involved codes initially associated with the domains 'inability to relax', 'effect on social life', and 'effect on work' being combined under a new domain named 'reduced quality of life'. Clinicians in the focus group felt that the codes associated with these initial domains were all simply different ways in which patients express tinnitus as having a general consequence for their quality of life. They considered the initial domain were not clinically meaningful and were all meaningfully captured as 'reduced quality of life'.

Secondary analyses

Because many patients reported more than one tinnitus-related problem we examined the degree to which problem codes related to different domains co-occurred within individual responses. Hierarchical cluster analysis was conducted in PAST version 3.06 (Hammer, Harper, & Ryan, 2001). In this analysis the likelihood of reporting different set of tinnitus related problems were estimated as Euclidean distances between problems when plotted per patient in an 18-dimensional space (representing the 18 domains in our data). We were interested in whether any of the domains identified more consistently grouped together, i.e. patients with problem *x* also generally report problem *y*. This would indicate tinnitus problems likely to co-occur, or potentially that there is redundancy of a domain, i.e. domain *x* and *y* are the same thing.

Results

Subjects

Of the 678 patients reporting reasons *why* tinnitus was a problem, the mean age was 57.2 years (SD = 14.0), 64% (432) were male and 36% (245) were female. Two-hundred and eighteen patients reported unilateral tinnitus, 302 reported bilateral tinnitus, and a further 90 reported hearing their tinnitus in their head (n=610, missing = 68). Over 70% of patients (n=503, missing = 83) reported fluctuations in their tinnitus, and 56% (n=382, missing =40) report sudden onset of tinnitus.

Percentage of time aware of tinnitus over the last month ranged from 0-100% (mean = 56%, SD = 30, n= 652). Percentage of time annoyed by tinnitus over the last month also varied from 0-100% of the time (mean = 39%, SD = 27, n=580). Two-hundred and sixty-five patients reported previously trying treatments for tinnitus, with the average number tried being two. However many patients had tried none (n=413), and some had tried as many as five.

Of the 678 patients reporting problems, 252 patients reported hearing problems (missing = 20), yet only 54 used hearing aids. When asked to rate severity, annoyance, and effect of tinnitus on life over the last month on 0-10 point scales, patients averaged 4.4 (SD = 2.3), 6.4 (SD = 2.5), 5.2 (SD = 2.8) respectively (n=669). When asked to rate how problematic tinnitus, sound tolerance, and hearing loss were on a 0-10 point scale, patients averaged 5.6 (SD = 2.8, n=649), 4.5 (SD = 3.0, n=353), and 3.0 (SD = 2.9, n=387) respectively.

Data Analysis

Four hundred and forty patients reported only one problem, 207 reported two problems, 30 patients reported three problems, and one patients reported four problems. The 994 problem codes were grouped, refined and finalised into 18 domains of tinnitus-related problem (Table 5.5-1).

Table 5.1-1 - The 18 domains of tinnitus handicap, the number of and examples of relevant codes

Domain number	Domain name	n codes in sample	Example codes
1	Reduced quality of life	125	Spoiling life; Interferes with everything, functional through emotional
2	Fear	107	Scares the life out of me; Fear of it always being there in future; I perceive it as a threat
3	Constant awareness	99	The focus of my life; Always there; constant sound
4	Annoyance	87	Annoying; Constant irritation; irritability; noise is really bothering me
5	Inability to concentrate	81	Wants all my attention; Cannot concentrate
6	Loss of quiet	72	Feel it will never be quiet again; Impacts my quiet time
7	Feeling deficient due to tinnitus	63	Wants to be perfect; Feels damaged. Based on own measure of before and after.
8	Loss of control	53	No choice; Can't do anything about it; don't have control over it; a problem I cannot solve
9	Effect on sleep and alertness	50	Difficult to sleep; Wake up tired
10	Emotional consequences of tinnitus	49	Very distressing; Makes me feel as if I'm falling apart
11	Effect on listening	44	Affects hearing of what's around; Noise gives me less opportunity to hear
12	Emotional reaction to tinnitus	39	Stresses me out; Driving me mad
13	Loss of sense of self	31	There and it wasn't before; Changing my personality
14	Physical Effects of Tinnitus	22	Makes me feel unwell; Tiring
15	Unpleasantness of Percept	22	Too loud to handle; Sharp frequency
16	Intrusiveness	19	Constantly invasive; Intrusion in my head
17	Need for Knowledge	17	Why do I have it?; Will it always be there?
18	Loss of Peace	14	Shattered my peace; Stops me finding peace

The domains 'Reduced quality of life' and 'Fear' included the highest numbers of problem codes, (125 and 107 respectively), whilst 'Need for knowledge' and 'Loss of peace' had the fewest (17 and 14 codes respectively) (Table 5.1-1). Cluster analysis revealed an apparent independence of the problem domains we defined (Figure 5.1-1). the smaller the 'distance' between domains the more common it was for these domains to be reported together by the same patient. Hence, 'Loss of peace' and 'Need for knowledge' were most commonly reported together, whereas 'Quality of life' and 'Constant awareness' were least often reported together.

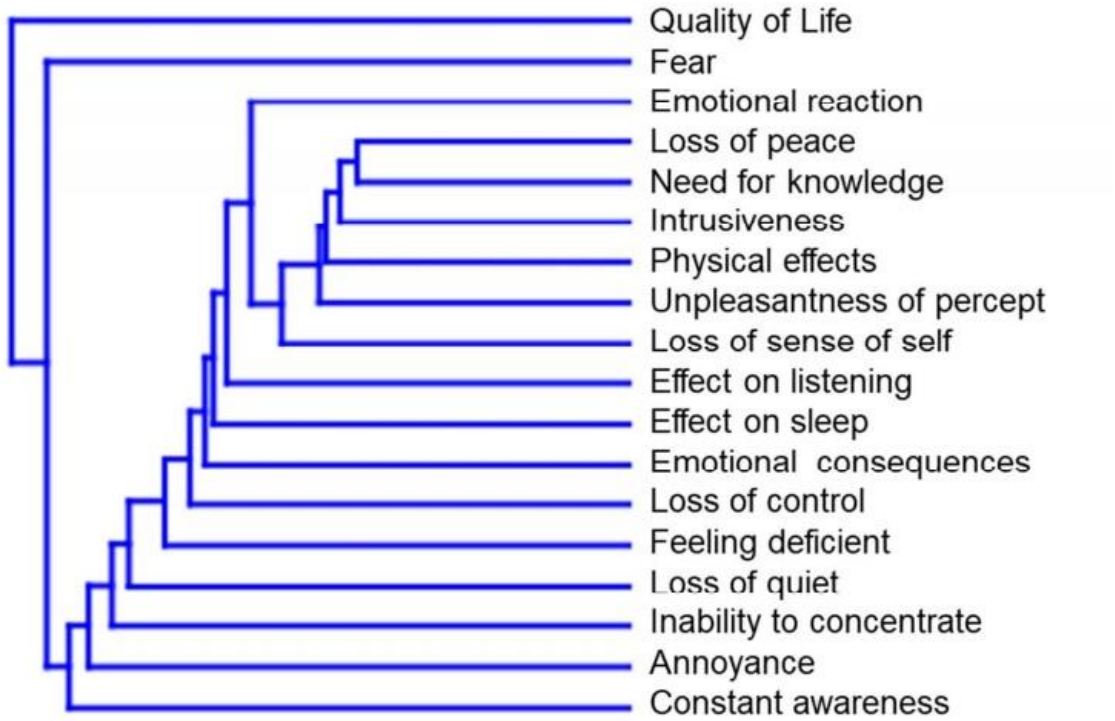

Figure 5.1-1 - Cluster analysis indicating relatedness of tinnitus problems within the responses from individual patients. Distances are Euclidean distances in an 18 dimensional space.

Discussion

In this largest study of its kind to date, we examined why tinnitus is reported as a problem in a clinical population. A retrospective analysis of data from 678 patients attending a tinnitus and hyperacusis clinic, whom identified one or more reasons why tinnitus was a problem, led us to identify 18 distinct problem domains.

Strengths and Limitations

Due to the subjective nature of qualitative research the involvement of four analysts, and our stepped process of identifying and verifying domains, supports there being rigour in the analytical process. The sample in this study is representative of a typical tinnitus patient population in terms of age and gender. However, patients were attending a private clinic, which would indicate at least some patients are from a more affluent socio-economic group than may have been represented if this study had been completed through a National Health Service clinic for example, where care is free at point of access. Variables such as tinnitus severity may differ between our sample and that in other populations and influence what is reported as a problem. If for example, our sample had more severe tinnitus then they would more likely have co-morbid anxiety or depression and potentially ascribe additional problems to their tinnitus (Bhatt, Bhattacharyya, & Harris 2017). Furthermore, the sample includes patients

attending the clinical over a 25-year period. It may be that some problems domains are much less an issue now than in previous decades.

A limitation of the Tinnitus and Hyperacusis Initial Interview Form may be that it is not a self-report questionnaire. It was completed by the clinician during initial consultation, whom recorded the exact wording of the patients' responses. However, the consultations are not audio recorded and as consequence the free-text responses cannot be verified. That being said, all forms were completed by the same clinician, so there is no discrepancy in user completion. There was also a small amount of demographic data missing.

It is acknowledged that reporting a reason why tinnitus is a problem does not imply causation or any relation between tinnitus and the problem reported at all. It may simply be that the problem is ascribed or confused with tinnitus. A full clinical evaluation is therefore indicated to disambiguate, for example, hearing problems due to hearing loss and those incorrectly ascribed by the patient to their tinnitus (Henry et al., 2015). That may be well be an issue in the current dataset given only a minority of those who reported hearing problems also reported using hearing aids.

Why tinnitus is a problem?

Of the 18 problem domains identified, the most common, collectively accounting for 53% of the total codes, were 'Reduced quality of life', 'Fear', 'Constant Awareness' 'Annoyance' and Inability to Concentrate'. Here we discuss evidence of those domains in the literature.

Reduced quality of life, emerged here as the commonest problem domain. This is in part due to its breadth; based on the clinician focus group it came to include a number of initial smaller domains relating to the effects of tinnitus on work, social life, and relaxation. It refers therefore to a general degradation or 'spoiling' of the quality of daily activities and experiences, relating most closely to the 'Effects on lifestyle' domain described by Tyler & Baker (1983). As a construct, quality of life is widely discussed in the tinnitus literature (Erlandsson & Hallberg, 2000; Nondahl et al., 2007; Härter, Maurischat, Weske, Laszig, & Berger, 2004). As a term it is broad ranging, from use to mean general wellbeing of individuals and society, to health specific, where for tinnitus it is used to describe tinnitus questionnaires quite generally (i.e. as measures of tinnitus-specific health-related quality of life). However, it also appears as a distinct construct of tinnitus problem as a subscale in the TFI, which has been shown to measure a different construct to general quality of life (Fackrell, Hall, Barry, & Hoare, 2016). In a similar fashion to how this domain emerged in the current study, in developing the TFI quality of life subscale, domains initially considered distinct termed 'Social Distress', 'Leisure', and 'Work' domains were grouped to form a single broad subscale (Meikle et al., 2012). As a domain therefore quality of life provides a subscale but not one that in itself is, for

example, indicative of the need for a particular intervention. Rather it is only useful as general marker of tinnitus problem level.

Fear, is a domain that has previously been underrepresented in analyses, questionnaires, and therapies. It includes, for example, a fear of the tinnitus itself, or a fear for a future with tinnitus, or fears of activities or sounds somehow making the tinnitus worse. Fears of the unknown are more specifically considered anxieties given they relate to imprecise or unknown threats (Öhman 2008). However, here we need to distinguish fear as a tinnitus domain from the construct of anxiety. Although reported as an issue by 12 of the 72 respondents (16.6%), fear is not featured as an individual problem within Tyler & Baker (1983). It is included within their 'Emotional Problems' domain, as part of the problem: 'Insecurity, Fear and Worry'. Sanchez & Stephens (1997, 2000), who build on Tyler & Baker's work, do not report fear as a specific problem domain. None of the tinnitus questionnaires mentioned previously explicitly use questions that would measure tinnitus-related fear. These questionnaires were developed prior to this study, and are based on the domains established by earlier works, which similarly do not single out problems related to fear. More recently developed tinnitus questionnaires either provide a composite measure of tinnitus-related fear, the Fear of Tinnitus Questionnaire (FTQ; Cima, Crombez, & Vlaeyen, 2011), and the Tinnitus Fear-Avoidance Cognitions and Behaviors Scale (T-FAS; Kleinstäuber et al., 2013), or include a number of relevant items, e.g. the Self-efficacy for Tinnitus Management Questionnaire (STMQ; Smith & Fagelson, 2011).

Fear is proposed to be a key factor in the maintenance of chronic tinnitus distress by Cima et al. (2011), as measured by a self-devised 'Fear of Tinnitus Questionnaire', developed for their study and yet to be validated. Cima et al. (2011) propose a fear-avoidance model for tinnitus, based on a model originally proposed for pain (Vlaeyen & Linton, 2000). This model predicts that the less tinnitus is experienced as a threat, the more accepted it becomes. Based on this concept, Cima et al. (2011) developed a Cognitive Behavioural Therapy (CBT) based treatment that includes elements of Tinnitus Retraining Therapy (TRT), with the aims of decreasing patients' fear of tinnitus and correcting their *"dysfunctional beliefs"* (p.1958) about tinnitus. Whilst their treatment has shown some benefit for patients in terms of reduced handicap even though the tinnitus percept might not have changed, the precise mechanism of benefit, e.g. extinction of fear, cognitive restructuring, requires evaluation using valid and specific questionnaire measures.

Constant awareness of tinnitus as a problem is not explicitly featured in previous studies, perhaps because awareness is considered to be an implicit problem. However, the codes in our study demonstrated that being aware of their tinnitus was, for many patients, their main issue. Reflective of this, there has been a growing recent interest in

mindfulness and acceptance based interventions for tinnitus management (Hesser, Westin, Hayes, & Andersson, 2009).

Awareness is captured to more or less a degree by different tinnitus questionnaires. For example, the THI asks "Do you feel as though you cannot escape your tinnitus?", whereas the TFI includes an explicit question about percentage awareness over the last week within its 'Intrusiveness' subscale. In clinical studies, awareness is sometimes captured through a tinnitus diary over a specified time period (Kröner-Herwig, Frenzel, Fritsche, Schilkowsky, & Esser, 2003; Zachriat & Kröner-Herwig, 2004), or more simply on a percentage of awake time awareness rating (e.g. Molini et al., 2010). Patient reports of awareness in our study were more related to the constancy of awareness; that it was inescapable, or permanent, although there were responses that simply stated tinnitus was a problem because "I'm aware of it". Interestingly in the study by Molini et al. (2010) there was an improvement in their primary measure for most patients after treatment yet percentage awareness did not change. There may be a disparity therefore between 'awareness' and what might make tinnitus clinically bothersome for an individual. Consequently, in terms of awareness, it would seem best to include a measure of it in the context of a multi-attribute questionnaire rather than relying on it alone as a measure of handicap or benefit.

Annoyance in the current study was determined from codes ranging from tinnitus being "a little annoying" to being a "constant irritation". It was included with 'Emotional Problems', as part of 'Annoyance, irritation and inability to relax' by Tyler & Baker (1983). Interestingly, Sanchez & Stephens (1997, 2000) did not report annoyance as one of their problem domains. Yet it does feature in a number of clinical questionnaires. One of the questions of the THQ asks (to what degree) "Tinnitus makes me feel annoyed" (Kuk et al., 1990).Item 3 in the TFI asks *"What percentage of your time awake were you annoyed by your tinnitus?"* This item is pooled with items related to awareness and tinnitus loudness in the 'Intrusiveness' subscale (Meikle et al., 2012). The THI (Newman et al., 1996) does not mention annoyance specifically but does question 'irritability' and 'upset' due to tinnitus.

In the literature, annoyance is acknowledged as an important issue. Hiller & Goebel (2006) comment that tinnitus annoyance contributes heavily to the level of tinnitus severity. Andersson (2002) suggests both psychological and educational aspects to a treatment plan to tackle annoyance. In the present study annoyance was quite consistently coded as tinnitus being annoying or initiating, suggesting it is an important construct to measure routinely and specifically in clinical practice and research.

'Inability to concentrate' was, as with other domains, reported across a spectrum from *"mildly distracting"* to *"permanent distraction"* and *"can't think about anything else"*. Problems of concentration and confusion are grouped under 'Emotional Problems' by Tyler & Baker (1983). Concentration problems ranked as the most common

problem in both studies by Sanchez & Stephens (1997, 2000). A number of questionnaires provide a measure of concentration problem. Conrad et al. (2015) reports the recently developed Tinnitus Cognitions Scale (T-Cog) which they found to provide subscale measures of tinnitus-related catastrophic thinking" and "tinnitus-related avoidance cognitions". The THQ asks (to what degree): "I cannot concentrate because of tinnitus". The THI asks "Because of your tinnitus, is it difficult for you to concentrate?", and "Because of your tinnitus, is it difficult for you to read?" which could be used as further insight into concentration issues. The TFI 'Cognitive subscale' provides a multi-item measure of this seemingly important domain (Meikle et al., 2012). More recently, Bankstahl & Görtelmeyer (2013) published a self-report tinnitus questionnaire specifically to measure the degree of cognitive 'failures and mishaps' that are relevant to performing adequately in daily life. This questionnaire is yet to be widely used.

A number of studies have also explored associations between tinnitus and performance on *behavioural* measures of memory (Hallam, McKenna, & Shurlock, 2004; Rossiter, Stevens, & Walker, 2006; Stevens et al., 2007) or attention (Hallam et al., 2004; McKenna & Hallam, 1999; McKenna, Hallam, & Shurlock, 1995; Stevens et al., 2007). These studies provide mixed evidence of any association and have particular methodological limitations that make further research warranted (Mohamad, Hoare, & Hall, 2016). As a result, a reliable link between performance-based and questionnaire-based measures is yet to be determined.

Conclusions

The current study points to 18 distinct domains of tinnitus problem that need to be considered in tinnitus assessment, and in the development of assessment tools or questionnaire measures of the impact of tinnitus. A single questionnaire of 18 domains would require at least 54 items (Meikle et al., 2012), however this would not be practical for use in every clinical or research situation. Furthermore, patients will not report problems in all 18 domains at pre-intervention assessment for example, making the same questions redundant in a post-intervention assessment. One possible action is to remove any domains considered irrelevant to an individual at pre-intervention, so they are not measured at post-treatment assessment (Tyler et al., 2014). An effective assessment needs to allow patients to express exactly what problems they are having, then more domain-specific questionnaires such as the Fear of Tinnitus Questionnaire (Cima et al., 2011) can be selected.

Current tinnitus questionnaires provide measures of various combinations of the domains identified here, but no single questionnaire covers all domains. A comprehensive measurement of all possible domains identified herein would require a combination of tinnitus questionnaires to be used.

5.1.2. A narrative synthesis of research evidence for tinnitus-related complaints as reported by patients and their significant others

The data presented has been published by Hall, D. A., Fackrell, K., Li, A. B., Thavayogan, R., Smith, S., Kennedy, V., Tinoco, C., Rodrigues, E. D., Campelo, P., Martins, T. D., Lourenço, C. M., Ribeiro, D., & Haider, H. F. (2018) as is possible to see at

Hall, D. A.*, Fackrell, K., Li, A. B.*, Thavayogan, R., Smith, S., Kennedy, V., Tinoco, C., Rodrigues, E. D., Campelo, P., Martins, T. D., Lourenço, C. M., Ribeiro, D., & Haider, H. F.* (2018). A narrative synthesis of research evidence for tinnitus-related complaints as reported by patients and their significant others. *Health and quality of life outcomes*, 16(1), 61.

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REVIEW Open Access

A narrative synthesis of research evidence for tinnitus-related complaints as reported by patients and their significant others

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Abstract

Background: There are a large number of assessment tools for tinnitus, with little consensus on what it is important to measure and no preference for a minimum reporting standard. The item content of tinnitus assessment tools should seek to capture relevant impacts of tinnitus on everyday life, but no-one has yet synthesised information about the range of tinnitus complaints. This review is thus the first comprehensive and authoritative collection and synthesis of what adults with tinnitus and their significant others report as problems in their everyday lives caused by tinnitus.

Methods: Electronic searches were conducted in PubMed, Embase, CINAHL, as well as grey literature sources to identify publications from January 1980 to June 2015 in which participants were enrolled because tinnitus was their primary complaint. A manual search of seven relevant journals updated the search to December 2017. Of the 3699 titles identified overall, 84 records (reporting 86 studies) met our inclusion criteria and were taken through to data collection. Coders collated generic and tinnitus-specific complaints reported by people with tinnitus. All relevant data items were then analyzed using an iterative approach to narrative synthesis to form domain groupings representing complaints of tinnitus, which were compared patients and significant others.

Results: From the 86 studies analyzed using data collected from 16,381 patients, 42 discrete complaints were identified spanning physical and psychological health, quality of life and negative attributes of the tinnitus sound. This diversity was not captured by any individual study alone. There was good convergence between complaints collected using open- and closed-format questions, with the exception of general moods and perceptual attributes of tinnitus (location, loudness, pitch and unpleasantness); reported only using closed questions. Just two studies addressed data from the perspective of significant others ($n = 79$), but there was substantial correspondence with the patient framework, especially regarding relationships and social life.

Conclusions: Our findings contribute fundamental new knowledge and a unique resource that enables investigators to appreciate the broad impacts of tinnitus on an individual. Our findings can also be used to guide questions during diagnostic assessment, to evaluate existing tinnitus-specific HR-QoL questionnaires and develop new ones, where necessary.

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Trial Registration: PROSPERO registration number: CRD42015020629. Protocol published in BMJ Open. 2016; 6e009171.

Keywords: Symptoms, Adults, Otology, Audiology, People important outcomes

Background

Tinnitus is a common condition which is usually described as a buzzing, ringing or hissing sound in the ears or in the head. Prevalence estimates vary from 11.9–30. 3% of the population depending on the question asked and the age of the population enrolled [1]. Davis and Rafaie [2] reported that tinnitus is "clinically significant" in about 20% of those who experience it. Data from UK clinical records concerning tinnitus that requires professional healthcare assistance indicates an incidence rate of 5.4 new cases for every 10,000 person-years (95% confidence interval: 5.3 to 5.5) [3].

In the case of tinnitus, both diagnostic assessment and evaluation of treatment-related outcomes rely on self-reports by patients because there are no observable clinical signs or objective tests of tinnitus. However, the range of potential tinnitus-related complaints is poten- tially extremely broad in scope and, because the tinnitus experience is very individualised and personal, those complaints tend to differ between individuals. To effectively discriminate problems experienced by different patients therefore, it is desirable to ask a comprehensive set of questions that are able to capture this diverse range of possible complaints [4]. This ensures that no important effects of tinnitus which might be important for personalizing individual patient management are missed. There has been no comprehensive collection about what all the possible complaints might be. The commonest problems have been proposed as aspects of quality of life such as psychological or emotional effects, impact on lifestyle, sleep disturbance, auditory and health effects [5], but this is by no means exhaustive. These five examples each describe a discrete dimension or aspect of tinnitus; which we call a "domain".

For the purposes of assessment, multi-item questionnaires have been developed and these ask questions relevant to numerous tinnitus domains. For the individual who seeks help, all the domains in which they experience problems, not just a limited subset, should to be explored to optimize diagnosis and rehabilitation [4]. Yet, since there has been no comprehensive collection about what all the possible complaints might be, there is little consensus among clinicians and researchers as to preference for a "standard" assessment [6]. Kennedy and colleagues [5] analyzed the item content of five common tinnitus-specific HR-QoL questionnaire instruments used for this purpose (Tinnitus Handicap Inventory THI, Tinnitus Severity Index TSI, Tinnitus Reaction Questionnaire TRQ, Tinnitus Handicap Questionnaire THQ, and the Tinnitus Questionnaire/Tinnitus Effects Questionnaire TQ/TEQ). For each questionnaire item, they classified what domain of tinnitus complaint it considered (see Table 1), using category labels that had previously been reported by three patient-centred studies [7–9]. The authors noted that there was a wide variation across the five questionnaires in the domains of tinnitus complaint that were assessed and in the proportion of items within each domain. These findings raise questions about whether these assessment tools are well-suited to effectively discriminate problems experienced by different patients. Certainly those assessing a limited number of domains could miss important aspects of an individual patient's difficulties. It is interesting to note that questionnaire developers draw on clinical experience, but tend not to provide adequate information on whether and how they established that the included items and subscales are important to patients (Table 1).

In this article, we therefore fill an important knowledge gap by conducting a comprehensive literature search and narrative synthesis to draw together an in-depth list of patient-reported domains describing different tinnitus-related problems. To date, no-one has yet conducted such a synthesis of the available data, despite the fact that this information is important for understanding the impact of tinnitus on an individual, for guiding patient assessment and for developing new and evaluating existing tinnitus-specific HR-QoL questionnaires.

None of the questionnaire developers listed in Table 1 formally acknowledged a conceptual framework guiding their development work, but it appears to us that tinnitus-specific HR-QoL questionnaire items tend to span the multi-dimensional categories of health captured by the conceptual framework for the World Health Organization (WHO) health-related Quality of Life-100 instrument; namely physical health, psychological state, level of independence, social relations, personal beliefs and their relationship to salient features of their environ- ment [10]. We therefore use this conceptual framework to organize and present the patient-reported domains that we identified from the literature.

Tinnitus affects not only the patient, but also those close to them (typically partners). The experiences of close relatives and friends therefore can provide

Table 1 Stakeholder input and data considerations during development of tinnitus-specific patient-reported questionnaire instruments. This table reported the top six most frequently used in clinical trials of tinnitus interventions; all developed in the English language [see 9]

Questionnaire instrument	Patient input	Professional input	Tinnitus constructs (domains or subscales)
Tinnitus Handicap Inventory [39]	Unclear	Yes	Tinnitus handicap (functional; emotional; catastrophic)
Tinnitus Functional Index [21]	No	Yes	Functional impact of tinnitus (intrusiveness; cognition; emotional; sleep; auditory; relaxation; sense of control; quality of life)
Tinnitus Severity Index [67]	Unclear	Unclear	Negative impact of tinnitus
Tinnitus Reaction Questionnaire [55]	Unclear	Yes	Psychological aspects of tinnitus
Tinnitus Handicap Questionnaire [43]	Yes	Yes	Tinnitus handicap (behavioural, emotional and social; auditory; outlook on tinnitus)
Tinnitus Questionnaire/ Tinnitus Effects Questionnaire [24, 22]	Unclear	Yes	Psychological aspects of tinnitus (intrusiveness; emotional and cognitive distress; sleep; auditory; somatic complaints)

important insight into the wider impact of tinnitus on everyday life and can be used as an "external barometer" for the needs of the patient. In the case of couples, it can even serve to identify therapeutic needs, to be directed towards either the couple or the spouse alone [11]. While there is a growing body of literature on the impact of tinnitus in those living with the condition; it is unclear what is known about the perspective of significant others.

This review answers the research question concerning what dimensions of tinnitus-related complaints patients and their significant others are reported as being a problem. The main objective of the present review is to collect and synthesise complaints in everyday life that have been reported by people with tinnitus, and also by their significant others. This process generates two perspectives about living with tinnitus: (i) the personal impact of tinnitus from the perspective of the person with tinnitus, and (ii) the personal impact of tinnitus from the perspective of the significant other. Clarifying what complaints are reported by individuals with tinnitus and by significant others would make it easier to identify any potentially important gaps in the content validity of current tinnitus-specific HR-QoL questionnaire instruments. Secondary objectives addressed whether people with tinnitus and their significant others have similar or different perspectives, and whether subtypes of tinnitus and health-related comorbidities influence the nature of the tinnitus complaints that are reported and which countries contributed data to our study findings.

Methods

We followed the search strategy, data collection and synthesis methods and the quality assessment as laid out in a predefined protocol [12]. Moreover, to aid later data synthesis, we separately recorded domains identified by open questions from those identified by closed questions (such as Numerical Rating Scales and questionnaires), and we recorded the evidence from closed questions such as if scores were elevated due to tinnitus, compared to controls. It is important to make distinctions between data gathering methods, since open questions best enable patients to have a voice about what is important to them, and not all closed questions necessarily reflect the tinnitus experience as seen from the patient perspective.

Eligibility criteria

Records were eligible for studies in which adults (≥18 years old) reported tinnitus as a primary complaint, irrespective of whether or not they were attending a clinic for treatment of those complaints, and those reporting data gathered from the significant others of adults with tinnitus. Studies reporting tinnitus as a secondary complaint were excluded. In the context of this review, we used the term "patient" to refer to anyone who has the lived experience of tinnitus. The review included studies reporting data gathered from the significant others of adults with tinnitus.

Records were eligible if tinnitus-related complaints had been collected as part of the screening or baseline assessments, prior to any tinnitus-specific intervention. To be eligible, specific complaints (such as "getting to sleep" and "waking up early") had to have been collected by the authors and sorted into domains (e.g. "Sleep difficulties") for reporting, or those complaints constituted items in a subscale or global questionnaire measure. In our review, a domain was defined as a discrete dimension or aspect of tinnitus that can encompass individual complaints with a similar conceptual theme which could be measured by a questionnaire subscale or single-construct questionnaire, but this was not a prerequisite. Collecting and synthesizing data corresponding to individual complaints was not the primary objective of this review and was deemed too great a task for the resources and time available.

Eligible study types were cross-sectional, non-intervention 'observational' designs, using techniques such as population surveys, questionnaires, interviews,

focus groups and case series. Records were excluded for studies reporting regression modelling predicting tinnitus severity, expert opinions, manufacturers' articles, practice guidelines, case reports, web-based patient discussion forums and any reviews. If systematic reviews were identified then all included records would be individually assessed for eligibility.

Eligible records were studies published on or after January 1980 conforming to our protocol [12]. To avoid language bias, articles that were not published in English were screened at full text or extracted by native speakers of the written language, using professional colleagues known to the authors.

Information sources

Information sources were published records which included grey literature, such as conference papers and postgraduate dissertations that had been archived on either Open Grey, PsycEXTRA, DART, ProQuest Dissertations and Theses, Networked Digital Library of Theses and Dissertations, Cos Conference Papers Index (ProQuest) and Web of Science (Thomson Reuters). Grey literature also included website content, searched using Google with the keywords page-by-page up to the point at which a page contains no eligible records. For peer-reviewed articles, electronic databases were searched: PubMed (National Center for Biotechnology Information), Embase (OVID), and Cumulative Index to Nursing and Allied Health Literature (CINAHL, EBSCO). Electronic searches identified publications from January 1980 to June 2015 and were conducted between 12 and 17 June 2015.

Four manual search methods were implemented to increase our confidence in a comprehensive coverage of the available literature and to ensure that all potentially eligible records had been identified. First, on 12 January 2016, we contacted 25 patient associations from across Europe, North America, and Oceania (see Box 2 [12]) to enquire about commissioned reports and unpublished reports relevant to the primary objective. Second, manual searches of reference lists of all articles identified as using open questions (14 March 2016) was conducted. Third, the bibliography of the 71 eligible records was circulated by email (8 July 2016) to 24 tinnitus experts who had previously developed patient-reported instruments (see Box 1 [12]) to identify any candidate records that were missing from our list. Finally, to ensure that the review was up-to-date, manual searches of the top five journals in which eligible records had been sourced (i.e. Ear and Hearing, International Journal of Audiology, Audiology, European Archives of Oto-Rhino-Laryngology and the Journal of Psychosomatic Research), and three additional journals in which eligible records using open questions had been sourced (International Tinnitus Journal, Journal of Speech and Hearing Disorders, and Hearing, Balance and Communication which was formerly the Journal of Audiological Medicine). The final manual search identified publications from June 2015 to December 2017 and was conducted on 20 January 2018.

Search strategy

The electronic database search strategy required ´tinnitus´ as a title or keyword, in conjunction with additional relevant search terms defined as relevant medical subject headings (MeSH) or text words, wherever possible. The search terms for PubMed and Embase used a combination of terms appearing in the title, keyword or subject, with terms as follows: ´(tinnitus) AND (problem OR complain* OR symptom)´ OR ´(tinnitus) AND (patient OR significant other OR partner OR family)´. For CINAHL and grey literature searches, "tinnitus" was defined as a keyword only. The only exception was ProQuest Dissertations and Theses in which keywords related specifically to co-morbidities, treatments, neural mechanisms and structures were excluded.

Study selection

Eligibility assessment was independently performed by two co-authors at each key step (i.e. title, abstract, and full-text screening). Discrepancies in title screening were resolved by DAH and HFH, while discrepancies at abstract and full-text stages were resolved by DAH and KF. Those discrepancies in eligibility assessment were predominantly concerned with evaluating the two criteria 'patient complaints not reported at the domain level' and 'factors predicting tinnitus severity'.

Data collection process

Two coders independently performed duplicate data collection for every study. Overall 13 coders shared the task, predominantly during a 5-day workshop. The number of studies per coder ranged from 5 to 66 (median 9). To minimize observer bias, the workshop included hands-on training with pre-prepared guidance material and electronic data collection form. To promote further data consistency, DAH and KF completed a post-hoc inspection of all 86 studies, collating one data record for each study. This eliminated minor discrepancies in data collection, in particular participant characteristics from the eligibility criteria (Methods) rather than from the reporting of findings (Results). We contacted the corresponding author by email (without reminder) to seek clarification of information, where required.

Data items

The electronic data collection form included a list of fields relating to eligibility criteria, characteristics of the

study population, relevant study findings and other details predefined in the protocol [12]. When information was not reported, the data field recorded 'not stated'. Tinnitus-related complaints were obtained from the measurement tools used for collecting individual complaints (transcribing the exact interview questions or scales, where relevant, and the responses or scores given). Tinnitus dimensions that included several domains describing patients and significant others' complaints, were labelled according to descriptions given by the original authors. For studies using closed questions to assess the impact of tinnitus, we did not simply extract data indiscriminately on all subscales or items of the questionnaire. Instead, we extracted data only for those subscales or items that had been highlighted by the study findings or conclusions as reflecting experienced complaints (i.e. those showing elevated scores in people with compared to controls, or demonstrating a substantive treatment-related change over time).

Synthesis of results

All included records were subjected to a qualitative synthesis that interpreted the data such that new conceptual understanding could emerge [13]. A variety of different terms were used to describe the same underlying theoretical construct, and so we needed to make grouping decisions across the data between studies to cluster together common domain-level concepts across studies. *Before* domain-level grouping of tinnitus-related complaints, t*hree corresponding authors responded to our query by confirming that* '*tinnitus intensity*' *was conceptually equivalent to the loudness of the tinnitus percept* [14–16].

Three coders used an iterative approach carefully considering the examples and explanations given by the study authors for each domain of tinnitus-related complaint. The first step required searching for and grouping the domain-level data reported by tinnitus patients under descriptive labels ("codes") that contained recurring keywords, such as "sleep" and "emotion". Preliminary domain groupings emerged from the given data taken directly from the full texts (without any abstraction). For the second step, the examples or quotes corresponding to these codes were considered too, and domain-level concepts were reviewed by the same three coders, and re-grouped, where necessary. The same coding scheme was then applied to the domain-level data and examples reported by significant others, in a third step, with new descriptive labels ("codes") added as required. Steps 2 and 3 involved constantly moving back and forth within the data to identify any overlap or differences in the emerging codes and domain-level groupings. Two new coders then reviewed the classification and made suggestions for revisions based on the domain keywords. Suggestions were shared among the coders, leading to a harmonization of the domain classification [17]. Considering the subjective nature of qualitative analysis, it was agreed that the coding and grouping process was complete once consensus was reached between all coders. The final set of domain labels were carefully reviewed with two lay representatives (native English speakers with tinnitus) to ensure they were understandable by the non-expert.

Results

Study selection

Figure 1 illustrates the flow diagram of all study records (see Additional file 1). Of the 3508 titles identified by electronic searches, 3201 were excluded ('out of scope'). This left 308 potentially relevant records for the next stage of abstract screening, including a small number of full-texts for those grey literature records. Fourteen records were translated for full-text screening and data collection (see Additional file 2) by trained native speakers. Overall, 212 records were excluded at abstract screening, and 52 records were excluded at the full-text screening and data collection stage with reasons for exclusion given in Fig. 1. Records were excluded for example because references were incomplete, abstracts were not accessible, or full texts were not accessible (see Additional file 3). Records classified as 'complaints not reported' all used standard questionnaires and closed questions, or objective quantitative measures; none used qualitative research methods asking open questions.

Manual search methods included contacting patient associations, searching reference lists, asking tinnitus experts for a bibliography list and searching selected journals. Only two associations responded (British Tinnitus Association and Belgian Tinnitus Association) and neither identified any commissioned reports relevant to the primary objective. For the reference list search, four articles were selected from the 96 full-texts assessed for eligibility specifically because they included open questions (see Fig. 1). One was based on authors' own single open question (Tinnitus Problem Questionnaire) [7], one was based on the authors' own structured interview [18], and two were based on the Structured Clinical Interview for Personality [19, 20]. From these, 38 potentially relevant records were identified. Three tinnitus experts sent references for potential records, and from these, ten potentially relevant records were identified. The manual search of the seven selected journals identified 13 additional eligible full texts.

The electronic and manual searches created a final list of 84 full-text articles that were included for data collection and data synthesis. References for all of these articles can be found in Additional file 4 and our complete dataset can be found in Additional file 5. Two articles

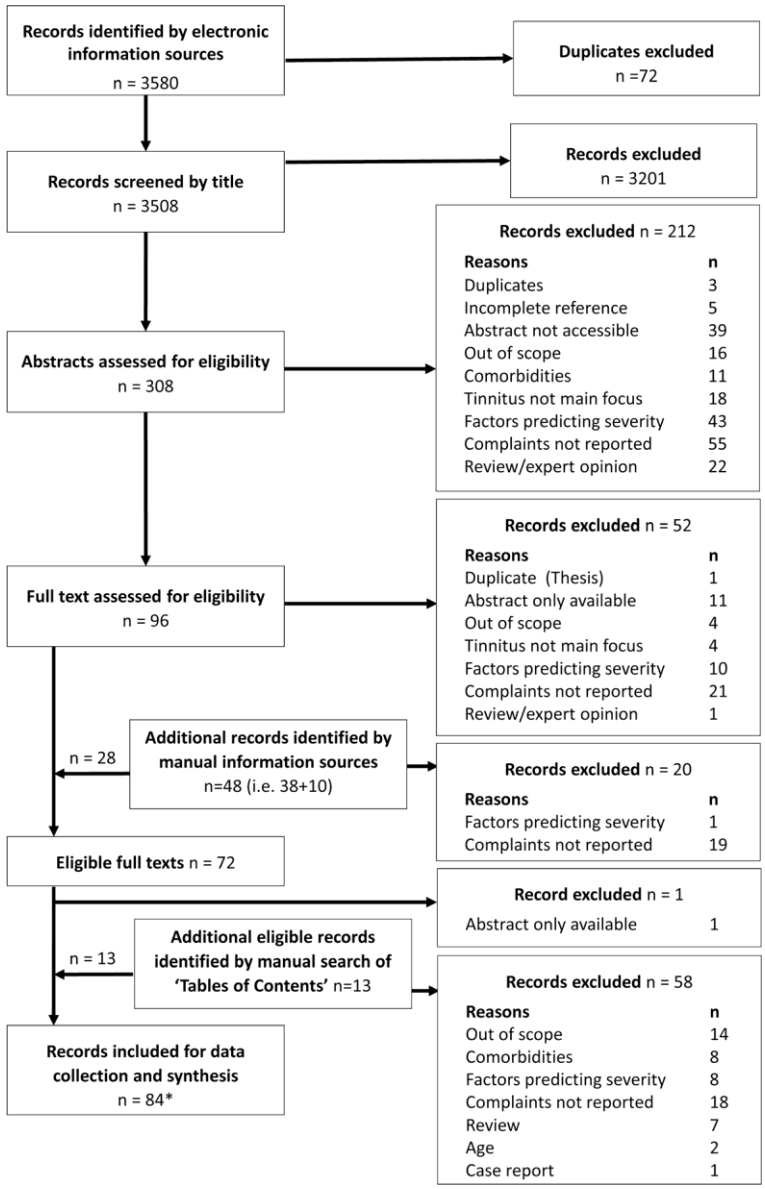

Fig. 1 Flow diagram of study records. Eighty-four records yielded 86 independent datasets for synthesis. Note that none of the review articles were systematic reviews

[21, 22] each reported two separate studies, and so this contributed two independent datasets to the synthesis (i.e. 86 studies in total).

Study characteristics

All 86 included studies assessed the patient experience, whilst two additionally questioned significant others. In total, our review considered data collected from 16,381 patients and from 79 significant others. El Refaie [23] confirmed by email that the number of participating significant others in his study was 57.

The majority of studies used closed questions (e.g. questionnaires, numerical rating scales) as the primary method of collecting individual complaints. Only eight studies asked open questions (885 patients). Open questions were used in the context of structured interviews. Two were based on the American Diagnostic and Statistical Manual of Mental Disorders [24, 25], one on the Psychological Impact of Tinnitus Interview [26], one on the authors' own structured interview [27], and one on the authors' own semi-structured interview [28]. The remaining three studies all asked the question from the Tinnitus Problem Questionnaire "Please make a list of the difficulties which you have as a result of your tinnitus. List them in order of importance, starting with the biggest difficulties. Write down as many of them as you

can" [7, 8, 29]. None of the included studies collected data using focus groups. Just two studies investigated the perspective of family members; one using a questionnaire about the quality of family life [23] and one using the author's own questionnaire [30]. Hence, all the data for significant others was collected using closed questions.

Synthesis of results

Complaints relevant to people with tinnitus

Overall 42 discrete unidimensional patient-reported domains were identified by the process of the data synthesis (highlighted by closed bullet points in Fig. 2), with nine multi-dimensional supra-level domains (highlighted in bold font in Fig. 2). A number of descriptors could not be fit into this domain framework because they were broad (e.g. Tinnitus handicap; Tinnitus problem; Tinnitus severity), described multiple theoretical constructs which do not group together (e.g. Effects of tinnitus on the patients social, emotional and physical behaviour), or described external modulators of the tinnitus (e.g. Stressors associated with onset or exacerbation of tinnitus). All of those descriptors that we were unable to allocate to one of the 51 domains are given in Additional file 6.

We have classified the domains into categories inspired by the conceptual framework of quality of life as measured by the WHOQOL-100 [10]. All domains are presented in Fig. 2, *ordered according to the frequency reported across all 86 studies. About two-thirds of domains were identified from both open- (□) and closed- (☒) question formats. Any notable exceptions are discussed below. For transparency of reporting,* Additional file 6 gives the domain grouping table that lead to the list of domains reported in Fig. 2. This table contains full details of our chosen domain label and all of the original domain-level terminology used by authors across the source information.

Negative attributes of the tinnitus sound

Data synthesis revealed numerous negative attributes of the tinnitus sound. Open-question formats elicited patient reports of '*Tinnitus awareness*' and '*Tinnitus intrusiveness*', also supported by the closed-question data. '*Tinnitus loudness*' was a recurring negative attribute, but this always arose from a closed question asked by the investigator (Fig. 2). Occasionally authors' reporting of loudness was intermixed with annoyance. For example, "subjects tended to report tinnitus that was perceived as moderately loud and annoying" [31]. '*Tinnitus pitch*', '*Tinnitus location*', and '*Tinnitus unpleasantness*' were also identified solely by closed questions (Fig. 2), but these rarely occurred across the 86 studies.

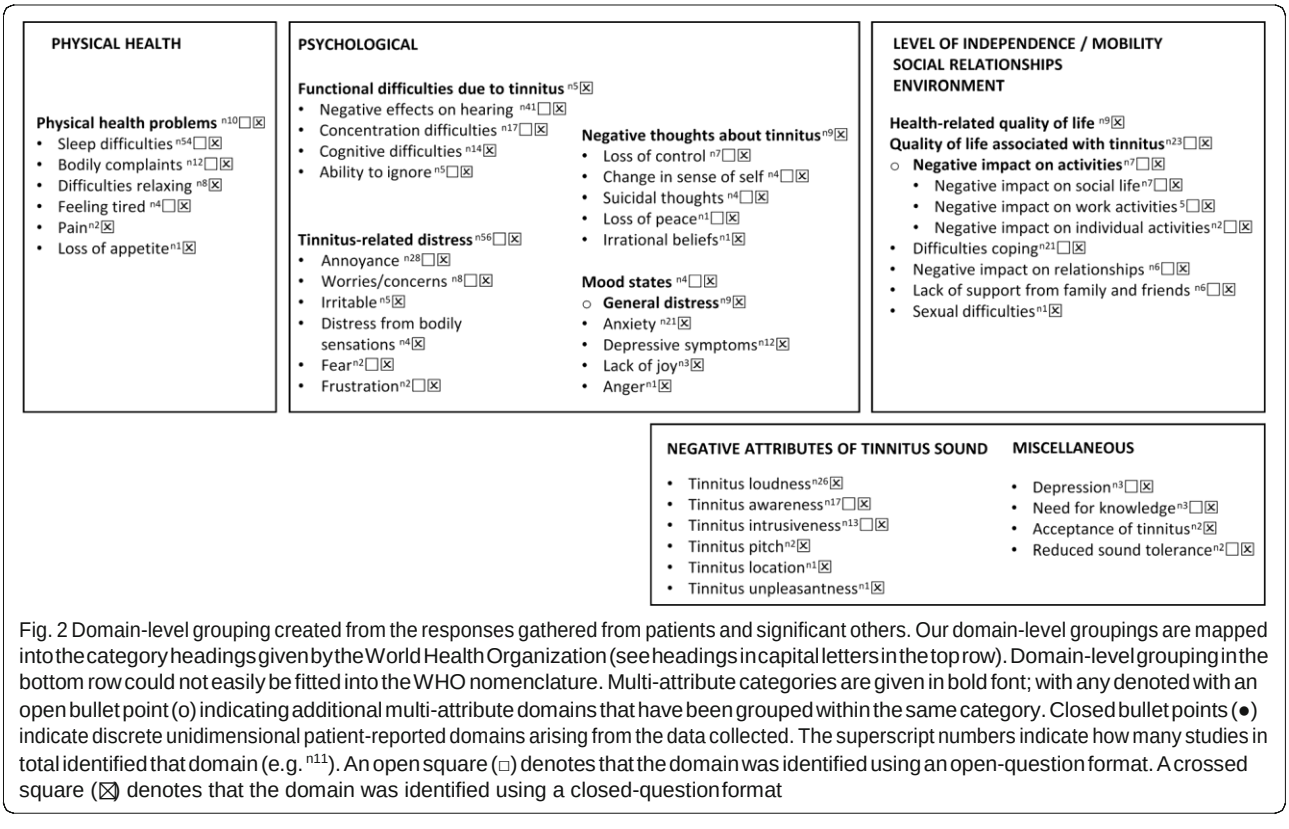

Fig. 2 Domain-level grouping created from the responses gathered from patients and significant others. Our domain-level groupings are mapped into the category headings given by the World Health Organization (see headings in capital letters in the top row). Domain-level grouping in the bottom row could not easily be fitted into the WHO nomenclature. Multi-attribute categories are given in bold font; with any denoted with an open bullet point (o) indicating additional multi-attribute domains that have been grouped within the same category. Closed bullet points (●) indicate discrete unidimensional patient-reported domains arising from the data collected. The superscript numbers indicate how many studies in total identified that domain (e.g. ^{n11}). An open square (□) denotes that the domain was identified using an open-question format. A crossed square (☒) denotes that the domain was identified using a closed-question format

Physical health problems

Patients often associated their tinnitus with their physical health. '*Sleep difficulties*' were the most common physical health difficulty in our dataset; identified using both open- and closed-question formats. Examples indicated difficulties in getting to sleep, in maintaining sleep and in the overall quality of sleep, for example "problems in getting to sleep, waking in the night, waking early" [22], and "tired during the day because tinnitus has disrupted sleep, lie awake at night because of tinnitus, difficult to get back to sleep after waking up at night" [32]. Complaints about physical health included pain, headaches, pressure in ears/head, nausea, dizziness and generally feeling unwell. These were collected and reported as a high-level multi-dimensional construct coded as '*Physical health problems*' [7, 8, 21, 33]. However, some studies separated out ear/head pain, headaches and muscle tension as symptoms of somatic complaints [34, 35]. We have therefore coded these as a single domain in its own right; coded as '*Bodily complaints*'. A few studies identified an individual physical symptom such as '*Feeling tired*' [16], '*Pain*' [16, 36], and 'Loss of appetite' [30].

Functional difficulties due to the tinnitus

Five studies clustered a broad range of functional difficulties into a high-level, supra-domain that we have called 'Functional difficulties due to tinnitus' [37–41]. This label came from the reporting of the functional subscale of the Tinnitus Handicap Inventory; a closed-question format. Different functional aspects were also coded as separate domains across studies. Most common in this category was '*Negative effects on hearing*'. This domain included any hearing problem that was attributed by patients to their tinnitus, over and above hearing loss per se. Examples were "difficulties in speech understanding" [42] our translated from Polish, interference with "ability to tell where sounds are coming from" [43], and "distortion of sounds" [28]. Patients also often attributed '*Concentration difficulties*' to their tinnitus, "being distracted by tinnitus" [44]. Related to the dimension of concentration difficulties was the domain that we have coded as '*Ability to ignore*' tinnitus, e.g. "Less able to divert their attention from their tinnitus" [27].

A number of studies using closed-question formats also reported '*Cognitive difficulties*' which encompassed problems with memory and/or attention, such as "Can't express/tip of tongue, Sudden forgetfulness; Difficulty thinking clearly or remembering" [21, 45]. Five studies grouped a broad range of functional difficulties into a high-level, supra-domain that we have called '*Functional difficulties due to tinnitus*' [37–41]. This label came from the reporting of the functional subscale of the Tinnitus Handicap Inventory; again a closed-question format.

Emotional complaints associated with tinnitus-related distress

Data synthesis revealed many different emotions experienced by patients, but the commonality was that they were all directly attributed to the tinnitus, or some other relevant sensation. Most commonly reported was a high-level construct that we have called '*Tinnitus-related distress*'. Distress is multi-dimensional in nature and encompasses a constellation of different emotions that other studies had coded as separate domains, such as "Inability to concentrate, Distress/upset, Stress/tension/inability to relax, Irritability, Isolation, Helplessness/frustration" [8] and "[…][…] depression […], anxiety […]" [18], and "loudness, unpleasantness of the noises; […] worries about the persistence of the noises; […] emotional effects (irritability, anger, sadness)" [22]. Annoyance was the most common specific emotion in our dataset. Again these codes predominantly arose from closed questions, but the authors of one of the open question studies [7] pooled "irritation" and "inability to relax" into the same domain as "annoyance" giving some insight into what this construct might mean to patients. A small number of studies did specifically capture, as a separate domain, the sense that tinnitus made them feel '*Irritable*'. For example, "Tinnitus causes me to feel irritated and angry" [42] translated from Polish. And perhaps related to this construct was also the feeling of '*Frustration*': "So ...everyone says 'you can't keep on focusing on tinnitus, you must do something nice instead, like travelling' [...] but I say 'Yes, but I cannot fly any more" [28]. '*Worries/concerns*' was another emotion directly associated with the tinnitus experience. The content of the worry appeared to differ from one patient to another, for example "I worry that there is something seriously wrong with my body" [22] and "It becomes a general feeling of worry in me that I did not have before when I did not know so much. It gets back at me, perhaps in many areas. It is how it feels, it sort of spreads in a way" [28]. Two studies reported '*Fear*,' and so we coded this as a separate emotion because it seemed to capture a stronger sense of emotion. For example, "I'm afraid that the tinnitus will become more disturbing and impairing in the long term" [38].

Finally, our data synthesis identified one negative emotion that was associated with bodily sensations (not with tinnitus per se), which we have termed '*Distress from bodily sensations*'. This domain was identified by four studies all using a closed-question format. It tended to be presented as a sign of a somatization or somatoform disorder, such as measured by the Tinnitus Questionnaire [14, 44].

Negative thoughts about tinnitus

A number of studies identified a construct associated with a '*Change in sense of self* '. Examples include "self-blame" and a negative "self-image"; "I am retired now, 54 years old and retired, it is not really fun is it, to think that thought?" [28]. Most of these examples arose in response to an open

question asked by the investigator. Open questions also identified '*Loss of peace*,' for example "Peace of mind" [46]. '*Suicidal thoughts*' was identified from both open and closed questions pertaining to suicide risk [26, 35]. Five studies captured high-level, supra-domain '*Negative thoughts about tinnitus*,' by asking closed questions. The sense of being unable to "escape tinnitus" and no longer coping with the tinnitus [37, 39], both questions that form part of the Catastrophic subscale of the Tinnitus Handicap Inventory. Similarly, the construct termed '*Loss of control*' forms a distinct domain of the Tinnitus Functional Index e. g. [21]. '*Irrational beliefs*' were also identified by a closed question in one study [47].

General mood states

In contrast to emotions, we have classified moods as general feelings that are not triggered by tinnitus, nor by any other sensation. Mood states tended not to be reported by patients using open questions, but rather in response to specific questionnaire items. A small num- ber of studies used '*Mood states*' as a high-level 'supra-domain' term, often measured using a generic mood questionnaire [26, 28, 30, 48]. Others used the term '*General distress*', a construct that is synonymous with 'stress' and was typically measured using a generic stress questionnaire e.g. [48–50]. From our dataset, a recurring specific mood state was '*Anxiety*'. '*Depressive symptoms*' was another. Typically, anxiety and depression were measured using a relevant (sub)scale of a closed-item questionnaire, such as the State Trait Anxiety Inventory [15, 51], Beck Depression Inventory e.g. [15, 52], or the Hospital Anxiety and Depression Scale e.g. [16]. Two other mood states relating to feelings not necessarily directed towards the tinnitus were '*Lack of joy*' (e.g. "frequency of loss of joy", 44) and '*Anger*' [48].

Health-related quality of life

Many study findings attributed a reduction in quality of life specifically to the tinnitus (i.e. '*Quality of Life associated with tinnitus*') [43]. This domain was identified using questionnaire items or subscales [Tinnitus Handicap Questionnaire, 21; Tinnitus Functional Index, 27], and also in response to an open question asked by the investigator [27, e.g. "Impairments in quality of life, reduced stress tolerance, psychosocial withdrawal"). A number of studies defined '*health-related Quality of Life*' as a generic domain relevant to people with tinnitus but not specifically attributed to the tinnitus. This was most commonly measured using a single-item Numerical Rating Scale e.g. [44, 53].

'*Negative impact on activities*' was another multi-dimensional domain that emerged from the data synthesis of both open- and closed-question formats. The type of activities were either unspecified "avoided otherwise enjoyable activities, more restricted in their activities" [27], or encompassed several different types of activity in the same author code, such as "interfered with work, less interested in going out" [54]. Within this supra-domain there were three unidimensional domains associated with specific categories of activity; namely social, work, and individual. '*Negative impact on social activities*' was illustrated by examples such as "General interference with leisure; Less interested in going out; Social life was limited" [21, 28]. '*Negative impact on work activities*' was exemplified by this description "Work as a situation in which tinnitus had a severe negative impact. Some had stopped working altogether, and others had reduced their working hours or changed workplace/work assignments" [28]. With respect to '*Negative impact on individual activities*', an illustrative example was "ability to concentrate, listen to music or read newspapers" [55]. Patients also reported issues relating to relationships with family and friends, for example "I become, yes, some kind of obstacle for, for some things my wife and I might have planned to do together" [28], and these were classified by the category '*Negative impact on relationships*'.

While some studies mentioned the use of coping strategies without giving examples, our data synthesis did indicate a diversity of cognitive and behavioural approaches to coping in use by patients, and which we have labelled as the domain '*Difficulties coping*'. Examples spanned avoidance ("I avoid noise due to tinnitus; I avoid silence due to tinnitus; Due to my tinnitus I avoid sporting activities" [38, 46]), using hearing protection ("Due to my tinnitus I try to protect my ears whenever it is possible" [38]), and what the authors called 'saving face' ("what is shown to other people" [28]). None seemed specific enough to separate into distinct domains with the exception of '*Lack of support from family and friends*' (e.g. "I notice people have a hard time trying to understand" [28] and "Family gets aggravated with me" [56]). A final domain in this category was '*Sexual difficulties*'. There was just one instance of this, and it was assessed in a study using the Chronic Illness Problem Inventory [24].

Miscellaneous domains

A small number of other domains were recorded, but could not easily be classified according to the above categories. Of note, a few studies included an assessment that indicated a clinical diagnosis of depression (e.g. Harrop-Griffiths [24] assessed patients against the American Diagnostic and Statistical Manual (DSM III) criteria). No studies reported on a formal clinical diagnosis of anxiety. Other domains were '*Need for knowledge*' which was exemplified by: "Explaining tinnitus to others" [7], and '*Acceptance of tinnitus*' which included

the example "Tolerance" [57]. These two domains were identified by few studies.

Also not easily classified in the WHOQOL-100 conceptual framework were two studies reporting a reduced sound tolerance, possibly associated with hyperacusis [28, 58].

Secondary analyses

Secondary objectives addressed whether people with tinnitus and their significant others have similar or different perspectives, whether subtypes of tinnitus and health-related comorbidities influence the nature of the tinnitus complaints that are reported, and which countries contributed data to our study findings.

Complaints reported by significant others

Only two studies addressed domains of tinnitus-related complaints reported by 79 significant others in terms of their own personal experience [23, 30]. These questionnaire studies identified ten domains which were all contained within the classification for people with tinnitus (see Additional file 7): '*Sleep difficulties*', '*Negative effects on hearing*', '*Mood states*', and '*General distress*', '*Negative impact on relationships*', '*Negative impact on activities*', '*Negative impact on social life*', and '*Difficulties coping*', '*Physical health problems*', and '*Need for knowledge*'. '*Negative impact on relationships*' was the only domain to be identified in both studies.

In addition to comparing the domains identified by patients and significant others, we had planned to conduct two further secondary analyses. The first was to explore whether different tinnitus subtypes might influence different patterns of reported domains. Pre-specified classifications were tinnitus duration (acute versus chronic), tinnitus presence (intermittent versus constant), tinnitus pulsatility (non-pulsatile versus pulsatile), tinnitus severity (mild versus moderate versus severe), co-morbid anxiety, depression and severity of hearing loss. Adequate analysis of each research required a sufficient number of studies either to have enrolled only participants according to individual subtypes, or to have separately characterized and reported complaints according to subtype. Unfortunately, none of the included studies did this for any of the pre-specified classifications. The second was to explore whether a health-related comorbidity might influence different patterns of reported domains. Seventy-one of the studies reported no co-morbidity re- lated inclusion criteria, 10 reported hearing loss, one insomnia, and one hyperacusis. Although this is insufficient to draw any strong conclusions, the form of the co-morbidity was associated with the reporting of the associated complaint. Notably, the study recruiting people with tinnitus and insomnia was one of the studies identifying the '*Sleep difficulties*' domain [59], and the study recruiting people with tinnitus and hyperacusis was one of the studies identifying the '*Reduced sound tolerance*' domain [58] (Fig. 2). In addition, three of the 10 studies actively recruiting people with hearing loss identified '*Negative effects on hearing*'. [47, 50, 60].

The final secondary analysis explored which countries contributed data to our findings. The complete dataset included the data item for the country where the study was conducted. Overall, the data predominantly came from UK, USA, Germany and Sweden (shown in Fig. 3), including the two studies investigating significant others (UK) and the four qualitative research studies reported below in Table 2 (UK, Sweden). This observed geographical bias was unlikely to be explained by our study design since we translated all eligible records.

Quality assessment

The protocol described three assessments of the quality of collecting, defining and reporting the domains of tinnitus-related complaints. We evaluated the extent to which investigators used an open questioning format, and then assessed quality for those studies using a qualitative research design. Qualitative research is valuable because it can illuminate the personal meaning of tinnitus without constraining findings by any investigators' preconceptions and can enable an in-depth exploration or relevant issues. Eight articles used open question interviewing, but four either used a closed format response scale [26, 27] or used patient responses only to make a psychiatric diagnosis [24, 25]. O*nly four remaining records* [7, 8, 28, 29] met the Critical Appraisal Skills Programme (CASP) [61] checklist screening questions confirming that they were qualitative research studies (610 patients in total). Two authors (KH and DAH) then subjected these records to a quality assessment using the remaining eight CASP checklist questions and agreed ratings are given in Table 2. While reporting of findings was adequately detailed, there was no confirmation of ethical approval, no consideration about whether or how data collection might have been affected by the investigator-patient relationship and incomplete reporting of how the text-based data had been analyzed to identify tinnitus domains.

The remaining 80 records (82 independent studies) were subjected to the quality assessment described in the protocol [12], relevant to the degree to which reported findings reflected the heterogeneity of a 'typical' tinnitus population. Records were assessed for: (i) justification of sample size, ii) reporting a wide variety of ages (mean and SD), (iii) gender balance (men and women), and (iv) no eligibility criteria that would exclude particular tinnitus subgroups. Each criterion was scored 0, 1 or 2 to give a composite score out of 8. The mean quality score was 5.24 (SD = 1.37). Most poorly handled was the

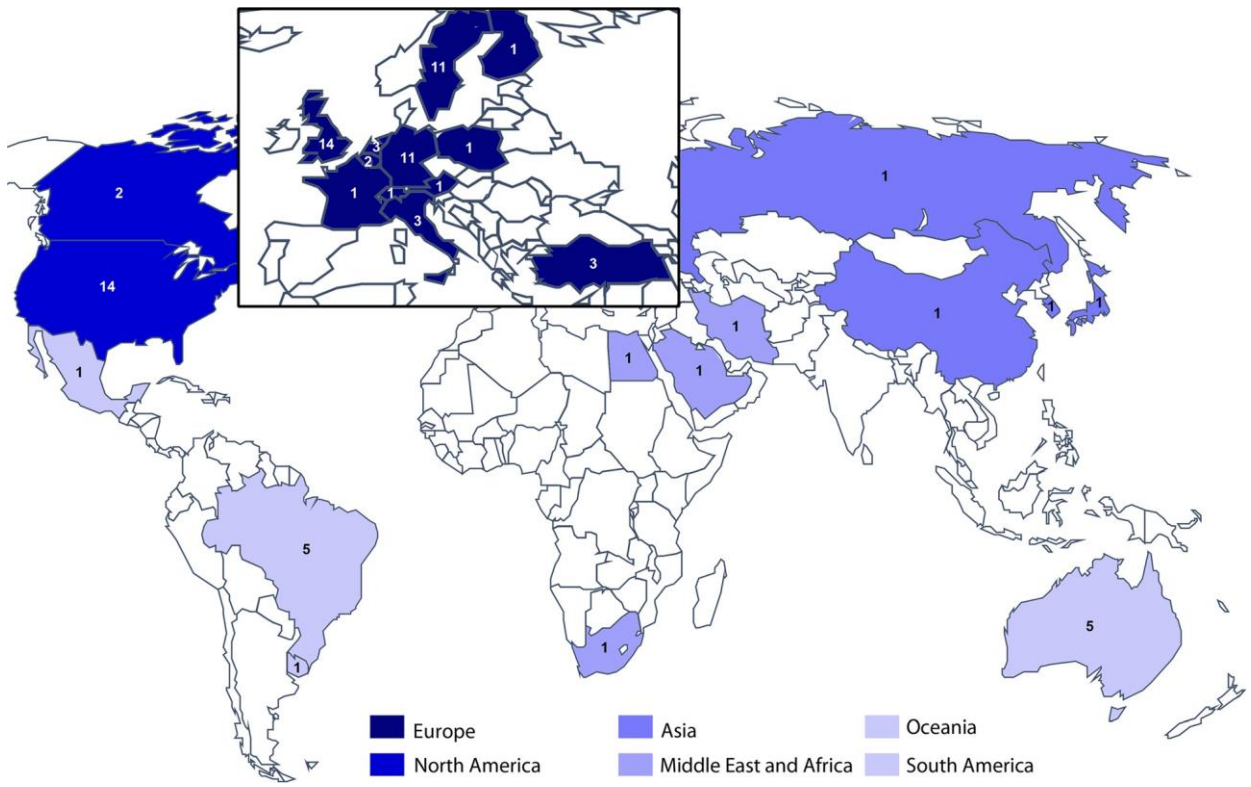

Fig. 3 World map illustrating the distribution of study sites for all included studies, inspired by the World Health Organization (WHO) regional classification, but with Region of the Americas separated into North and South and with Australia and separated from Western Pacific region, because of cultural and language differences. Regions are colour coded in different shades of blue and the values denote the number of studies contributing to the review from that country

justification for sample size, with 69 studies not given any explanation for why the numbers recruited were sufficient to address the primary question.

Discussion

The effects of tinnitus on the person with the condition and on their significant others are pervasive and affect the quality of life for all involved. This comprehensive review is important because it has collated and synthesized, for the first time, everyday life tinnitus-related complaints that have been reported by people who have the lived experience of tinnitus and their significant others.

Comparison with other studies

To our knowledge, no other study has achieved a comprehensive qualitative synthesis of patient-reported complaints associated with tinnitus. Perhaps two of the closest are a systematic review of clinical trials of tinnitus in adults [6], and a qualitative content analysis of tinnitus problems and effects on everyday life according to the International Classification of Functioning, Disability and Health [62]. The findings of these two studies

Table 2 CASP checklist for records that passed the first two screening questions. √ = checklist criterion was met, ✗ = not met, and ? = can't tell

	Research design	Recruitment strategy	Data collection	Relationship/ bias	Ethical issues	Data analysis	Statement of findings	Value of the research
Tyler & Baker 1983 [7]	?	?	√	✗	✗	✗	√	√
Sanchez & Stephens 1997 [8]	√	√	?	✗	✗	✗	√	√
Sanchez & Stephens 2000 [29]	√	?	?	✗	✗	✗	√	?
Andersson & Edvinsson 2008 [28]	?	√	√	?	✗	✗	√	√

are consistent because they map very well onto concepts that are equivalent to the domains that have emerged from our qualitative data synthesis, albeit sometimes with a slightly different choice of wording (e.g. '*Tinnitus-related cognitions*' [6] became '*Negative thoughts about tinnitus*' and '*Sustaining attention*' [62] became '*Concentration difficulties*'). The main reason for these differences in wording can be attributed to the novel influence of involving lay people with tinnitus whose role has been to challenge us to find domain labels that were as jargon free as possible. Members of the public were not explicitly involved in our review team, but did influence our choice of domain labels at the reporting stage because of their involvement in an ongoing study as part of the next step in our research programme [63]. Despite our rather strict definition of a domain, our synthesis identified a large number of discrete unidimensional constructs associated with tinnitus complaints. This lengthy classification list differs markedly from all previous studies, but perhaps surprisingly even for those published studies which surveyed tinnitus patients using an open question and then analyzed the resulting patient narratives. For example, Tyler and Baker's landmark study [7] of 72 patients reported only four domains (hearing, lifestyle, general health, emotional problems). The examples that they gave for the hearing domain map well onto our domain '*Negative effects on hearing*', and so do those for health (see '*Physical health problems*'). However, lifestyle and emotional problems do not, perhaps because Tyler and Baker [7] intermingled a range of different concepts. For example, the examples that they gave for lifestyle we have coded under numerous discrete domains (Sleep difficulties; Tinnitus awareness; Difficulties coping; Negative impact on social life; Negative impact on relationships; Negative impact on work activities; Negative impact on individual activities; Need for knowledge). Indeed, Sanchez and Stephens [8, 29] seem to have also recognized a difficulty with multidimensional constructs because in their analysis of data collected using the same procedure as Tyler and Baker [7] they created the additional domain '*Sleep*'. We consider there to be scientific value in reporting patient-related complaints at the level of individual, discrete health-related constructs, not high-level broad categories. In our experience, both patients and healthcare professionals find these both understandable and highly relevant to their own personal experiences [63].

Limitations of the study

We acknowledge a potentially limiting factor is that our search identified *only four* qualitative research studies assessing 610 patients [7, 8, 28, 29], and no new qualitative studies were identified in the manual search update but see [62]. Geographical bias was avoided since no records were excluded because of an inability to adequately translate into English.

Given the relative paucity of qualitative methodology to understand tinnitus complaints, as experienced by the patient and their significant others, it is possible that additional complaints might emerge from new research. For example, we are aware of one unpublished study, presented at a recent conference [64], in which the authors collected tinnitus-related complaints from 988 patients using a single open question: "Why is tinnitus a problem?". However, this new study does not add any new information to the domain-level grouping represented in Fig. 2.

Future directions

Our findings highlight a number of knowledge gaps each of which be a promising future direction for research. First, the tinnitus-related complaints spanned aspects of physical health, psychological health (i.e. functional, cognitive and emotional), independent activities, social relations, and leisure activities. For the majority of these, we found converging evidence for their relevance to people with tinnitus, through responses to open-format questions as well as group differences in scores on closed-format questionnaires. Although patients typically attributed direct causality to the tinnitus, we noted that these domains are also generic components of well-being that are represented within the WHO conceptual framework of quality of life [10]. This raises an important unanswered question about whether or not a profile of the impact of tinnitus could adequately be measured by a standardised, generic quality of life instrument.

Second, we observed that a small number of patient-reported domains were identified only by directed, closed questions asked by the investigator and were never 'spontaneously' reported by patients in response to an undirected, open question. Notably, these included general moods (not triggered by tinnitus) and also the four major perceptual attributes of the tinnitus sound (i.e. its location, loudness, pitch and unpleasantness). These domains highlight discrepancies between the perspectives of patients and healthcare professionals; while they appear to be valued by clinical practitioners, this is not true for patients. This finding also raises a specific dilemma because loudness is a common primary outcome measure in clinical trials [6], and yet it may not be so relevant to patients. Importantly, this review raises concerns about whether tinnitus loudness has sufficient content validity to be an essential item for inclusion, certainly as part of a patient-reported primary outcome instrument when determining the clinical efficacy of an intervention. Again, further research is warranted.

Third, the impact of tinnitus on the patient's significant other may provide clues on how a couple or family

deal with tinnitus in their daily routine and considering such challenges may contribute a more complete clinical profile of a patient undergoing clinical assessment and management. Our review highlights a gap in our knowledge concerning third-party disability because there is a paucity of literature about the effect of tinnitus on significant others. Like hearing loss, many fewer studies are directed at investigating the impact of the condition on close friends and family than on patients themselves. For hearing loss, a recent review identified 24 articles reporting the impact of tinnitus on significant others [65]. However, in the case of tinnitus, third-party disability does not appear to be a topic of growing research interest because we identified only two articles, with the most recent having been published over 10 years ago [23]. This lack of data means that our findings are unlikely to capture all domains relevant to this stakeholder group, and so further research is warranted.

Finally, our findings make a specific contribution to the ambitious roadmap for developing a Core Outcome Set for tinnitus which would set minimum standards for collecting and reporting outcomes in all clinical trials of tinnitus [63]. This review identifies all those patient-reported domains that could be candidates for a Core Outcome Set, thus giving credence to the patients' viewpoint.

Conclusions

There is a recognition that measurement instruments used for clinical diagnosis and for evaluation of the outcome of tinnitus interventions should have good content validity (i.e. that their content is an adequate reflection of complaints that are relevant to tinnitus) [66]. The findings of this comprehensive review therefore contribute fundamental new knowledge and a unique resource that will enable investigators to evaluate the relevance to patients of any multi-item patient-reported questionnaire for tinnitus. Clarifying the types of tinnitus-related complaints that are often reported enhances our understanding of the lived experience of patients and highlights important gaps in content validity of current tinnitus-specific HR-QoL questionnaire instruments.

Additional files

Additional file 1: Table summarising the electronic information sources used to identify the 3580 records. For a description of the abbreviations, see text. (DOCX 17 kb)

Additional file 2: Fourteen non-English language records that were screened and either excluded or included at the full-text stage by native language speakers. 'Complaints not reported' = authors reported the global tinnitus score calculated from a multi-attribute questionnaire without reporting the component domains or subscales. (DOCX 18 kb)

Additional file 3: Records that were excluded because either the abstracts and/or full-texts were not accessible, or there was an incomplete reference which meant that the article could not be traced. (DOCX 35 kb)

Additional file 4: References for all 84 full-texts included for data collection and synthesis. (DOCX 22 kb)

Additional file 5: Complete dataset, including our domain coding and quality assessment. (XLSX 520 kb)

Additional file 6: Grouping table reporting the different terminology used by authors to describe the same theoretical constructs. Grouping considered the examples and explanations given by the study authors for each domain of tinnitus-related problem (examples not reported here). Domains that could not be coded either because they were not well-defined, described multiple theoretical constructs which did not group together, or described external modulators of the tinnitus were as follows: *Ability to mask the tinnitus sound; Aggravated by noise; Auditory perceptual characteristics of tinnitus; Catastrophic; Changes for loud background noise; Changes in perception over time; Effects of tinnitus on health; Effects of tinnitus on the patients social, emotional and physical behaviour; Emotional; Emotional reaction, social activities and communication, and focused attention; Emotions; Factors that aggravate tinnitus; Functional; Functional handicap caused by tinnitus; Illness focusing; Masking effects; Medical interaction; Most problematic situation; Other people; Overall patient stress and severity of tinnitus; Psychological; Relax; Relief from tinnitus; Self-perceived tinnitus handicap; Sensations in the presence of such sounds; Situational difficulties; Situational effects; Stressors associated with onset or exacerbation of tinnitus; The extent of problems due to tinnitus; Tinnitus loudness/strength, annoyance, impact on life and severity; Tinnitus handicap; Tinnitus problem; Tinnitus sensation; Tinnitus severity; Tinnitus burden and severity*. (DOCX 18 kb)

Additional file 7: Grouping table reporting the different terminology used by authors to describe the same theoretical constructs describing complaints reported by significant others who are members of the family of a person with tinnitus. Each grouping considered the examples and/or explanations given by the study authors for each problem domain (examples reported in the text). All data come from just two studies [23, 31]. (DOCX 14 kb)

Abbreviations

CINAHL: Cumulative Index to Nursing and Allied Health Literature; HR-QoL: Health-related quality of life questionnaire; MeSH: Medical subject headings; PICOS: Patient, Intervention, Comparison, Outcome, Setting; TEQ: Tinnitus Effects Questionnaire; THI: Tinnitus Handicap Inventory; THQ: Tinnitus Handicap Questionnaire; TQ: Tinnitus Questionnaire; TRQ: Tinnitus Reaction Questionnaire; TSI: Tinnitus Severity Index; WHO: World Health Organization

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Availability of data and materials

The dataset used for the qualitative synthesis during the current study are available from the corresponding author, on reasonable request.

Authors' contributions
ABL and DAH conducted the electronic searches, KF conducted the manual searches. Screening and eligibility steps were carried out by ABL and RT, under guidance from DAH and KF. The workshop (15–20 April 2016, Lisbon) was organized by HFH and led by KF and data collection was conducted by KF, DAH, ABL, HFH, DER, TDM, SS, PC, VK, RT, CT, DR, and VML. KF and DAH led the domain-level grouping step, and DAH and KF conducted the data synthesis and CASP quality assessment. DAH and HFH contributed equally to all other stages of the protocol development, produced and approved the manuscript. All authors read, edited and approved the final manuscript. HFH and KF are the data guarantors.

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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5.2. Diagnosis and treatment of Tinnitus

In the present study we intend to contribute to increase knowledge regarding tinnitus in order to allow improvements in its diagnosis and treatment.

We have a subchapter regarding TINNET an European research network with the goal of coordinating 235 researchers, mathematicians and clinicians from different disciplines out of 30 participating countries in order to establish standards for tinnitus diagnosis and improve treatment efficacy.

We had an international systematic review (SR) of existing tinnitus guidelines (Fuller et al., 2017). Another SR of outcome domains and instruments used in clinical trials of tinnitus treatment effectiveness in adults, the protocol for this SR was previously published (Hall et al., 2015).

In order to demonstrate the diversity regarding tinnitus symptom also included in this subchapter is a scoping review about somatosensorial tinnitus and a study about the Delphi methodology used in order to obtain a consensus diagnosis criterion on this subtype of tinnitus.

5.2.1. TINNET as an European research network for tinnitus

TINNET is the acronym that stands for TINnitus research NETwork or TINnitus's NEw Treatments. It was a Cost Action BM1306 - 'Better understanding the Heterogeneity of Tinnitus to Improve and Develop New Treatments'.

COST (Cooperation in Science and Technology) is a working network whose mission is to strengthen joint technical and scientific research in Europe. It is based on an intergovernmental cooperation network agreed by the representatives of 19 European countries during the Ministerial Conference held in Brussels in November 1971. In order to better manage the resources available in Europe, both in terms of scientific and technological knowledge as financial. Cost actions also have the mission of supporting young researchers.

TINNET was a pan-European multidisciplinary network with the aim to identify pathophysiologically and clinically meaningful subtypes of tinnitus and their neurobiological underpinnings. It was launched on April 2014 and has lasted during four years (Figure 5.2-1). Specifically, this action had the major goal to facilitate (1) the identification of meaningful criteria for tinnitus subtyping, (2) the neurobiological underpinnings of the different tinnitus subtypes and (3) their relevance for response to treatment. This will be facilitated by standards for clinical assessment and outcome

measurement, by large-scale multicentre data assessment and by data management in a quality-controlled database.

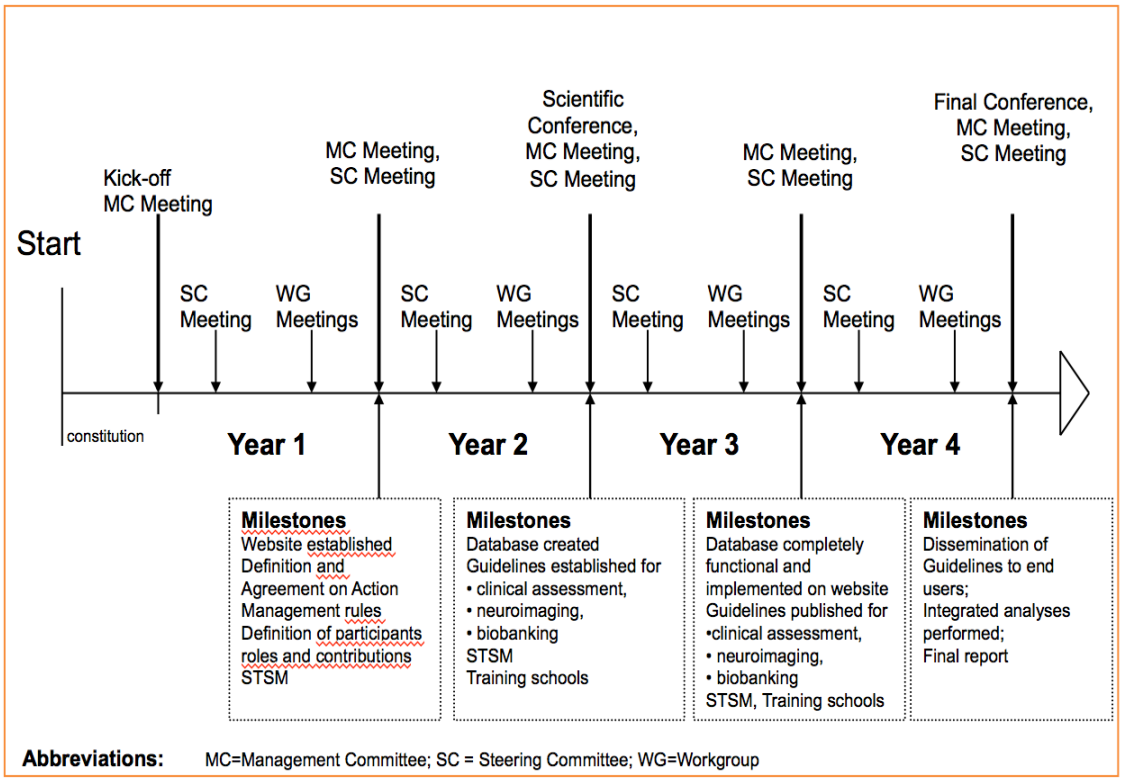

Figure 5.2-1 - Organization and objectives for TINNET Working Groups.

COST Action BM1306 comprised 5 working groups (WG): WG1 Clinical, WG2 Database, WG3 Neuroimaging, WG4 Genetics and WG5 Outcomes (Table 5.2-1).

Table 5.2-1 - Five interactive working groups: objectives.

WG 1 Clinical:	Standard for phenotypes; disclosure of guidelines.	**WG 2** **Database**
WG3 Neuroimaging	Standard – Neuroimaging studies (EEG, MRI ...); Mechanisms of neuro-biological subtypes of tinnitus.	
WG 4 **Genetics**	Standard – collection, storage, sharing - Biobank; Genetic biomarkers.	Management and quality control; statistical analysis; Deploying the database on the site.
WG 5 **Results**	Standard – clinical trials, evaluation results; identification of clinical predictors.	

WG 1 Clinical: Establishment of a standard for patient assessment and characterization

Experts from different disciplines have joined forces to develop easy and meaningful guidelines for detailed clinical and phenotypic characterization of tinnitus cases and controls (symptom scoring instruments and specific validated self-report questionnaires) (Figure 5.2-3).

Tinnitus evaluation (e.g. audiologic assessment, loudness, frequency, modulatory factors) but also comorbid conditions (e.g. hearing impairment, hyperacusis, depression, anxiety) and impact (tinnitus related handicap, quality of life) were be assessed.

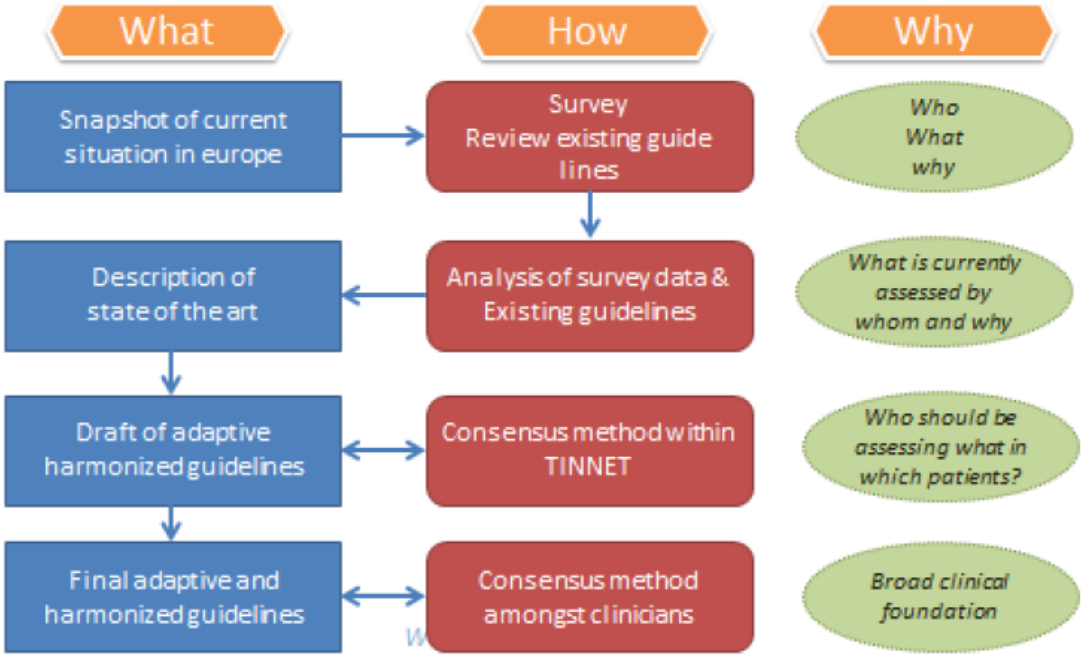

Figure 5.2-2 - Network Management and Organization.

WG 2 Database establishment and implementation on the website

Combining complemented disciplines such as clinicians, clinical trial and data documentation specialists, biostatisticians and mathematicians this WG had the goal of standardisation of data management (statistical analysis) and quality control, for development of strategies for hypothesis driven data analyses and for data driven analyses. They planned to extend the database to accommodate longitudinal data and develop statistical methods for analysing longitudinal data, to identify clinical, neuroimaging or genetic treatment predictors and strategies to advance the database in a self-learning expert system to assist clinicians in treatment decisions.

WG 3 Neuroimaging

In this WG have participated clinical researchers and specialists in neuroimaging. They have established standard operation procedures (SOPs) for data acquisition, analysis development and standardization of innovative data-analysing methods (e.g. MRI (magnetic resonance tomography) - connectivity analysis, individual component analysis and EEG (electroencephalography), in order to identify the neurobiological mechanisms of the different forms of tinnitus (clinically relevant) and test neuroimaging as an endophenotypization strategy in tinnitus research.

WG 4 Genetics

This WG has joined clinicians, experts from molecular genetics, statistics and bioinformatics with the goal of creating a pan-European biobank to study the underlying genetic basis of tinnitus. They had the responsibility of establishment of SOPs for sample (blood) collection, storage, sharing and genetic analysis of the human genome as well for statistical analysis of genotypic data and correlation with clinical and neuroimaging data and for gene-gene and gene-environment interaction studies.

WG 5 Standards for Treatment outcome measurement and central collection of results

This WG brought together clinicians, experts for clinical research methodology, statisticians, and representatives of the health industry and patient organisations (for specification of outcome measurements relevant for patients). WG5 has established standards for outcome measurements in clinical trials to enable data collection of treatment results in the central database. And also defined standards for clinical trials in tinnitus and for outcome measurement both in clinical trials and in clinical routine (Figure 5.2-3).

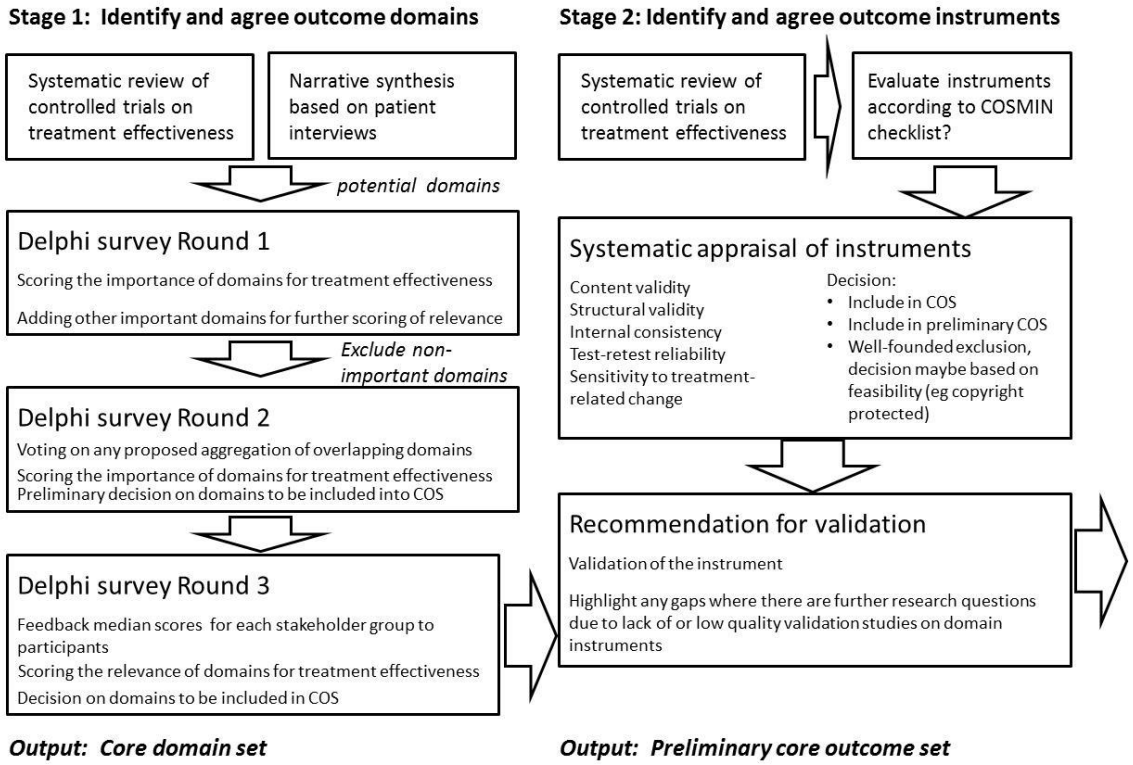

Figure 5.2-3 - Roadmap of WG5 activities.

This European Concerted Research Action (COST Action) intended a stepwise approach which involved identification of (1) meaningful clinical and demographic characteristics for tinnitus subtyping, (2) tinnitus related changes of brain activity in the different forms of tinnitus, (3) intermediate genetic phenotypes for the identification of genetic factors in the pathogenesis of tinnitus and (4) predictors for response to various treatments. This approach required a coordinated effort from basic scientists, technicians and clinicians of different disciplines working together in ongoing close collaboration. Up to now 235 researchers out of 30 participating countries registered for being a participant in the TINNET Action.

Haúla Haider has actively participated since the beginning of the TINNET (April/2014) as co-chair of clinical working group and member of the working group 5. She has participated in a Short Time Scientific Mission at University of Nottingham (UK), NIHR Nottingham Biomedical Research Centre, Hearing Sciences group, Division of Clinical Neuroscience, School of Medicine. She has also organized a training school and TINNET meetings and was member of WG1 and WG5 steering groups dynamically participating at scientific activities of both groups. The principal delivers from WG1 were the European survey about tinnitus assessment and treatment, a systematic review about existing tinnitus guidelines (Fuller & Haider et al., 2017), the Multidisciplinary European Guidelines for Tinnitus: diagnostics, assessment and treatment.

WG3 has established a consensus for neuroimaging data acquisition based on input from tinnitus experts in neuroimaging from across the world. Recommendations for electroencephalography (EEG) and magnetoencephaology (MEG) are already available on the TINNET website. The recommendations for magnetic resonance imaging (MRI) are in the final stages of dissemination.

WG4 have published an updating study exposing that the available evidence for genetics in tinnitus is scarce and addressing the ideal design of studies concerning tinnitus genetic should use concordance twin studies and optimize patient selection according to phenotype and/or etiology to avoid genetic interpretation bias (Lopez-Escamez et al., 2016).

From WG5, a systematic review about outcome domains and instruments used in clinical trials of tinnitus treatments in adults and a narrative synthesis of research evidence for tinnitus-related complaints as reported by patients and their significant others, member of Delphi steering group COMiT'ID, participating as key advisor involved in activities that conducted to the Delphi process of achievement of a core outcome set of recommended outcome that should be evaluated in tinnitus effectiveness treatment on sound, psychologic or pharmaceutics therapies. The action had its final meeting in March 2018 but there are still ongoing research networking activities.

5.2.2. Different Teams, Same Conclusions? A Systematic Review of Existing Clinical Guidelines for the Assessment and Treatment of Tinnitus in Adults

The data presented has been published by Fuller, T. E., Haider, H. F., Kikidis, D., Lapira, A., Mazurek, B., Norena, A., Rabau S., Lardinois R., Cederroth C.R., Edval, N.K., Brueggeman P.G., Rosing, S. N., Kapandais, A., Lungaard, D., Hoare D.J. & Cima, R.F.F. (2017) as is possible to see at

Fuller, T. E.*, Haider*, H. F., Kikidis, D., Lapira, A., Mazurek, B., Norena, A., ... & Cima, R. F. F. (2017). Different teams, same conclusions? A systematic review of existing clinical guidelines for the assessment and treatment of tinnitus in adults. *Frontiers in psychology, 8*, 206.

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Different Teams, Same Conclusions? A Systematic Review of Existing Clinical Guidelines for the Assessment and Treatment of Tinnitus in Adults

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Background: Though clinical guidelines for assessment and treatment of chronic subjective tinnitus do exist, a comprehensive review of those guidelines has not been performed. The objective of this review was to identify current clinical guidelines, and compare their recommendations for the assessment and treatment of subjective tinnitus in adults.

Method: We systematically searched a range of sources for clinical guidelines (as defined by the Institute of Medicine, United States) for the assessment and/or treatment of subjective tinnitus in adults. No restrictions on language or year of publication were applied to guidelines.

Results: Clinical guidelines from Denmark, Germany, Sweden, The Netherlands, and the United States were included in the review. There was a high level of consistency across the guidelines with regard to recommendations for audiometric assessment, physical examination, use of a validated questionnaire(s) to assess tinnitus related distress, and referral to a psychologist when required. Cognitive behavioral treatment for tinnitus related distress, use of hearing aids in instances of hearing loss and recommendations against the use of medicines were consistent across the included guidelines. Differences between the guidelines centered on the use of imaging in assessment procedures and sound therapy as a form of treatment for tinnitus distress respectively.

Conclusion: Given the level of commonality across tinnitus guidelines from different countries the development of a European guideline for the assessment and treatment of subjective tinnitus in adults seems feasible. This guideline would have the potential to benefit the large number of clinicians in countries where clinical guidelines do not yet exist, and would support standardization of treatment for patients across Europe.

Keywords: tinnitus, clinical guidelines, assessment, treatment, systematic review

INTRODUCTION

Tinnitus is essentially made up of two components, the phantom perception of a sound in the ears or head, and the degree of emotional reaction to that percept. Tinnitus can co-occur with several medical-otological disorders such as presbycusis, though etiology is unknown for the majority of tinnitus patients (Baguley et al., 2013b). In rare cases tinnitus indicates a serious underlying pathology such as vascular troubles, vestibular schwannoma (VS), or otosclerosis (Baguley et al., 2013a). In most cases however subjective tinnitus is a benign symptom. In many patients co-morbidities exist such as anxiety, depression, insomnia, and concentration problems, all of which severely impair quality of life (Langguth et al., 2011). In 1–3% of cases tinnitus causes severe health problems, with a wide range of effects on daily life functioning (Davis and Refaie, 2000; Fujii et al., 2011; Kim et al., 2015). Evidence corroborates that the aversive psychological reactions, such as cognitive problems, negative emotions, and dysfunctional attentional processes are of main importance in leading to a severe tinnitus condition (Erlandsson and Hallberg, 2000; Andersson et al., 2006; Cima et al., 2011; Kleinstauber et al., 2013; McKenna et al., 2014; Handscomb et al., 2017).

During the last decades, efforts have been made to better understand tinnitus pathophysiology and provide specialized treatments to patients (Kamalski et al., 2010; Cima et al., 2012; Langguth et al., 2013; Hoekstra et al., 2014). A large number of management strategies including various assessment and treatment procedures exist and have evolved but lack empirical support. For example, there is no evidenced treatment or licensed pharmacological therapy to eliminate the tinnitus percept (Langguth and Elgoyhen, 2012). The Cochrane Library lists 10 completed systematic reviews on different tinnitus treatments, all of which reported small numbers of studies of variable quality (e.g., Martinez-Devesa et al., 2010). These facts combined makes it difficult for healthcare professionals to decide what is best for which tinnitus patient. This is evidenced by the discrepancy between scientific and clinical perspectives on the management of tinnitus and the *actual* day-to-day practice in European healthcare settings (Hoare et al., 2012); tinnitus patient care is fragmented and *ad hoc* (Hoare and Hall, 2011; Hoare et al., 2012). To date there has been no overview of the number of existing clinical practice guidelines for tinnitus, the details included, their comparability, or their purpose. Clinical practice guidelines are defined as systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances (Field and Lohr, 1990). They have the benefit of simplifying and standardizing assessment and treatment options for clinicians and patients. A European Union guideline would extend this benefit to 28 countries. This systematic review aims to identify, review, and examine the clinical guidelines which do exist for tinnitus. The tinnitus assessments (diagnostics and measures), processes, and treatment options recommended by the respective guidelines will be compared and summarized.

METHODS

The aims, the work plan, and the protocol for this systematic review were developed by TINNET Working Group 1, a COST Action BM1306 (2014–2018) to create a pan-European tinnitus research network (http://tinnet.tinnitusresearch.net/). This review was registered with PROSPERO, the international register of systematic reviews (protocol number: CRD42016038588) prior to commencing the literature search. The review was exempt from human ethics procedures as there were no human participants and only secondary sources of data (the clinical guidelines) were used.

Eligibility Criteria

Records were considered eligible for inclusion if they fit the definition of a guideline by describing and making recommendations on the assessment, diagnosis, and or treatment of subjective tinnitus for adults (i.e., people aged 16 years or older). Those records were required to identify or describe themselves as guidelines, and be the most recent guideline form the country of origin. No publication date or language restrictions were imposed on the eligibility of the guidelines.

Guidelines were excluded if they were for objective tinnitus, pediatrics, referred only to the triage or referral pathways for assessing and treating tinnitus, or if they were a guide for only one specific type of assessment or treatment procedure for tinnitus.

Literature Search

The literature search for clinical guidelines included the Medline, PubMed, and the Cumulative Index to Nursing and Allied Health Literature (CINAHL), and EMBASE databases. In addition to these the National Guideline Clearinghouse (www.guideline.gov), National Institute for Health and Clinical Excellence (NICE; https://www.nice.org.uk/), Guideline International Network (GIN; http://www.g-i-n.net/), Google, and hand-search of reference lists of any included guidelines was undertaken. International experts were also contacted to ask if they were aware of any guidelines that had not already been identified from

the search results. The date that the search for guidelines was first conducted was 2 May 2016 and was undertaken by TEF and HH using "tinnitus" and "guideline" as the two key terms. The final search was conducted on 24 June 2016.

Study Selection

Two reviewers independently screened search results by title and abstract, and then by full text if required. The first 20 pages of search results from Google, and all search results from GIN, NICE, and the National Guideline Clearinghouse were screened. In the event of disagreements, a third reviewer (BM) acted as an arbiter. As an additional check and in line with other systemic review searches using internet search engines, a *post-hoc* rule of stopping searching after three consecutive pages without new search results was applied. In this case, no new search results were identified after the first eight pages.

Data Extraction

Data extraction was undertaken using a tailored form that had been pilot tested and was emailed to reviewers in the form of an Excel spreadsheet. A document with guidance on the extraction of information for each of the items was provided to each of the reviewers to improve consistency of data extraction. Data extraction from each guideline was undertaken by at least two reviewers who were native speakers of or fluent in the language in which the guideline was published. Reviewers extracted information from the guidelines regarding items about the: country and year of publication, availability, author details, sponsor/funder involved, scope, target audience, developers and process related to the guideline, recommendations for assessment and treatment procedures, the level of evidence and type of rating system used (e.g., Oxford) related to the recommendations, and items related to the implementation and revision of the guideline.

Data Management

HH and TEF were responsible for data management and maintained editorial rights. All identified records were saved into a Microsoft word master file and then saved in pdf-copy.

Quality Assessment and Risk of Bias

All reviewers of the guidelines also completed the AGREE II tool (Brouwers et al., 2010) to assess the quality of the guidelines. AGREE II is an international tool to assess the quality and reporting of practice guidelines (www.agreetrust.org). It contains 23 items grouped under six guideline domains. Each item is scored on a 1–7 scale where 1 = "Strongly disagree" and 7 = "Strongly agree." Scores are standardized to provide an overall percentage score. Previous reviews have used a 60% marker to distinguish high and low quality guidelines (Sanclemente et al., 2014; Ruszczynski et al., 2016).

Details relating to the sources of funding, professional affiliations, and editorial independence of the guideline developers were extracted as indicative of risk of bias.

Data Synthesis

Data extracted by the reviewers were collated and integrated into summary tables and a narrative synthesis describing the similarities and differences between the clinical practice guidelines was completed.

RESULTS

Five clinical guidelines for tinnitus were ultimately included in this review (see **Figure 1** for details of the search and selection process). They were guidelines from Denmark (Jørgensen et al., 2007), Germany (The Association of the Scientific Medical Societies, 2015), The Netherlands (Dutch Association for Ear Nose Throat and Head surgery [Nederlandse Vereniging voor Keel – Neus – Oor heel kunde en Heelkunde van het Hoofd – Halsgebied], in press), Sweden (Idrizbegovic et al., 2011), and United States (Tunkel et al., 2014). Several documents were excluded as by definition not providing a guideline. For example, the Australian audiology clinical practice standards (Audiology Australia, 2013) underwent full-text screening but was not included as it only related to audiological management and had a brief section on tinnitus assessment. The UK Good Practice Guide (Department of Health, 2009) also was excluded as it explicitly states: "This Good Practice Guide to the delivery of services is not, and does not aim to be, an evidence-based guideline for clinical practice with individual patients" (p. 5). The Tinnitus Research Initiative (TRI) algorithm (Biesinger et al., 2010), after some debate within the review team, was also excluded because it was judged not to be a "clinical guideline." A list of full text documents considered but excluded is in Appendix 1.

Although there was not a restricting time period for the guidelines, no guidelines older than 10 years were identified. With exception of the Danish guideline (published in 2007) all were developed during the last 5 years.

Details about Development of the Clinical Guidelines

Table 1 provides detailed information about the stakeholder involvement, rigor of development, and the editorial independence associated with the respective clinical guidelines.

All the guidelines included information on the professional backgrounds of the participants in the respective development groups and in three out of the five cases (American, Dutch, and German), provided information on how views of funding bodies and competing interests were addressed. Although patient groups and the public were consulted in the development of three guidelines (American, Dutch, and German), the actual expected users of the guidelines were health professionals.

Details were provided in all guidelines (with the exception of those from Sweden which did not provide methodological information) about how literature was located and used to inform the respective recommendations. That is, details of search strategies using MeSH and other search terms and databases such as Medline and PsychInfo were included. Tools and criteria used to assess the evidence included the: Oxford Centre for Evidence Based Medicine (U.S. and German guidelines) and American Academy of Paediatrics' (American guideline) evidence criteria respectively, the AMSTAR checklist (Dutch guideline), and

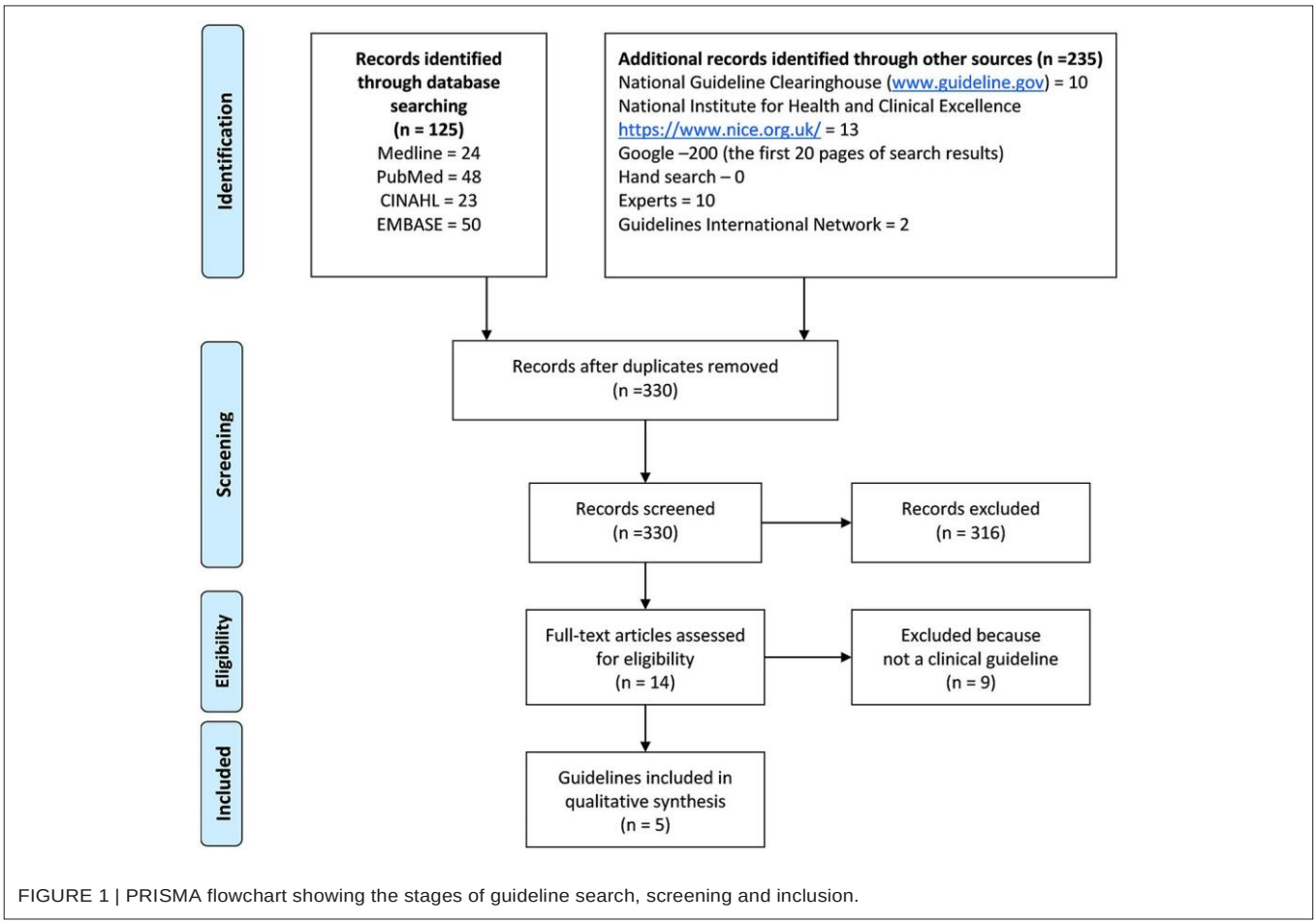

FIGURE 1 | PRISMA flowchart showing the stages of guideline search, screening and inclusion.

the GRADE ranking system of trust in conclusions of the literature (Dutch guideline). American, Danish, Dutch and German guidelines all provided information and referred to the research literature associated with each recommendation as well as describing their methods for reaching consensus on each recommendation. The Dutch, German and U.S. guidelines consider the strengths and limitations of the research literature and were reviewed externally prior to publication. Similarly, those three guidelines also state a year by and/or describe conditions under which they would be revised.

Assessment Recommendations in the Clinical Guidelines

Table 2 compares assessment recommendations between the respective national tinnitus guidelines. All guidelines, except the Danish, recommend a clinical history (anamnesis/targeted history/special tinnitus anamnesis) be taken.

All guidelines describe the need for physical examination by an ENT doctor, although physical examination is not explicitly referred to in the Swedish guideline. The American guideline recommends examination to exclude objective tinnitus, cardiovascular disease and vascular lesions, neurologic diseases, middle or outer ear infection/disease, vertigo, head-neck masses, or other treatable conditions. The German guideline additionally mentions cervical, dental, and temporomandibular joint functional exploration in a silent environment to evaluate tinnitus modulation.

Audiological assessment was recommended in all the included guidelines. The majority refers to audiometry as a general category, but the German guideline provides most detail. For example, it specifies details relating to the assessment of oto-acoustic measurements, brainstem auditory evoked responses, caloric tests, determination of tinnitus loudness and frequency using narrow-band noise and pure tones, residual inhibition, Feldmann masking curves (Feldmann, 1984), and loudness discomfort level. None of the other guidelines included in this review recommend psychoacoustic measurements of tinnitus frequency or intensity.

The German guideline does not refer to specific psychological assessments though the other guidelines do in varying terms. For example, when tinnitus is severe or accompanied by psychological factors, the Swedish guideline recommends psychological assessment while the Danish guideline recommends a structured interview. The American guideline on the other hand recommends that clinicians distinguish between patients with or without bothersome tinnitus for subsequent referral (when necessary) to a psychologist or psychiatrist.

TABLE 1 | Summary of guideline development by country.

Country	Professionals involved	Views of patients considered	Target users	Views of funding body	Competing interests
Germany	Audiologists, psychiatrist, psychologists, otolaryngologists, dentists, pediatricians, neurologists, and patient representative groups	Patient representative groups were included in the guideline development group; contributed to external review on draft documents, and patient related information was also considered from the results of a literature review	Physicians (especially ENT), phoniatry and pediatric audiology, psychiatry, psychosomatic, neurology, mouth, jaw, and facial surgeons and dentists, psychologists, general practitioners	A statement concerning financial and other interests and editorial independence is included.	Competing interests are declared and when relevant, stakeholders with competing interests were excluded
Denmark	Speech Pathologist and hearing therapists	NS	Hearing therapists	NS	NS
Netherlands	Details provided. ENT-doctors, psychologist, clinical physicist-audiologist	Dutch Association of the Hearing Impaired consulted. A literature review regarding patient preferences was also conducted	ENT doctors, audiology centers, GP's, psychologists, psychiatrists	A statement of independence was signed by professionals involved	Competing interests are declared
USA	Paediatric and adult otolaryngologists, otologists/neurotologists, geriatrician, behavioral neuroscientist, neurologist, audiologist, family physician, radiologist, psychiatrist, psycho-acoustician, nurse, physician, and consumer advocates	Yes: also included a draft of the guideline being made available for public comment	Any clinician, health care provider, specialty physicians, and non-physician providers such as audiologists and mental health professionals	Funded by American Academy of Otolaryngology—Head and Neck Surgery Foundation but no statement of independence from the process	Competing interests are declared
Sweden	Partial details provided—included medical doctors, and professional representatives from the tinnitus teams for diagnostics and rehabilitation	NS	Staff at the audiology and balance clinic at Karolinska University Hospital and professionals that might refer to the clinic (GPs, ENTs or audiologist)	NS	NS

(Continued)

TABLE 1 | Continued

Country	Methods used	Evidence criteria	Category of evidence*	Strengths and limitations	Methods for reaching consensus	Consequences of the recommendations	Link between evidence and recommendations	Peer review?	Update of the guidelines?
Germany	Systematic methods used, details provided in the guideline	Classified according to Oxford Centre of Evidence-based Medicine criteria	1a	Strengths and limitations of the body of evidence are clearly described	Formal consensus technique	The guideline includes health benefits, side effects and risks formulating the recommendations	There is an clear link between the recommendations and the supporting evidence	External review	Due in 2020.
Denmark	Systematic methods used, details provided in the guideline	NS	The guidelines are based on literature, and articles based on the consensus of leading professionals in the field of audiology (evidence level IV)	NS	Informal consensus. All recommendations are based on the ICF model	NS	Each recommendation is provided with an argument based on relevant literature	Not peer reviewed	NS
Netherlands	Systematic methods used, details provided in the guideline	Based on AMSTAR checklist	1a, 1b, IV	The strength of the evidence is specified according to GRADE. Evidence tables describe limitations and strengths of the included studies	Recommendations were evidence based and the importance the workgroup gave to them conforms to GRADE	Recommendations were made considering the scientific value, preferences of the patient, costs, and availability of the organization	There is a clear link between the recommendations and the supporting evidence	External review	Update due in 2020 or sooner if new compelling evidence warrants earlier consideration
USA	Systematic methods used, details provided in the guideline	Based on criteria from the Oxford Centre for Evidence-Based Medicine	American Academy of Pediatrics Categories of evidence (A, B, C, D, and X) updated to be in accordance with Oxford Centre for Evidence-Based Medicine	Strengths and limitations of the body of evidence are clearly described	This guideline was developed using an explicit and transparent a priori protocol for creating actionable statements based on supporting evidence and the associated balance of benefit and harm	The benefits and harms of the recommendations have been considered for each recommendation.	There is an clear link between the recommendations and the supporting evidence	External review	Update due in 2018/9 or sooner if new compelling evidence warrants earlier consideration
Sweden	No method reported	No evidence criteria	No evidence provided	None provided.	NS	NS	NS	NS	NS

**Unless stated, the level of evidence refers to/uses the Oxford Centre for Evidence Based Medicine criteria (GRADE system consists of 4 grades of degree of trust in conclusions of the literature: high, moderate, low, and very low) NS, not specified; ENT, Ear Nose Throat; GP, General Practitioner.*

TABLE 2 | Clinical guideline recommendations regarding assessment of patients with tinnitus.

Guideline	Physical examination	Hearing and audiology tests	Psychological assessment	Assessment tools/questionnaires recommended	Other assessment procedures	Procedures not recommended
Germany	Orientating neurological assessment of cervical spine, vestibular is with examination of denture (including TMJ) in silence to screen modulation of tinnitus • Orientating examination of functioning of N. facialis • ENT examination including tympanic membrane microscopy, asopharyngoscopy and eustachian respectively • stethoscopic examination of the ear and of the carotid artery, particularly in pulsatile tinnitus	• Pure tone audiometry • discomfort, possibly with categorical loudness scaling • determining of tinnitus loudness and frequency using narrow-band noise and pure tones • residual inhibition • determining the minimum masking level by white noise and pure tones; masking curves according to Feldmann • tympanometry and acoustic reflex including recording possible changes due to breathing or heart rate • TEOAE and/or DPOAE • brainstem auditory evoked response (BAER) • preliminary vestibular examination possibly including caloric testing • Brainstem audiometry (BERA) when medically justified, economically viable and likely to be useful in informing counseling might be of potential benefit	NS	• Goebel-Hiller Tinnitus Questionnaire, • VAS or other validated scales	• Special tinnitus anamnesis (see Structured Tinnitus Interview (Goebel and Hiller, 2001) • X-rays of the cervical spine, if further indicated also functional images	• Acoustic examination with more than 84 dB 1 week after acute tinnitus or tinnitus exacerbation
Denmark	NS	• Audiometry (performed by ENTs) • LDL/UCL • If necessary also: ABR	• Structured interview	• THI-DK • VAS-scale for hyperacusis • Tværfaglig Tinnitus Screening (Danish tool assessing signs of anxiety)	If necessary also: • ABR, • CT/MRI, • blood samples, • other neurological tests	NS
Netherlands	• Anamnesis, • ENT-assessment inclusive otoscopy and tuning fork tests, • Blood pressure measurement, • Flexible nasofaryngoscopy, • Palpation of neck and area around ear	• Audiometry (Air and bone conduction) • Speech audiometry	• Detailed assessment regarding the nature how tinnitus impacts on daily life and functioning, comorbid symptoms	• TQ, mini-TQ • THI • TFI • THQ • HADS	• MRI/MRA, • CT, • DSA (angiography)	• Not to use MRI with every patient with non-pulsatile, unilateral tinnitus.

(Continued)

TABLE 2 | Continued

Guideline	Physical examination	Hearing and audiology tests	Psychological assessment	Assessment tools/questionnaires recommended	Other assessment procedures	Procedures not recommended
USA	• Targeted history and physical examination of the head and neck including otoscopy and neurologic examination. • When pulsatile tinnitus is reported, the examination should focus on identification of cardiovascular disease and vascular lesions	• Prompt, comprehensive audiological examination (Tonal and Speech audiometry and Immittance) in patients with tinnitus that is unilateral, persistent (≥6 months), or associated with hearing difficulties (Strong recommendation); • Initial comprehensive audiological examination (including ear specific masked air and bone conduction) in patients who present with tinnitus regardless of laterality, duration, or perceived hearing status (Option)	• Distinction between patients with bothersome tinnitus from patients with non-bothersome tinnitus. • Assess degree of tinnitus related disability (including baseline measurement for the purpose to establish effects of treatment). • Assess if further psychological treatment required	• TQ, • TEQ, • THQ, • TRQ, • THI, • TFI	NS	• Imaging studies unless patients have one or more of the following: tinnitus that localises to one ear, pulsatile tinnitus, focal neurological abnormalities, or asymmetric hearing loss
Sweden	NS	• Audiometry (including LDL when necessary) • Speech and speech in noise test and impedance audiometry • ABR and MRI when necessary	• In case of severe tinnitus: the first encounter with the psychologist/ psychiatrist is investigative and informative. (1) symptoms tinnitus, (2) individual's mental status, (3) the overall life situation	• BAS (basic own questionnaire), • THI • HADS (when necessary)	• Anamnesis focused on tinnitus onset, laterality, character and patients' problems. • Consideration of psychological factors and somatosensory factors	NS

ABR, Auditory brainstem response; BAER, Brainstem auditory evoked response; CT, Computer tomography; DSA, Digital subtraction angiography; DPOAE, Distortion product optoacoustic emission; ENT, Ear nose throat; GP, General Practitioner; HADS, Hospital Anxiety and Depression Scale; LDL, Loudness discomfort level; MRA, Magnetic resonance angiography; MRI, Magnetic resonance imaging; TEOAE, Transient evoked optoacoustic emission; TEQ, Tinnitus evaluation questionnaire; TFI, Tinnitus functional index; THI, Tinnitus handicap inventory; THQ, Tinnitus handicap questionnaire; TMJ, Temporomandibular joint; TQ, Tinnitus questionnaire; TRQ, Tinnitus reaction questionnaire; UCL, Uncomfortable listening level; VAS, Visual analog scale.

The Tinnitus Handicap Inventory (THI; Newman et al., 1996, 1998) is the most frequently referred to assessment questionnaire followed by Tinnitus Questionnaire (TQ; Goebel and Hiller, 1994). Visual Analog Scales (VAS; e.g., Germany, Denmark) and the Hospital Anxiety and Depression Scale (HADS; Zigmond and Snaith, 1983, e.g., The Netherlands, Sweden) were referred to by at least two guidelines. The American guideline referred to a large number of questionnaires including the: TQ (Goebel and Hiller, 1994), THI (Newman et al., 1996, 1998), Tinnitus Effects Questionnaire (TEQ; Hallam et al., 1988), Tinnitus Handicap Questionnaire (THQ; Kuk et al., 1990), Tinnitus Reaction Questionnaire (TRQ; Wilson et al., 1991), and Tinnitus Functional Index (TFI; Meikle et al., 2012).

Several guidelines make recommendations for or against the use of other assessment related procedures. For example, the German guideline refers to X-rays of the cervical spine. Although three guidelines recommend magnetic resonance imaging (MRI) as an assessment of tinnitus, The American and the Dutch guideline recommend against it, unless patients have one or more of: tinnitus that localizes to one ear, pulsatile tinnitus, focal neurological abnormalities, or asymmetric hearing loss. The German guideline also recommends against acoustic examination using sound pressure levels more than 84 dB 1 week after acute tinnitus or tinnitus exacerbation.

Summary of Recommendations Regarding the Assessment of Subjective Tinnitus

- Conduct a thorough physical examination to exclude possible (physical) causes of tinnitus (three of five guidelines; not stated in Danish and Swedish).
- Complete a thorough audiological assessment (all guidelines).
- Establish the degree to which a patient experiences subjective tinnitus as bothersome or distressing using a validated and reliable multi-item questionnaire such as the TQ, THI, TFI, or HADS (all guidelines).
- In situations where patients appear to be experiencing a degree of distress or difficulties related to living with tinnitus, consider making a referral for an assessment by a psychologist or psychiatrist (four of five guidelines; not stated in German guideline).
- Variation exist in recommendations regarding the use of imaging studies (e.g., MRI).

Treatment Recommendations across the Guidelines

Table 3 compares therapeutic recommendations for the treatment of subjective tinnitus between the respective national tinnitus guidelines; note the Danish guideline is not included in this table as it provides only recommendations regarding assessment procedures. Across the guidelines there is generally a high degree of consistency in the recommendations for or against: the use of medicines (prescribed drugs and herbal supplements); audiological and psychological interventions; and, transcranial magnetic stimulation. Greatest variation occurs in the recommendations concerning the use of therapies involving sound such as Tinnitus Retraining Therapy (TRT).

There is a consensus that medicines should not be prescribed for the treatment of subjective tinnitus, though some variation in the level of specificity that each guideline has. For example, the German guideline lists specific medicines that should not be prescribed for the treatment of tinnitus. The German and Swedish do however note that medicines such as antidepressants might be prescribed to treat comorbid conditions. Herbal supplements such as Gingko biloba are also specifically recommended against being used in all guidelines except for Sweden which does not make recommendations for or against their use.

The use of hearing aids is recommended by all guidelines but only when clinically meaningful hearing loss is also present in people suffering from tinnitus. The use of a cochlear implant is mentioned in the Dutch and German guidelines and only recommended when there is profound hearing loss or deafness in addition to tinnitus. The Dutch guideline is the only one to provide scores on tinnitus questionnaires (e.g., TQ, THI) for when such interventions should be considered (e.g., it recommends referral to specialized stepped-care CBT for tinnitus in cases where TQ score is greater than 30, in combination with a clinically relevant request for healthcare by the patient, as is judged by the referring party).

Psychological interventions for tinnitus can potentially include a wide range of components but there is general consensus on the use of two of them. In particular, the provision of information and education about tinnitus and treatment options is consistently recommended across the guidelines although there is some variation in the specificity of the content that each provides. Second, specialized CBT for tinnitus is specifically recommended by all the guidelines except for Sweden which mentions it only in relation to the presence of stress, anxiety, or depression.

Least consistency exists across the guidelines in relation to TRT. Specifically, the Dutch guideline recommends that TRT can only be contemplated if tinnitus is very mild (TQ $<$ 30) and the patients specifically asks for TRT, the American guideline indicates that sound therapies "may" be recommended to patients with tinnitus, while the Swedish guideline recommends that sound stimulation be used as part of TRT for people without hearing loss. The German guideline recommends the use of notched music therapy, but recommends against the use of TRT.

In relation to other less commonly used treatments (such as acupuncture or hyperbaric oxygen), the guidelines mostly indicate that there is an insufficient body of evidence to be able to make recommendations for or against their use.

Lastly, three guidelines (Germany, The Netherlands, and U.S.) either caution that there is insufficient evidence, or make additional recommendations against the use of transcranial magnetic stimulation (rTMS), transcranial direct current stimulation (tDCS), dietary supplements, neuromodulation treatments, and hearing aids for tinnitus patients without hearing loss.

Summary of Therapeutic Recommendations regarding the Treatment of Subjective Tinnitus

- Provide information about tinnitus and treatment options (all guidelines).
- Use hearing aids only when patients also experience hearing loss (all guidelines).

TABLE 3 | Tinnitus guideline recommendations regarding treatments for tinnitus.

Country	Medicine	Audiological	Psychological	Sound therapies	Other	Treatments recommended against using
Germany	• None for tinnitus but refers to, for example, some for co-morbid depression, e.g., glutamate-antagonists.	• Hearing aids for patients with hearing loss; Cochlear Implants for patients with deafness.	• General counseling (including information provision). • Tinnitus specific CBT (aimed at reducing attention focusing toward the ear noise, reappraisal of the tinnitus and its consequences) individual or group-settings, also treatment for comorbidities. • Hospital treatment for decompensated tinnitus and/or with severe psychiatric comorbidity. • An absence of conclusive evidence of effectiveness for self-help groups.	• Audio therapy including "notched music," "coordinated reset" or music therapy.	• Absence of evidence of effectiveness for: acupuncture, cervical vertebral spine therapy/ physiotherapy, hyperbaric oxygen; and, electric stimulation (e.g., transcutaneous electric stimulation, ear and cervical spine; vagus stimulation); Acoustic Coordinated-Reset Neuromodulation. • Uncertain recommendation for rTMS.	• Sound therapy including Noiser and TRT. • Hearing aids for patients with only tinnitus. • Medicines (including: steroids, melatonin, antidepressants, Sulpirid, Apraxolam, Sertraline, Botox A, Pramiprexol, Nortriptyline, Piribedil, Vardenafil, Trazodone, Atorvastatin, Gabapentin, anticonvulsants, Paroxetine, Lamotrigine, Cyclandelat, Baclofen, Nicotinamide, Tocainid, Misoprostol, Egb 761, Amitriptyline, Misoprostol, Pramipexole Dopamine. • Herbal medicines and vitamins (including Ginkgo biloba zinc).
Netherlands	None	• Consider a trial of hearing aids. • In patients with high TQ (>60) or THI (>78) scores, and have severe hearing loss or deafness and have not responded to CBT, consider Cochlear Implant.	• Educational material about tinnitus and treatment options considered essential. • Specialised CBT for patients with TQ > 30 or THI >36.	• TRT can only be contemplated in case tinnitus is very mild (TQ<30) and the patients specifically asks for TRT.	None	• rTMS. • TDCS. • Gingko biloba. • Acupuncture. • Auditive perceptual training. • Hyperbaric oxygen.
Sweden	• None for tinnitus specifically but does state that if necessary, sleeping pills or antidepressants, can be used to treat sleep disorders or depression (no drug types, names, or dosage provided).	• For people with tinnitus and hearing loss hearing aids are fitted.	• Individual or group tinnitus information meetings. For patients without hearing loss, this is based on a modified version of TRT protocol. • There is reference to CBT in case of stress/ anxiety/ depression, but no clear recommendation.	• Sound stimulation as part of TRT for people without hearing loss.	• For middle ear dysfunctions such as otosclerosis, surgery is possible – no clear recommendation is provided. • For tensions or pain in the jaw, neck, shoulders or back, referral to "bite" therapist, or physiotherapist.	None

(Continued)

TABLE 3 | Continued

Country	Medicine	Audiological	Psychological	Sound therapies	Other	Treatments recommended against using
USA	None	• Clinicians *should* recommend a hearing aid evaluation for patients with hearing loss and persistent, bothersome tinnitus.	• Clinicians *should* educate patients with persistent, bothersome tinnitus about management strategies. • Clinicians *should* recommend cognitive behavior therapy to patients with persistent, bothersome tinnitus.	• Clinicians *may* recommend sound therapy (e.g., TMT, TRT) to patients with persistent, bothersome tinnitus but patients must be informed of potential outcomes as well as costs associated with sound therapy.	• *No recommendation* can be made regarding the effect of acupuncture in patients with persistent bothersome tinnitus based on the poor quality of trials, no benefit, and minimal harm.	Clinicians *should not* routinely recommend: • Medicine (including antidepressants, anticonvulsants, anxiolytics, or intratympanic medications for a primary indication of treating persistent, bothersome tinnitus. • Dietary supplements and herbal medicines (e.g., Ginkgo biloba, melatonin, zinc). • TMS

CBT, Cognitive behavioral therapy; NS, not specified; rTMS, repetitive Transcranial magnetic stimulation; TCDS, Transcranial direct stimulation; THI, Tinnitus handicap inventory; TMS, Transcranial magnetic stimulation; TMT, Tinnitus management therapy; TQ, Tinnitus questionnaire; TRT, Tinnitus retraining therapy.

- Specialised CBT for tinnitus should be offered to patients (three of four guidelines; Sweden refers to use of CBT in context of co-morbid anxiety or depression).
- There is a lack of consensus on the use of TRT for tinnitus.
- Prescribed medicines and herbal supplements should not be used for the treatment of tinnitus (all guidelines).
- Treatment with TMS is recommended against by Dutch and U.S. guidelines, and German guidelines give an "uncertain" recommendation.

Quality Assessment of the Guidelines

The AGREE II tool was used by the authors who undertook data extraction of the respective guidelines and the summarized results are shown in **Table 4**. In general the domains of "stakeholder involvement" and "clarity of presentation" respectively by guideline developers were rated high (good quality). Conversely, ratings on the domain of "applicability" which refers to how the guidelines might be disseminated, implemented and evaluated were low. For the domains addressing the scope and purpose of the guidelines, rigor of development and editorial independence, a pattern emerged whereby the American, Dutch and German guidelines were rated considerably higher (AGREE II scores >60% on all domains) than the Danish and Swedish guidelines (AGREE II scores <60% on all domains).

DISCUSSION

This systematic review aims to compare existing clinical guidelines for the assessment and treatment of subjective tinnitus in adults. Five guidelines, developed in the last 10 years within Europe, Scandinavia, and North America were included in the review. Although there are differences in some specific recommendations for assessment and treatment procedures across the guidelines, in general, commonalities across guidelines were high. The fact that there are differences in some of the recommendations is not surprising and appears to reflect the relatively young state of the field and the evolving nature of assessment and treatments for subjective tinnitus—a symptom with a high level of heterogeneity. On the other hand, the level of agreement, for example, in the recommendation of specialized cognitive behavioral therapy reflects the growing evidence base for the effectiveness of this treatment to alleviate patients' distress and impairment, even though significant changes in the tinnitus percept itself as a result of CBT have been proposed, though not yet assessed across studies.

When the methods of the development of guidelines were reported, it was clear that the respective groups were making efforts to be transparent, systematic, and using the best available evidence base, and frequently linking recommendations to specific research literature. For example, systematic reviews and meta-analyses were referred to whenever available to inform recommendations. It should be noted though that there is a lack of high quality studies or powered randomized trials of some treatments either for practical or methodological reasons. Regardless, the strengths and limitations of the evidence for particular recommendations were included for the majority

TABLE 4 | Summary of AGREE II domain scores (%) by country.

	Scope & Purpose	Stakeholder involvement	Rigour of development	Clarity of presentation	Applicability	Editorial independence
Germany	61	94	83	89	71	67
Denmark	52	44	24	59	2	17
Netherlands	81	100	97	100	9	100
USA	86	97	93	100	71	88
Sweden	42	42	1	33	2	13
Median	61	94	83	89	9	67
Average	64	75	60	76	31	57

of the guidelines and thus enable the user/reader to make informed decisions about following the recommended actions. Furthermore, target users were generally clearly defined and the development groups were comprised of a range of the health professionals often involved in the assessment and treatment of tinnitus. These two factors are important not only for providing expert input into the guideline, but also for garnering "buy-in" from potential users of the guidelines and focussing the content.

Differences between the Guidelines

Differences in recommended assessment procedures tend to relate to specific techniques (questionnaires, diagnostic tests, types of scanning techniques) rather than general principles [e.g., trying to establish tinnitus severity, hearing loss, psycho-social problem(s)], or the presence or absence of severe physical pathology that might be causing the tinnitus. Differences related to, for example, the recommended questionnaires for assessing tinnitus related interference and distress. While all the guidelines referred to the THI (the German guideline indirectly refers to this), only the American, Dutch and German guidelines referred to the TQ. Recommendations for specific questionnaires to measure psychological distress (especially symptoms of anxiety and depression) also varied with some guidelines not mentioning any (e.g., United States) and others such as the Dutch and Swedish guidelines which referred to the HADS. Differences also existed between the recommendations to assess loudness discomfort levels with the American and Dutch guidelines not recommending the use of such tests while the other guidelines did.

With regard to treatments, differences are found primarily regarding recommendations for the use of sound therapies. TRT specifically is not recommended by the German guideline, conditionally by the Dutch guideline and the American guideline indicates that clinicians "may" recommend it; TRT is currently being tested in a large multicenter trial in the U.S. (clinical trials ID: NCT01177137). A lack of evidences about other treatments such as acupuncture, hyperbaric oxygen and some herbal supplements leads most groups to recommend against them. The American guideline though is more cautious and simply states that because there is a lack of evidence they can neither recommend for or against the use of such treatments.

Differences in the recommendations of assessment and treatment procedures could be explained by a combination of factors including the time of the development of the guideline and availability of translated versions of the questionnaires (e.g., the TFI was published in 2012 which was after that of the Danish and Swedish guidelines), the known psychometric properties of the questionnaires themselves [e.g., concerns have been raised about the cross-cultural use of the HADS (Maters et al., 2013)], and the different methods used to reach consensus by the different guideline groups.

Consistencies across the Guidelines

Across the guidelines consensus appears to exist on a number of important general features of assessment relating to subjective tinnitus. Specifically, there is consensus about the initial need for excluding a physical cause of the tinnitus, conducting an audiometric assessment of the patient, using standardized questionnaires to measure degrees of tinnitus related distress, and when relevant, making referrals for further psychological assessment.

Regarding the therapeutic recommendations for the treatment of subjective tinnitus, all guidelines recommend against the use of medicines for the treatment of the tinnitus specifically but note that medicines are appropriate for treating co-morbid conditions. There is also agreement in the recommendations to use hearing aids for patients experiencing hearing loss and CBT to facilitate adjustment to the symptom, alleviate distress and tinnitus-related interference in daily life.

As a group of tinnitus researchers and clinicians, we endorse the specific principles and practices of assessment and treatment that are consistently found across the guidelines. Further, while a treatment for removing the tinnitus percept does not exist, we reiterate the importance of providing patients with bothersome tinnitus, evidence based cost-effective treatment(s) in a way (such as stepped care) that is minimally burdensome to the patient. That is patients who are assessed as having relatively little tinnitus related distress and interference should receive less intensive treatment in the first instance, than someone who is assessed as having severe levels of distress and interference in activities of daily living.

Strengths and Limitations of the Review

There are two critical factors that affect the conclusions that can be drawn from the included guidelines. Firstly, and as with all systematic reviews, the search strategy and inclusion criteria used determine what is located and subsequently included. In this review, we used the search terms "tinnitus" and "guideline" to conduct the search in a wide range of databases, repositories of clinical guidelines, and search engines, with the intention of being focussed enough to identify the most relevant documents within a manageable number of

search results. Only including the term "guideline" though might have resulted in relevant documents, albeit not called "guidelines," being omitted from search results. Similarly, our use of inclusion/exclusion criteria that led to the decision to exclude documents such as the TRI flowchart could be problematic as it (the TRI flowchart) is a comprehensive document potentially used in many situations to inform assessment and treatment decisions.

To minimize the risk of omitting relevant search results we contacted a range of international experts and members of guideline development groups. In addition to this, we conducted hand searches of the references lists of included guidelines for relevant sources. We also recruited native speakers to extract data from the respective guidelines in an effort to ensure that data collection was as accurate as possible. It is possible, that different search and inclusion criteria might have led to different documents being included. However, given the large range of assessment and treatment options and the limited evidence base around many treatments in particular, it is unlikely that our conclusions would differ significantly if further guidelines had been identified at this time. Future systematic reviews though will be able to use this as a reference point.

IMPLICATIONS AND CONCLUSIONS

As researchers from around the world are collecting and making efforts to better understand the heterogeneity of subjective tinnitus in adults and systematically evaluate assessment and treatment options, we have, for the first time, described the major similarities and differences between existing clinical guidelines for subjective tinnitus in adults. The results reveal true guidelines from only five countries and thus highlight a need to develop guidelines that are endorsed by the range of professionals involved in assessing and treating tinnitus. Although we do not place a great deal of weight on the quality assessment ratings of the guidelines, they do suggest that there is room for improvement particularly with regard to implementation and evaluation. The absence of guidelines contributes to the variations that exist in assessment and treatment of tinnitus internationally.

While it would be tempting to do so, it is beyond the scope of this paper to formulate a new or composite guideline based on the results that we have obtained. Instead, the results of this review in conjunction with those from a survey of European tinnitus healthcare providers and researchers (Cima et al., 2016) will form the basis of further work on the development of a set of European clinical guidelines for the assessment and treatment of tinnitus being undertaken by the COST-action TINNET: Working Group I "*Clinical*." As with existing clinical guidelines, attention will need to be given to how the future European guideline is disseminated, subsequently evaluated, and the implications for resource management considered. We expect it to be challenging task but one that will hopefully result in a more reliable and equitable assessment and treatment of tinnitus patients across Europe.

AUTHOR CONTRIBUTIONS

TF, HH, DK, AL, BM, AN, SR and RC, conceived and designed the study. TF and HH wrote and revised the protocol and manuscripts, conducted the literature search, data extraction, and data synthesis. SR, RL, CC, NE, PB, SNR, AK, DL, and BM extracted data. DH, AN, DK, and RC reviewed and edited manuscripts and contributed intellectually to the content of the manuscript. All authors approved the manuscript for submission.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

APPENDIX

Excluded full text documents

1. UK Good practice guide for adults with tinnitus (Department of Health, 2009).
2. Audiology Australia Professional Practice Standards - Part B Clinical Standards (Audiology Australia, 2013).
3. American Speech language and hearing association Tinnitus triage guidelines (American Speach Language Hearing Association, 2016).
4. Ear care, NHS Scotland General practice guide for ear care. (NHS Scotland, 2006).
5. TRI flowchart (Biesinger et al., 2010).
6. Clinical guide for audiologic tinnitus management: Assessment and Clinical guide for audiologic tinnitus management: Assessment (Henry et al., 2005a) and Treatment (Henry et al., 2005b).
7. Adult Tinnitus Management Clinical Practice Recommendation (Henry et al., 2015).
8. American Academy of Audiology Audiologic Guidelines for the Diagnosis and Management of Tinnitus Patients (American Academy of Audiology, 2000).

5.2.3. Systematic review of outcome domains and instruments used in clinical trials of tinnitus treatments in adults

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Systematic review of outcome domains and instruments used in clinical trials of tinnitus treatments in adults

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Abstract

Background: There is no evidence-based guidance to facilitate design decisions for confirmatory trials or systematic reviews investigating treatment efficacy for adults with tinnitus. This systematic review therefore seeks to ascertain the current status of trial designs by identifying and evaluating the reporting of outcome domains and instruments in the treatment of adults with tinnitus.

Methods: Records were identified by searching PubMed, EMBASE CINAHL, EBSCO, and CENTRAL clinical trial registries (ClinicalTrials.gov, ISRCTN, ICTRP) and the Cochrane Database of Systematic Reviews. Eligible records were those published from 1 July 2006 to 12 March 2015. Included studies were those reporting adults aged 18 years or older who reported tinnitus as a primary complaint, and who were enrolled into a randomised controlled trial, a before and after study, a non-randomised controlled trial, a case-controlled study or a cohort study, and written in English. Studies with fewer than 20 participants were excluded.

Results: Two hundred and twenty-eight studies were included. Thirty-five different primary outcome domains were identified spanning seven categories (tinnitus percept, impact of tinnitus, co-occurring complaints, quality of life, body structures and function, treatment-related outcomes and unclear or not specified). Over half the studies (55 %) did not clearly define the complaint of interest. Tinnitus loudness was the domain most often reported (14 %), followed by tinnitus distress (7 %). Seventy-eight different primary outcome instruments were identified. Instruments assessing multiple attributes of the impact of tinnitus were most common (34 %). Overall, 24 different patient-reported tools were used, predominantly the Tinnitus Handicap Inventory (15 %). Loudness was measured in diverse ways including a numerical rating scale (8 %), loudness matching (4 %), minimum masking level (1 %) and loudness discomfort level (1 %). Ten percent of studies did not clearly report the instrument used.

Conclusions: Our findings indicate poor appreciation of the basic principles of good trial design, particularly the importance of specifying *what* aspect of therapeutic benefit is the main outcome. No single outcome was reported in all studies and there was a broad diversity of outcome instruments.

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PROSPERO registration: The systematic review protocol is registered on PROSPERO (International Prospective Register of Systematic Reviews): CRD42015017525. Registered on 12 March 2015 revised on 15 March 2016.

Keywords: Adult otolaryngology, Audiology, Clinical trials, Methods

Background

Tinnitus is an auditory percept – often described as a 'ringing in the ears'– in the absence of a corresponding auditory stimulus and is experienced by approximately 10–20 % of the population [1]. As a symptom there is a no consensus on its aetiology [2, 3] and work is ongoing to profile tinnitus so that interventions can be more specifically targeted [4]. For a subset of individuals, tinnitus severely interferes with activities of daily life, but its impact is wide-ranging and heterogeneous across individuals. Patients report problems in getting to sleep, the need to avoid noisy situations, hearing difficulties, difficulties with concentration, and experience despair, frustration, irritation, depression, fear and worry [5]. Currently, no cure exists for tinnitus but many interventions are being tested [6]. There is reasonable evidence to suggest that cognitive behavioural-based psychological treatments are effective at improving quality of life [7], negative mood, dysfunctional beliefs and tinnitus-related fear [8].

Despite some optimism for treating tinnitus-related distress [9] the field is plagued by a number of fundamental and recurring problems that limit the evidence base and ultimately affect patient care and policy-related decisions. From a trialists' perspective there is disagreement on what tinnitus-related problems constitute distinct elements of tinnitus, such as perceived loudness or emotional distress, and which are sufficiently important to be considered as domains that should be measured in all studies [10]. This situation has contributed to the high level of diversity in, for example, trial design and measurement of outcomes in confirmatory randomised controlled trials, which hinders comparison and meta-analysis across studies [6]. A recent systematic review examined outcomes of randomised controlled trials of interventions for adults with tinnitus up to March 2013 [11]. However, the review was not concerned with evaluating what was measured, nor the choice of outcome instruments. Rather, it focused on evidence for treatment-related benefits and harms, using this information to develop a clinical practice guideline [12]. Hence, further investigation is warranted to determine more generally what outcomes (namely domains and instruments) are being used in trials of tinnitus interventions.

The difficulties in synthesising evidence from tinnitus trials has negative implications for the provision of effective clinical care since clinicians, insurers, healthcare commissioners, regulatory bodies and other policymakers cannot make informed decisions without good evidence. There are very few practice guidelines and so in the UK and other countries care is not delivered to tinnitus patients in a standardised way [13]. Rather it tends to be driven by reimbursement policies and by which clinical profession (general practitioner, ENT specialist, audiologist, clinical psychologist, etc.) delivers the care.

In sum, the variations in research and in clinical methodologies used to assess, treat, and study tinnitus form a problematic circle, where an incomplete evidence base means that clinical guidelines are developed with limited knowledge, and the lack of standardised clinical practices cannot reliably feed back into addressing important research questions. This scenario is ultimately likely to contribute to an inefficient use of scarce healthcare resources and unnecessary suffering for patients. At present we attempt to break this circle by examining what outcome domains have been defined, and what outcome measures have been used in studies of treatments for adults with tinnitus, by means of a systematic review of publicly available trial protocols. This should ultimately lead to a description of a minimum standard for trialists to choose outcome measures for use in clinical trials that evaluate a tinnitus intervention [10]. A core set would enable results to be more easily compared and synthesised and the most effective interventions to be identified [14].

Objectives

The primary objective of this systematic review is to identify and evaluate the current reported outcome domains in clinical and experimental studies of adults with tinnitus, with a focus on trial designs investigating the treatment of tinnitus, and published between the date of an international consensus meeting in July 2006 [15] and March 2015. Data collection considered both which domain of tinnitus was identified as important for demonstrating therapeutic benefit and which instrument was used to assess that domain. Three secondary objectives considered the choice of instruments with respect to identifying patterns: (1) across continents to determine whether there are geographical preferences for using one primary outcome instrument over another, (2) across years to determine changes over time in the uptake of outcome instruments as a primary outcome, and (3) across interventions to determine whether particular

classes of intervention favour using one primary outcome instrument over another.

Methods

Details of the study eligibility criteria, information sources, search strategy, selection and data collection processes, as well as data synthesis methods were published as a protocol in advance of completing the data collection [16]. Reporting is guided by the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) [17] and are described using the PRISMA checklist (see Additional file 1).

Eligibility criteria

Study eligibility was defined according to PICOS (Patient, Intervention, Comparison, Outcome, Setting) and there were no modifications to the published protocol [16]. All included studies assessed adults (men and women) aged 18 years or older who reported tinnitus as one of their primary complaints, irrespective of whether they were recruited from clinical or non-clinical populations. There were no restrictions on the type of intervention as long as the main motivation was to bring about a therapeutic benefit for people with tinnitus. Studies in which the impact on tinnitus was of secondary relevance (e.g. where reducing hearing problems was the primary aim) were excluded. Consistent with this approach, only those studies reporting tinnitus-related changes as a primary outcome were included, irrespective of how those changes were measured. The systematic review included randomised controlled trials, before and after studies, non-randomised controlled trials, case-controlled studies and cohort studies. There were no restrictions on research settings.

To be included in this report, articles were required to be written in English and published in or after July 2006 [15]. These decisions were motivated by resource limitations. Furthermore, to improve clinical and scientific value, any studies either recruiting fewer than 20 participants with tinnitus or having fewer than 20 at the end point of the study were excluded. This cut-off was selected in advance, following Needleman et al. [18]. We included published systematic reviews and meta-analyses that considered tinnitus trials meeting the above criteria. These reviews and meta-analyses were not subject to the data collection process itself, but we did a hand-search and include any additional eligible studies reported within them.

During the data collection process, a small number of studies were identified where age-related eligibility or target sample size were missing. In cases where neither pieces of information were reported, the corresponding author was contacted for more details by email, with one reminder.

Information sources

Studies were identified by searching electronic databases of research literature (Table 1). The following list details the database, as well as the number of records identified by the search strategy (in parentheses): PubMed (National Centre for Biotechnology Information) (n = 759), EMBASE (Ovid) (n = 244), Cumulative Index to Nursing and Allied Health Literature (CINAHL, EBSCO) (n = 145) and the Cochrane Central Register of Controlled Trials (CENTRAL) (n = 560). A number of different electronic trial registers were also searched: ClinicalTrials.gov (n = 141), the International Standard Randomised Controlled Trial Number registry (ISRCTN, BioMed Central) (n = 22), the International Clinical Trials Registry Platform (ICTRP, World Health Organisation) (n = 183), and the Cochrane Database of Systematic Reviews (CDSR) (n = 23). Electronic searches were run on 12 and 13 March 2015 by authors DAH and AJS, and were not updated.

Table 1 Table summarising the electronic information sources used. For a description of the abbreviations, see text

Type of electronic search	Database	Number of items (n)
Academic databases	PubMed	759
	EMBASE	244
	CINAHL	145
	CENTRAL	560
Clinical trial registers	ClinicalTrials.gov	141
	ISRCTN	22
	ICTRP	183
	CDSR	23

In addition, a hand-search was conducted using the 251 published records that had met eligibility at the abstracts and full-text screening stages. Specifically, we hand-searched the set of registered clinical trials to identify any further registers of the same trial and also to identify any published protocols or study findings that were indexed to that trial by its unique study identifier. We also manually searched the 18 systematic review articles to look for any overlooked studies for inclusion. An additional 52 records were identified by these approaches. Following this step, the systematic review articles themselves were not included for data collection purposes.

Search strategy

The search strategy used in this systematic review was previously published [16]. Search terms for PubMed, EMBASE, and CINAHL were informed by the PICOS criteria and were: (1) tinnitus AND (2) stud* OR clinical trial* OR therap* OR treatment* OR intervention*. Where possible the search was limited to humans (not animals), adults (not paediatric), English language and 2006-date of search. The syntax for the subsequent search of the CENTRAL trials registry of the Cochrane Collaboration was: #1 tinnitus; #2 Paediatric:TI,AB,KY; #3 Pediatric:TI,AB,KY; #4 child*:TI,AB,KY; #5 #1 NOT

#2 NOT #3 NOT #4, #6 english:LA, #7 #5 AND #6, #8 (2006–2015):PD NOT IN MEDLINE NOT IN EMBASE AND 2006 TO 2015:YR, and #9 #5 NOT INMEDLINE NOT INEMBASE. Electronic trial registers all used 'tinnitus' as the main search term.

Data management

DAH was responsible for data management and maintained the editorial rights. All identified records were saved into a Microsoft Excel master file where records were tracked through the screening and data collection process by a unique study identification code. A simple system of record annotation was implemented to capture reasons for exclusion. At the end of data collection, checking and formatting, a pdf copy of the master file was created as a 'locked' record so that there is a version of the data that cannot be edited in error (7 December 2015). An editable Excel version of this document can be downloaded (see Additional file 2).

Selection process

Endnote was used to remove 141 duplicate records from the PubMed, EMBASE and CINAHL searches, while the remaining 362 duplicates were manually identified within the Excel master file by DAH and HH using author names, study title and trial registration number. This gave a total of 1574 records for eligibility screening. Screening steps were carried out DAH, HH and AJS. Following the pre-specified protocol, a two-step process was implemented to decide eligibility: first by reading the title, and second by reading the abstract and full text. It was possible to exclude 1153 records by title and summary information alone (see Fig. 1). Full texts were obtained for the 421 remaining records that potentially met the inclusion criteria or for which there was insufficient summary information to make a clear decision. From this step, a further 170 records were excluded, leaving 251 for data extraction. It is interesting to note that almost one third of those records excluded at this step was due to the small sample size of the study (see Fig. 1). Twenty-two records were excluded because they recruited participants below 18 years of age. Moreover, 55 full texts were excluded because the sample size was less than 20 participants and 11 full texts were excluded because they were not available in English. Instead, these were published in national journals written in the native language. So that the reader can scrutinise the data for evidence of geographical bias in these three full-text exclusion criteria, details are broken down by country in Table 2. This information gives some indication for a risk of bias excluding tinnitus studies conducted in the USA since 21 were removed on the basis of small sample size, leaving only 39 records from the USA contributing to the systematic review. There is also a risk of bias excluding tinnitus studies conducted in China since six were removed because they were published in Chinese, leaving only three records from China contributing to the systematic review. Note that language bias was avoided for studies registered on ISRCTN and ICTRP since an English language translation is given. Ten trials in Iran, seven in Japan and two in China, two in Brazil and one in the Republic of Korea were included via this route.

At least two co-authors performed each key step (i.e. title screening, full-text screening, and data collection) independently for every record. Due to an error in allocating full texts to co-authors, some records had data collection by more than two co-authors (31 were completed by three co-authors, 11 by four and 9 by five). Discrepancies between independent co-authors were rare and were mostly

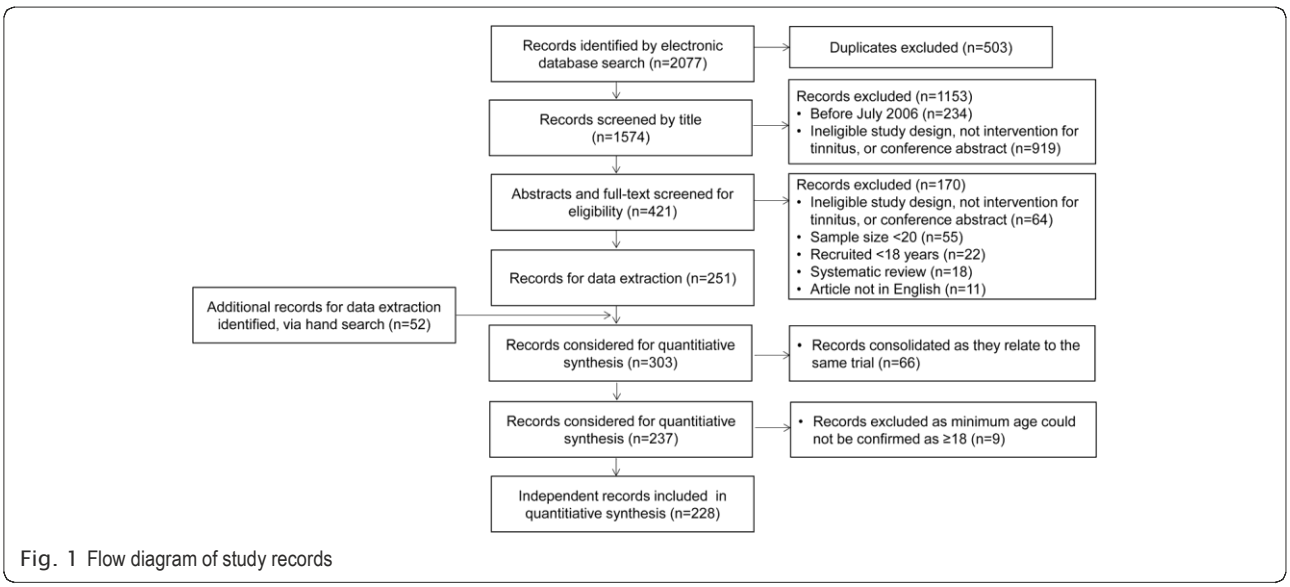

Fig. 1 Flow diagram of study records

Table 2 Summary of those records excluded at the full-text screening stage because (1) the sample size was less than 20 participants, (2) because the articles were not available in English, or (3) they recruited participants below 18 years of age. Details are broken down by country

	Sample size <20	Non- English language	Minimum eligibility (age in years)				
			13	14	15	16	17
Austria				1			1
Belgium	2					1	
Czech Republic							
Finland	2						
France	3						
Germany	4	1		2		4	
Italy	3						1
Spain	1						
Sweden	3						
Switzerland	1					1	
The Netherlands	3						
Turkey					1		
Iran		2			1		
Iraq			1				
Israel	1						
Egypt				1			
Brazil	2	1			1		
Uruguay	1						
China		6			1		1
Japan	3						
Republic of Korea	1	1		1		1	
Australia	2						1
New Zealand	1					1	
USA/Canada	22						

accountable by differences in terminology. These were resolved by DAH who was responsible for data management. As per the protocol [16], inter-rater agreement was not calculated, but all co-authors reviewed and approved the master file before data lock.

We pieced together data from multiple reports of the same study by manually screening all included records using author names, study title and trial registration number. This step of consolidating records happened throughout the data collection process, and in particular during the data formatting check. Where there were multiple reports, the data extraction reflects the information provided in the report with the latest publication date. Any discrepancies between information reported in the different articles were noted under the data item heading 'intention versus reporting'.

Data collection process

We contacted 29 trialists to request missing information about the minimum age for inclusion and two investigators to request missing information about sample size. With respect to age, 20 confirmed that all participants were 18 years of age or older, two authors could no longer be contacted, two responded but were unable to confirm the minimum age, one responded but said he was too busy to provide the information, and four did not respond. On the basis of this, nine records were excluded. Both investigators who were contacted about sample size were able to provide the required information and so these records were included. A summary of those relevant records are provided in more detail in an additional Table (see Additional file 3). After exclusion, 228 records were included for data collection. A further Table provides full references (see Additional file 4). Data items gave rise to headings in a data collection sheet. Data collection was guided by an electronic form (Excel spreadsheet) that was also used to collate all responses. Data collection was conducted by a pool of 20 project team members (number of extracted studies ranged from 5 to 228, median 19.5). The primary reason for not limiting data collection to a smaller pool was to

lessen the resource burden since we received no grant funding to conduct the research activity. To mitigate against observer bias, a full set of guidance notes was produced for the data collection procedure and calibration exercises were conducted with new members of the review team prior to any individual contribution to this review. Both the sheet and the guidance notes were developed and revised across several review authors during a 3-day workshop and through two iterations of piloting. Data collection was conducted independently and with at least two team members for every included record. In an amendment to the pre-specified protocol, DAH verified the data collection for all included records to ensure consistency in approach and in terminology; the latter being necessary for automated data counting. Another step to mitigate against observer bias during the data collection process was by avoiding any instance where an individual extracted data relating to one of their own trials.

Data items

Data items included all of the fields reported in the published protocol [16]. A majority of data items fall within the PICOS framework. Participant data items relating to the inclusion criteria for each trial record were: (1) minimum age, (2) maximum age (if any), (3) tinnitus duration, (4) intermittent or constant tinnitus, (5) pulsatile or non-pulsatile tinnitus, (6) tinnitus severity, (7) any other subtypes of tinnitus, and (8) any other health-related comorbidities. Participant data items relating to the exclusion criteria for each trial record were: (9) any other subtypes of tinnitus, and (10) health-related comorbidities. Intervention data items recorded the (11) type and (12) duration of intervention in each arm of the trial. Data items describing the study design (i.e. 'comparison') comprised: (1) a pull-down list of study design options (randomised controlled trials, before and after studies, non-randomised controlled trials or case-control studies and cohort studies) and (2) a record of the duration of each intervention, separately for each arm of the trial. Outcome data items were: (1) the outcome domain(s) specified by the investigators, (2) the instruments specified by the investigators, and (3) time frame. Information relating to these three data items was recorded separately for all primary and secondary outcomes. Where authors were not explicit about this distinction, we tried to tease this information out of the article by reading the Methods and Results sections of each record. But if this was not possible, then all information was entered as a primary data item. A 'setting' data item reported the country where the study was conducted. Supplementary information was also extracted from each included trial on: (1) the name and email address of the corresponding author, (2) the date of study start, (3) the aim of the trial, (4) sample size calculation, with a full-text extraction of the reported details, (5) the sample size, (6) a description of any modifications to the methods, particularly any discrepancies between the trial protocol and the subsequent report of the findings, and (7) the date of publication. The protocol was amended so that if minimum age of eligibility or sample size estimate was not reported, then the data collection recorded the minimum age of the recruited participants or the recruited sample size as the 'next best alternative,' where this information was given. An additional data item not planned in the protocol recorded whether the study authors specified any minimal clinically important difference, or related construct that was used to interpret the clinical significance of the findings. For example, Cima and et al. [8] specified a pre- versus post-intervention change of 0.065 (SD 0.15) in health utility score measured using the 36-item short form Health Survey. This information is not reported here, but will be presented in a separate manuscript. If any information is not reported, then 'not stated' was recorded in the corresponding field.

Where a trial record consolidated several pieces of information (such as a protocol and the published findings), the data items reported in the synthesis related to the most recent publication. For those records in which several pieces of information are consolidated into a single record, we sought to detect any modifications to the methods leading to inconsistencies between the protocol and the final reported study. Given that the review focused on the design of clinical trials, wherever possible information relating to each data item was taken from the study design reported in the most recent publication, not from any report of the study results. For example, sample size recorded the estimated sample size not the number of participants actually enrolled into each intervention arm. And, the date of publication recorded the date of the print copy, not the date of first submission, acceptance or the date of 'online first' publication.

Outcomes and prioritisation

The primary research question in this review concerned the outcome domains (and instruments) being used in clinical trials of tinnitus treatment. Therefore, the priority for data synthesis and reporting of findings was data relating to all primary outcomes. Where authors failed to distinguish between primary and secondary outcomes, we classified them all as primary. Those outcomes explicitly defined as secondary were also examined, but as a secondary research question.

Risk of bias in individual studies

Given that the primary objective of this systematic review concerns methodology (not therapeutic effects), we limited the assessment of risk of bias to the data collection methods for consolidated records rather than any analysis of those data. In particular, we investigated where there were inconsistencies between the outcomes

defined in the trial registration and/or protocol and those given in the subsequent study report. Of the 228 studies selected for inclusion, 60 (26 %) had multiple records. We examined only those consolidated records with a protocol and study report(s) comparing data items across records. From this set, 21 were found to have descriptions of eligibility criteria (inclusion or exclusion), primary outcome measures, and/or secondary outcome measures that were altered retrospectively in the final report. An additional Table gives more details about the findings from the risk of bias assessment (see Additional file 5). None of the studies reported a justification for the changes, but insufficient information was given in the publications to determine any instances of intentional deception (i.e. outcome-reporting bias) where outcomes had been selected on the basis of the results, for inclusion in the publication of trial findings [19–21]. We did not contact authors to examine reasons for altered reporting.

Results

The primary objective was to identify and evaluate the current reported outcome domains and instruments in designs of intervention studies of adults with tinnitus, published since July 2006.

Domains

For the first part of the analysis, we scrutinised the data collected under the data item relating to the primary outcome domain(s) specified by each set of investigators. There were 505 data entries describing 35 different types of primary domain (Table 3). Domain grouping was conducted by a subgroup of tinnitus experts (three ENT surgeons, one audio-vestibular physician, and two researchers) and was broadly informed by the Cochrane Effective Practice and Organization of Care (EPOC) recommendations [22]. Patient outcomes concerned with health status, well-being and health behaviours constituted the largest category by far and so we expanded this into domains relating to (1) the tinnitus percept, (2) the impact of tinnitus, (3) other co-occurring complaints, (4) health-related quality of life, and (5) body structures and functions (Table 3). Remaining EPOC categories were (6) adverse events or harms and (7) satisfaction, with further categories for (8) treatment-related outcomes, and (9) for domains that were unclear or not specified by the author. The most popular primary outcome domain directly relating to tinnitus was 'tinnitus loudness' ($n = 70$, 14 % defined as primary outcome domain in all studies), with 'tinnitus distress' ($n = 33$, 7 %) and 'tinnitus annoyance' ($n = 21$, 4 %) following.

Over half (n = 279, 55 %) of the data entries did not clearly describe the complaint of interest. Since this was such a large percentage, we chose to examine this in more detail rather than simply report as a quantitative summary of quality, as per the protocol [16]. Instead, we sought to describe the ways in which the authors' specification of each primary outcome domain appeared to be inadequate using a narrative approach. Primary outcome domains in category 7 were classified into five subheadings (Table 3). On 128 occasions (25 %), the investigators did not explicitly state which domain their trial intended to assess and so we refer to these as 'not specified'. 'Tinnitus severity' was the next most common phrase used to define the outcome domain of interest (n = 69, 14 %). We note that in our protocol [16], we had stated that this is not an adequate domain because it does not explain the dimension of complaint on which severity should be considered. The same applies to 'tinnitus handicap' (n = 14, 3 %). We also experienced difficulty in interpreting a further 58 (12 %) data entries because the terminology was indeterminate (referred to as 'cannot code'). We are confident that this is not a coding issue, as DAH verified that the data collection for all included records captured the text as reported by the authors. Examples include 'improvement,' 'treatment responder,' 'change,' 'tinnitus impact,' 'size of tinnitus problem,' 'tinnitus impairment,' 'problems associated with tinnitus,' 'difficulties due to tinnitus,' 'degree of tinnitus,' 'sensation of tinnitus,' and 'tinnitus characteristics'. Again, none of these clearly explain the dimension of complaint on which improvement or problems should be considered. 'Multi-domain specification' refers to composite measures describing several different complaints such as 'tinnitus annoyance and distress' and 'internal thoughts, sensations and feelings' (n = 10, 2 %).

There were 579 data entries describing 60 different types of secondary domain. Again, Table 3 indicates similar patterns, with 'tinnitus loudness' (n = 42, 7 %), with 'tinnitus distress' ($n = 18$, 3 %) and 'tinnitus annoyance' ($n = 15$, 3 %) being the most popular. Safety (n = 43, 7 %), Quality of life (n = 20, 3 %), and depression (n = 18, 3 %) were also popular as secondary outcome domains.

Instruments

The second part of the primary objective was to identify and evaluate the current reported outcome instruments and for this we interrogated the data collected under the data item relating to the primary outcome instrument(s) specified by each set of investigators. Overall, there were 505 data entries describing 78 different types of instrument (Table 4). We used a categorisation scheme based on the one for domains. Instruments were grouped according to whether the tests relate to: (1a) the tinnitus percept (investigator-administered), (1b) the tinnitus percept (numerical rating scale), (2a) the impact of tinnitus (patient-reported questionnaire), (2b) the impact of tinnitus (numerical rating scale), (3) other co-occurring complaints, (4a) health-related quality of life (patient-reported questionnaire), (4b) health-related quality of life (numerical rating scale), (5)

Table 3 Summary of all primary and secondary outcome domains across all 228 clinical trials. Domains have been grouped according to eight major topic categories. Categories 1–5 relate to different types of 'patient outcomes', categories 6–7 relate to 'adverse events' and 'satisfaction', following the Effective Practice and Organisation of Care classification scheme [22]. Categories 8 and 9 best describe the remaining outcomes reported in the included records. Percentages are rounded so, for example, 0 % denotes a value that is <0.5 %

	Primary domains		Secondary domains	
	Number	Percentage (%)	Number	Percentage (%)
(1) Domains relating to the tinnitus percept:				
Tinnitus loudness	70	14 %	42	7 %
Tinnitus pitch	12	2 %	13	2 %
(2) Domains relating to the impact of tinnitus:				
Tinnitus distress	33	7 %	18	3 %
Tinnitus annoyance	22	4 %	15	3 %
Tinnitus awareness	10	2 %	2	0 %
Cognition	2	0 %	4	1 %
Behaviour	1	0 %	0	-
Acceptance of tinnitus	0	-	3	1 %
Catastrophising	0	-	1	0 %
Concentration	0	-	2	0 %
Tinnitus intrusiveness	0	-	2	0 %
Tinnitus-related cognitions	0	-	1	0 %
Tinnitus-related fear	0	-	1	0 %
(3) Other co-occurring complaints:				
Depression	8	2 %	18	3 %
General distress	5	1 %	5	1 %
Anxiety	4	1 %	6	1 %
Anxiety and depression	4	1 %	13	2 %
Hearing threshold	4	1 %	11	2 %
Hearing loss	2	0 %	1	0 %
Speech perception	2	0 %	0	-
Hearing handicap	1	0 %	3	1 %
Hearing loss annoyance	1	0 %	0	-
Sleep quality	1	0 %	12	2 %
Somatic sensations	1	0 %	1	0 %
Fear (anxiety)	0	-	1	0 %
Hyperacusis	0	-	3	1 %
Mood	0	-	2	0 %
Sound tolerance	0	-	1	0 %
Speech discrimination	0	-	3	1 %
(4) Health-related quality of life (QoL):				
QoL (tinnitus)	16	3 %	13	2 %
QoL	9	2 %	20	3 %
Coping	3	1 %	0	-
Occupational health	0	-	1	0 %
QoL (hearing)	0	-	1	0 %
Sense of control	0	-	1	0 %

Table 3 Summary of all primary and secondary outcome domains across all 228 clinical trials. Domains have been grouped according to eight major topic categories. Categories 1–5 relate to different types of 'patient outcomes', categories 6–7 relate to 'adverse events' and 'satisfaction', following the Effective Practice and Organisation of Care classification scheme [22]. Categories 8 and 9 best describe the remaining outcomes reported in the included records. Percentages are rounded so, for example, 0 % denotes a value that is <0.5 %
(Continued)

(4) Body structures and functions:				
Neck mobility	1	0 %	1	0 %
Neural activity	1	0 %	2	0 %
Oxidative stress	1	0 %	0	-
Active myofascial trigger points	0	-	1	0 %
Blood parameters	0	-	1	0 %
Gene expression	0	-	1	0 %
Metabolism	0	-	4	1 %
Neck pain	0	-	1	0 %
Neuroendocrine hormones	0	-	1	0 %
Pharmacokinetics	0	-	1	0 %
Structural brain change	0	-	1	0 %
(6) Adverse events or harms:				
Safety and tolerability	6	1 %	4	1 %
Safety	2	0 %	43	7 %
Drug safety and tolerability	1	0 %	4	1 %
Side effects	1	0 %	15	3 %
Headache	0	-	1	0 %
Pain frequency	0	-	1	0 %
Pain intensity	0	-	1	0 %
(7) Satisfaction:				
Treatment satisfaction	1	0 %	5	1 %
(8) Treatment-related outcomes:				
Withdrawals	1	0 %	0	-
Adequacy of blinding	0	-	1	0 %
Credibility (sham)	0	-	1	0 %
Credibility (treatment)	0	-	2	0 %
Needling sensation (acupuncture)	0	-	1	0 %
Therapeutic alliance	0	-	1	0 %
Tolerability	0	-	5	1 %
(9) Domain of interest unclear or not specified by the authors:				
Not specified	128	25 %	140	24 %
Cannot code	58	11 %	76	13 %
Multi-domain specification	10	2 %	10	2 %
Tinnitus severity	69	14 %	29	5 %
Tinnitus handicap	14	3 %	5	1 %
Total	505	100 %	579	100 %

body structures and functions, (6) adverse events or harms, (7) satisfaction, (8) treatment-related outcomes, or (9) were unclear or not specified by the authors. Twenty-eight different instruments were used only once as a primary outcome and these are listed in an additional Table (see Additional file 6).

Instruments assessing the impact of tinnitus were most common and of these, the Tinnitus Handicap

Table 4 Summary of all primary and secondary outcome instruments used across all 228 clinical trials. Instruments have been grouped according to the major domain categories reported in Table 3, as well as those instruments that were not clearly specified by the authors. Note that the total refers to the number of instruments across all 228 trials. The remainder are reported in Additional file 6. Percentages are rounded so, for example, 0 % denotes a value that is <0.5 %

	Primary outcome instruments		Secondary outcome instruments	
	Number	Percentage (%)	Number	Percentage (%)
(1a) Investigator-administered tests relating to the tinnitus percept:				
Tinnitus loudness matching	20	4 %	16	3 %
Tinnitus pitch matching	9	2 %	22	4 %
Minimum masking level	5	1 %	12	2 %
Loudness discomfort level	3	1 %	2	0 %
Tinnitus bandwidth matching	0	-	2	0 %
(1b) Patient-reported numerical rating scales relating to the tinnitus percept:				
Tinnitus loudness	37	8 %	24	4 %
Tinnitus pitch	0	-	2	0 %
(2a) Patient-reported questionnaire instruments relating to the impact of tinnitus:				
Tinnitus Handicap Inventory	77	15 %	31	5 %
Tinnitus Questionnaire (German version)	29	6 %	11	2 %
Tinnitus Questionnaire (English version)	5	1 %	0	-
Tinnitus Functional Index	18	4 %	3	1 %
Tinnitus *Beeinträchtigungs Fragebogen*	13	3 %	8	1 %
Tinnitus Severity Index	12	2 %	1	0 %
Tinnitus Reaction Questionnaire	11	2 %	2	0 %
Tinnitus Handicap Questionnaire	8	2 %	5	1 %
Mini-Tinnitus Questionnaire	6	1 %	0	-
Tinnitus Effects Questionnaire	2	0 %	0	-
Tinnitus diary	2	0 %	0	-
Tinnitus Psychological Impact Questionnaire	2	0 %	0	-
Tinnitus Severity Scale	0	-	6	1 %
Tinnitus Acceptance Questionnaire	0	-	6	1 %
(2b) Patient-reported numerical rating scales relating to the impact of tinnitus:				
Tinnitus distress	7	2 %	8	1 %
Tinnitus annoyance	21	4 %	14	2 %
Tinnitus awareness	10	2 %	2	0 %
(3) Patient-reported questionnaire instruments relating to other co-occurring complaints:				
Beck Depression Inventory	7	1 %	13	2 %
Hospital Anxiety and Depression Scale	7	1 %	27	5 %
Perceived Stress Questionnaire	3	1 %	0	-
Spielberger State and Trait Anxiety Inventory	3	1 %	3	1 %
Brief-Coping with Problems Experienced	2	0 %	0	-
Pittsburgh Sleep Quality Index	2	0 %	2	0 %
Hearing Handicap Inventory	1	0 %	4	1 %
Hyperacusis questionnaire (undefined)	1	0 %	2	0 %
Attention and Performance Self-assessment Scale	0	-	2	0 %
Befindlichkeitsskala	0	-	2	0 %
Cognitive Failures Questionnaire	0	-	2	0 %
Depression Anxiety and Stress Scale	0	-	3	1 %

Table 4 Summary of all primary and secondary outcome instruments used across all 228 clinical trials. Instruments have been grouped according to the major domain categories reported in Table 3, as well as those instruments that were not clearly specified by the authors. Note that the total refers to the number of instruments across all 228 trials. The remainder are reported in Additional file 6. Percentages are rounded so, for example, 0 % denotes a value that is <0.5 % *(Continued)*

Insomnia Severity Index	0	-	7	1 %
Major Depression Inventory	0	-	5	1 %
Sleep Questionnaire (undefined)	0	-	3	1 %
(4a) Patient-reported questionnaire instruments relating to health-related quality of life:				
Clinical Global Impression Scale	4	1 %	14	2 %
36-item short form Health Survey	2	0 %	6	1 %
WHOQOL-BREF	0	-	10	2 %
EuroQoL	0	-	2	0 %
Quality of Life Inventory	0	-	2	0 %
(4b) Patient-reported numerical rating scales relating to health-related quality of life:				
Quality of Life (tinnitus)	5	1 %	6	1 %
Quality of Life	4	1 %	1	0 %
(5) Technical and laboratory measurements relating to body structures and functions:				
Pure tone audiometry	15	3 %	23	4 %
Speech audiometry (various types)	6	1 %	11	2 %
Electroencephalography	4	1 %	5	1 %
Blood chemistry	2	0 %	10	2 %
Positron Emission Tomography	2	0 %	0	-
Electrocardiogram	1	0 %	4	1 %
Digit symbol test	1	0 %	2	0 %
Blood drug levels	0	-	4	1 %
Magnetic Resonance Imaging	0	-	3	1 %
Otological examination	0	-	4	1 %
Otoscopy	0	-	2	0 %
Psychoacoustic assessment (undefined)	0	-	3	1 %
Tympanometry	0	-	2	0 %
Urine analysis	0	-	2	0 %
(6) Measures of adverse events or harms:				
Adverse events/Side effects	4	1 %	30	5 %
(7) Measures of satisfaction:	No instruments reported			
(8) Measurement instruments of treatment-related outcomes:				
Withdrawal rate	2	0 %	2	0 %
(9) Measurement of interest unclear or not specified by the authors:				
Cannot code	20	4 %	52	9 %
Other numerical rating scale (undefined)	18	4 %	29	5 %
Questionnaire (authors' own)	15	3 %	13	2 %
Numerical rating scale of tinnitus severity	12	2 %	1	0 %
Not specified	8	2 %	18	3 %
Total	505	87 %	579	85 %

Inventory was the most popular (n = 77, 15 %) [23] and was one of the instruments recommended by the 2006 consensus meeting [15]. Other recommended questionnaires were the Tinnitus Questionnaire (n = 34, 7 %), the Tinnitus Reaction Questionnaire (n = 11, 2 %), and the Tinnitus Handicap Questionnaire (n = 8, 2 %). However, our review

indicates that the Tinnitus Functional Index, Tinnitus *Beeinträchtigungs Fragebogen* (a shortened version of the Tinnitus Handicap Inventory translated into German) and Tinnitus Severity Index were just as widespread.

Tinnitus loudness matching was a popular tool for assessing the tinnitus percept (n = 20, 4 %). A numerical rating scale of loudness was also a common approach (n = 37, 8 %), but there was little consistency in the measurement scale used (e.g. Table 5). Other domains relating to the impact of tinnitus were evaluated using a numerical rating scale predominantly annoyance (n = 21, 4 %), awareness (n = 10, 2 %), and distress (n = 7, 1 %). Numerical rating scales with 0–10 and 0–100 point scales were popular.

About 16 % (n = 78) of the data entries did not clearly report the instrument used. These were classified into five subheadings under Table 4, category 9. On 20 occasions (4 %), we experienced difficulty in interpreting the data entry (referred to as 'cannot code'). One recurring example was where investigators did not state the provenance of the 'tinnitus questionnaire' which could be either a published Tinnitus Questionnaire [24, 25], or a translation of one of these or to an authors' own instrument. We observed 15 instances (3 %) where investigators reported using their own (unpublished) questionnaire, which limits reproducibility.

There were 579 data entries describing 108 different types of secondary instrument (Table 4). Of those, 49 instruments were used only once as a secondary outcome and these are listed separately in a Table (see Additional file 6). Although the Tinnitus Handicap Inventory remained a common choice as a secondary outcome (n = 31, 5 %), other tinnitus-related questionnaires were much less so. Instead, adverse events (n = 30, 5 %) and the Hospital Anxiety and Depression Scale (n = 27, 5 %), pure tone audiometry (n = 23, 4 %), tinnitus pitch matching (n = 22, 4 %), the Clinical Global Impression Scale (n = 14, 2 %) and the WHOQOL-BREF (n = 10, 2 %) were some of the more popular choices for secondary outcomes.

The protocol did state that the timing of the primary end point would be examined [16], but we did not pursue this analysis because the timing of the end point was reported inconsistently across studies (some relative to the start of the treatment and others relative to the end of the treatment) and the duration of treatment varied so greatly (some were just a few days, some extended up to 1 year, and others did not clearly specify). Moreover, the time frame of surveillance for adverse events was rarely stated.

Pattern of primary outcome instruments across world regions

The first secondary analysis assessed how the pattern of primary outcome instruments varied across world regions. Countries recruiting into identified clinical trials were categorised into six world regions using the World Health Organisation (WHO) as a guide [26]. Findings are summarised in Fig. 2. The 'European region' represented the greatest research activity with 151 sites recruiting across all 228 trials. Most research was conducted in Germany (n = 48), Belgium (n = 20), and UK (n = 12). In the Middle East and Africa region, most trials were conducted in Iran (n = 17), while in Asia most research was conducted in Japan (n = 11) and the Republic of Korea (n = 9).

With respect to patient-reported questionnaires relating to the impact of tinnitus, the Tinnitus Handicap Inventory was the most common one used as a primary outcome across all world regions, except for Oceania where the Tinnitus Reaction Questionnaire was preferred. Since few clinical trials were conducted in South America or Oceania, findings for these world regions should be interpreted with caution. The Tinnitus Questionnaire was common in Europe, especially in Germany, but not in the rest of the world. Even in Europe, it is used in different forms

Table 5 Summary of the different formats for numerical rating scales used across all 228 clinical trials. These are used to assess a wide range of domains including tinnitus loudness annoyance, awareness, distress and tinnitus-related quality of life

	Primary outcome instruments		Secondary outcome instruments	
	Number	Percentage (%)	Number	Percentage (%)
Numerical rating scale (0–3)	1	0 %	0	-
Numerical rating scale (0–10)	49	10 %	18	3 %
Numerical rating scale (0–100)	18	4 %	13	2 %
Numerical rating scale (1–9)	0	-	1	0 %
Numerical rating scale (1–10)	12	2 %	0	-
Numerical rating scale (1–100)	1	0 %	0	-
Numerical rating scale (4 points)	3	1 %	2	0 %
Numerical rating scale (5 points)	2	0 %	2	0 %
Numerical rating scale (7 points)	3	1 %	0	-
Numerical rating scale (10 points)	0	-	1	0 %
Numerical rating scale (10-cm line)	5	1 %	14	2 %

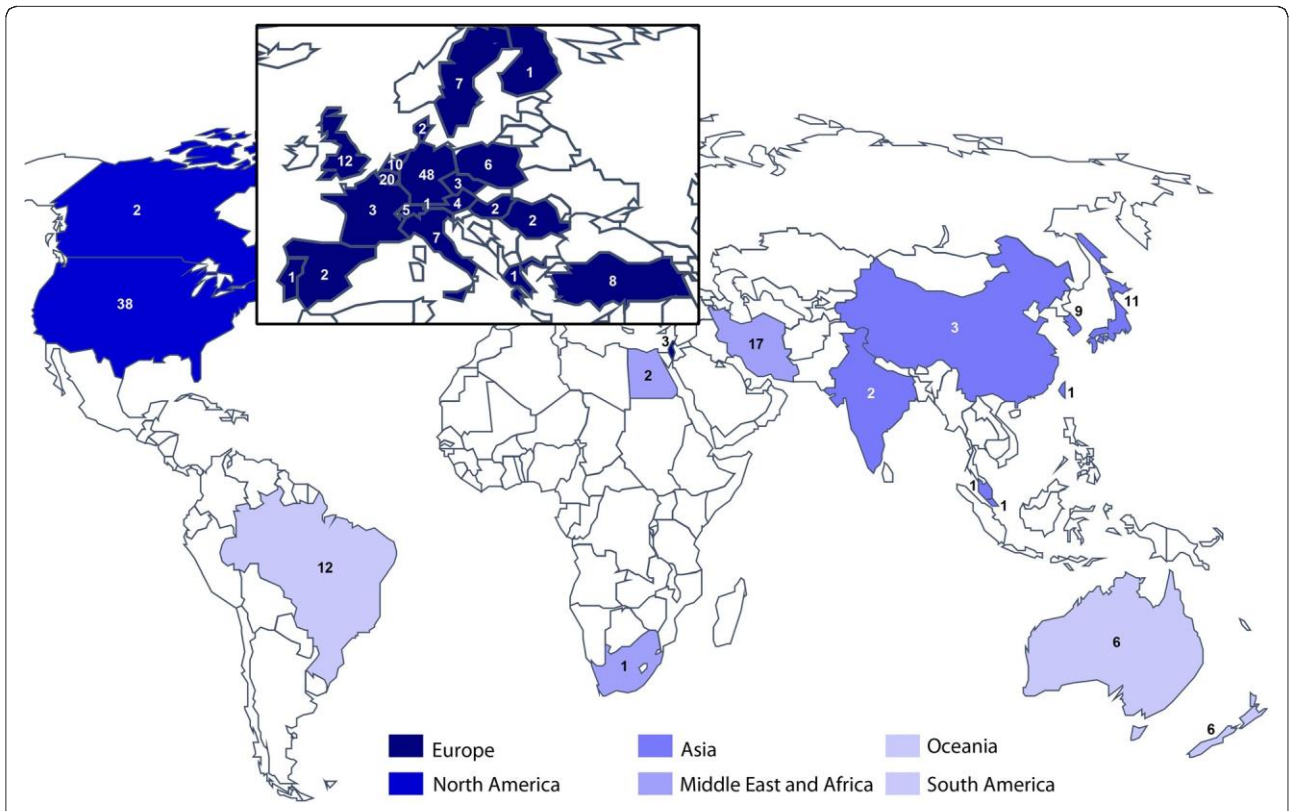

Fig. 2 World map illustrating the distribution of recruiting sites for all included studies, inspired by the World Health Organization (WHO) regional classification. Figures within each country indicate only one trial [27] had a recruiting site in South Africa and so this was combined with countries in the WHO Eastern Mediterranean region to create the Middle East and Africa region ($n = 20$). The WHO Region of the Americas was separated into North and South America because we anticipated that language differences might influence choice of outcome instruments. Similarly, Australia and New Zealand were considered separately from Western Pacific region, as Oceania, while other countries were combined with the WHO Southeast Asia region to create a single Asian region

because the English and German versions differ from one another [28]. The Tinnitus Severity Index was common in the Middle East and Africa region, but not in other parts of the world. Measures of tinnitus loudness were also most common in the Middle East and Africa region (both using loudness matching and numerical rating scales), with countries in Asia also favouring a loudness numerical rating scale.

Pattern of usage of primary outcome instruments across years

We also examined the status of selected primary outcome instruments over the time frame of the review (Fig. 3). Due to the wide variety of instruments, analysis focused on the most frequently used that were highlighted in the previous section (Instruments). For meaningful analysis, we split the total time frame into three periods, using the best available information. The first period was from 1 January 2011 to 12 March 2015 (i.e. the date of the electronic searches) ($n = 102$). The second period was from 1 August 2006 to 31 December 2010 ($n = 99$). The third period included any remaining studies in which data was collected on or before 31 July 2006 (i.e. before the Tinnitus Research Initiative (TRI) consensus meeting) [15], but not published until after this date ($n = 27$). Here we describe the patterns for the first two periods because of the comparable sample size and more robust definition, but all data are presented in Fig. 3. The Tinnitus Handicap Inventory, the Tinnitus Handicap Questionnaire, and the Tinnitus *Beeinträchtigungs Fragebogen* were equally popular across both 5-year periods. The Tinnitus Functional Index and numerical rating scales of tinnitus loudness increased in popularity, while the Tinnitus Questionnaire (German version), the Tinnitus Reaction Questionnaire, the Tinnitus Severity Index and tinnitus loudness matching all seemed to decrease in popularity.

Pattern of usage of primary outcome instruments across interventions

All records were coded according to eight broad classes of procedure either as part of the intervention of interest or the control. These were: pharmacology ($n = 66$), electrophysiology ($n = 59$), sound therapy ($n = 56$), psychological therapy or counselling (referred to as 'talking') ($n = 47$),

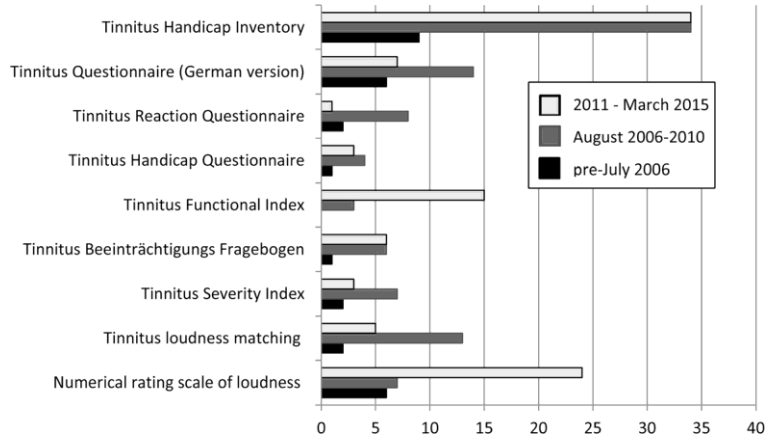

Fig. 3 Pattern of usage over time for selected primary outcome instruments. Note that the identification of studies categorised as 'pre-July 2006' may not be representative as many records relating to this period would have been excluded according to our search criteria

complementary therapy (n = 33), surgery (n = 10), manual physical therapy (n = 7), and relaxation (n = 3). Where interventions involved more than one procedure, all procedures were coded. For example, an intervention involving an intra-tympanic injection was coded as both pharmacology and surgery, and Tinnitus Retraining Therapy with a *Ginkgo biloba* supplement was coded as talking, sound and pharmacology.

The domain of tinnitus loudness was least frequently assessed in talking therapies (3 %), with the other major classes of intervention all assessing this perceptual characteristic more frequently: pharmacology (17 %), electrophysiology (16 %), sound therapy (15 %), and complementary therapy (14 %). In contrast, talking therapies favoured assessments of tinnitus distress (13 %) more than the other intervention classes: pharmacology (2 %), electrophysiology (6 %), sound therapy (7 %), and complementary therapy (4 %). Numerical rating scales and the Tinnitus Handicap Inventory were commonly used as outcome instruments for all types of interventions.

Quality assessments

Following the protocol, we assessed the quality of defining and reporting outcomes in three ways. The first quality assessment considered the degree to which primary outcome instrument(s) in each study were appropriate and consistent with the authors' choice of primary outcome domain(s). For example, the Tinnitus Severity Index would not be considered an ideal measure for quality of life, nor would 'psychophysical method' as a measure of tinnitus loudness. Within each study, we counted the number of consistent primary outcomes, calculated as a function of the proportion (%) of primary outcomes in that study. Overall, 31 (14 %) studies achieved a 100 % score, with 16 of those specifying a single primary outcome. In contrast to this, 133 (58 %) studies scored 0 %, with 52 of those failing to specify the primary domain and five not specifying the primary instrument. The remaining studies reported only partially correct outcomes: n =5 scored 1−25 %, n = 31 scored 26−50 %, n = 21 scored 51−75 % and n =7 scored 76−99 %.

The second quality assessment demonstrated that few trial designs were informed by a sample size calculation based on previous data for the primary outcome instrument. We excluded from this analysis 91 records that were trial registrations because a sample size calculation was not required for reporting. Of the remaining 137 records, sample size calculation was reported in only 37 of them (27 %). A sample size calculation requires specification of the primary outcome instrument, the expected difference between the treated and untreated groups, the pooled standard deviation, the desired statistical power, whether the hypothesis testing is one- or two-sided and the significance level (alpha). Over the 37 studies reporting sample size calculation, 31 (83 %) and 32 (86 %) studies reported statistical power and alpha value respectively, but the primary outcome instrument, the expected difference between groups and whether the test was one- or two-sided were mentioned in only 17 (46 %), 19 (51 %) and 14 (38 %) studies respectively. From the 17 studies reporting the primary outcome instrument, the Tinnitus Handicap Inventory was the most popular choice (n = 8). However, the magnitude of the expected change varied from study to study. It ranged from 6.55 to 20 points, but was also expressed as 50 % of reduction. Note that the developers of the Tinnitus Handicap Inventory recommend that a 20-point or greater change is required to account for test-retest variability [29].

The third quality assessment highlighted that many of the studies are suboptimal in terms of clearly defining what end point is the most important with respect to drawing a conclusion about treatment efficacy. For assessing whether

an intervention has therapeutic benefit to patients, it is good practice to state a priori one outcome instrument [30]. Figure 4 illustrates the number of primary outcome instruments administered in each study. Just over half of all studies (118/228, 52 %) reported only one primary measure. However, the remainder reported multiple measures without distinguishing primary from secondary outcomes, with 70 studies (31 %) reporting two or three potential primary measures, 29 studies (13 %) reporting four or five and 11 studies reporting more than this. Two studies reported 12 measures without distinguishing primary from secondary outcomes [31, 32].

Exploring the pattern of primary outcomes across tinnitus subgroups

A final analysis pre-specified in the published protocol was an exploratory one to address the question about whether a particular outcome domain (or instrument) was preferentially selected in trials enrolling a particular tinnitus subtype [16]. Here we considered tinnitus severity (as denoted by the authors), hearing loss, depression and anxiety because these are most relevant for determining choice of a tailored intervention.

Tinnitus severity

With respect to the primary domains, 96 out of the 505 came from studies that specified a severe tinnitus as an inclusion criterion. In those studies, an objective criterion was defined as some sort of minimum score on a published tinnitus questionnaire. For this subgroup compared to all 228 studies, we expected there would be a greater proportion of primary domains evaluating the functional impact of tinnitus, but this was not the case. The pattern was not noticeably different from the full dataset.

Hearing loss

Forty-seven of the 505 primary domains came from studies that specified a hearing loss as an inclusion criterion. Again, only studies were considered where an objective criterion had been defined and this was typically a minimum hearing level in dB at particular frequencies. Compared to the full dataset reported in Table 3, the proportion assessing tinnitus distress was slightly lower (4 % compared to 7 %). We also noted that the only study to report on a speech-based primary measure was part of this hearing loss subgroup [33]. Other audiological domains such as loudness and pitch had the same pattern of usage as the full dataset.

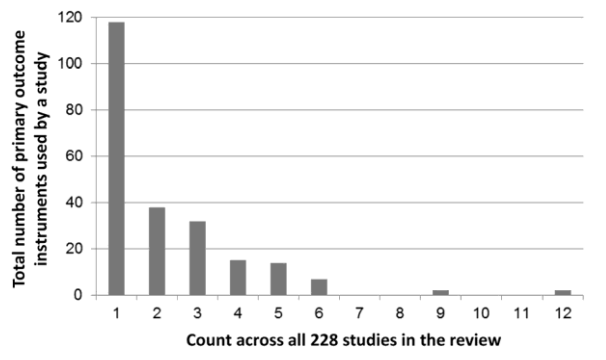

Fig. 4 Number of primary outcome instruments reported across the 228 studies included in the review

Depression and anxiety

Only one registered clinical trial actively recruited participants experiencing a comorbid depressive state [34], and no studies specified an inclusion criterion for a comorbid generalised anxiety. It is not possible, therefore, to consider any patterns within these subgroups.

Discussion

There is a growing general recognition that insufficient attention has been paid to the outcomes measured in clinical trials [14]. Specifically, for tinnitus these limitations have been acknowledged in a number of systematic reviews, especially those published by the Cochrane Centre [35, 36], and have been highlighted by an international working party of the Tinnitus Research Initiative [15].

Principal findings

No single outcome was reported in all studies. Instead a diverse range of outcomes were measured and reported. There are three key messages from our work.

First, over half of all studies did not adequately describe the domain for which they were predicting a predominant therapeutic benefit. In these cases, primary (and secondary) outcome domains were either not specified at all or were unclear. We believe that non-reporting mainly reflected a poor understanding of how important it is for individual trials to pre-specify the expected outcome. When conducting the review, we observed that the headings used within trial registries promote the reporting of instrument choice, rather than the outcome domain.

Second, there was extremely broad diversity of outcome instruments. Loudness was the most popular perceptual attribute of tinnitus described at the domain level, but there was no agreement on how to measure it and the precise methodology was often under-reported. Examples of descriptions for loudness matching included 'matching at 1 kHz', 'psychoacoustical measure', and 'by audiometry'. Patient-reported questionnaires relating to the impact of tinnitus were the most common primary outcome instruments, but again there was no consensus about which one should be chosen. The Tinnitus Handicap Inventory remains the most popular questionnaire instrument simply because it is translated into the greatest number of languages. Certainly, it has limitations for the purpose of

outcome measurement [28]. Worthy of note, we advise caution if pooling findings from the Tinnitus Handicap Inventory in a meta-analysis since it is unclear whether all translations achieve equivalence with the British original [37]. In compiling the list of tinnitus-related questionnaires (Table 4), it was striking how uninformative are the questionnaire names in helping trialists to choose between them. All include the word 'tinnitus' but rarely qualify that with a description of which tinnitus-related domains or constructs are assessed by the tool. Generic names and terms such as 'handicap' and 'severity' perpetuate the difficulty that many trialists experience in understanding what construct(s) a particular questionnaire instrument measures. For example, the Tinnitus Handicap Inventory [23] predominantly measures the construct of tinnitus-related distress, while the Tinnitus Handicap Questionnaire [38] measures the physical, emotional *and* social consequences of tinnitus, as well as hearing ability.

Third, treatment-related outcomes were rarely recorded. Safety, tolerability, side effects and withdrawals might be domains that all inform the measurement of adverse events, but these accounted for less than 2 % of primary outcome domains and 12 % of secondary outcome domains. Again, non-reporting mainly reflected a poor understanding of how important it is for individual trials to investigate and report harms, as well as benefits [39].

Comparison with other studies

Our work provides the first detailed set of information on the selection and reporting of outcome domains and outcome instruments in clinical trials of tinnitus. One previous systematic review examined outcomes of randomised controlled trials of interventions for adults with tinnitus [11], but outcome data collection and reporting was restricted to 'use of validated instruments for assessing tinnitus symptoms … any audiometric data … length of follow-up, and adverse event reporting.' pp. 2–3, not the full set of outcomes considered in the present review. Reported findings indicated only that 20 % of studies used a validated tinnitus instrument, 79 % of studies used audiometric measurements, 42 % of studies specified adverse events, and the median follow-up time was 3 months. No further details were given and what constitutes a 'validated instrument' was not defined, so comparisons are restricted. Our study findings at least confirm the limited use of patient-reported questionnaire instruments relating to the impact of tinnitus. While we find little consistency across studies in reporting adverse events, our findings suggest that adverse event reporting is about 5 %, markedly less than the 42 % reported by Plein et al. [11].

Our review identifies limitations in the range of reported outcomes in clinical trials that are reflected more broadly across the field of audiological research. Here two reviews have been undertaken to identify outcome measures used in research on adults with hearing loss. In the first, Granberg et al. [40] conducted a systematic review of published articles, including a range of study designs. The authors found 51 different patient-reported questionnaire instruments relating to the impact of hearing loss out of the 122 studies included, with only 16 being used twice or more. Our review confirmed similar diversity (24 different tinnitus-related questionnaire instruments) and lack of consensus (14 used twice or more). In the second, Barker and et al. [41] conducted a scoping review to document the range and nature of outcome measurement in the context of adult auditory rehabilitation. Like us, they included registered trials and published studies. The most common outcome domain was 'hearing handicap' which was measured in 23 out of the 37 studies included, using five different patient-reported questionnaire instruments. Again, the use of generic terms such as 'handicap' perpetuate the difficulty that many trialists experience in understanding what construct(s) are measured by a particular questionnaire instrument. The frequency of reporting adverse events was not given by Granberg et al. [40], but Barker et al. [41] stated that no studies reported on adverse events. Poor reporting of harms-related data is not restricted to clinical trials in the hearing sciences [42].

Strengths and limitations of the study

The strengths of our study rest on the inclusion of both registered (ongoing) clinical trials of tinnitus, as well as published study findings and on the broad-ranging and comprehensive evaluation of both the outcome domains and the outcome instruments used. Several potential limitations were unavoidable due to limited resources. These were the use of a pre-defined time window and the exclusion of non-English language records. While the search strategy excluded trials that were registered or published prior to July 2006, it is likely to have included trials designed prior to this date. However, there was insufficient information reported to ascertain this with any degree of certainty. Whether or not any systematic bias was introduced by the use of an English-language restriction is also uncertain, and may not affect systematic review conclusions [43].

Our study adds new insights to an emerging body of empirical evidence on outcome reporting within ENT and audiology trials [40, 41]. Our findings should help to steer trialists in these disciplines about good reporting practice, as well as to inform Cochrane and other systematic reviewers on the choice of outcomes for their work. Our study leads us to agree with Hoare et al. that 'To be useful, future studies should … be consistent in their use of outcome measures' [35].

The longer-term intention for this work is to develop a core outcome set that identifies by consensus a minimum

standard for reporting in clinical trials of tinnitus in adults. This review makes a specific contribution to that ambitious endeavour by identifying which domains have been defined in relevant clinical trial designs to date. When developing a core outcome set, it is important to capture in the long list of potential outcome domains all those that need to be considered for inclusion [44]. For that long list to be truly comprehensive, it is important to capture relevant information that is contained within those studies. A limitation of the current review concerns those domain definitions that were unclear or not specified by their authors. This is especially important where the domains relate to patient-reported outcomes of the impact of tinnitus. One way to address the current gap is to deconstruct the patient-reported outcome instruments by creating a list of all questionnaire items, grouping individual items into simi- lar constructs or domains and then cross-checking them against the current domain list reported here [44].

Conclusions

We are the first group to conduct a systematic review that targets the reporting of outcome domains and instruments in clinical trial designs that evaluate interventions for tinnitus. The findings of this review have produced an extremely rich dataset that has enabled us to address a number of different primary and secondary questions concerning different aspects of good trial design. Our findings add important new insights pointing to the lack of awareness and understanding of good trial design in so far as this relates to outcomes. A general lack of consensus regarding the choice of outcomes did affect trial design, conduct and reporting with particular reference to lack of sample size calculation, and lack of robust interpretation of whether the intervention was therapeutically beneficial or not.

Our findings emphasise the need to improve trial design and reporting. A small number of the included studies in our review acknowledged Consolidated Standards of Reporting Trials (CONSORT) guidelines for reporting [45], but this is more the exception than the rule. Using such guidelines would improve definitions of all outcome measures including pre-specifying the time point of primary interest as well as detailed reporting of any important changes to methods or outcomes after the trial commenced with reasons for such changes. To improve reporting, we draw attention to the specialised CONSORT guidelines for reporting harms-related issues in a randomised controlled trial [39].

Additional files

Additional file 1: PRISMA checklist of items to include when reporting a systematic review. (DOCX 19 kb)

Additional file 2: TINNET WG5 master file. An editable version of the data master file. (XLSX 313 kb)

Abbreviations

CDSR, Cochrane Database of Systematic Reviews; CENTRAL, Cochrane Central Register of Controlled Trials; CINAHL, Cumulative Index to Nursing and Allied Health Literature; CONSORT, Consolidated Standards of Reporting Trials; COST, Cooperation in Science and Technology; EBSCO, Elton Bryson Stephens COmpany; EMBASE (Ovid), Excerpta Medica Database; EPOC, Effective Practice and Organization of Care; ICTRP, International Clinical Trials Registry Platform; ISRCTN, International Standard Randomised Controlled Trial Number registry; PICOS, Patient, Intervention, Comparison, Outcome, Setting; PRISMA, Preferred Reporting Items for Systematic reviews and Meta-analyses; PROSPERO, International Prospective Register of Systematic Reviews; PubMed, database maintained by the United States National Library of Medicine at the National Institutes of Health; QoL, quality of life; TINNET, TINnitus research NETwork; TQ, Tinnitus Questionnaire; WHO, World Health Organisation; WHOQOL-BREF, World Health Organisation Quality of Life (brief version)

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Authors' contributions

DAH managed the review process and, as data guarantor, verified the data collection for all records. AJS conducted the electronic search. DAH, HH and AJS conducted the selection process. DAH and JJD conducted the risk of bias analysis. DAH, HH, AJS, PL, SR, JJD, AL, NKE, CRC, MM, TF, ABC, PB, DMT, AN, RFFC, and BM contributed to the data collection, analysis, and manuscript writing with authorship order reflecting the relative size of that contribution. DAH, HH, SR, DMT and BM conducted the domain grouping. RLM conducted a blinded assessment of the sample size calculation. DAH, HH, AJS, PL, SR, JJD, AL, NKE, CRC, MM, TF, ABC, PB, DMT, AN, RFFC, RLM and BM read and approved the manuscript.

Competing interests

Alain Londero reports non-financial support from Grand Audition, Audionova, Audition Libre and Amplifon France unrelated to this work. All other authors declare that they have no competing interests.

Additional file 3: Table S1. Table of records containing missing data that was queried to the corresponding author by email. (DOCX 21 kb)

Additional file 4: Table S2. Table reporting the full reference list for all 228 included records. (DOCX 39 kb)

Additional file 5: Table S3. Tabulation of the evaluation of outcome reporting bias. '✓' denotes consistent reporting across publications; '✗' denotes inconsistent reporting; 'o' denotes partial reporting whereby the instrument remains consistent but the time frame does not; 'P-only' denotes that the outcome was specified in the protocol, but not reported as a study finding; 'F-only' denotes that the outcome was not specified in the protocol, but was reported as a study finding. For P-only, we cannot distinguish cases where an outcome was measured and analysed but not reported, measured but not analysed or reported, or not measured. (DOCX 25 kb)

Additional file 6: Table S4. Outcome instruments used only once either for primary or secondary outcomes. (DOCX 19 kb)

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5.2.4. Pathophysiology, diagnosis and treatment of somatosensory tinnitus

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REVIEW
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Pathophysiology, Diagnosis and Treatment of Somatosensory Tinnitus: A Scoping Review

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Somatosensory tinnitus is a generally agreed subtype of tinnitus that is associated with activation of the somatosensory, somatomotor, and visual-motor systems. A key characteristic of somatosensory tinnitus is that is modulated by physical contact or movement. Although it seems common, its pathophysiology, assessment and treatment are not well defined. We present a scoping review on the pathophysiology, diagnosis, and treatment of somatosensory tinnitus, and identify priority directions for further research.

Methods: Literature searches were conducted in Google Scholar, PubMed, and EMBASE databases. Additional broad hand searches were conducted with the additional terms etiology, diagnose, treatment.

Results: Most evidence on the pathophysiology of somatosensory tinnitus suggests that somatic modulations are the result of altered or cross-modal synaptic activity within the dorsal cochlear nucleus or between the auditory nervous system and other sensory subsystems of central nervous system (e.g., visual or tactile). Presentations of somatosensory tinnitus are varied and evidence for the various approaches to treatment promising but limited.

Discussion and Conclusions: Despite the apparent prevalence of somatosensory tinnitus its underlying neural processes are still not well understood. Necessary involvement of multidisciplinary teams in its diagnosis and treatment has led to a large heterogeneity of approaches whereby tinnitus improvement is often only a secondary effect. Hence there are no evidence-based clinical guidelines, and patient care is empirical rather than research-evidence-based. Somatic testing should receive further attention considering the breath of evidence on the ability of patients to modulate their tinnitus through manouvers. Specific questions for further research and review are indicated.

Keywords: somatosensation, somatosensory, tinnitus, physical therapy, physiotherapy, cross modal

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INTRODUCTION

Tinnitus is defined as the conscious perception and reaction to a sound in the absence of a matching external acoustic stimulus, commonly described as a *phantom* perception. It is considered a symptom rather than a disease *per se* (Jastreboff and Hazell, 1993; Bürgers et al., 2013). Tinnitus is present in more than 10% (11.9–30.3%) of the adult population (McCormack et al., 2016), although only 0.5–3% refers to it as a problem that decreases quality of life (Coles, 1984; Swain et al., 2016).

Although tinnitus has been the subject of much research, its pathophysiology remains poorly understood. It is well-accepted that many social factors, such as poor education, lower income, or occupational and recreational activity associated with high noise exposure, influences the prevalence and risk of tinnitus (Hoffman and Reed, 2004). Moreover, it is regularly associated with hearing loss, trauma, or ototoxic medication triggering cochlear damage, with sustained neural changes in the central auditory system that succeeds such lesions (Møller, 2011a; Langguth et al., 2013). Tinnitus prevalence is believed to increase with age up to 65 years, where after it decreases (Hoffman and Reed, 2004; Shargorodsky et al., 2010). It is also a widespread symptom among children with hearing loss (Coelho et al., 2007) and many causes of hearing loss and tinnitus are thought to be the same (Crummer and Hassan, 2004).

Recent neuroimaging and animal model studies suggest that tinnitus-related neural activity may involve complex interactions between several sensory modalities, sensorimotor, somatomotor, and visual-motor systems, neuro-cognitive, and neuronal-emotional networks (Cacace, 2003; Sanchez and Rocha, 2011a,c; Ostermann et al., 2016). Signs of interactions between the auditory system and the somatosensory system include gaze-evoked tinnitus (Cacace et al., 1994; Pinchoff et al., 1998; Lockwood et al., 2001), cutaneous-evoked tinnitus (Cacace et al., 1999a,b), motor manipulation or forceful muscle contractions of head, neck and limbs that induce or suppress tinnitus, or affect tinnitus loudness (Sanchez et al., 2002, 2007; Simmons et al., 2008). Pressure on myofascial trigger points (Travell, 1960; Wyant, 1979; Fricton et al., 1985; Bjorne, 1993; Rocha et al., 2006, 2008; Rocha and Sanchez, 2007), electrical stimulation of the median nerve and hand (Moller and Rollins, 2002), finger movements (Cullington, 2001), orofacial movements (Pinchoff et al., 1998), and pressure applied to the temporomandibular joint (i.e., Bjorne, 1993) are also observed to modulate tinnitus in some people. Such "somatosensory tinnitus" is supposed to be a prevalent tinnitus subtype (for review see Ralli et al., 2016) and prevalence may even be under-estimated because it relies on self-report that tinnitus is modulated by touch or movement (Ward et al., 2015). For example, the prevalence of somatic modulation is higher when the patients are questioned specifically about it rather than spontaneous reports (Sanchez et al., 2002).

For clarity we will use the following definitions: Tinnitus Modulation is the human capability of changing the tinnitus perception (frequency or intensity) by means of performing a certain manouver or movement of the head or neck or jaw or limbs or the eyes. Triggers is the phenomenon that acivates tinnitus modulation, examples: gaze movement, some tactile stimulous, performing a certain manouver or movement of the head or neck or jaw or limbs or the eyes. So the peripheral activity or stimulation are the primary single sources of a precise modulation of the tinnitus sound and it is described as trigger activity and the term modulation is reserved solely for describing the central neural activity that affect changes in tinnitus percept. In the most comprehensive literature review to date on somatosensory tinnitus, Sanchez and Rocha (2011a,b) spoke of the need to establish evaluation protocols and specific treatments for somatosensory tinnitus that focus on both the auditory pathway and the musculoskeletal system. Yet there has never been a scoping review or systematic review on the topic. In this review, we scope the primary research literature on the pathophysiology, diagnosis, and treatment of somatosensory tinnitus. The aims of the review are to account the breadth and current state of knowledge on somatosensory tinnitus, to consider priority directions for research, and to identify whether any systematic reviews would be informative to the field.

MATERIALS AND METHODS

Literature searches were conducted in November 2016 in Google Scholar, PubMed, and EMBASE databases using the search terms somato* AND tinnitus (see Appendix 1 in Supplementary Material for an example search). Search results were screened to identify original articles and case reports for review. For Google Scholar, results were screened until five consecutive results pages yielded no new potentially relevant results. Additional hand searches of publications were conducted in the same databases using the additional broad search terms etiology, diagnose, treatment. Records were independently reviewed by at least two authors. In cases of disagreement, opinion of a third reviewer was taken as consensus. Inclusion criteria were: somatosensory tinnitus as main or secondary study objective, inclusion of at least one group with patients or case study suffering from somatosensory tinnitus, definition of somatosensory tinnitus, description of somatosensory tinnitus diagnostic approach or treatment. If the focus of the study was somatosensory tinnitus pathophysiology, diagnosis, or management, and at least one of the study groups or case study consisted of somatosensory tinnitus patients, the study was included; otherwise it was excluded. Exclusion criteria were articles written in languages other than English, and records relating solely to objective tinnitus.

Initial screening was based on abstract reading. Where there was uncertainty whether or not a record was relevant the full text record was screened. Records were grouped into three categories: pathophysiology, diagnosis, and treatment. One record could be relevant to more than one category. All records included patients with somatosensory tinnitus (P). Interventions (I) and their effects were recorded. Outcome measures were also identified (O), and comparisons (C) were described either between patients and controls, groups of patients divided by tinnitus type or intervention, as well between groups of patients before and after intervention for somatosensory tinnitus (see **Figure 1**).

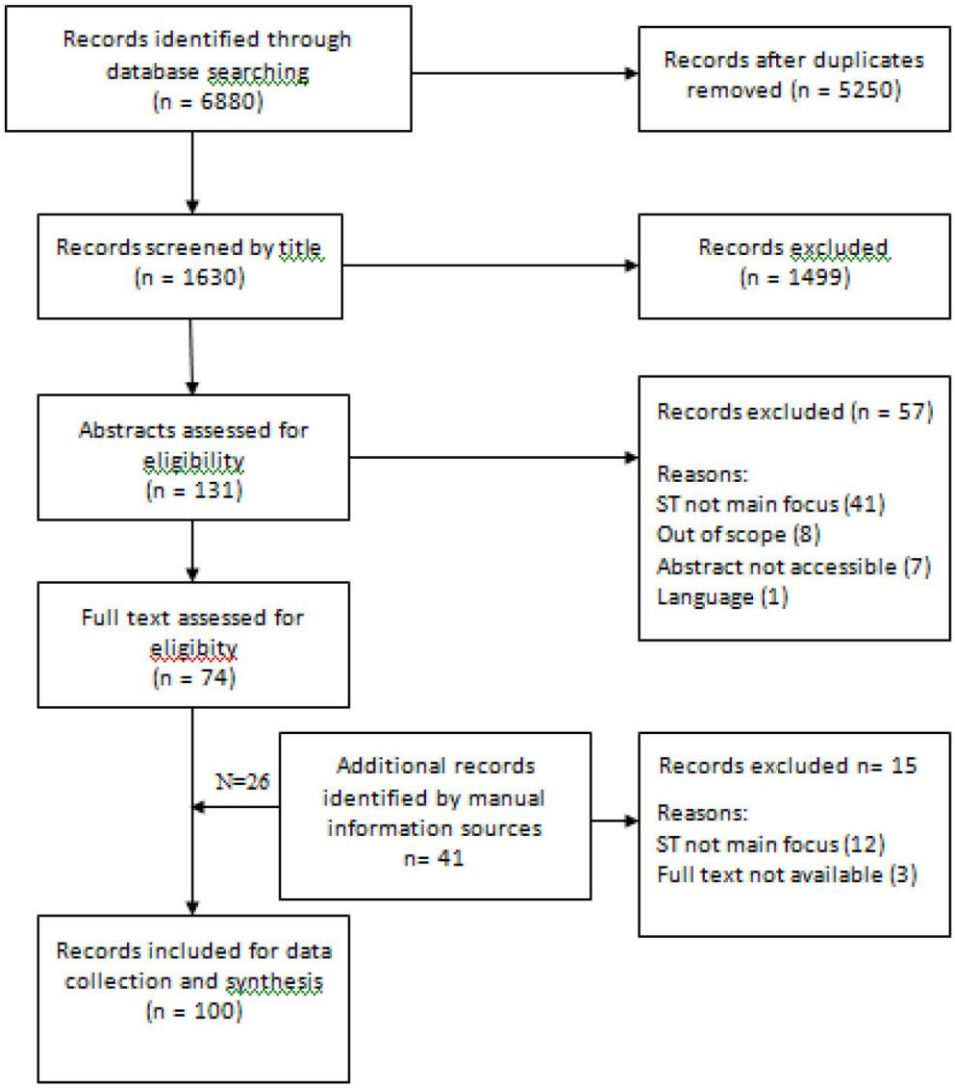

FIGURE 1 | Flow chart of study records.

RESULTS

The initial searches for somat * o AND tinnitus yielded 1,630 records of which 100 were suitable for inclusion in the review. Records are subdivided for review according to pathophysiology, diagnosis, and management.

Pathophysiology and Etiology

Records describing studies on the pathophysiology and etiology of somatosensory tinnitus were included and are reviewed here. A table compiling the case controlled studies and cross-sectional studies were summarized in Appendix 2 in Supplementary Material (case reports, reviews and book chapters were excluded). A number of authors suggest the somatosensory stimuli inducing tinnitus are deeply related to abnormal cross-modal plasticity of somatic-auditory interactions (Cacace, 2003; Levine et al., 2007; Herraiz, 2008; Rocha et al., 2008; Koehler and Shore, 2013) whereby somatic modulations of tinnitus results from abnormal auditory neural interactions—distortion of the normal synaptic activity—within the central nervous system, as Sanchez et al. (2007) describes, *"The information triggered by muscle contractions is carried by the somatosensory system and, upon reaching the cuneiform nucleus, may influence tinnitus through its projection over the auditory pathway due to an overactivitiy in the cochlear nucleus."* In particular, modulation of hyperactivity of neurons in the dorsal cochlear is triggered by the stimulation of specific ipsilateral cranial nerves, i.e., branch of the trigeminal nerve, explaining how ipsilateral tinnitus may be modulated by head and neck's manipulation (see a review, Kaltenbach, 2006). In guinea pigs, it was demonstrated that DCN bimodal plasticity is stimulus timing-dependant and implicated as an underlying mechanism in tinnitus (Shore et al., 2007; Koehler and Shore, 2013).

Levine et al. (2003) found somatic modulation in patients with tinnitus and deafness patients, identifying neural interactions in the central nervous system as the main protagonists in this process. Levine et al. (2008) also suggest that pulsatile tinnitus is modulated by the somatosensory system of the head or upper lateral neck, presenting two mechanisms; (1) cardiac synchronous somatosensory activation of the central auditory pathway, or (2) distortion of the normal synaptic activity between the somatosensory and auditory central nervous system. Simmons et al. (2008), studying patients who could modulate tinnitus with jaw clench found that an alteration in tinnitus loudness related to a variation in neural activity in the auditory cortex, concluding that tinnitus originates in the central auditory pathway. The same effect has been observed in patients who can modulate their tinnitus with eye movements (Lockwood et al., 2001; Sanchez and Akemi, 2008), and in patients whose tinnitus is modulated by intravenous administration of lidocaine (Reyes et al., 2002). Modulation of tinnitus with oral-facial movements suggest that the classical auditory system is not implicated in tinnitus because limbic structures respond to sound stimulation in patients with tinnitus (through hypoactivity localized in the hippocampus), further indicating the central auditory system and not the cochlea as the origin of tinnitus (Lockwood et al., 1998; Cacace, 2003; Schaette and McAlpine, 2011). In his studies, Levine found that patients could better detect changes in their tinnitus when using isometric maneuvers of the extremities, compared to head/neck maneuvers, suggestive of a major role of the central neural pathway as opposed to the auditory periphery (Cacace, 2003). In fact, a higher prevalence of somatoform disorders in individuals with tinnitus may also relate to certain craniocervical pathological features (e.g., herniated discs or temporomandibular joint syndrome; Chole and Parker, 1992; Rubinstein, 1993; Gelb et al., 1997; Levine, 1999b) and dental and jaw diseases (Han et al., 2009). For example, there is a higher than general incidence of tinnitus in patients and normal hearing who have temporomandibular disorder (TMD) (Levine, 1999b), suggesting that it may be associated with other symptoms of TMD (Chole and Parker, 1992; Bernhardt et al., 2011). The temporomandibular joint (TMJ) is thought to be commonly involved in the ability to modulate tinnitus, particularly its loudness (Ralli et al., 2016). Recently the risk of tinnitus was established as 8.37 times higher for patients with TMD (Bürgers et al., 2013), and unilateral tinnitus is even reported to be on the same side as unilateral TMD (Bürgers et al., 2013). These patients are also reportedly able to regulate their tinnitus through certain jaw or neck movements (Wright and Bifano, 1997a; Vielsmeier et al., 2011, 2012; Bürgers et al., 2013). Since tinnitus is normally related to the opposite risk factors (i.e., older males with hearing loss), such findings postulate that TMJ may be the cause and maintenance of tinnitus (Vielsmeier et al., 2011). It is proposed that TMD can cause tinnitus through the disruption of the trigeminal input (Vielsmeier et al., 2012; Ostermann et al., 2016). Another indication supporting the role of TMD in tinnitus is that the two conditions occur simultaneously. Evidence also shows that worsening of tinnitus coincides with aggravation of TMD (Wright and Bifano, 1997b).

Diagnosis

Records describing studies on diagnosis or rate of diagnosis of somatosensory tinnitus were included and are reviewed here. A table compiling the case controlled studies and cross-sectional studies were summarized in Appendixes 3, 4 in Supplementary Material (reviews, thesis, and book chapters were excluded), concerning both epidemiology and diagnosis fields, respectively. Common attributed risk factors for any subtype of tinnitus are male gender, older in age and hearing problems (i.e., Hazell, 1991; Abel and Levine, 2004; Eggermont and Roberts, 2004; Hoffman and Reed, 2004; Oostendorp et al., 2016), except for TMD-tinnitus patients (Chole and Parker, 1992; Wright and Bifano, 1997b; Vielsmeier et al., 2011; Bürgers et al., 2013). Recent evidence in a British cohort study shows that somatic tinnitus is more common among younger people and it is unrelated to hearing loss or tinnitus severity (Ward et al., 2015). Some of these audiological and demographic traits, may be indeed useful in informing therapy (Won et al., 2013) through the identification of "clinical criteria for useful subtyping of tinnitus patients" (Vielsmeier et al., 2012).

Signs of somatosensory tinnitus include head or neck problems (i.e., temporomandibular joint syndrome, osteophits, arthorosis, spondylosis, myofascial trigger points, etc.), dental or jaw diseases, frequent pain in head, neck, or shoulder girdle, aggravation of events of simultaneous pain and tinnitus, incorrect body postures, and severe bruxism (Sanchez and Rocha, 2011b,c). Such complexity demands a multidisciplinary team (i.e., dentist, physiotherapist) to diagnose.

Somatosensory tinnitus is strongly evidenced when the patient can modulate the loudness or intensity of their tinnitus (Abel and Levine, 2004; Latifpour et al., 2009; Sanchez and Rocha, 2011b,c; Oostendorp et al., 2016). Hence somatic testing may identify patients who could be treated with somatosensory system-related therapies. However, this type of testing receives little attention (Won et al., 2013).

There are various presentations of somatosensory tinnitus to be aware of. Typical cases include gaze-evoked or modulated tinnitus, cutaneous-evoked tinnitus, and tinnitus modulated by movement of corporal elements (i.e., head, fingers, jaw). Gaze-evoked/modulated tinnitus, the modulation of tinnitus by eye movement, provides clues on the potential cortical role in tinnitus (Lockwood et al., 2001). Simmons et al. (2008) found a large sample of patients who were capable of modulating their tinnitus by eye movement, half of whom had developed this ability after undergoing surgery for removal of an acoustic neuroma; these patients were able to change the tinnitus loudness and pitch through eye movement.

Studies of cutaneous-evoked tinnitus (using magnetoencephalographic signals and tactile discrimination tests) have found that cutaneous stimulation of skin on the hand region (specifically palm and fingers) activates the somatosensory system along with the auditory cortical areas in congenitally deaf individuals (Cacace et al., 1999a,b; Cacace, 2003).

In respect to modulation of tinnitus through of head and neck, Levine (1999a) reported that 68% of 70 patients could modulate tinnitus through maneuvers of the head, neck, or

less intensely, maneuvers of limb. Similarly, Sanchez et al. (2002) found both patients with tinnitus (65.3% of 121 persons) and healthy subjects (14% of 100) could modulate or develop, respectively, tinnitus through 16 different maneuvers, and later found 57.9% of a study population could modulate tinnitus using nine different maneuvers (Sanchez et al., 2007). Simmons et al. (2008) found that, in 93 subjects able to modulate tinnitus by jaw clench, 90% could increase the loudness of their tinnitus, and 50% could alter the pitch. In a different assessment, the same authors found that 78% of their sample of 45 subjects could modulate their tinnitus with movement of the head or neck, mainly using the cranial and cervical nerves and using forceful maneuvers. In another study, Won et al. (2013) found that in 57% of tested ears in a population sample of 163 patients, tinnitus (especially unilateral tinnitus) was modulated through neck maneuvers or jaw maneuvers, decreasing and increasing tinnitus loudness respectively. The authors also reported that in their sample bilateral and low-pitch tonal tinnitus was rarely modulated by movement and may even be aggravated by somatic therapy. More distal movement is also observed to modulate tinnitus. Cullington (2001) reported the case of a 78-year-old man with severe hearing loss implanted with a cochlear implant in his right ear was able to modulate his tinnitus by moving his finger. Fascinatingly, this patient reported that the quicker the movement, the more intense was tinnitus loudness; passive or isometric movement did not modulate the tinnitus (Sanchez and Akemi, 2008). See **Table 1** for a summary of somatic maneuvers.

Even when the patient cannot self-modulate tinnitus, it may be altered by other kinds of stimuli, using maneuvers to increase activity of the trigeminal nerve such as passive muscular palpation to find myofascial trigger points (MFT), relaxation, and massage (Simmons et al., 2008; Sanchez and Rocha, 2011b; Shore, 2011; Won et al., 2013).

Treatment

Records describing studies on the treatment of somatosensory tinnitus were included and are reviewed here by treatment category. Case controlled studies and cross-sectional studies were summarized in Appendix 5 in Supplementary Material.

Physiotherapeutic Treatment

Studies have accounted the benefits for tinnitus of treating (temporomandibular disorder) TMD. Wright and Bifano (1997a) studied tinnitus in TMD patients and reported that 56% had been cured and 30% had a significant improvement with cognitive therapy and modulation through maneuvers. However, it has also been found that that severe tinnitus is less likely to improve with TMD therapy (Wright and Bifano, 1997a). Another similar study has shown that younger patients with moderate tinnitus were more likely to experience relief of their tinnitus through TMD therapy (Wright and Bifano, 1997b). Tinnitus severity as a predictor of the effectiveness of TMD therapy has already been proposed by others including Erlandsson et al. (1991) and Bush (1987).

The presence of fluctuating tinnitus is another factor that may associate with TMD treatment effectiveness (e.g., Tullberg and Ernberg, 2006).

One form of TMD treatment is occlusal splint therapy (Attanasio et al., 2015). In their study involving this treatment in patients presenting with chronic subjective tinnitus Attanasio et al. (2015) divided patients into three groups according to whether TMD was absent, present, or the patient was considered predisposed to TMD. Patients were subjected to treatment with

TABLE 1 | Summary of somatic manouvers.

Authors	Body part	Maneuvres (examples)
Cullington, 2001	Finger	Moving up and down the middle finger of left hand**[#]
Levine, 1999a; Sanchez et al., 2002, 2007; Abel and Levine, 2004; Levine et al., 2007	Extremities	Locking the fingers of the two hands together and pulling as hard as possible, or resisting maximal pressure to. Shoulder abduction. Flexion or abduction of the hip. Resisting or not an applied force.
Lockwood et al., 2001; Sanchez and Akemi, 2008; Simmons et al., 2008	Eye	Moving in the vertical or horizontal axis**
Cacace et al., 1999a,b; Cacace, 2003; Sanchez and Akemi, 2008	Cutaneous	Stimulation of a well-defined region—various regions of the hand and fingers (e. g., palm, dorsal web regions, and fingertips)**[&]
Pinchoff et al., 1998; Sanchez et al., 2002, 2007; Abel and Levine, 2004; Levine et al., 2007; Simmons et al., 2008; Latifpour et al., 2009; Won et al., 2013	Jaw	Clench the teeth, open and close mouth, protrude jaw, slide jaw. Resisting or not an applied force.
Levine, 1999a; Sanchez et al., 2002, 2007; Abel and Levine, 2004; Simmons et al., 2008; Latifpour et al., 2009; Won et al., 2013	Head and neck	Moving the head back and in front and laterally, resisting or not an applied force (against the head in a neutral position or turned to one of the sides).
		Applying pressure on muscle insertions–esternocleidomastoid, splenius capitis, and posterior auricular.

All the different voluntary muscle contraction manouvers should be sustained during 5–10 s and performed using a moderate degree of force in a silent environment (Levine, 1999a). The idiopathic somotosensorial tinnitus will present more relevant modulation with jaw and head-neck manouvers.

***Very specific to certain cases of patients subjected to brain neurosurgery or cochlear implantation only rarely is it spontaneous.*

[#] The patient reported that the quicker the movement, the more intense the tinnitus loudness, passive or isometric movement did not modulate the tinnitus.

[&] Studies of cutaneous-evoked tinnitus, (using magnetoencephalographic signals and tactile discrimination tests) have found that electrical stimulation of the median nerve and hand region or cutaneous stimulation of skin on various regions of the hand including dorsal web regions and fingertips activate the somatosensory system along with the auditory cortical areas in congenitally deaf individuals (Cacace et al., 1999a,b; Cacace, 2003).

a neuromuscular occlusal splint for 6 months (using the splint at night time) and rated for the severity of tinnitus using 10-point visual analog scale and Tinnitus Handicap Inventory (THI; Newman et al., 2004) questionnaire. Post-treatment THI scores were reduced in all groups but was most pronounced in the TMD (experience or predisposed) groups. The authors concluded that, once otologic disorders and neurological diseases are excluded, that clinicians should refer patients for an evaluation of the temporomandibular joint and subsequently to treat patients with TMD or a predisposition to it.

Wright (2000) suggested oro-myofunctional therapy as an effective alternative to occlusal splints therapy. Their study involved patients from the US air force seeking treatment for tinnitus, dizziness, and/or nonotologic otalgia without an identifiable cause and presenting with TMD symptoms in the temple, jaw, or preauricular area. Patients were provided a dental orthotic and TMD self-care instructions. After 3 months of orthotic wear, the percentages of patients reporting at least moderate symptom improvement of their tinnitus, dizziness, otalgia, and/or TMD were 64, 91, 87, and 92%, respectively. Follow-up telephone calls 6 months after completion of TMD therapy revealed that all patients maintained their symptom improvements. These findings imply that TMD was affecting the patients' otologic symptoms.

Stomatognathic Therapy

Usually it includes splints therapy, therapeutic exercises for the lower jaw and occlusal adjustment in combination with counseling.

For a long time, scientists have investigated the effects of dental and stomatognathic therapies in tinnitus (Junemann, 1941; Gelb and Arnold, 1959; Dolowitz et al., 1964; Kelly and Goodfriend, 1964; Gelb et al., 1967; Koskinen et al., 1980; Ioannides and Hoogland, 1983; Cooper et al., 1986; Bush, 1987; Rubinstein and Erlandsson, 1991). According to the findings of Rubinstein (1993), almost one-third of patients report improvement in their tinnitus after mandibula movements and/or pressure on their TMJs. More recently, Bürgers et al. (2013) found that stomatognathic therapy had a positive effect on tinnitus symptoms in 44% of their TMD-tinnitus patients (n = 25), up to 3–5 months after the first intervention; while promising it is noted that there was no control group in this study. Using dental functional therapy, the authors found an improvement on acute or subacute tinnitus in 100% of the patients but little improvement in patients with chronic tinnitus. It is important to note that the authors discussed an individual therapeutic strategy with each patient before the start of treatment. The authors suggested long term studies are conducted to assess the outcome and advised caution when interpreting current epidemiological data.

Chiropractic Therapy

Chiropractic therapy is a correction therapeutic treatment of an abnormal movement pattern through the manipulation of the vertebral column and extremities. Only three studies related to chiropractic treatment of tinnitus were identified and all three were case studies. Alcantara et al. (2002) described the chiropractic therapy in a 41-year-old woman with history of ear pain, tinnitus, vertigo, altered hearing, ear infections, and headaches, and who was diagnosed TMD and cervical subluxation. The authors reported a complete relief from the TMD symptoms, including tinnitus, after only 9 treatments (2 months). The treatment involved the application of high-velocity low amplitude adjustments. Kessinger and Boneva (2000) also reported progress in a 75-year-old patient who received upper cervical specific chiropractic care which resulted in improvements in vertigo, tinnitus, and hearing loss. These authors concluded that the success of chiropractic therapy was due to improvement in cervical spine function.

DeVocht et al. (2003) also describes the chiropractic management of a 30-year-old woman with TMJ pain. The patient suffered daily from unremitting jaw pain for 7 years accompanied by headache, tinnitus, decreased hearing, and a feeling of congestion in her right ear. Twenty months of chiropractic treatment resulted in total resolution of all symptoms except fullness of the right cheek.

Muscle Relaxation

Combined with chiropractic care, muscular relaxation (through massage and stretching exercises) is used in clinical practice. For instance, evidence suggests that palpation of masseter, pterygoid, and sternocleidomastoid muscles or myofascial trigger points can modulate tinnitus (Rocha et al., 2008; Teachey et al., 2012). Björne (2007) reported on the effectiveness of stretching exercises targeting the suboccipital muscles, along with rotation movements in the atlanto-occipital joint and relaxing exercises, on a TMD patient population (no control group). Bjorne notes that patients with Ménière's were more likely to present with TMJ and cervical spine disorder's symptoms (including tinnitus), than people who do not have Ménière and using a coordinated therapy of TMJ and cervical spine disorder (relaxation and posture) found improvements in self-reported tinnitus severity that were retained up to 3 year follow up.

Latifpour et al. (2009) evaluated 24 subjects from an original pool of 41 subjects (non-randomized), divided into two groups: treatment and control group. The authors compared self-training of stretching, posture training, and acupuncture, targeting muscle symmetry and balance in the jaw and neck, and later reported an improvement of tinnitus in the treatment group. In this blinded study they observed immediate and long term (3 months) improvements in the treatment group.

Another therapy worth noting here; in a pilot study with 11 patients, Kaute (1998) reported improvement in vestibular disturbances through the method of Arlen's Atlas Therapy, normally applied to whiplash-injured patients, concluding it to be indicated where tinnitus may be caused by neck muscle tension. This study suggest that muscular relaxation may play a significant role in the treatment of tinnitus but high quality explanatory studies (i.e., comparison with a control, blinded, randomized allocation), are needed.

Somatic Modulation Therapy

Somatic modulation therapy (treatment aiming to modulate the intensity of a given symptom, by movement) has rarely been

studied beyond case studies. Sanchez et al. (2007) were the first to investigate the effect of repetitive training maneuvers with head and neck muscle contractions, focusing on its value as a tinnitus retraining therapy. The authors found it to have a significant effect on the modulation patterns but not in the daily perception of tinnitus.

In the case of a 39-year-old woman who developed gaze-evoked tinnitus after surgery to remove a left vestibular Schwannoma, therapy consisted of a repetitive gaze training and tinnitus was resolved after 14 weeks (Sanchez and Akemi, 2008). Interestingly, there was both a "horizontal" and "vertical" gaze effect on tinnitus and the vertical component responded more quickly to treatment suggesting more than one neural network or process was involved in this case.

In another case, a 54-year-old man with severe tinnitus noticed an improvement through tactile stimuli to the ipsilateral postauricular area, head rotation, opening of the mouth, and clenching teeth and mandible lateralization (Sanchez and Akemi, 2008). In another case of tinnitus improvement through tactile stimulation was reported in a single patient by Emmert et al. (2014); the patient reported a decrease in tinnitus intensity in the left ear when a tactile stimulus was applied (block-design using EPI sequence—the patients touched on the right cheek on seven blocks of 25 s, intercalating with 25 vs rest).

Electrical Stimulation

Recent evidence reported a significant improvement in tinnitus using transcutaneous electrical nerve stimulation (Herraiz et al., 2007; Vanneste et al., 2010). Trans-electrical nerve stimulation (TENS) of areas of skin close to the ear increases the activation of the dorsal cochlear nucleus through the somatosensory pathway and may augment the inhibitory role of this nucleus on the CNS and thereby ameliorate tinnitus (Herraiz et al., 2007).

Vanneste et al. (2010) applied transcutaneous nerve stimulation in the upper cervical nerve in 240 patients with the ability to modulate tinnitus and found a significant suppression of tinnitus. Although only 18% of the patients responded to the treatment, 43% declared an improvement and six patients reported a total suppression of tinnitus (Vanneste et al., 2010). Herraiz et al. (2007) showed that trans-electrical nerve stimulation led to improvements in 46% of somatic tinnitus patients (reduced VAS tinnitus severity scores) after 2 weeks of treatment. Intermittent "typewriter"—sound like tinnitus was the most responsiveness. Herraiz et al. (2007) also noted that tinnitus caused by a somatosensory injury had a better response than somatic tinnitus with an otologic disease.

Standardizing the indications and method could increase the efficacy of electrical stimulation in somatic tinnitus according to most authors. These results are promising so further controlled trials are warranted.

Pharmaceutical Treatment

Only one relevant record describing a pharmaceutical treatment was included. In this case study McCormick and Walega (2015) reported the successful treatment of refractory somatic tinnitus with cervical epidural injection of 80 mg triamcinolone acetonide. The patient was 61-year-old male with previous history of bacterial otitis media.

Surgical Treatment

No surgical treatment studies specific to somatosensory tinnitus were identified. One case study worth mentioning however was that of a 65 years old patient with left sided tinnitus and with left sided cervical neck pain who experienced a complete resolution of somatic tinnitus for over 1 year through radiofrequency ablation of the left C2–C3 medial branches of the dorsal ramus ipsilateral to tinnitus symptoms (Gritsenko et al., 2014).

DISCUSSION

Tinnitus is complex in nature and so ideally, and to achieve the best results, diagnosis and treatment should be specific to an individual patients experience. Further research on the physiological processes that lead to somatosensory tinnitus would facilitate the development of a specific protocol and therapy targeting the auditory pathways and musculoskeletal disorders (Sanchez and Rocha, 2011c). Indeed, any holistic view of tinnitus needs to take into consideration the auditory system as a dynamic and active structure, integrating systems of reaction, stimulation, and emotion and tinnitus itself as a symptom with complex causes that indicate hyperactive neural activity (Møller, 2011a) and activation of neural plasticity (Moller and Rollins, 2002; Møller, 2011b; Smith et al., 2013), without the participation of the ear (Møller, 2016).

Evidence points to a high prevalence of somatosensory tinnitus, but that it is under-investigated by clinicians and the processes underlying are still poorly studied. For instance, only very recently have the first steps been made toward understanding the genetic underpinnings of subjective tinnitus (Lopez-Escamez et al., 2016) or the social context and environment which may influence tinnitus, following the new Social-Neurophysiological Model of Tinnitus. This model proposes the integration of the neurophysiological system (Jastreboff, 1990; Jastreboff and Jastreboff, 2000) the relation between psychophysiological and behavioral systems) and the social information system, associated with the emotional experience of tinnitus (Li et al., 2015). These avenues may help develop clinical strategies that adapt to patient's understanding and attitudes toward tinnitus, through social learning. What these will mean for somatosensory tinnitus is an open question.

It is important to note that an early and precise diagnosis, presents the best outcomes for the patient treatment (Herraiz, 2008). Recent research on the treatment of somatosensory tinnitus has focused on bone and muscular disorders, on each structure independently or using multimodal approach including manual therapy and exercise (Michiels et al., 2014, 2016). This demands different practitioners (dentists, neurologists, audiologists, physiatrist etc.) to be involved in treatment. Although such strategies do not target tinnitus directly, such therapies are shown to ameliorate its side effects.

It is not possible to cure tinnitus through dental and TMD therapies. But these same therapies may contribute to a multidisciplinary methodology of tinnitus treatment (Herraiz, 2008; Bürgers et al., 2011). It is a priority to establish how TMD and somatosensory tinnitus are related and what criteria should be used to select tinnitus patients for different TMD therapies.

Further research is needed to attest the efficacy of TMD therapy on tinnitus and to access the placebo effect (Rubinstein, 1993; Tullberg and Ernberg, 2006).

A multidisciplinary approach to managing somatosensory tinnitus may result in different strategies being used by different teams of clinicians if there is poor interdisciplinary communication and the lack of large-scale controlled trials to inform evidence-based clinical guidelines (Møller, 2007). In addition, standardization of core measures hinders the process of any potential meta-analysis on the large datasets, which would aid the development of clinical interventions for tinnitus. However, it will need to be tested whether these standardized outcomes are sensitive to treatment related changes in groups of patients or trail participants who have somatosensory tinnitus.

CONCLUSION

Because somatosensory tinnitus is not judged a disease *per se*, but instead it is considered a symptom, its diagnosis and treatment were related to other disorders. Connection to hearing loss and bone and muscular disorders are evident.

With this scoping review, we intended to give the reader a broad overview of findings to date concerning somatosensory tinnitus, and encourage new systematic and integrative analyses which will hopefully bring the much-needed order to the field of tinnitus research.

We propose several outstanding studies on somatosensory tinnitus:

1. There is some discrepancy over the prevalence of somatosensory tinnitus; a systematic review is needed.
2. The etiology of somatosensory tinnitus needs continued investigation. Particularly, and considering the involvement of neural plasticity, it is necessary to determine the exact processes that initiate the abnormal cross-modal plasticity of somatic-auditory interactions. Moreover, it is important to determine the exact relation between the head/neck maneuvers in the central neural system.
3. There is a lack of objective diagnostic methodology, which may misguide clinical management. Clinical guidelines that consider or are specific to somatosensory tinnitus are needed.
4. There are many and different strategies for managing tinnitus, originating in different clinical fields (audiology, neurology, psychology, etc.), and not all strategies have been trialed in somatosensory tinnitus. Integrating such strategies, and having in mind that each patient is a singular case, may increase the success of clinical management practices for tinnitus.
5. To support further trials and data synthesis in somatosensory tinnitus there needs to be standard research methodologies. Theses should be developed through consensus.
6. A therapeutic intervention combining simultaneously several types of treatment approaches may bring the best results for tinnitus relief, but such combinations may also be individual specific.

AUTHOR CONTRIBUTIONS

HH is the guarantor of the review. DH and DK created the search strategies. DK and CN created the tables in appendix. IP contributed in data extraction and initial manuscript. HH, DH, and RC contributed equally to all other stages of the manuscript development, produced, and approved the manuscript. NT, HC, AL, and JP provided consultative advice and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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5.2.5. Diagnostic criteria for Somatosensory tinnitus: A Delphi consensus and Face-to-Face Meeting to Establish Consensus

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Diagnostic Criteria for Somatosensory Tinnitus: A Delphi Process and Face-to-Face Meeting to Establish Consensus

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Abstract

Since somatic or somatosensory tinnitus (ST) was first described as a subtype of subjective tinnitus, where altered somatosensory afference from the cervical spine or temporomandibular area causes or changes a patient's tinnitus perception, several studies in humans and animals have provided a neurophysiological explanation for this type of tinnitus. Due to a lack of unambiguous clinical tests, many authors and clinicians use their own criteria for diagnosing ST. This resulted in large differences in prevalence figures in different studies and limits the comparison of clinical trials on ST treatment. This study aimed to reach an international consensus on diagnostic criteria for ST among experts, scientists and clinicians using a Delphi survey and face-to-face consensus meeting strategy. Following recommended procedures to gain expert consensus, a two-round Delphi survey was delivered online, followed by an in-person consensus meeting. Experts agreed upon a set of criteria that strongly suggest ST. These criteria comprise items on somatosensory modulation, specific tinnitus characteristics, and symptoms that can accompany the tinnitus. None of these criteria have to be present in every single patient with ST, but in case they are present, they strongly suggest the presence of ST. Because of the international nature of the survey, we expect these criteria to gain wide acceptance in the research field and to serve as a guideline for clinicians across all disciplines. Criteria developed in this consensus paper should now allow further investigation of the extent of somatosensory influence in individual tinnitus patients and tinnitus populations.

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Introduction

Tinnitus is the phantom sensation of sound in the absence of overt acoustic stimulation (Landgrebe et al., 2012). It occurs in approximately 10% to 15% of adults and is experienced as severely annoying by 1.6% (Baguley, McFerran, & Hall, 2013). Reported prevalence ranges can vary, depending on the way tinnitus is diagnosed and the age and gender of the assessed population (McCormack, Edmondson-Jones, Somerset, & Hall, 2016). Tinnitus is mostly subjective, as only the patient experiences it, and it is generally described as whistling, hissing, sizzling, or ringing (Baguley et al., 2013). Typically, tinnitus is related to hearing loss or a noise trauma, where cochlear abnormalities are the initial source, and neural changes in the central auditory system maintain the tinnitus (Baguley et al., 2013).

In the 1990s, the first researchers (Hiller, Janca, & Burke, 1997; Pinchoff, Burkard, Salvi, Coad, & Lockwood, 1998) started to mention a possible influence of the somatosensory system on tinnitus complaints, but it was only in 1999 that Levine (1999) first described a hypothesis for this tinnitus subtype, which he named *somatic tinnitus* (ST).

ST (also called somatosensory) is a subtype of subjective tinnitus, where altered somatosensory afference from the cervical spine or temporomandibular area causes or changes a patient's tinnitus perception.

Since Levine's first publication (1999), several animal and human studies have found connections between the somatosensory system of the cervical or temporomandibular area and the cochlear nuclei (CN), offering a physiological explanation for ST (Lanting, de Kleine, Eppinga, & van Dijk, 2010; S. E. Shore, 2011; Zhan, 2006). According to these studies, cervical or temporomandibular somatosensory information is conveyed to the brain by afferent fibers, the cell bodies of which are located in the dorsal root ganglia or the trigeminal ganglion. Some of these fibers also project to the central auditory system. This enables the somatosensory system to influence the auditory system by altering spontaneous rates or synchrony of firing among neurons in the CN, inferior colliculus or auditory cortex. In this way, the somatosensory system is able to alter the pitch or loudness of the tinnitus (S. Shore, Zhou, & Koehler, 2007).

Sanchez and Rocha (2011) proposed a set of diagnostic criteria to help recognizing patients with ST in clinical practice. According to these criteria, ST is suspected when the medical history shows at least one of the following: (a) evident history of head or neck trauma; (b) tinnitus association with some manipulation of the teeth, jaw, or cervical spine; (c) recurrent pain episodes in head, neck, or shoulder girdle; (d) temporal coincidence of appearance or increase of both pain and tinnitus; (e) increase in tinnitus during inadequate postures during rest, walking, working, or sleeping; and (f) intense bruxism periods during the day or night (Sanchez & Rocha, 2011). In addition, Sanchez and Rocha (2011) mention that ST often changes its loudness, pitch, or localization during stimulation in the head or neck region. Others (Biesinger, Groth, Hoing, & Holzl, 2015; Ward, Vella, Hoare, & Hall, 2015) state that the presence of this *somatic modulation*, through voluntary movements or specific resistance tests, is very important, if not the most important criterion, in diagnosing ST. These differences in diagnostic criteria might, at least partially, explain the large differences in prevalence of ST, which vary from 16% to 83% in different studies (Abel & Levine, 2004; Levine, Abel, & Cheng, 2003; Michiels, De Hertogh, Truijen, & Van de Heyning, 2015; Ralli et al., 2017; Simmons, Dambra, Lobarinas, Stocking, & Salvi, 2008; Ward et al., 2015; Won et al., 2013).

The lack of any agreed standards for clinical assessment make it unclear how to diagnose ST. Therefore, we aimed to reach a consensus on diagnostic criteria for ST among professional experts with current experience in assessing and managing ST. To reach this goal, we conducted a systematic review of the literature, followed by a modified two-round Delphi survey and a face-to-face meeting.

Methods

We used a Delphi process to gain consensus on a set of diagnostic criteria for ST among a panel of experts (scientists and clinicians). The Delphi technique, originally developed by the RAND Corporation, is a structured process that uses a series of questionnaires or rounds to gather and to provide information on a certain topic (Keeney, Hasson, & McKenna, 2001).

Systematic Review

A modified Delphi technique (Fackrell et al., 2017) was used, asking participants to review a *long list* of potential diagnostic criteria for ST rather than asking

participants to nominate criteria from scratch. This long list was created using data collected by a systematic review of the relevant literature. A search of the online search engine PubMed was performed up until October 2017. PubMed searches biomedical literature from MEDLINE, life science journals, and online books. A lenient search strategy was performed to identify the following terms appearing in all fields—(''Tinnitus''[Mesh])AND (Somatosensory OR somatic). Studies were eligible if they contained information on specific clinical features or diagnostic criteria of ST or inclusion criteria relating to ST. Screening and selection of eligible articles and data extraction were conducted by the first author. Data extraction was limited to assessment information only, which was then used to create a long list of potential diagnostic criteria for ST (Table 1).

Modified Delphi Survey

Panel selection. Experts in ST were identified if they were a senior (i.e., first or last) author of an included publication that had been identified in the systematic review and were able to understand written English. Responsibility for conducting and managing the Delphi process was not an exclusion criterion for panel membership. In addition, those experts were each asked to recommend other ST experts from academic or clinical fields. This process identified 18 individual experts from 10 countries (Belgium, Brazil, France, Germany, Israel, Italy, Portugal, United Kingdom, South Korea, and United States) and 16 universities or hospitals. Of those, 15 agreed to participate in the Delphi panel. Two answered they did not feel confident enough with the subject to be part of the survey and one did not respond to the invitation.

The Delphi survey. The two-round Delphi survey was managed using Qualtrics® Survey Software to support the international reach of the study. Academic and clinical experts were pooled to create a single professional stakeholder group. To promote retention of panel members, each round was open for a short time (4 weeks) and the time between rounds was kept to a minimum (2 weeks). Response rates were regularly monitored, email reminders were sent to target individuals who had yet to complete the round.

In Round 1, 15 panelists were asked to evaluate the level of importance of each potential diagnostic criterion for ST from the long list. The order of items was fixed across rounds. Participants scored each outcome domain inspired by the GRADE scale of 1 to 9 (Guyatt et al., 2011). Scoring used a Likert-type scale with additional interpretation categories; 1 to 3 indicated that the item was *not essential* for diagnosing ST, 4 to 6 indicated it *may be present, but not essential*, and 7 to 9 indicated that it was *essential*. *Unable to score* was always an option. Participants were also able to suggest additional diagnostic criteria in a free-text comment.

In Round 2, those panelists who completed at least 80% of the Round 1 survey received the same long list, plus the additional items suggested by at least one panelist. Participants were presented with graphical feedback (a bar chart) to summarize the panel results from Round 1. The purpose of Round 2 was to enable the participants to reflect on their answers, taking into account the opinion of their peers, and to score the different items again. From Round 2, a recommendation for inclusion as a diagnostic criterion for ST was predefined as at least 70% of the panelists scored 7 to 9, and fewer than 15% scored 1 to 3. Conversely, a recommendation for exclusion was at least 70% of the panelists who scored 1 to 3 and fewer than 15% scored 7 to 9.

Consensus Meeting

The 14 panelists who completed Round 2 of the Delphi survey were invited to participate in a face-to-face consensus meeting that took place on March 13, 2018, prior to the *Tinnitus Research Initiative Conference* 2018 in Regensburg, Germany. A group of six clinicians or academic professionals with expertise on ST attended the meeting. The panel included three clinicians (one audiologist and two ear, nose, and throat [ENTs]) and three scientists (one neurologist, one ENT, and one physical therapist). Authors 1 to 5 served on this panel. The meeting lasted 3 h, and the discussion was semistructured according to the nominal group technique (Harvey & Holmes, 2012). Participants were encouraged to voice their opinions. All strongly dissenting opinions were considered.

The starting point for the consensus discussion was guided by the recommendations from the Delphi survey. First, participants were asked to consider those items where, after Round 2 of the survey, the recommendation was for exclusion as a diagnostic criterion for ST. The remaining items were individually discussed and voted for, with voting options being *include* or *exclude*. Again the predefined definition of consensus was for at least 70% of the participants to agree.

Results

Systematic Review

The search strategy identified 167 articles, of which 18 were eligible for inclusion. A detailed overview of the selection process is shown in Figure 1. Synthesis of the data extracted from those 18 articles related to patient assessment for ST yielded 34 potential diagnostic criteria. A list of these can be found in Table 1, along with references to the source of that information.

Table 1. Overview of the "Long List" of 41 Potential Diagnostic Criteria.

References	Potential diagnostic criterion	Voting results consensus meeting
Biesinger et al. (2015), Haider et al. (2017), Ward et al. (2015), Vielsmeier et al. (2012), Sanchez and Rocha (2011), Levine and Oron (2015), and Bechter, Wieland, and Hamann (2016)	The patient is able to modulate the tinnitus by voluntary movements of the head or neck.	100% inclusion
Biesinger et al. (2015), Ward et al. (2015), and Sanchez and Rocha (2011)	The patient is able to modulate the tinnitus by voluntary movements of the jaw	100% inclusion
Ward et al. (2015) and Kapoula, Yang, Vernet, Bonfils, and Londero (2010)	The patient is able to modulate the tinnitus by eye movements	100% inclusion
Biesinger et al. (2015) and Ward et al. (2015)	The patient is able to modulate the tinnitus by clenching the teeth	100% inclusion
Biesinger et al. (2015) and Haider et al. (2017)	Tinnitus is modulated by pressure on myofascial trigger points	100% inclusion
Biesinger et al. (2015), Ward et al. (2015), Ralli et al. (2016), and Ostermann et al. (2016)	Tinnitus is modulated by resistance tests of the cervical spine (somatic maneuvers)	100% inclusion
Biesinger et al. (2015), Ward et al. (2015), Ralli et al. (2016), and Ostermann et al. (2016)	Tinnitus is modulated by resistance tests of the jaw (somatic maneuvers)	100% inclusion
Haider et al. (2017)	Tinnitus is modulated by resistance tests of the arm (somatic maneuvers)	100% "can be present occasionally"
Bechter et al. (2016), Ralli et al. (2016, 2017), Sanchez and Rocha (2011), and Erlandsson, Rubinstein, and Carlsson (1991)	Tinnitus is accompanied by frequent pain in the cervical spine, head or shoulder girdle	100% inclusion
Bechter et al. (2016)	Tinnitus is accompanied by muscular tension of the upper posterior cervical muscles of the head-neck transition	100% inclusion
Haider et al. (2017), Ward et al. (2015), Ralli et al. (2016) , Vielsmeier et al. (2012), Erlandsson et al. (1991), Tullberg and Ernberg (2006), and Buergers, Kleinjung, Behr, and Vielsmeier (2014)	Tinnitus is accompanied by temporomandibular disorders (pain in the jaw or masticatory muscles)	100% inclusion
Haider et al. (2017)	Tinnitus is accompanied by signs of osteophytes or spondylosis on radiography	100% exclusion
Haider et al. (2017)	Tinnitus is accompanied by the presence of pressure tender myofascial trigger points	100% inclusion
Haider et al. (2017)	Tinnitus is accompanied by dental diseases	75% inclusion 25% exclusion
Haider et al. (2017), Ralli et al. (2017), and Michiels, Van de Heyning, Truijen, Hallemans, and De Hertogh (2017)	Tinnitus and pain symptoms aggravate simultaneously	100% inclusion
Haider et al. (2017)	Tinnitus is accompanied by poor body posture	100% exclusion
Haider et al. (2017), Ralli et al. (2017), and Bosel, Mazurek, Haupt, and Peroz (2008)	Tinnitus is accompanied by bruxism	100% inclusion
Ralli et al. (2017)	Tinnitus is accompanied by teeth clenching	100% inclusion
Ward et al. (2015)	Presence of a pulsatile tinnitus, not synchronous with the heartbeat	100% "can be present occasionally"
Ward et al. (2015)	Tinnitus loudness is reported to vary from day to day	100% inclusion
Ralli et al. (2017) and Sanchez and Rocha (2011)	Tinnitus is preceded by a head or neck trauma	100% inclusion
Ralli et al. (2017), Sanchez and Rocha (2011), and Michiels et al. (2017)	Tinnitus increases during bad postures (while resting, walking, working or sleeping)	100% inclusion
Vielsmeier et al. (2012)	Tinnitus is maskable by music or sounds	100% exclusion
Sanchez and Rocha (2011) and Michiels et al. (2017)	Tinnitus and pain complaints appeared simultaneously	100% inclusion

(continued)

Table 1. Continued

References	Potential diagnostic criterion	Voting results consensus meeting
Tullberg and Ernberg (2006) and Bosel et al. (2008)	Tinnitus is accompanied by malocclusion of the teeth	100% "can be present occasionally"
Bosel et al. (2008)	Tinnitus is accompanied by oral parafunctions (such as: bruxism, teeth clenching, biting nails, . . .)	Item is covered by including bruxism and teeth clenching
Peroz (2003)	Tinnitus is accompanied by muscular dysfunction of the masticatory area	Item is covered by including temporo-mandibular disorders
Bosel et al. (2008)	Tinnitus is accompanied by noises of the temporo-mandibular joint	Item is covered by including temporo-mandibular disorders
Bosel et al. (2008)	Tinnitus is accompanied by palpation pain in the masticatory muscles	Item is covered by including temporo-mandibular disorders
Ostermann et al. (2016)	Tinnitus is accompanied by fascial dysesthesia (such as a tingling or numb feeling in the face)	100% "can be present occasionally"
Kapoula et al. (2010)	Tinnitus is accompanied by deficits in eye fixation, smooth pursuit tests or optokinetic nystagmus	100% exclusion
Michiels et al. (2017)	Tinnitus is low pitched (<1000 Hz)	100% exclusion
Levine, Nam, and Melcher (2008)	Constant pulsatile tinnitus, synchronous with the heartbeat, that can momentarily be abolished by a strong muscle contraction of the head or neck or a strong pressure applied to the same muscles	40% inclusion and 60% exclusion
Levine et al. (2008)	In case of a unilateral tinnitus, the audiogram does not account for unilateral tinnitus (e.g.,: normal audiogram, symmetric hearing loss or hearing loss greater in the contralateral ear)	100% inclusion
Suggested by panel	Patient indicates a relationship between the sleep quality at night and the tinnitus during the day	100% "can be present occasionally"
Suggested by panel	Taking a nap during the day affects the tinnitus	60% inclusion and 40% exclusion
Suggested by panel	Tinnitus is accompanied by increased muscle tension in the suboccipital muscles	100% inclusion
Suggested by panel	Tinnitus appearance is preceded by orthodontic procedures	60% inclusion and 40% exclusion
Suggested by panel	Tinnitus is intermittent or has large fluctuations in loudness	100% inclusion
Suggested by panel	Soft unilateral tinnitus or loud tinnitus throughout the head	100% "can be present occasionally"
Suggested by panel	Tinnitus is accompanied by lack of molar support	100% exclusion

Delphi Survey

Each round of the Delphi survey was open for 4 weeks, with 2 weeks in between both rounds.

Seven additional items were suggested by at least one panelist in Round 1 (see Table 1). These were added to Round 2 of the Delphi survey.

At the end of Round 2, scores for the expert panel indicated support for the inclusion of two diagnostic criteria since more than 70% of the Delphi panel members scored them 7 to 9 and fewer than 15% scored them 1 to 3 (see Table 1). Conversely, scores indicated the exclusion of six diagnostic criteria since more than

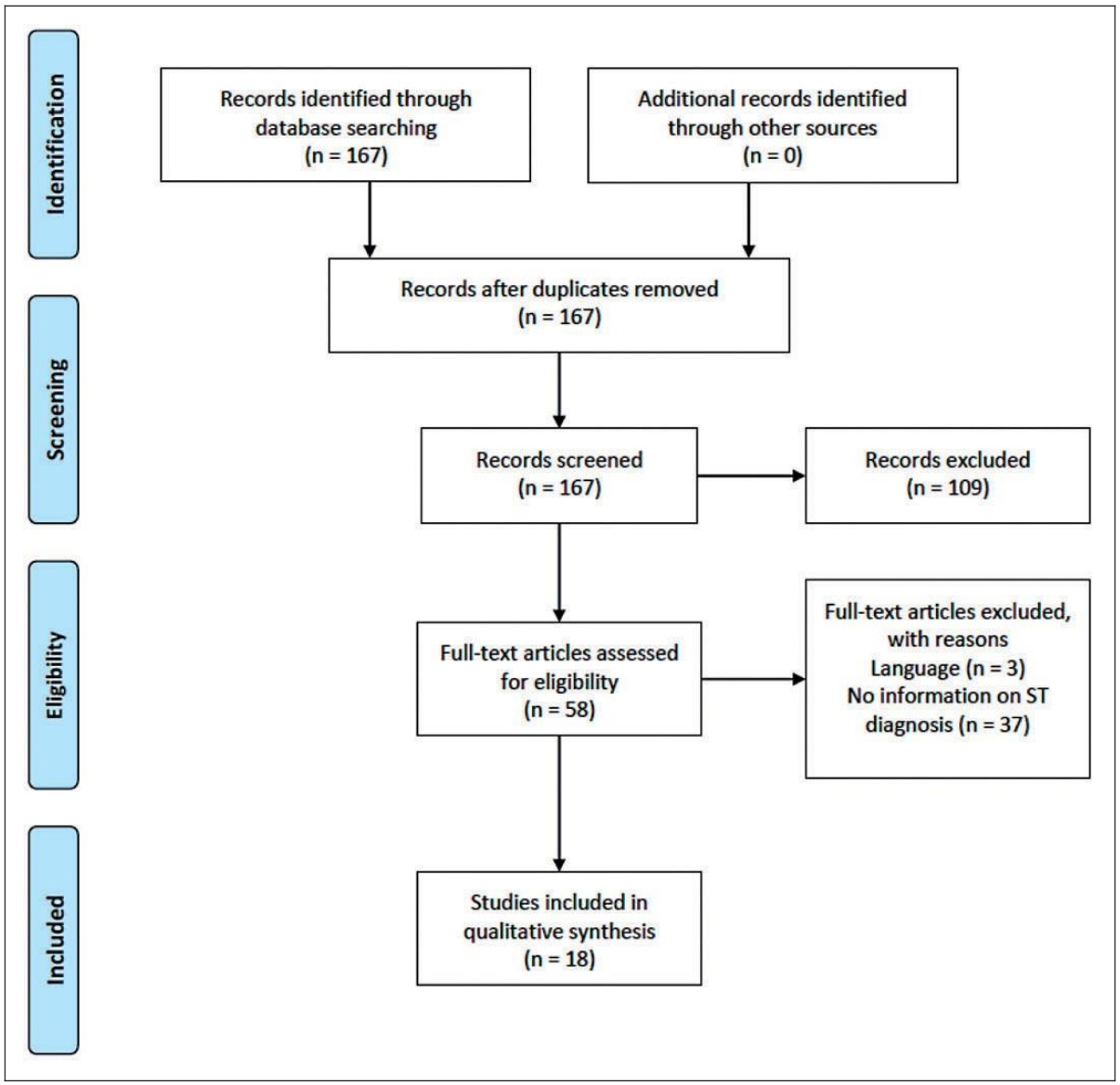

Figure 1. Overview of the inclusion process of articles in the systematic review.

70% of the Delphi panel members scored them 1 to 3 and fewer than 15% scored them 7 to 9 (see Table 1).

Consensus Meeting

The intended goal of the meeting was to agree on a list of assessment criteria that should be present in every single patient receiving a clinical diagnosis of ST. At the start of the consensus meeting, participants urged caution that this goal would not be possible. The reasoning for this caution was that, according to their extensive clinical experience, individual patients with ST can present with a large set of different symptoms. As an alternative goal, the group instead agreed to provide a list of items that, if present, would strongly suggest an influence of the somatosensory system on the patient's tinnitus.

The panel was first asked to consider those 2 items that had been identified as *essential* by the Delphi survey participants in Round 2 and 5 items that had been identified as *not essential* in Round 2. They agreed to, respectively, include the 2 and exclude the 5 presented items (100% agree). The remaining 34/41 items were then discussed and voted for (see Table 1 for details). In cases where at least four of the six participants voted for inclusion, a diagnostic criterion was added to the final assessment list.

The items that were agreed upon for inclusion are presented in Tables 2 to 4, according to features of tinnitus modulation, tinnitus characteristics, and accompanying symptoms, respectively. The first set of items to be discussed was the patient's ability to modulate his or her tinnitus by voluntary movements, somatic

Table 2. Items on Tinnitus Modulation That, If Present, Strongly Suggest Somatosensory Influence of Tinnitus.

Criteria on tinnitus modulation
The patient is able to modulate the tinnitus by voluntary movement of the head, neck, jaw or eyes
The patient is able to modulate the tinnitus by somatic maneuvers
Tinnitus is modulated by pressure on myofascial trigger points

Table 3. Tinnitus Characteristics That, If Present, Strongly Suggest Somatosensory Influence of Tinnitus.

Tinnitus characteristics
Tinnitus and neck or jaw pain complaints appeared simultaneously
Tinnitus and neck/jaw pain symptoms aggravate simultaneously
Tinnitus is preceded by a head or neck trauma
Tinnitus increases during bad postures
Tinnitus pitch, loudness and/or location are reported to vary
In case of unilateral tinnitus, the audiogram does not account for unilateral tinnitus

Table 4. Accompanying Symptoms That, If Present, Strongly Suggest Somatosensory Influence of Tinnitus.

Accompanying symptoms
Tinnitus is accompanied by frequent pain in the cervical spine, head or shoulder girdle
Tinnitus is accompanied by the presence of pressure tender myofascial trigger points
Tinnitus is accompanied by increased muscle tension in the suboccipital muscles
Tinnitus is accompanied by increased muscle tension in the extensor muscles of the cervical spine
Tinnitus is accompanied by temporomandibular disorders
Tinnitus is accompanied by teeth clenching or bruxism
Tinnitus is accompanied by dental diseases

maneuvers, or pressure on myofascial trigger points (8/34 items). Seven criteria reached consensus for inclusion. The ability to modulate the tinnitus by resistance tests of the arm was not included. This item was labeled as *can be present occasionally, but not systematically enough to be on the list*. All six participants agreed that a patient's ability to modulate his or her tinnitus strongly suggests an ST, but that ST can also exist without this ability to modulate the tinnitus. Some participants strongly cautioned that the use of somatic maneuvers as a single criterion can potentially lead to overdiagnosis. The second set of items (11/34 items) to be discussed were tinnitus characteristics that often exist in patients with ST. Items that were considered important to include were the simultaneous onset and aggravation of tinnitus and pain symptoms in the neck or jaw area, potentially preceded by a head or neck trauma. In addition, the increase in tinnitus during certain postures (such as bad posture during computer work or sleep) and the presence of variations in pitch, loudness, and location of the tinnitus were pointed out as items that strongly suggest ST. Another typical tinnitus characteristic is that, in case of a unilateral tinnitus, the audiogram does not account for a unilateral tinnitus. One item on this list *a specific type of constant pulsatile tinnitus, synchronous with the heartbeat, that can momentarily be abolished by a strong muscle contraction of the head or neck muscles or by a strong pressure applied to the same muscles* (Levine et al., 2008) caused a prolonged discussion. Due to dissenting views on this topic, there was no consensus (after voting) to either definitively include or exclude the item.

The third set of items (15/34 items) to be discussed were those symptoms that can accompany the patient's tinnitus. Items that were considered important to include were frequent pain in head, neck, or shoulder girdle; temporomandibular disorders; pressure-tender myofascial trigger points in the head–neck region; increase in muscle tension in the neck extensor muscles; bruxism or teeth clenching; and dental diseases. The group agreed that whenever one or more of these symptoms are present, this strongly suggests an influence of the somatosensory system on the patient's tinnitus.

In total, six items were identified as *can be present in a single patient, but not systematically enough to be on the list of diagnostic criteria*.

Discussion

This study aimed to reach an international consensus on diagnostic criteria for ST. Up until now, academics and clinicians have often used their own criteria to include patients in trials on ST. For the first time, experts in ST were gathered together to create a consensus statement about the diagnostic assessment of ST.

This consensus recommends aspects of tinnitus modulation, tinnitus characteristics (such as varying pitch and loudness), and accompanying symptoms that are strongly suggestive of ST in an individual patient while acknowledging that the individual presentation of the condition can vary from patient to patient.

In agreement with the diagnostic criteria given by Sanchez and Rocha (2011), the experts in ST agreed that rather than a definitive set of diagnostic features, clinical assessment should instead look for evidence of certain features that, if present, would strongly suggest an influence of the somatosensory system on the patient's tinnitus. The list proposed in this consensus study confirms many of the same diagnostic criteria provided by Sanchez and Rocha (2011) but also adds some new items.

Implications of Our Findings for the Tinnitus Community

From the literature, many authors have diagnosed a patient with ST according to whether the patient could modulate the tinnitus by either voluntary movements or somatic maneuvers (Biesinger et al., 2015; Haider et al., 2017; Ward et al., 2015). Our consensus meeting panel recognized the importance of somatic modulation, especially by voluntary movements, for the ST diagnosis but added that *the absence of this ability does not rule out ST*. Hence, somatic modulation should not be used as a simple *yes or no* criterion for diagnosing ST. Although the use of somatic maneuvers to assess tinnitus modulation was voted in, some participants believed that the use of these maneuvers as a single criterion can potentially lead to overdiagnosis. For example, a study of Abel and Levine (2004) showed that not only were 83% of patients with tinnitus able to modulate their tinnitus through somatic maneuvers, but in addition, 65% of nonclinical *healthy* participants perceived a tinnitus-like sound during somatic maneuvers.

It must be noted that certain items, such as *Tinnitus accompanied by frequent pain in the head, neck or shoulder girdle* or *Tinnitus accompanied by temporomandibular disorders,* should be used with a certain prudence if they are the only criterion present in a patient. This is because tinnitus and neck or jaw problems can also co-occur without a causal relation (Michiels et al., 2015). On the other hand, when these items are combined with another criterion, such as *Tinnitus and neck or jaw pain complaints appeared simultaneously* or *The patient is able to modulate the tinnitus by voluntary movement of the head, neck, jaw or eyes,* the ST diagnosis gets stronger.

Strengths and Limitations of the Study

Our Delphi survey was completed by a relatively small number of experts, which might have influenced the decision-making. On the other hand, we were able to identify only 18 potential ST experts in our literature search, of which 14 (78%) completed both rounds. We would have liked to have all of them in our consensus meeting, but unfortunately only six were able to attend the meeting. Because there was no financing for this study, we decided to host the consensus meeting prior to the tinnitus research initiative conference to enable as many of our experts as possible to attend this meeting without extra travel costs. Several panelists, however, had other engagements at the time of the meeting. Although a larger sample of consensus meeting panelists would have been preferred, a representative sample of ST experts, from four different countries and six different institutions, was present at the meeting. This is far more than in most consensus meetings in larger scientific fields.

The multifactorial causes of tinnitus in most patients can probably explain the differences in experience. The panel members agree that cases where the somatosensory system is the main cause of the tinnitus exists but are rare. On the other hand, a large group of patients have secondary somatosensory influence on their tinnitus to a certain degree. This somatosensory influence can be combined with other influences such as increased stress levels, anxiety, or depression. All these influences can also increase a tinnitus that is strongly associated by auditory deafferentation, such as noise exposure.

Future Research Directions

Although the item concerning presence of a constant pulsatile tinnitus, synchronous with the heartbeat, reached no consensus for either in- or exclusion, the group advised that the examiner should keep in mind that in some cases such a pulsatile tinnitus can be affected by somatic maneuvers. Further research is, however, needed to describe the characteristics and treatment opportunities for these patients.

Now that a set of criteria to recognize ST is agreed upon by an international panel of ST experts, clinicians can use these criteria to determine the extent to which the somatosensory system influences an individual patient's tinnitus. ST should not be seen as a specific category of tinnitus, but more as a factor that can influence a patient's tinnitus in a larger or smaller degree.

The next step should be to find the most effective treatment for patients with ST. It must be noted that this *most effective treatment* might not be the same for all patients with ST. As in all musculoskeletal conditions, the most appropriate treatment is often a combination of treatment modalities tailored to the individual patient's needs. Since psychological factors, such as stress, anxiety, and depression, influence both tinnitus and neck or jaw problems, it might also be interesting to investigate the effect of a combined treatment comprising physical therapy modalities and psychological techniques on tinnitus severity in future studies.

Conclusion

This study used an international Delphi survey and consensus meeting to agree upon a set of criteria that strongly suggest ST. Because of the international nature of the survey, we expect these criteria to gain a wide acceptance in the research field and to serve as a guideline for clinicians. The criteria developed in this consensus paper now allow to further investigate the extent of somatosensory influence in individual tinnitus patients and tinnitus populations.

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5.3. Biomarkers of tinnitus

5.3.1. Evidence for biological markers of tinnitus: A systematic review

Submitted in Trends in Hearing

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Abstract

Subjective tinnitus is a phantom sound heard only by the affected person and may be a symptom of various diseases. Currently, diagnosis and monitoring of tinnitus are based on subjective audiometric and psychometric measurements, there are no objective methods. The aim of this review was to synthesize the evidence for the existence and clinical usefulness of biomarkers of the development or severity of tinnitus.

We conducted a systematic search of several databases. The initial searches were complemented by scanning reference lists from relevant systematic reviews and the included primary studies; citation searching of the included primary studies using Web of Science, and hand searching the last 6 months of key otology journals. All systematic review stages were carried out by at least two authors. Forty-six records were included in the review and were categorized according to the biological variable measured. There was no evidence for an association between tinnitus and thyroid function, glucose blood level, sedimentation velocity, C-reactive Protein, or unspecific serum Immunoglobulins. The results showed conflicting evidence for the association between tinnitus and full blood count, lipid profile, oxidative stress, vitamins, neurotrophic factors and inorganic ions. However, there was a negative correlation between steroid levels and tinnitus. Neurotransmitters as tinnitus biomarkers are a promising line of investigation. Biological markers may provide an easier means for determining the diagnosis and prognosis of tinnitus, as well as a measure of treatment effectiveness. However, larger studies, with stricter exclusion criteria and powerful harmonized methodological design are needed.

Protocol published on PROSPERO (CRD42017070998).

Key words: Tinnitus, biomarker, genetic, oxidative stress, inflammation.

Introduction

Tinnitus is defined as the perception of sound in the absence of external noise, commonly described as a phantom sound perception. Subjective tinnitus is perceived only by the patient and can manifest as ringing, buzzing or hissing (Kraus et al, 2011; Salvinelli et al, 2003). This type of tinnitus affects 5-15% of adults, having a negative impact on quality of life. In may induce stress, depressive symptoms, anxiety, sleep disturbance or work impairment (Kraus et al, 2011). Tinnitus can be triggered by various causes and is associated with multiple comorbidities, which confounds a precise phenotype and hampers research (Vona et al, 2017). It is very often associated with hearing loss due to the inner, middle or outer ear pathology and neuronal plasticity induced by the lesion (Kraus et al, 2011; Henry et al, 2005; Yüksel and Karatas, 2016). Additional factors, including ageing, head and neck diseases, noise exposure, systemic diseases, infectious diseases, autoimmune diseases, ear diseases, stress and temporomandibular joint disorders (TMD) have been associated to tinnitus (Yüksel and Karatas, 2016).

To date, the diagnosis of tinnitus and measurement of therapeutic success relies on the patient self-report. Although very meaningful, these subjective measures must be strengthened by the development of objective, unbiased tests using biomarkers.

There are several definitions of biomarker. The "*Biomarkers Definitions" Working Group of the National Institute of Health* (2001) defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". The International Programme on Chemical Safety, led by the *World Health Organization* (WHO, 1995) in coordination with the United Nations and the International Labor Organization, has defined a biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease". The WHO (1995) has stated that a true definition of biomarkers includes "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological". Studies using animal models provided important insight on tinnitus biological markers (Hu et al, 2014; Kraus et al, 2011).

Identification of tinnitus-specific biological factors and their potential relationship with various disorders and tinnitus could provide the identification of specific markers for tinnitus. These biomarkers would strengthen the existing diagnostic methods and could contribute to the development of successful therapeutic approaches.

Aims and objectives

The aim of this systematic review was to synthesise the evidence for the existence and clinical usefulness of biomarkers of the development or severity of tinnitus.

Methods

This systematic review is reported according to the PRISMA guidelines (Liberati et al., 2009). The protocol was published on PROSPERO (Hoare et al., 2017, registration ID: CRD42017070998).

Eligibility criteria. Eligible information sources were assays, investigational studies, exploratory studies, and basic science studies. We included studies about subjective tinnitus and the association between biological factors and the presence or severity of tinnitus. We excluded studies involving populations with Ménière's disease, otosclerosis, vestibular schwannoma, chronic otitis media, history of tumour and chemotherapy, ototoxic drugs intake, autoimmune diseases, neurodegenerative or demyelinating disease. Study reports not available in English, relating solely to objective tinnitus, and animal studies were also excluded.

Information sources. Initial literature searches were conducted in July 2017 in CINAHL, PsychINFO, EMBASE, ASSIA, PubMed, Web of Science, Science Direct, and EBSCO Host. The search terms were tinnitus* AND gene* OR protein OR hormone OR immunoglobulin OR enzyme OR cytokine OR interleukin OR lipid OR vitamin OR marker. The initial search was complemented by scanning the reference lists of related reviews and of the included studies. Citation searching of the included studies was conducted using Web of Science.

An update search was conducted in January 2018 (searching key journals and Google Scholar) and using the same search terms as in the initial search. We also conducted a manual search of the Table of Contents from the last two issues of key journals from which these eligible records had been sourced (i.e., Frontiers, The Laryngoscope, Hearing Research Journal and International Journal of Audiology).

Study selection. Eligibility of records was independently reviewed by at least two authors at each key step (i.e. abstract screening, full-text screening, and data extraction). Where disagreement emerged, consultation with a third reviewer was taken to reach consensus.

Initial screening was based on abstracts analysis. Full text record was screened whenever either reviewer was uncertain about exclusion. Records were grouped into eleven categories: protein markers, vitamin markers, hormone markers, inorganic ions markers, lipid markers, interleukin markers, immunoglobulin markers, enzyme markers, general markers, and genetic markers.

Data extraction. A data extraction form (Excel) and guidance notes (word document) were developed for the review, independently piloted on two included records, and revised for clarity before formal data extraction. Data extraction of all included records was performed independently by two reviewers. A third reviewer was recruited in case of disagreements between the two initial reviewers. Extracted data items included: study title, corresponding author details, country where study was performed, date of publication, date of study start, study design, aim, type and duration of intervention (if applicable), sample characteristics (population, age, sample size, sample size calculation, gender, education), duration of tinnitus, intermittent or constant tinnitus, tinnitus severity, presence of hearing loss, inclusion/exclusion criteria (subtypes of tinnitus, co-morbidities), biological variable measured (gene, protein, hormone, immunoglobulin, enzyme, interleukin, lipid, mineral salt, vitamin, other), measurement tools, tinnitus assessment, timing of measurements, main findings (associations between biological factors and tinnitus), and any further notes.

Risk of bias in individual studies. Risk of bias was assessed for five key categories of medical test performance as reported by Santaguida et al. (2012). Each study was assessed for (1) Population bias (Spectrum effects, context or selection bias), (2) Test protocol (variations in test execution or test technology), (3) Reference standard and verification (inappropriate reference standard, differential or partial verification bias, (4) Interpretation (review or incorporation bias, observer variability), and (5) Analysis (handling of indeterminate results, arbitrary choice of threshold values). For each category a quality judgement was made, scored as either 'Yes' (low risk of bias), 'No' (high risk of bias), or 'Unclear' (insufficient information in the record to make a judgement). When rating a study 'No' on any domain reviewers noted reasons why. At least two reviewers independently assessed risk of bias in each record. A third reviewer was nominated to arbitrate any disagreements in judgements that were not resolved through discussion between the two reviewers.

Summary measures. Data from individual studies are reported descriptively (mean values from individual measures, and correlations between biological factors and presence or severity of tinnitus within studies, as reported).

Synthesis of results. Included records were categorised according to whether they reported data on the same biological variable. Sixteen categories (Table 1) were identified and records were coded as 'Category_record number' (e.g. Proteins_3). Some categories include only one study (e. g. Angiotensin converting enzyme, Alpha- Adducin, Genome Wide association) and some studies were included in several categories.

There was insufficient consistency in study design or population to perform a meaningful quantitative synthesis of results. We therefore included all studies in a narrative synthesis. Common areas of bias across studies were also described in a narrative synthesis.

Results

Study selection. The initial search retrieved 3801 records. After removal of duplicates, 3770 records remained for abstract screening. Of the 3770 records, 3689 were excluded as not related to review question or not meeting the inclusion criteria. Seventy-eight full-text records were screened and 44 of them were included. A further two included records were identified in update searches. Reasons for exclusion at the full text screening stage are presented in the record flow chart (Figure 5.3-1). Thus, 46 records were included in the review.

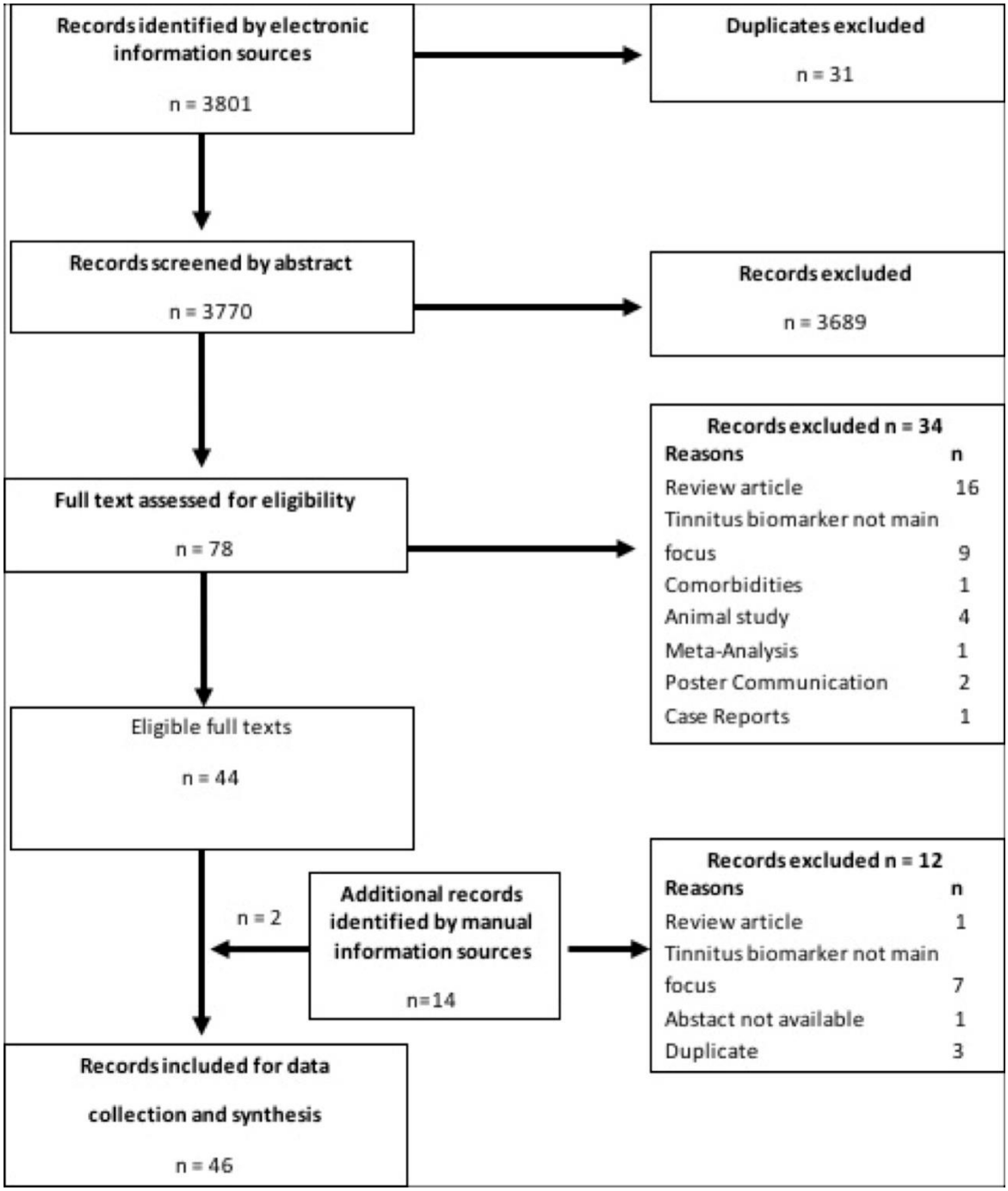

Figure 5.3-1 - Flow diagram of study records.

Study characteristics. Forty-six studies from authors in 17 countries were included (Figure 5.3-2). Most studies were conducted in Turkey or Germany (10 in each) followed by Italy (four included records). Thirty-one included studies were investigational, five were exploratory, five were assays, two were case-controlled studies, one was a prospective randomized single-blinded sham-controlled cross over study, and one was a genome wide association study.

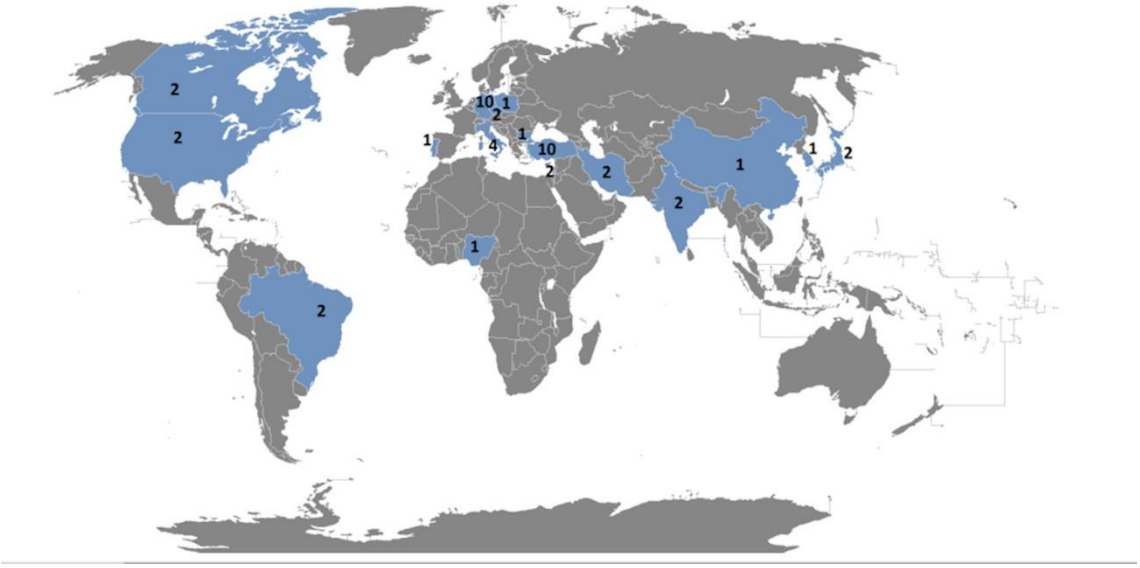

Figure 5.3-2 - World Map with the countries of origin of the included records in our review. Legend: Turkey (10), Germany (10), Italy (4), Japan (2), Israel (2), India (2), Canada (2), Iran (2), Brazil (2), Czech Republic (2), USA (2), China (1), Nigeria (1), Korea (1), Bulgaria (1), Poland (1) and Portugal (1).

Narrative description

For brevity here we describe only the results from studies with statistical relevant results (Table 5.3-1). Conclusions however are based on a synthesis of all included studies, including those not findings any significant associations.

Table 5.3-1 - Categories of included records.

Category of biomarker	Biological variable measured	Record code	Studies with statistical relevant results
Full blood count	Neutrophil-to-lymphocyte ratio	General_3, 13	General_13
	Platelet Indices	General_3, 16; Lipid_3	General_16; Lipid_3
Lipid profile	Cholesterol	Lipid_1, 2, 6; General_8	Lipid_1, 6; General_8
	Low-density lipoprotein	Lipid_2, 3, 4, 6; General_13	Lipid_4, 6;
	High-density lipoprotein	Lipid_2, 3, 4, 6; General_13	Lipid_4, 6;
	Triglycerides	Lipid_1, 2, 6	Lipid_1, 6
Glucose	Blood glucose level	General_13; Lipid_1	Lipid_1
Thyroid function	Thyroid function	General_13, Lipids_1, 3	-
Cortisol and products	Cortisol	Hormone_2, 3, 4	Hormone_2, 3
	Stress Hormone	Hormone_4	-
	Neurosteroids and bioactive substances	General_4	General_4
Other hormones	Melatonin	Vitamins_3	Vitamins_3
Inorganic ions	Serum zinc	Inorganic_ions_1, 3	Inorganic_ions_1, 3
	Copper, zinc, chromium, magnesium	Inorganic_ions_3	Inorganic_ions_3
Oxidative stress	Reactive oxygen metabolites	General_8, 9, 10, 11	General_10, 11
	N-Acetyltransferase 2 (NAT2)	Genetic_28	-
Vitamins	Vitamin B1	Vitamins_1	Vitamins_1
	Vitamin C	Vitamins_3	-
	Vitamin B12	Vitamins_2, 3, 4 and 5	Vitamins_3, 4, 5
Interleukins	Interleukin-6	Interleukins_1, 2; General_18	Interleukins_1
	Interleukin-1b	Interleukins_2	Interleukins_2
	Interleukin-10	General_18	-

	Tumor necrosis factor alpha (TNF-α)		General_18
Sedimentation Velocity, C - reactive Protein and unspecific Immunoglobulins;	Erythrocyte sedimentation, C-reactive protein, B2 Glycoprotein antibodies, Prothrombin antibodies, Anticardiolipin antibodies, Annexin antibodies, Antinuclear antibodies and Antineutrophil cytoplasmic antibodies	Protein_3	-
Neurotransmissors	Gamma aminobutyric acid (GABA)	General_5, Genetics_19, 20	Genetics_19, 20
	Serotonine	General_14, Genetic_3, 11	General_14, Genetics_3
	Glutamate	General_25, Genetics_ 28	General_25, Genetics_28
	Choline, n-ac-Aspartate	General_25	-
Neurotrophic/ protective factors	Brain-derived neurotrophic factor (DBNF)	Protein_1, 4; Interleukines_2, Genetics_2, 23	Protein_1, 4, Genetics_2
	Glial cell-derived neurotrophic factor (GDNF)	Genetics_11, 23, 17	Genetics_23, 17
	Heat Shock Protein 70 (HSP70)	Protein_3	Protein_3
Ion channels	ClC-Kb	Genetics_5	-
	Potassium voltage-gated channel (KCNE1)	Genetics_18, 21, 24	Genetics_18
Single studies: Angiotensin converting enzyme and Alpha- Adducin	Allele frequencies of ACE I/D and ADD1 Gene polymorphisms.	Genetics_27	Genetics_27

Full blood count

Three investigational studies reporting full blood count were included (Table 5.3-2). In those studies a total of 569 participants were included.

'General_13' (Ozbay, et al., 2015) compared the Neutrophil-to-Lymphocyte Ratio (NLR) in patients with severe tinnitus to healthy participants. Tinnitus severity was measured using the Tinnitus Handicap Inventory (THI; Newman et al, 1996). They found that mean NLR was significantly higher in patients with severe tinnitus than in control participants ($p<0.05$).

'General_16' (Yüksel & Karatas, 2016) investigated whether there was a relationship between platelet indices and subjective tinnitus. Assessments included magnetic resonance, biochemical and haematological analyses and auditory evaluation. The THI was used to measure tinnitus severity. Mean Platelet Volume (MPV), platelet distribution width (PDW), and platelet count (PC) were measured in venous blood samples. They found that MPV levels were significantly lower in patients with subjective tinnitus than the control group and negative correlations were observed between MPV, PDW, and PC levels. PDW and PC levels were significantly higher in tinnitus participants than in controls. Authors conclude that tinnitus may be characterized by autoimmune and/or inflammatory events, and that measurement of platelet indices may be ca useful guide in assessing the aetiology and severity of subjective tinnitus.

'Lipid_3' (Sarıkaya et al., 2016) compared MPV and platelet levels in patients with idiopathic subjective tinnitus to healthy controls. Complete blood count (CBC) and lipid profile were also evaluated. The authors found that there was no difference in platelet levels between groups, but MPV was significantly higher in the patient group.

In the Full Blood Count category, demographics were comparable in terms of age and gender, but MPV differed significantly. In 'General_16' MPV was significantly lower in the tinnitus group, whereas in 'Lipids_3' authors found MPV values to be significantly higher in the tinnitus group. In 'General_markers_13' (Ozbay et al., 2015) did not find statistical differences for MPV values (that were higher for tinnitus group) among tinnitus and non-tinnitus groups. Demographics did not differ between 'General 16' and 'Lipid 3', whereas 'General markers 3' included a younger population and excluded participants with moderate to severe hearing loss (Ozbay et al., 2015). There is therefore no indication what relationship, if any, exists between MPV and /or hearing loss. Differences across the three studies which may explain the varied findings included differences in sample size and inclusion criteria (hearing loss, tinnitus severity), and so further more consistent studies are needed.

Table 5.3-2 - Descriptive Analyses of Full Blood Count

Record code: Title of the study	Study Design (setting)	Sample Size	Age	Gender	Tinnitus severity (tinnitus duration - weeks)	Biological variables (Measure)
General_13: Neutrophil-to-lymphocyte ratio in patients with severe tinnitus: prospective, controlled clinical study	Investigational studies (University hospital)	107 TP, 107 C.	TP= age 38.7±12.7; C=35.8±13.9.	TP=72 W and 35 M; C= 83 W and 24 M	THI - Only patients with moderate, severe or catastrophic tinnitus symptoms (at least two weeks)	**NLR**: TP=2.067±1.4; (hemogram and full biochemistry profile analyses)
General_16: Can Platelet Indices Be New Biomarkers for Subjective Tinnitus?	Investigational study (Hospital)	100 TP, 100 C	TP= 50,95±14.6C=44. 3±8.9	TP=43 M and 57 W; C= 39 M and 61 W	The average of THI was 41.62± 14.65. Grade I: 2 patients, II: 37 patients, III: 43 patients, IV: 18 patients. There was no patient in the grade V (NS)	**MPV**: TP=8.27±0.6fl **PDW:** TP=19.21±2.2fl **PC:** TP=271.75±54.62/u (Venous blood samples)
Lipid_3: "Increased mean platelet volume in patients with idiopathic subjective tinnitus"	Investigational studies (University Hospital)	101 TP, 54 C	TP= 40.87 ± 14.13; C= 42.35 ± 8.94	TP= 54 W and 47 M; C= 36 W and 18 M	NS (average 135.44 weeks months, range 12–960 weeks)	**MPV**: TP= 9.69 ± 1.30 (fl) **PL:** TP=260.970 ± 59.700 (10^3/mm^3) (CBC)

Note. TP – Tinnitus Patients; C – Control Group; W – Women; M – Men; THI – Tinnitus Handicap Inventory; NLR - neutrophil-to-lymphocyte ratio; MPV - mean platelet volume; PDW – platelet distribution width; PC – Platelet Count; PL - platelet levels; CBC – Complete blood count.

Lipid Profile

Four studies reporting lipid profiles were included (Table 5.5-3). These studies reported a total of 351 participants (193 participants with tinnitus and 158 controls). All studies were investigational, and one additionally presented a matched case-control.

'Lipid_1' (Almeida et al., 2009) compared tinnitus severity (using the Brazilian Portuguese version of the THI) pre and post a 7-month nutritional intervention program for Tinnitus (NIPT), in subjects with metabolic disorders. Laboratory tests included cholesterol levels and fractions, and triglyceride tests. They found that 56.9% of patients with tinnitus had hypercholesterolemia, and 17.5% had hyperglycemia; both rates were higher than are found in a general population.

'Lipid_4' (Sutbas et al., 2007) investigated the prevalence of hyperlipidemia in patients who had high-frequency hearing loss and tinnitus due to chronic noise exposure and evaluated the risk of developing sensory hearing loss in those patients compared to control subjects. The intervention consisted of a low-cholesterol diet and anti-hyperlipidemic therapy. Lipid profiles were established through a CBC. According to the response to therapy the participants were divided into the "responsive" group and the "unresponsive" group. The main findings in the "responsive" group were (1) a reduced mean tinnitus score associated with a reduction in triglyceride levels from high to normal, and (2) a statistically significant change in self-reported tinnitus severity after therapy.

'Lipid_6' (Rajesh, 2016) estimated prevalence of hyperlipidemia in patients with tinnitus, and assessed improvements in tinnitus after hyperlipidemia treatment in those positively identified. Treatment consisted of oral atorvastatin, 10 mg daily. They measured changes in tinnitus symptomatology but did not provide details about the tinnitus severity measurement tool used. A fasting lipid profile was evaluated in all the patients. Data showed that diabetes and hyperlipidemia were statistically significantly associated with tinnitus. Among the patients with an altered lipid profile, after 2 months of treatment an improvement in lipid profile was seen in 51 (94.5%) patients and a symptomatic improvement in tinnitus was reported by 22 (40.8%) patients.

In 'General_8' (Martines et al., 2015) the aim of the study was to determine the risk factors for tinnitus, and the effects of their interactions. The THI was used to measure tinnitus severity, and peripheral blood samples were collected to analyze several variables. The findings indicated an increased risk of tinnitus in those with hypercholesterolemia. Risk of tinnitus increased 8-fold when patients were smokers and had hypercholesterolemia, and 3.5-fold in patients with diabetes and hypercholesterolemia.

Table 5.3-3 - Descriptive Analyses of Lipid Profile.

Record code: Title of the study	Study Design (setting)	Sample Size	Age	Gender	Tinnitus severity (tinnitus duration - weeks)	Biological variables (measure)
Lipid_1: "Tinnitus sensation pre and post nutritional intervention in metabolic disorders"	Investigational studies (University Hospital)	21 TP	60 years old (SD = 10,8).	7 M and 14 W	THI - Brazilian Portuguese version. Slight:2; Mild: 6; Moderate: 7; Severe/catastrophic: 6 (NS)	**HyperCho** (>200 mg/dl)**:** A= 66,7%; B= 68,75% Pt= 62,5%, **HyperTri** (>170 mg/dl): – A= 28,6%; B=25%; Pt= 12,5% (CBC)
Lipid_4: "Low-Cholesterol Diet and Antilipid Therapy in Managing Tinnitus and Hearing Loss in Patients with Noise-Induced Hearing Loss and Hyperlipidemia"	Investigational studies (Military personnel)	42 TP: - UG: 22; RG: 20	TP= age range, 19– 60 years; mean 45. UG= mean age 39; RG= mean age 42	All males.	NS (8 weeks to 576 weeks, mean 264 weeks)	**UG (mean):** **HDL** (mg/dl): A=44.5; Pt=48.9; **LDL** (mg/dl): A=144; Pt=153; **VLDL** (mg/dl): A=39; Pt=37; **RG (mean):** **HDL** (mg/dl): A=43.5; Pt=43.1; **LDL** (mg/dl): A=144; Pt=128; **VLDL** (mg/dl): A=38.7; Pt=35.5 (CBC)
Lipid_6: "A study on the relationship of hyperlipidaemia with tinnitus among patients in a tertiary care centre"	Investigational studies (Tertiary care centre)	84 TP, 84 C	TP= 8, 16, 20, 18, 14, and 8 people belonged to the age groups 21-30, 31-40, 41-50, 51-60, 61- 70, and 71-80 years; C= 6, 10, 23, 19, 20, and 6 people belonged to these age groups.	TP= 39 (46.42%) M and 45 (53.58%) W; C= 51 (60.71%) M and 33 (39.29%) W.	NS (NS)	**HyperLip**: TP=64.29%; **Diabetes:** TP=15.48%; **P:** improvement in **lipid profile**= 94.45%; improvement in **tinnitus**=40.75% (Fasting lipid profile – serum samples)
General_8: "Clinical observations and risk factors for tinnitus in a Sicilian cohort"	matched case–control study; Investigational studies (University Hospital)	120 P - 46 TP, 74 C	TP= age range 14 to 85, with a mean age of 57.6 years ± 13.15. The 79.16 % of subjects were 50 years old.	TP= 31M and 15 W (M/W ratio 2.06). 74C= 46 M and 28 W (M/W ratio 1.64).	THI score: mean value of 41.04 ± 21.12); Slight: 15.22 % (7/ 46), mild: 32.6 % (15/46); moderate: 21.73 % (10/46), severe 26.08 % (12/ 46) and catastrophic 4.35 % (2/46) (NS)	**HyperCho** (mg/dl): TP= 47.82%; **Serum cholesterol** (mg/dl): TP=199.24 ± 48.45; (blood samples)

Note. TP – Tinnitus Patients; C – Control Group; P – Participants; W – Women; M – Men; UG – Unresponsive Group; RG – Responsive Group; THI – Tinnitus Handicap Inventory; NS – Not Stated; HyperCho – hypercholesterolemia; HyperTri – hypertriglyceridemia; A= Pre intervention; B= Pre intervention II; Pt= Post intervention; HDL – high-density lipoprotein; LDL – low-density lipoprotein; VLDL – very-low-density lipoprotein; HyperLip – Hyperlipidemia.

There is good evidence therefore for an association between the presence or severity of tinnitus and hyperlipidaemia. Despite methodological differences across studies there is a consistent finding that hyperlipidaemia is associated with the presence or severity of tinnitus, and that treatment of hyperlipidaemia is associated with improvements in tinnitus.

Glucose and Thyroid function

There were no statistically significant relevant results in this category.

Cortisol and products

Three studies of cortisol (two investigational studies and one assay) involving a total of 104 participants (66 tinnitus patients and 38 controls) were included (Table 5.3-4).

'Hormone_2' (Hébert & Lupien, 2007) and 'Hormone_3' (Hébert & Lupien, 2009) assessed reactivity of hypothalamic-pituitary-adrenal (HPA) axis by measuring salivary cortisol level in tinnitus patients and age-matched controls after exposure to stressors. In 'Hormone 2' they used the Trier Social Stress Test (TSST) as a stressor, and in the 'Hormone 3' participants were exposed to noise for 20 minutes with salivary cortisol samples taken at baseline and after 10, 20, 30, 40, and 60 minutes. In both studies, salivary cortisol analysis was measured by radioimmunoassay. Participants also completed the Tinnitus Reaction Questionnaire (TRQ), and a question about subjective feelings of stress. In 'Hormone 3', a self-devised tinnitus intensity scale was used.

In 'Hormone_2', salivary cortisol increased significantly 20 minutes after exposure to the TSST in the control group and returned to baseline at 40 minutes. The tinnitus group demonstrated a delayed and blunted cortisol response with no increase in cortisol level at 20 min after exposure to TSST. In 'Hormone_3' tinnitus patients had a lower salivary cortisol level than controls. Cortisol at 30 minutes (10 minutes after the end of noise) was significantly higher than at 10, 40, or 60 minutes but not higher than 20 minutes or baseline. The main findings of this study highlighted the effect of noise exposure on cortisol secretion in both tinnitus and non-tinnitus participants. Noise increased cortisol secretion in both groups, increase subjective stress levels, and tinnitus intensity, especially in tinnitus patients who are already distressed by tinnitus.

Table 5.3-4 - Descriptive Analyses of Cortisol and Products.

Record code: Title of the study	Study Design (Setting)	Sample Size	Age	Gender	Tinnitus Severity (Tinnitus Duration - Months)	Biological variable (measure)
Hormone_2: "The sound of stress: Blunted cortisol reactivity to psychosocial stress in tinnitus sufferers"	Investigational studies (GP and SHG)	18 TP, 18 C	TP= 68.8 (5.7); C= 68.9 (5.5)	TP= 10 M and 8 W; C= 8 M and 10 W.	TRQ: Mean of 20.2 (17.7) (Mean 176 months range 18 - 420 months)	**Cortisol levels** (μg/dl)**:** Group×Time interaction, F (5,170) = 5.92 (saliva samples – specific radioimmunoassay)
Hormone_3: "Salivary cortisol levels, subjective stress, and tinnitus intensity in tinnitus sufferers during noise exposure in the laboratory"	Assays (NS)	20 TP, 20 C	TP= 56-78, mean 67.9 SD 6; C= 61-77, mean 68.8 SD 5.5	TP=10 W and 10 M; C=9 W and 11 M.	Mean 19.9 (10.3) from the Tinnitus related distress using the TRQ Validated French version (18 to 420 months, mean 14.5 SD 9.7, range 1.5 – 35)	**Cortisol levels** (μg/dl)**:** TP= 0.079; effect of group: F(1,38)=4.4; effect time: F(5,190)=8.81 (saliva samples – specific radioimmunoassay)
General_4: "Circulating steroids negatively correlate with tinnitus"	Investigational studies (NS)	28 TP	M=52.5 ± 19.2; W=5.2 ± 16.5	12 W, 16 M	NS (NS)	**Pregnenolone (**nmol/L) =1.4 (0.77, 1,7) and **its sulfate (**nmol/L)= 140 (85, 240), **DHEA (**nmol/L)=1600 (920, 2700), 7α **Hydroxy-DHEA (**nmol/L)= 0.49 (0.35, 0.79) **Androstenediol (**nmol/L)**=** 1.2 (0.74, 1.9), **Progesterone (**nmol/L)**=** 1.8 (1, 2.8), **Cortisol (**nmol/L)**=** 480 (380, 610), 20α-**Dihydroprogesterone (**nmol/L)**=** 1.1 (0.71, 1.8), **Androstenedione (**nmol/L)**=** 3.4 (2.8, 4.4), **Allopregnanolone sulfate (**nmol/L)= 7.3 (4.6, 14), **Isopregnanolone (**nmol/L)= 0.19 (0.1, 0.29), **its sulfate (**nmol/L)= 11 (7.1, 16), **Androsterone (**nmol/L)**=** 0.37 (0.23, 0.68), **Epiandrosterone (**nmol/L)**=** 0-6 (0.34, 0.94) (CBC)

Note. TP – Tinnitus Patients; C – Control Group; W – Women; M – Men; GP – General Population; SHG – Self Help Group; NS=Not Stated; TRQ – Tinnitus reaction questionnaire; DHEA – dehydroepiandrosterone; CBC – complete blood count.

In 'General_4' (Chrbolka et al., 2017) a wide spectrum of circulating steroids in middle-aged tinnitus patients with hearing loss not greater than 40 dB were quantified. The study involved tinnitus patients with depression. Using chromatography and immunoassay, pregnenolone and its sulfate, dehydroepiandrosterone (DHEA), 7α-hydroxyl-DHEA, androstenediol, progesterone, cortisol, 20α-dihydroprogesterone, androstenedione, allopregnanolone sulfate, isopregnanolone, its sulfate, aldosterone, and epiandrosterone were quantified. All variables exhibited a significant relationship to tinnitus intensity, mostly at significance levels of $p<0.01$. Steroid levels negatively correlated with both tinnitus intensity (dB) and frequency (kHz). They reported that 22.2% of the variability in tinnitus intensity and frequency was explained by the levels of circulating steroids.

From this category we highlight that steroid levels negatively correlate with tinnitus, i. e. the three included studies in this category consistently show that tinnitus patients have lowers cortisol saliva and blood levels compared to healthy controls or normative values.

Melatonin

One investigational study of melatonin, involving 139 participants (81 tinnitus patients and 58 controls) was identified (Table 5.3-5).

Table 5.3-5 - Descriptive Analyses of other hormones (melatonin).

Record code: Title of the study	Study Design (setting)	Sample Size	Age	Gender	Tinnitus Severity (Tinnitus duration)	Biological variable (measure)
Vitamin_3: "The role of plasma melatonin and Vitamins C and B12 in the development of idiopathic tinnitus in the elderly"	Investigational studies (Community Programme)	139P - 81TP 58C	Mean age=66,9 (0,77) ranging from 60 to 98 years	78 W and 61 M	NS (NS)	**Melatonin** (pg/mL): TP= 11.1+3.4;(Plasma levels)

Note. TP – Tinnitus Patients; C – Control Group; P – Participants; W – Women; M – Men; NS – Not Stated;

'Vitamins_3' (Lasisi, Fehintola & Lasisi, 2012) was a pilot investigational study which found a correlation between low plasma melatonin and self-reported tinnitus among the elderly.

Inorganic ions

Two investigational studies of inorganic ions and tinnitus, involving 213 participants (123 tinnitus patients and 85 controls) were included (Table 5.3-6).

Table 5.3-6 - Descriptive Analyses of inorganic ions.

Record code: Title of the Study	Study Design (setting)	Sample Size	Age	Gender	Tinnitus Severity (Tinnitus Duration)	Biological variable (measure)
Inorganic_ions_1: "Zinc deficiency and tinnitus"	Investigational studies (University Hospital)	73 TP, 38 C	TP= 42.7 (S.D., 11.0); C= 42.4 (S.D., 9.7); Inclusion criterion: (20-59 years).	TP= 50 W (68.5%); 23 M (31.5%); C= 26 W; 12 M	NS (NS)	**Zn** (µg/dl)**:** TP= 88.19±12.4; (blood sample)
Inorganic_ions_3: "Serum iron, copper, zinc, chromium, manganese levels in idiopathic tinnitus patients in comparison with healthy individuals"	Investigational studies (Hospital)	55 TP, 47 C	TP= 47.34 years (range:22 - 75 years);	TP= 53.1% W and 46.9% M	NS (NS)	**Zn** (µg/dl)**:** TP=100/87±19/00; **Cu** (µg/dl)**:** TP=112/48±27/59; **Fe** (µg/dl)**:** TP=95/68±35/97; (flame atomic absorption spectrometry. **Mn** (µg/dl)**:** TP=12/52±3/35; **Cr** (µg/dl)**:** TP=26/71±5/39; (Serum Samples – Flameless Atomic Absorption Spectrophotometer)

Note. TP – Tinnitus Patients; C – Control Group; W – Women; M – Men; NS – Not Stated; Zn – Zinc; Cu – Copper; Fe – Iron; Mn – Manganese; Cr – Chromium.

'Inorganic_ions_1' (Ochi et al., 2003) examined the correlation between serum zinc (Zn) levels and audiometric performance, measuring serum Zn levels in patients suffering from tinnitus. The frequency and loudness of tinnitus were measured using an audiometer, and several characteristics of tinnitus including ear side, continuity, and duration were assessed. Serum Zn levels were measured from peripheral blood samples. Patients were divided into two groups depending on their serum Zn levels: hypozincemia (n=24) and normal serum Zn (n=49). Authors did not describe the measurement tool. They found no statistically significant differences between their two groups on any variables except for tinnitus loudness which was higher in patients with hypozincemia.

In 'Inorganic_ions_3' (Yazdkhasti, Abtahi & Mozafarinia 2016) the serum concentration of chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), and Zn in patients with idiopathic tinnitus were measured and compared with those of healthy subjects. Tinnitus evaluation was not described. The concentration of Fe, Cu and Zn were measured by flame atomic absorption spectrometry and concentration of Cr and Mn were measured by Flameless Atomic Absorption Spectrophotometer. Mean serum iron and manganese did not differ between groups. However, mean serum Zn concentrations were significantly higher in patients with tinnitus than in controls, and mean serum copper and chromium levels were significantly lower in tinnitus patients than in the control group.

Only Zn was measured in both studies, and despite differences in study design there is evidence for an association between this inorganic ion and the presence or severity of tinnitus. Further studies of this and other inorganic ions are indicated.

Oxidative stress

Two investigational studies of oxidative stress, with a total of 64 tinnitus patients and 47 control participants, were included (Table 5.3-7).

Table 5.3-7 - Descriptive Analyses of Oxidative Stress.

Record code: Title of study	Study Design (setting)	Sample Size	Age	Gender	Tinnitus Severity (Tinnitus Duration – days)	Biological variable (measure)
General_10: "Tinnitus and oxidative stress in selected series of elderly patients"	Investigational studies (NS)	20 TP, 22 C	TP= 67+-3; C=66+-3.	All males.	NS (Less than 180 days)	**MDA** (µmole/dl): TP: B=1.97±0.15; J=2.60±0.04; **4-HNE** (µmole/dl): TP: B=1.44±0.02; J=2.42±0.01; **MPO** (µmole/dl): TP: B=0.30±0.05; J=0.73±0.04; (All above by HPLC) **GSH-PX** (IU/ml): TP: B=5.36± 0.03; J=2.22±0.04; (blood samples – calorimetric method)
General_11: "Oxidative stress, nitric oxide, endothelial dysfunction and tinnitus"	Investigational studies (University Hospital)	44 TP, 25 C	TP= age range 36–52; C= age range 34–50.	TP= 21 M and 23 W; C= 15 M and 23 W	NS (not more that 10 days, mean 7±2 days)	**NOx** (µmol/dl)**:** TP: B= 55±1.7; J= 48.17±1.57; **L-arginina** (µmol/dl)**:** TP: B=30.5±0.9; J=38.05±1.61; **L-citrulina** (µmol/dl)**:** TP: B=40±2.9; J=25.73±2.26; **vWF:A** (IU/ml): TP: B=82±11; J=116±5.2; **TM** (IU/ml): TP: B=40.2±1.4; J=54±2.1 (Plasma concentrations)

Note. TP – Tinnitus Patients; C – Control Group; W – Women; M – Men; NS – Not Stated; MDA – plasma malonaldehyde;4-HNE – 4-hydroxynonenal; MPO – mieloperoxydase; GSH -PX – Plasma glutatione

peroxidase; NOx – nitrite plus nitrate; vWF:Ag – von Willebrand factor antigen; TM – trombomodulin; B – Brachial vein; J – Jugular vein; HPLC – high performance liquid chromatography.

'General_10' (Neri et al., 2002) investigated whether reactive oxygen species (ROS) are involved in the pathogenesis of tinnitus. Methodology included an interview and audiological examination. Blood levels (from brachial and internal jugular vein) of some oxidative markers were determined including plasma malonaldehyde (MDA), 4-hydroxynonenal (4-HNE), mieloperoxydase (MPO), and plasma glutathione peroxidase (GSH-PX). There was a significant difference in the concentration of oxidative markers (MDA, 4-HNE, MPO) and antioxidant GSH-PX between brachial and jugular vein in tinnitus patients. Namely, the jugular vein values of oxidative markers were all higher, while the antioxidant GSH-Px was lower than in the brachial vein within the same group. At the same time, in the group without tinnitus, there was no significant difference between the brachial and jugular blood concentrations on the other parameters measured. However, there was little between group differences, with the exception of GSH-Px which was higher in those without tinnitus. The study results indicate that a state of oxidative stress is present in reflux blood of cerebral region jugular vein in patients affected by tinnitus.

'General_11' (Neri et al., 2006) asked whether pathogenic endothelial dysfunction is involved in idiopathic tinnitus. All participants had recent onset tinnitus (less than 10 days). Assessments included plasma concentrations (blood from the internal jugular vein and brachial vein) of nitrite plus nitrate (NOx), L-arginine and L-citrulline, and von Willebrand factor antigen (vWF:Ag) to determine endothelial cell activation, thrombomodulin plasma concentrations of MDA, 4-HNE and MPO to determine oxide-reductive status as stable lipoperoxidation products in vivo, and GSH-Px to determine oxygen radical scavenging enzyme activity. NOx was measured using a photometric method and L-citrulline was measured using high performance liquid chromatography (HPLC). Patients with idiopathic tinnitus were found to have significantly ($p<0.005$) higher levels of oxidative damage maker (MDA, 4-HNE, and MPO) concentrations and plasma scavenger activity in jugular compared to brachial blood, accompanied by increased free radical (FR) concentrations and reduced plasma glutathione peroxidase activity. They also displayed a significant reduction in peripheral and jugular concentrations of NOx, L-arginine, and L-citrulline and increased L-arginine in jugular vein reflux blood compared with peripheral blood ($p <0.05$ in all cases). Regarding the oxidant/antioxidant profile markers, no significant differences between tinnitus patients and control group in the asymptomatic period were observed in brachial blood whereas mean values of oxidant/ antioxidant profile markers were significantly higher in tinnitus patients compared to controls and subjects in the symptomatic period.

Both studies favor oxidative stress as a potential tinnitus biomarker, especially for recent onset tinnitus. However, the study populations were not general;

'General_10' only included male participants and 'General_11' included those with acute tinnitus (less than 10 days). Neither study included audiological nor tinnitus severity measures so, those results have to be interpreted with caution. Replication studies with a general tinnitus population are needed.

Vitamins

Two investigational studies and two assays, involving a total of 2520 participants (532 tinnitus patients and 1988 controls) of vitamins were included (Table 5.3-8).

'Vitamin_1' (Attias et al., 2002) measured Vitamin B1 and Vitamin B12, in peripheral blood samples. The main finding in the study was a sudden significant, stepwise increase in tinnitus after 10 years of service, as opposed to an almost linear increase in the noise-induced hearing loss with age. The study population included only male soldiers exposed to noise. They concluded that the low levels of vitamins B1 and B12 in the studied population favored tinnitus being of central origin.

'Vitamin_2' (Berkiten et al., 2013) aimed to determine vitamin B12 levels in patients with non-pulsatile tinnitus and to assess the efficacy of replacement treatment on tinnitus and hearing in patients with vitamin B12 deficiency. Vitamin B12 was measured in blood . Patients with vitamin B12 deficiency started vitamin B replacement therapy. The results showed no significant relationship between tinnitus and vitamin B12, and vitamin B12 replacement treatment was not found to affect tinnitus. The authors concluded that vitamin B12 therapy for tinnitus remains controversial.

'Vitamin_4' (Shemesh et al., 1993) aimed to determine the incidence of vitamin B12 deficiency in patients with chronic tinnitus and noise-induced hearing loss (NIHL), NIHL only, and those without tinnitus or NIHL. Differences in severity of tinnitus and hearing sensitivity were compared across participants with and without vitamin B12 deficiency. In addition, the effects of vitamin B12 replacement therapy on tinnitus in a subgroup of cobalamin deficient tinnitus sufferers was evaluated. Levels of vitamin B12 were evaluated through serum cobalamin levels. The results suggested a relationship between vitamin B12 deficiency and dysfunction of the auditory pathway. Some improvement in tinnitus and associated complaints were observed in 12 patients following vitamin B12 replacement therapy ($p = 0.023$). These results indicate a high prevalence, with more severity of serum Cobalamin deficiency among tinnitus patients. There was a tendency toward more severe tinnitus complaints from patients with vitamin B12 deficiency than from patients with normal cobalamin levels.

In 'Vitamin_5' (Singh et al., 2016) the objective was to assess the prevalence of Vitamin B12 deficiency in chronic subjective tinnitus patients in an Indian population, and the therapeutic effect of parenteral vitamin B12 on tinnitus. Vitamin B12 levels were evaluated using a chemiluminescence method. Patients were followed for a period of 1

month after 6 weeks of interventional therapy. Of the patients with tinnitus, 17 (50%) were vitamin B12 deficient. In the control group 35% were vitamin B12 deficient. Patients with vitamin B12 deficiency showed a significant improvement in tinnitus severity score after vitamin B12 therapy ($p = 0.016$). However, there was no significant improvement in severity scores of patients without B12 deficiency. The results favour Vitamin B12 therapy in vitamin deficient subjects. Though tinnitus severity was highest in the treatment group with normal cobalamin levels, the improvement in tinnitus severity levels was significant in the cobalamin-deficient group. In view of the findings of this study, cobalamin deficiency could also be present with tinnitus only in the absence of other manifestations and the authors suggest serum cobalamin determination in chronic tinnitus patients. There is mixed evidence therefore that vitamin B12 levels are a biomarker for tinnitus, and that vitamin B12 supplementation in cases of deficiency will lead to improvements in tinnitus severity.

Table 5.3-8 - Descriptive Analyses of Vitamins.

Record code: Title of the study	Study Design (setting)	Sample Size	Age	Gender	Tinnitus Severity (Tinnitus Duration - Months)	Biological variable (measure)
Vitamin_1: "Support for the central theory of tinnitus generation: a military epidemiological study"	Investigational studies (soldiers/randomly selected)	TP= 355, NTP=1892	Range from 18-50 years (random group 18-60)	All males	NS (NS)	**Vit B1** TP: N=44.6%; MD=30.5%; SD=24.8%; **Vit B12** TP: N=64%; MD=14%; SD=22%; (Blood samples)
Vitamin_2: **"**Vitamin B12 levels in patients with tinnitus and effectiveness of vitamin B12 treatment on hearing threshold and tinnitus"	Assays (NS)	TP=100, C= 20	TP=17 to 66, mean 43.87± 10.12); C=19-59, mean 33.85± 11.16	TP= 38 M and 62 W; C= 9 M and 11 W	NS (3 months to 120 months)	**Vit B12** (pg/mL): **TP=** 63 low levels, and 37 normal levels. (Blood samples)
Vitamin_4: "Vitamin B_{12}* Deficiency in Patients With Chronic-Tinnitus and Noise-Induced Hearing Loss"	Investigational studies (army personnel)	57 TP, C: 29 subjects with NIHL and 27 normal audiograms	mean age of 39.4± 10.5 years)	NS	TQ (lasting more than 6 months)	**Vit B_{12} deficiency** (pg/mL) **TP and NIHL**=47% (Serum cobalamin levels)
Vitamin_5: "Therapeutic role of Vitamin B12 in patients of chronic tinnitus: A pilot study"	Assays (Medical College)	20 TP, 20 C	TP= 38.37 (±12.40)	M/W ratio 2:3.	TSIQ: B12 deficiency (Pret:36.50±7.6 Postt:28.30±6.2 Without B12 deficiency (Pret:38.16±12.0 Postt:37.23±11.2) (lasting more than 6 months duration, 16.32 (±15.6) months)	17TP were **Vit B12** (pg/ml) deficient that is 42.5% showed deficiency. **Prevalence of Vit B12 deficiency TP=**50% (serum cobalamin deficiency levels – chemiluminescence method)

Note. TP – Tinnitus Patients; C – Control Group; NTP – Non-Tinnitus Patients; NIHL – noise-induced hearing loss; W – Women; M – Men; NS – Not Stated; Vit – Vitamin; TQ – tinnitus questionnaire; TSIQ - tinnitus severity index questionnaire; Pret – Pretherapy; Postt – Posttherapy; N – Normal; MD – mild deficiency; SD – severe deficiency.

Interleukins

Three studies of interleukins were included, one cross-sectional, one exploratory, and one investigational study (Table 5.3-9). In total, 266 participants, with 116 tinnitus patients, 13 non-tinnitus patients and 137 healthy control participants were included.

'Interleukin_1' (Doi et al., 2015) evaluated the association between the polymorphism of the Interleukin-6 (IL-6) gene in the region 174G/C and the presence of tinnitus in elderly participants with a history of occupational noise exposure. The study included 179 participants, 33.5% of who reported noise exposure history, with 42.5% having tinnitus. Of the 66.5% without noise exposure history, 28.5% had tinnitus. The findings showed a significant association between the genotype and allele frequencies of the IL6–174 gene and tinnitus among those with a history of exposure to noise. Participants with tinnitus with the C allele were less likely to have tinnitus associated with the history of exposure to occupational noise (OR = 0.167, CI 95% 0.167-0.749) compared to those carrying the G allele. No association was found between the genotypic frequency of the IL6 -174 gene (rs1800795) and tinnitus among participants without exposure to occupational noise.

'Interleukin_2' (Szczepek et al., 2014) explored whether the profile and concentrations of circulating cytokines and neurokines could reflect tinnitus-related distress. Interleukin-1b (IL-1b) and IL-6 levels in blood were measured. IL-1b was found to correlate with tinnitus-related distress, whereas IL-6 concentrations were below the detection threshold. A positive correlation between the concentration of IL-1b and a visual analogue scale measure of tinnitus awareness was also reported.

'General 18' (Weber et al., 2002) asked whether improving stress management would influence psychological and stress-related immunological parameters – IL-6, interleukin-10 (IL-10) and Tumor necrosis factor alpha (TNF-α) – in chronic tinnitus patients. The tinnitus patients (n = 26) and non-tinnitus participants (n = 13) took part in a standardized 10-week relaxation program. An additional group of tinnitus patients (n= 18), randomly assigned, served as a further control (C). The hypothesized baseline differences between tinnitus and non-tinnitus participants were not verified on most parameters. The relaxation program resulted in positive changes in psychometric measures, notably a reduction of tinnitus-related distress. It was concluded that decreased type 1 (Th1)-derived cytokine TNF-α may be characterized as a biomarker for improving stress-management in tinnitus. There were no differences in IL-6 levels between tinnitus patients and and controls. In summary, across three studies only IL-6 was studied in all with inconsistent results. This may be because the measurement tools differed between studies. Any role of Interleukine-6 as a biomarker for tinnitus is therefore still unclear and further studies are advised.

Table 5.3-9 - Descriptive Analyses of Interleukins.

Record code: Title of the study	Study Design (setting)	Sample Size	Age	Gender	Tinnitus Severity (Tinnitus Duration – Months)	Biological variable (measure)
Interleukines_1: "Association between polymorphism of interleukin-6 in the region 174G/C and tinnitus in the elderly with a history of occupational noise exposure"	cross-sectional study (public)	179P= 33.5% (60P) with NE (26TP, 34C); 66.5% (119P) without NE (34TP, 85C)	68.9 ± 5.25 years	65.4% of female elderly	NS (NS)	**Genotype frequency** (ng/µL)**:** **No NE: TP**: GG=2(06.5); CC=15(48.4); GC=14(45.2). **With NE: TP:** GG=13(86.7); CC=00(0.0); GC=02(13.3); **Allelic frequency: No NE TP**: G=18(29.0); C=44(71.0);. **With NE: TP:** G=28(93.3); C=02(06.7); (Allele presence/absence, and genotypic frequency)
Interleukines_2: "Biological correlates of tinnitus-related distress: An exploratory study"	Exploratory studies (tinnitus center)	30 TP	age between 18 and 67 years - mean age 47 years,	14 W and 16 M	German version of TQ: pure tone (17) or a narrow-band noise (13) (mean 60 months, ranging from 9 months to 336 months)	**IL-1b** (pg/ml)= 4.00 ± 0.43; **IL-6** (pg/ml) = 0.38 ± 0.06; (Blood collection and serum processing)
General_18: "Impact of a relaxation training on psychometric and immunologic"	Investigational studies and assays (relaxation programme)	26 TP; 13 NTP and 18 C	TP=43.2 ± 9.7; NTP= 32 ± 6.7; C= 42.0 ± 11.3	T= 16 M+10 W; NTP= , 8 M+5 W; C= 8 M+10 W	NS (NS)	**TNF-α** (pg/ml)= significantly decreased in TP (p=.001), **IL-6** (pg/ml)= higher IL-6 levels than **NTPs** at T1 (P=.002) and T2 (P=.015), but not at T3 (P=.092) in **TP**; **IL-10** (pg/ml)- NS (Serum level evaluation)

Note. TP – Tinnitus Patients; C – Control Group; NTP – Non-tinnitus Patients; W – Women; M – Men; NE – Noise Exposure; NS – Not Stated; IL-1b – Interleukin-1b; IL-6 – Interleukin-6; IL-10 – Interleukin-10; TNF-α – Tumor necrosis factor alpha; T1 – Time 1; T2 – Time 2; T3 - Time3.

Sedimentation Velocity (SV), C - reactive Protein (cRP) and unspecific Immunoglobulins

There was one study in this category with no statistically significant results.

Neurotransmitters

Six studies of neurotransmitters were included. Four were investigational studies, one was an exploratory study, and one was a prospective randomized controlled trial. Those studies include a total of 741 participants (334 tinnitus patients, 35 non-tinnitus patients and 372 healthy controls) (Table 5.3-10).

'Genetic_19' (Rottenberg et al., 2014) explored the association between tinnitus and gamma-aminobutyric acid type A (GABA(A) receptors. The polymorphism in GABA(A) Beta-4 subunit gene was measured by PCR products. Findings suggested two main regulatory mechanisms of tinnitus generation. First, a brainstem mechanism that depends on severity of hearing loss. And second, a cortical mechanism dependent on the genotype of (CA)n tandem repeat in GABA(A) beta-3 subunit gene. However, regulation of GABA signaling as well as the role of GABA(A_ receptors in chronic tinnitus remains unclear.

'Genetic_20' (Sand et al., 2012) also evaluated the GABA receptor, aiming to determine whether there was an association between the gene KCTD12 and tinnitus. The GABA-B receptor subunit gene was measured by Sanger sequencing of PCR products. A positive family history of tinnitus in first-degree relatives did not predict rs34544607 genotype. When allele frequencies were compared to data from a large reference population of European ancestry, rs34544607 was associated with tinnitus ($p=0.04$). However, KCTD12 genotype did not predict tinnitus severity ($p=0.52$) and the association with rs34544607 was weakened and no longer significant after screening 50 additional cases ($p=0.07$). The results imply genetic variation in a GABA-B receptor auxiliary subunit may be a risk modifier in chronic tinnitus.

In 'General_5' (Daftary et al., 2004) authors evaluated the cortical benzodiazepine receptor distribution (BZR) in patients with tinnitus, using venous blood samples after radiolabeling with 123I-iomazenil, single-photon emission computed tomography (SPECT) and magnetic resonance. Comparison of homotypic brain regions showed statistically significant asymmetry in the distribution volumes (V3) data in the superior temporal cortex ($p = 0.03$ for both). Comparison of tinnitus patients to healthy controls showed a trend toward reduced BZR density in the frontal lobes bilaterally ($p < .001$) and a reduction in the cerebellum ($p = 0.045$). This preliminary study of BZR distribution in a cohort experiencing severe, chronic tinnitus supports temporal, frontal lobe, and cerebellar involvement in the disorder. The results of the voxel-based statistical parametric mapping (SPM) analysis also suggest changes in BZR distribution in the frontal lobes and, to a lesser extent, in the cerebellum. The frontal lobe showed

the least asymmetry; for the volume of interest assessment, however, this analysis does not account for the symmetrical relative reduction in frontal lobe receptor concentration. They also saw an insignificant decrease in receptor concentration in the cerebellum.

Two investigational studies evaluated the serotonin receptor. 'General_14' (Sachanska, 1999) included patients with vestibular disturbances, healthy controls, and patients with tinnitus. The purpose was to evaluate the concentration of serotonin levels through blood samples by the Snyder method. The findings showed that patients with tinnitus have significantly higher blood serotonin than reference values. Seven patients (29.17%) had a mean serotonin value of 1.111 nmol liter; 9 (37.50%) had a value of 660 nmol liter; and 8 (32. 80%) had standard serotonin values (459 nmol-liter).

'Genetic_3' (Deniz et al., 2010) assessed the association between tinnitus and the serotonin transporter gene (SLC6A4 polymorphism) as measured by the SLC6A4 polymorphism – variable number tandem repeats (VNTR) and the promoter region (5-HTTLPR), in blood samples, measured by PCR products. There was no difference between genotypes of patients and controls regarding VNTR (W 2= 0.012 and p = 0.994) or 5-HTTLPR (W 2 = 0.262, p = 0.877) polymorphisms. There was no association between the visual analog scale ratings of tinnitus of the patients and VNTR polymorphism ($p > 0.05$) but there was an association between the visual analog scale scores of the patients and 5-HTTLPR polymorphism. For the first visual analog scale parameter (severity), the scores of patients with "ll" genotype were significantly higher than the scores of patients with "ls" genotype ($p = 0.004$). For the third visual analog scale parameter (tinnitus discomfort level), the scores of patients with "ll" genotype were significantly higher than the scores of patients with "ls" genotype and "ss" genotypes ($p = 0.002$ and $p = 0.03$, respectively). For the fourth (attention-deficit) and the fifth parameters (sleep disorder), the scores of patients with "ll" genotype were significantly higher than the scores of patients with "ls" and "ss" genotypes ($p=0.04$ and $p=0.03$, respectively). This finding suggests that SLC6A4 polymorphism, especially "ll" variant of the 5-HTTLPR polymorphism, is associated with the limbic and autonomic system symptoms such as tinnitus discomfort, attention-deficit, and sleep disorder. SLC6A4 polymorphism may be a marker of tinnitus distress.

'General_25' (Cacace et al., 2017) assessed the efficacy of low frequency (1-Hz) repetitive transcranial magnetic stimulation (rTMS) over auditory cortex of the left temporal lobe as an experimental treatment modality for noise-induced tinnitus. The study measured glutamate, choline, and n-acetylaspartate using the magnetic resonance spectroscopy. A significant reduction in tinnitus handicap was observed following active rTMS stimulation ($p < 0.005$). There was a significant pair-wise correlation between the metabolite loudness variables; noteworthy was the reduction in tinnitus loudness level that correlated positively with down regulation of glutamate.

The importance of this finding was that it only occurred in the active rTMS condition and that it was specific to the hemisphere that was stimulated ($r = 0.77$, $p < 0.05$).

For the last in this category, the record 'Genetic_28' (Haider et al., 2017) metabotropic glutamate receptor subtype 7 (GRM7) analyses was performed by qPCR in a sample of 78 older adults with age-related hearing loss and with or without tinnitus.

Findings suggest that individuals with a T/T genotype have 33% lower risk of tinnitus compared to individuals with A/A or A/T genotype. There was a statistically significant association between the presence of the allele A/T of GRM7 and severe tinnitus. The likelihood of severe tinnitus (scoring ≥ 56 on the THI) was 14.2 higher for those carrying the allele A/T compared to T/T. The study concluded that allele A/T of GRM7 is associated with greater severity of tinnitus.

Taken altogether the three studies concerning GABA are not consistent. 'Genetic_19' examined GABA-A, 'Genetic_20' focuses on GABA-B and replication studies are needed. As for serotonin, the two included studies suggest relevance, namely the variant "ll" of the SLC6A4 polymorphism seems to relate to tinnitus distress. Both studies involving glutamate suggest an important role of this neurotransmitter in tinnitus, namely a correlation between glutamate down-regulation and symptoms improvement, and that GRM7 allele A/T is predictive of tinnitus severity.

Table 5.3-10 - Descriptive Analyses of Neurotransmissors.

Record code: Title of the study	Study Design (setting)	Sample Size	Age	Gender	Tinnitus Severity (Tinnitus Duration - Months)	Biological variable (measure)
Genetic_19: "The Significante of (CA)n Tandem Repeat in GABA (A) Beta-3 Subunit Gene in Tinnitus Manifestation"	Exploratory studies (NS)	131 TP	52 ± 13.8 years	61 M and 70 W	TS (NS)	**polymorphism in GAB(A) Beta-3 subunit gene** – **TP:** allele A1 frequency of 0.41 (Blood samples – PCR and sequencing PRC product)
Genetic_20: "Resequencing of the auxiliary GABAB receptor subunit gene KCTD12 in chronic tinnitus"	Investigational studies (NS)	95 C, 50 TP	C= 50.6± 12.1; TP= 49.3±11.3	C= 67 M and 28 W; TP= 40 M and 10 W	TQ: averaged 37.1±16.3 (mean±SD) out of 84 points (N =144) (NS)	**KCTD12:** F87F (rs73237446) and T178T (rs34544607) variants- rs34544607 was associated with **TP** (p=0.04); minor allele for T178T (0.0494 vs. 0.0263, p=0.04); (GABAB receptor) (blood samples – sanger sequencing of PCR products)
General_14: "Changes in Blood Serotonin in Patients with Tinnitus and Other Vestibular Disturbances"	Investigational studies (NS)	134 P – 35 patients with vestibular disturbances; 75 C; 24 TP	NS	NS	NS (NS)	**Serotonin levels** (nmol/liter)**:** TP: 12% increase/ 23%decrease; Seven of the patients (29.17%) had a mean serotonin value of 1,111 nmol liter; 9 (37.50%) had a value of 660 nmol liter; and 8 (32. 80%) had standard serotonin values (Blood samples)
Genetic_3: "Significance of Serotonin Transporter Gene Polymorphism in Tinnitus"	Investigational studies (NS)	54 TP, 174 C	TP= between 20 and 51	TP= 33 W (61%) and 21 M (39%)	**THI:** 38.8 + 24 **VAS1: VNTR – TP:** 10/10=7.5±2.6; 10/12=5±2.3; 12/12=4.6±2.1;	**SLC6A4 polymorphism** – **VNTR** (mmol/L): TP: 12/12= 55.6%; 12/10= 37%; 10/10= 7.4%; **5-HTTLPR** (mmol/L): TP: *ll*= 22.2%; *ls*= 48.1%; *ss*= 29.6%; (Blood samples)

					5-HTTLPR – TP: *ll*=6.9±1.7; *Ls*=4.3±2.2; *ss*=5±2.2 (34.7(53.7); (1-340months)	
General_25: "Glutamate is down-regulated and tinnitus loudness-levels decreased following rTMS over auditory cortex of the left hemisphere: A prospective randomized single-blinded sham-controlled cross-over study"	Prospective randomized single-blinded sham-controlled cross-over design (NS)	25 TP	ranging in age from 24 to 80 years (mean age: 54.2 ± 14.2 years)	All males	THI (score 38) and TQ (NS)	**Glutamate** (rTMS)= main effect of hemisphere (F=2.01, p > 0.15), there was a significant main effect of condition (F=10.4, p=.002), **choline** (rTMS)= main effect of hemisphere (F=0.062, p > 0.78) or condition (F=0.713, p=0.4) and **n-acetyl aspartate** (rTMS)= main effect of hemisphere (F=0.286, p=0.594) or condition (F=0.010, p=0.922) (Magnetic resonance spectroscopy)
Genetic_28: "Biomarkers of Presbycusis and Tinnitus in a Portuguese Older Population"	Investigational studies (Hospital)	78 P – 50TP, 28C	age 55 to 75 and mean 64.6± 5.58) years	33 M and 45 W	THI: Slight:10; Mild: 15; Moderate:13; Severe: 10; Catastrophic: 2 (NS)	**TP: GMR7** (SNPs)**=** A/A= 5P; A/T= 26P; T/T= 47P; (PCR amplification followed by Sanger sequencing (Blood samples – PCR)

Note. TP – Tinnitus Patients; C – Control Group; P – Participants; W – Women; M – Men; NS – Not Stated; TS – Tinnitus score; TQ – Tinnitus questionnaire; THI – tinnitus handicap inventory; VAS1- Visual Analogue Scale for rating tinnitus severity; GABA – gamma-aminobutyric acid; KCTD12 – potassium channel tetramerization domain-containing protein; SLC6A4 – serotonin transporter gene; 5-HTTLPR – 44-bp insertion deletion in the promoter region; VNTR – 17-bp variable number tandem repeats; GRM7 - metabotropic glutamate receptor subtype 7;

Neurotrophic/protective factors (BDNF, GDNF, HSP70)

Five investigational studies and one exploratory study including a 684 participants (518 tinnitus patients and 166 controls) were included (Table 5.3-11).

In 'Protein_1' (Goto et al., 2012) the objective was to investigate the peripheral plasma levels of Brain-Derived Neurotrophic Factor (BDNF) in patients with tinnitus, and to evaluate possible correlations between plasma BDNF levels and tinnitus severity and comorbid conditions. Patients with mild tinnitus (THI score <36) had significantly higher concentration of BDNF in plasma (1,321.9 ± 1266.1 pg/mL) than patients with severe tinnitus (385.1 ± 524.9 pg/mL; $p < 0.01$) or the controls ($p < 0.01$). Plasma BDNF levels ranged from 48.6 to 4045.4 pg/mL (average, 768.7 ± 961.4 pg/mL) in tinnitus patients and from 44.8 to 1289.9 pg/mL (average, 338.5 ± 287.7 pg/mL) in controls. In that study, comorbid depressive symptoms were measured using the Hospital Anxiety and Depression Scale (HADS). No significant differences were found in BDNF levels, HADS scores, or THI scores between the three groups Interestingly, those patients who had few or no depressive symptoms (HADS scores ≤14) also had significantly lower THI scores ($p < 0.05$) and higher BDNF levels ($p < 0.01$). After adjusting for possible effects of HADS scores, partial correlation coefficients for BDNF levels and THI scores indicated that there was no relationship between BDNF levels and THI scores. The results showed that plasma BDNF levels may reflect the comorbid depressive symptoms occurring in tinnitus patients and therefore may be a useful tool for objective evaluation of the health status of tinnitus patients.

The authors of 'Protein_4' (Xiong et al., 2016) evaluated the correlation between BDNF levels and tinnitus severity. In addition, alteration in plasma levels of BDNF before and after Tinnitus Retraining Therapy (TRT) in patients with severe tinnitus was analyzed. The plasma concentration of BDNF in patients with severe tinnitus decreased significantly after effective TRT. However, the BDNF concentration did not correlated with tinnitus loudness or severity.

'Genetic_2' (Coskunoglu et al., 2017) was designed to detect a possible contribution of BDNF mutations to tinnitus pathophysiology by examining the relationship between BDNF polymorphisms, mutations, and BDNF serum levels. The average serum BDNF level in tinnitus patients was significantly lower than in the control group ($p < 0.0001$). No statistically significant relationships were found between tinnitus handicap and BDNF gene polymorphisms or serum levels ($p = 0.30$).

'Genetic_23' (Sand et al., 2012) examined the role of genetic variants of glial cell-derived neurotrophic factor (GDNF) and BDNF in tinnitus. The allele frequencies were determined for three GDNF and two BDNF markers measured by restriction fragment polymorphisms analyses of PCR amplicons . The authors concluded no main effect of individual BDNF or GDNF variants on the susceptibility to the phenotype. However,

multiple regression analysis identified genotypes that predicted tinnitus severity in women (but not in men). There was no significant difference in mean tinnitus severity scores between carriers and non-carriers of the minor alleles ($p>0.19$), nor did a positive family history of tinnitus in first-degree relatives predict minor allele carrier status ($p=0.08$). Minor allele carrier status explained 19.1% of the variance in tinnitus severity of the female subgroup. The investigation provides original information regarding the contribution of neurotrophic factor genes to the intensity of tinnitus.

'Genetic_17' (Orenay-Boyacioglu et al., 2016) examined the relationship between GDNF polymorphisms and tinnitus. Although no correlation was found, heterozygosity was significantly lower for GDNF rs1110149 polymorphism in tinnitus group than in the controls group ($p<0.05$).

'Protein_3' (Savastano et al., 2006) was an exploratory study that analyzed the presence of auto-antibodies against Heat Shock Protein-70 (HSP-70) and circulating immune complexes using Western blot immunoassay The mean concentration of circulating immune complexes in tinnitus patients (4.2 μg/ml) was significantly higher ($p = 0.012$) than in the control group (0.9 μg/mlp). Thirteen of 36 tinnitus patients (36%) and none (0%) in the control group had circulating anti-HSP-70 antibodies. In 10 of 13 HSP70-auto-antibody positive patients, the concentration of circulating immune complex was significantly elevated. In the tinnitus group, the mean concentration of circulating immune complex was 6.9 μg/ml, in the HSP-70- auto-antibody positive subgroup and 2.6 μg/ml in the HSP-70-negative subgroup ($p =0.024$). Thus, the presence of circulating complexes and anti-HSP70 antibodies could be a biomarker of tinnitus.

Four studies involved BDNF and two involved GDNF, with contradictory results. One possible explanation in the case of BDNF is that some studies used serum (Coskunoglu A et al., 2017) while others used plasma. Further research is necessary to clarify.

Table 5.3-11 - Descriptive Analyses of Neurotrophic and Protective Factors.

Record code: Title of study	Study Design (setting)	Sample Size	Age	Gender	Tinnitus Severity (Tinnitus Duration - Days)	Biological variable (measure)
Protein_1: "Various levels of plasma brain-derived neurotrophic factor in patients with tinnitus"	Investigational studies (hospital)	43 TP, 30 C	TP= 57.1 ± 15.2; C= 50.7 ± 10.1	TP= 14 M and 29 W; C= 15 M and 15 W	THI: ranged from 2 to 90 (average: 38.2± 23.4). 25 patients with mild tinnitus (THI scores of less than 36) and 18 with severe tinnitus (THI score was more than 38) (ranged from 2 days to 9360 days (average duration: 765±1788 days)	**BDNF concentration** (pg/ml)**:** TP**:** ranged from 48.6 to 4045.4 (average, 768.7 ± 961.4); (plasma levels)
Protein_4: "Plasma brain-derived neurotrophic factor levels are increased in patients with tinnitus and correlated with therapeutic effects"	Investigational studies (hospital)	82 TP, 32 C	TP= 42.7 ± 14.2; C= 40.1 ± 11.9	TP= 36 M and 46 W; C= 17 M and 15 W	THI 32.4 ± 16.8 VAS Awareness 6.7 ± 1.5; Annoyance 6.3 ± 1.0; Loudness 6.3 ± 1.2 (mean 1800 days, range from 540 to 5400 days)	**Plasma BDNF** (pg/ml): **TP=** 1076.1 ± 495.9 ranged from 96–2475) was significantly higher than those in **C** (plasma levels)
Genetic_2: "Evidence of associations between brain-derived neurotrophic factor (BDNF) serum levels and gene polymorphisms with tinnitus"	Investigational studies (university hospital)	65 TP, 42 C	TP= 22 to 55 - 43.6 ± 10.7; C= 23 to 55, 39.3 ± 9.8	TP= 30 W and 35 M; C= 13 W and 29 M	THI - 37.4 ± 20.0; VAS VAS-1 6.1 ± 2.3, VAS-2 7.9 ± 2.7, VAS-3 5.8 ± 2.8, VAS-4 3.2 ± 1.7, and VAS-5 3.7 ± 1.9 (for at least 90 days)	**BDNF level** (pg/ml): **TP=** 1374 ± 326; **BDNF gene polymorphisms (**μl): **TP=** 84.6% GA and 15.4% AA for rs6265; **TP=** 32.7% CC, 49.9% CT, 17.4% TT for rs1491850 (**T AF TP=** 42.3%; **C AF TP=**57.7%; for rs1491850). **TP=** 23.4% CC, 42.0% CT, and 34.6% TT for rs203024; (**TP=AF** was 55.6%) (serum levels, enzyme-linked immunosorbent assay and PCR)

Genetic_23: "GDNF and BDNF gene interplay in chronic tinnitus"	Investigational studies (NS)	240 TP	age 50.3 ±12.9	171 M and 69 W	German version of TQ: scores averaged 41.0 ± 1.2 out of 84 points (N=236); mild in 73 subjects (30.9%), moderate in 73 (30.9%), severe in 45 (19.1%), and extreme in 45 (19.1%) (More than 180 days)	**AF: GDNF** – rs1110149 (MAF - 0.490; MMA - G:C), rs884344 (MAF - 0.269; MMA - T:G) and rs3812047 (MAF - 0.143; MMA – G:A). Two **BDNF** markers - rs2049046 (MAF - 0.494; MMA - A:T) and rs6265 (MAF - 0.196; MMA - G:A) (Restriction enzyme analses of PCR amplicones - DNA was extracted from lymphocytes)
Genetic_17: "Relationship Between Chronic Tinnitus and Glial Cell Line-Derived Neurotrophic Factor Gene rs3812047, rs1110149, and rs884344 Polymorphisms in a Turkish Population"	Investigational studies (University Hospital)	TP=52; C=42	TP= 43.6 ± 10.7; C= 39.3 ± 9.8	TP= 19 W and 33M; C= 13W and 29M	THI: 37.4 ± 20.0 VAS VAS-1 6.1 ± 2.3; VAS-2 7.9 ± 2.7; VAS-3 5.8 ± 2.8; VAS-4 3.2 ± 1.7; VAS-5 3.7 ± 1.9 (at least 90 days)	**GDNF gene polymorphisms** (µmol/µl)**:** **rs884344**: **TP=**5.8 % T:T, 92.3 % T:G, 1,9 % G:G;. **rs3812047**: **TP=** 67.3 % G:G, 28.8 % G:A, 3.9 % A:A; **rs1110149**: **TP=** 32.7 % C:C, 55.8 % C:G, 11.5 % G:G. **AF**: **TP=**51.9 % T, 48.1 % G; **for rs884344**; **TP=**81.7 % G, 18.3 % A; **for rs3812047**; **TP=**60.6 % C, 39.4 % G; **for rs1110149** (blood samples, PCR-based restriction fragment length polymorphism)
Protein_3: "Western Blot Immunoassay for HSP-70 Antibodies in Idiopathic Tinnitus: A Preliminary Report"	Exploratory studies (University/ blood donors)	36 TP, 20 C	TP= 20 and 65 mean age 41.0; C= 20 and 65 mean age 43.9	TP= 17 W and 19 M; C= 9 W and 11 M	NS (NS)	**HSP-70** (ug/ml): 13 of the 36 **TP**: HSP-70-positive/ none of the **C** HSP-70-positive. Ten of the 13 HSP70-positive patients had CIC values higher than normal values. CIC= 6.9 ug/ml, in the HSP-70-positive subgroup and 2.6 ug/ml. in the HSP-70-negative subgroup (p =.024). (Serologic tests – serum and blood samples)

Note. TP – Tinnitus Patients; C – Control Group; W – Women; M – Men; NS – Not Stated; ; THI – tinnitus handicap inventory; VAS – Visual Analog Scale; VAS-1 – severity of tinnitus; VAS-2 – frequency and duration of tinnitus;VAS-3 – discomfort level; VAS-4 – attention deficit; VAS-5 – sleep disorders; TQ – Tinnitus Questionnaire; BDNF – brain-derived neurotrophic factor; GDNF – Glial Cell Line-Derived Neurotrophic factor; HSP-70 – heat shock protein 70; MAF – minor allele frequency; MMA - major:minor allele; AF – Allele frequency; CIC - circulating immune complex

Ion channels (K+ and Cl-)

One exploratory study of potassium and chloride channels had statistically significant results (Table 5.3-12).

The authors of 'Genetics_18' (Pawełczyk et al., 2012) tested the hypothesis that genetic variability in genes of the potassium-recycling pathway is associated with increased susceptibility to tinnitus in male subjects. The study analysed the occurrence of Single Nucleotide Polymorphism (SNP) in ten genes encoding potassium-recycling proteins. The selected genes were five connexin genes (Cx26 (GJB2), Cx30 (GJB6), Cx30.3 (GJB4), Cx31 (GJB3), Cx32 (GJB1)), four potassium channels or channel subunits genes (KCNJ10 – potassium inwardly-rectifying channel, subfamily J, member 10, KCNQ4, KCNE1, KCNQ1 – potassium voltage-gated channel, potassium voltage-gated channels, KQT-like family and Na+/2Cl–/K+ cotransporter gene (SLC12A2 – solute carrier family 12, member 2). The SNP analyses demonstrated that in subjects with tinnitus, polymorphisms in KCNE1 (rs915539, p = 0.005) were associated with the resistance to developing noise-related hearing loss whereas polymorphism in Slc12A2 (rs10089, p = 0.016) were associated with susceptibility to developing noise-related hearing loss.

Table 5.3-12 - Descriptive Analyses of Ion Channels.

Record code: Title of the study	Study Design	Sample Size	Age	Gender	Tinnitus Severity (Tinnitus Duration)	Biological variable (measure)
Genetic_18: "Analysis of inner ear potassium recycling genes as potential factors associated with tinnitus"	Exploratory studies (previous association analysis/ database)	128 TP, 498 C	TP= mean age: 42; C=41	Only males	NS (NS)	**KCNE1 OR(95% CI): TP=** 1.1230 (0.7890–1.5982); **Slc12A2 OR(95% CI): TP=** 1.6899 (0.9991–2.8582); (analysis of Single Nucleotide Polymorphisms)

Note. TP – Tinnitus Patients; C – Control Group; NS – Not Stated;

Single studies: Angiotensin converting enzyme and Alpha- Adducin

'Genetics_27' (Yüce et al., 2016) considered whether the polymorphism in genes encoding Angiotensin-Converting Enzyme (ACE) and α-adducin (ADD1) could contribute to the development of tinnitus, in a sample of 89 tinnitus patients and 104 controls (Table 5.3-13). The authors found no differences in the distribution of ACE genotypes between tinnitus patients and control subjects. However, the ADD1 GW genotype was found to be significantly more frequent in tinnitus patients than in controls (p>0.009).

In addition, the results demonstrated that persons carrying ADD1 G460W polymorphism had a 2.5-fold increased risk of developing tinnitus than the persons not carrying that polymorphism.

Table 5.3-13 - Descriptive Analyses of Single studies: Angiotensin converting enzyme and Alpha- Adducin.

Record code: Title of the Study	Study Design (setting)	Sample Size	Age	Gender	Tinnitus Severity (Tinnitus Duration - Months)	Biological variable (measure)
Genetic_27: "Angiotensin-Converting Enzyme (ACE) I/D and Alpha-Adducin (ADD1) G460W Gene Polymorphisms in Turkish Patients with Severe Chronic Tinnitus"	Investigational studies (Hospital)	193 – 89 TP, 104 C	TP= 48.1±13.5; C= 45.0±16.0	TP= 48 W (53.9%) and 41 M (46.1%); C= 50 W (48.1%) and 54 M (51.9%)	THI (for more than 6 months)	**Genotype distribution and allele frequencies ACE I/D II**: **TP**=18 (20.2%); **ID**: **TP**=41 (46.1%); **DD: TP**=30 (33.7%); **Alleles: I: TP**=77 (43.25%); **D: TP**=101 (56.75%); **ADD1 Gene polymorphisms**: **GG: TP**=47 (52.8%), **GW: TP**=41 (46.1%), **WW: TP**=1 (1.1%), **Alleles: G: TP**=135 (75.8%), **W: TP**=43 (24.2%), (Blood samples – PCR and RFLP)

Note. TP – Tinnitus Patients; C – Control Group; W – Women; M – Men; THI – Tinnitus Handicap Inventory; RFLP – restriction fragment length polymorphism.

Single studies: Genome Wide Association Study

The record 'Genetic_8' (Gilles et al., 2017) involved a genome-wide association study (GWAS) but found no statistically significant genome-wide association with tinnitus.

Risk of bias across studies

Risk of bias according to our five key categories of medical test performance are In general, population bias was judged to be low, i.e. studies were judged to include a 'homogenous' tinnitus patient population that would not lead to spectrum effects, context or selection bias. For some studies however, population bias was rated unclear, e.g. where sample size was small (as in 'General_3', 'General_4'), or where the selection of the control group was unclear (as in 'Inorganic_ions_1', 'Inorganic_ions_3'). Risk of bias due to test protocol (variations in test execution or test technology) was also rated low for most studies. Risk of bias was rated as unclear for some studies however, e.g.

where there was insufficient description of the tinnitus assessment procedure for it to be reproduced (as in 'Lipid_6'). Reference standard and verification bias was rated as low for most studies, i.e. the reference standard used was judged appropriate, with no evidence of differential or partial verification bias. Nevertheless, for some studies there was insufficient information to make a judgement (rated unclear) or evidence of bias (rated no). For example, in 'Inorganic_ions_3' there was no reference to normative values, and in 'Lipid_4' they used a tinnitus questionnaire without a reference standard. Risk of bias in interpretation was low in most studies and unclear or high in a small number. For example, in 'Inorganic_ions_1' effects were interpreted from statistical difference only without use of a reference standard. Bias due to analysis was low for most studies but unclear or high for a small number of studies. For example, 'Genetic_9' was judged unclear as different definitions of tinnitus were used that could have explained some of their findings, and in 'Genetic_29' it was judged that a lot of confounding factors were not considered in analysis. For others, reasons or rationale for the thresholds chosen unclear (e.g. 'Inorganic_ions_1').

Discussion

In this systematic review we collated evidence for biological markers of tinnitus and provided a narrative synthesis of the results. A number of strong and complementary findings were observed, as were many unanswered conflicts, so there are opportunities for replication of studies and further research on this exciting topic.

Candidate tinnitus biomarkers

1. Vitamins

It was observed that low concentration of vitamin B_{12} correlates with the development of subjective idiopathic tinnitus among the elderly and with impaired hearing function. Correction of vitamin B_{12} deficiency also appears to improve tinnitus. These findings suggest tinnitus could be an early neurologic feature of vitamin B_{12}-deficiency, especially in the elderly for whom vitamin B_{12} screening is advised. However, vitamin B_{12}-deficiency can also emerge because of nutritional habits. The choice of method to measure vitamin B_{12} is an important issue. Elevated B_{12} in serum can accompany B_{12}-deficiency resulting from the defects in tissue uptake. A more sensitive method of diagnosis would be to evaluate one of the metabolites that accumulate because of vitamin B_{12} cellular deficiency. Vitamin B_{12} is required for enzymatic conversion of methylmalonic acid (MMA) to succinyl-CoA, and in combination with folic acid, for homocysteine (HC) to be converted to methionine. Although elevated MMA is more specific indicator of vitamin B_{12} deficiency, elevated MMA and HC levels together have been found to be 99.8% sensitive for diagnosing functional vitamin B_{12} deficiency (Vashi et al. 2016). Future studies addressing vitamin B_{12} as a possible biomarker for tinnitus should include larger populations, with stricter exclusion criteria regarding co-

morbidities, with methodological design that allows better control of possible confounding variables namely noise exposure or associated hearing loss. Issues regarding the need for more standardized operational cut-off limits to set for the definition of Vitamin B_{12} deficiency in future studies might overcome some of the apparent diverging results between studies addressing the role of Vitamin B_{12} in the aetiology and pathophysiologic mechanisms for tinnitus. So, the role of vitamins as potential tinnitus biomarkers is still a matter of debate.

2. Lipid profile

Studies involving treatment for hyperlipidaemia consistently found an improvement in tinnitus severity in line with improvements in lipid levels (Sutbas, A. et al, 2007; Rajesh, R. 2016). Evidence therefore favours an association between the two variables.

3. Cortisol and products, and Interleukins

Every included study of cortisol showed lower level for tinnitus participants regardless of the biological product being saliva or blood. So far, studies suggest a role of cortisol in the response of those with tinnitus to acute stress (Herbert & Lupien, 2007, 2009). The long term cortisol profile in tinnitus patients is yet to be studied. Hormones_2 and 3 (Herbert & Lupien, 2007, 2009) evaluate cortisol levels at baseline and several time-points after stress exposure, but in General_4 (Chrbolka et al., 2017) is not recorded the timing of single blood sample measurement so is not possible to anticipate the influence of circadian rhythm in this last study. One of the main finding with respect to cortisol was a significant and consistent correlation with tinnitus frequency and intensity, indicating a significant link between suppressed adrenal steroidogenesis and tinnitus supporting the probability that tinnitus can be a stress-related disorder. The topic of tinnitus and stress (dually intervening as causal and consequence) related to the activation of HPA axis, has been studied by several authors (for a review see Szczepek et al., 2017). Cortisol may influence the immune system, the gut microbiome-brain axis, the autonomic nervous system, the renin-angiotensin-aldosterone system (RAAS), and the energy metabolism (Dallman 2010). On the cellular level, cortisol induces two types of responses: rapid (non-genomic, dependent on activation of cell signaling pathways) and slow (genomic, in which cortisol acts as transcription factor). Stress interferes with the production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1beta (IL-1beta), and interleukin-6 (IL-6) (Szczepek et al. 2014; Weber et al. 2002) possibly implicating the involvement of inflammatory mechanisms in tinnitus pathogenesis. The literature refers to the possibility of a pathogenic connection with nonspecific laboratory indicators of immune disease and the neuro-sensorial hearing loss, referring to the role of these indicators in the inner ear (Matsuoka & Harris, 2013; Mijovic et al, 2013). This relationship between immunologic and auditory systems is a starting point for new studies that consider the possibility of this biomarker in the

diagnosis of tinnitus. In this review concentrations of Il-1b, Il-6 and TNF-α differed significantly between tinnitus patients and controls, implicating these biological variables as possible tinnitus biomarkers. However, the measurement tools differed between studies and the role of Interleukine-6 is still unclear. These limitations dictate the need for large-scale studies, better confounding factors control and consistent methodology.

4. Neurotransmitters

Neurotransmitters also appear a fruitful line of enquiry, particularly as markers of tinnitus severity or distress level. Concerning serotonin, tinnitus patients have significantly higher levels (Sachanska, 1999) and the SLC6A4 polymorphism, especially the "ll" variant of the 5-HTTLPR polymorphism, are associated with tinnitus severity (Deniz et al., 2009). Serotoninergic activity, particularly 5-HTTLPR activity, is associated with a variety of physiological diseases such as anxiety and depression (Yilmaz et al., 2001; Lesch et al., 1996). Serotonin therefore may be involved in depression and tinnitus. Studies involving glutamate also showed interesting results (Cacace et al., 2017; Haider et al., 2017).

5. Angiotensin converting enzyme and Alpha-Adducin

In this category most relevant finding is that ADD1 G460W polymorphism carriers had 2.5 fold increased risk of developing tinnitus compared to those not carrying that polymorphism.

6. Oxidative stress

One potential cause of tinnitus is oxidative stress, resulting from an imbalance between the production of reactive oxygen species (ROS) and the detoxification of their reactive intermediates. Under physiological conditions, ROS are neutralized by antioxidant enzymatic scavengers including superoxide dismutase (SOD), catalases, glutathione S-transferase (GST), and glutathione peroxidase (GPX). With age, the production of ROS increases and so does oxidative damage. Consequently, the increase of oxidative stress contributes to aging and age-related diseases including tinnitus (Fujimoto and Yamasoba, 2014). Our findings confirm oxidative stress as a candidate tinnitus biomarker albeit in subpopulation studies of acute tinnitus and an all-male population (Neri, S. et al 2002, 2006). Another interesting finding is that, assuming effluent blood from the inner ear represents a small proportion of the whole brain flux then those results favor the involvement of peripheral and central auditory pathways, and certainly central non-auditory related areas in tinnitus etiology. All studies considered, there is some evidence that oxidative stress plays a role in acute or sub-acute tinnitus, but there is a need for studies replication in chronic tinnitus. Studies associated with cell cultures are indicated to establish more conclusive translational findings about the role of oxidative stress in tinnitus.

7. Other hormones

Data from one study of elderly patients suggest a correlation between tinnitus presence and lower melatonin plasma levels (Lasisi et al, 2012). Moreover, some studies have showed great improvement, after melatonin treatment, in patients with severe tinnitus and insomnia (Pirodda et al., 2010), as well as another study in tinnitus patients with sleep disturbances (Megwalu et al., 2005), but the latter lacked randomization, blinding, or placebo control. For this reason further studies with stronger methodological design are advised.

Less likely candidates as tinnitus biomarkers

1. Neurotrophic/Protective factors

A number of studies evaluated neurotrophic factors reporting varying findings. One possible explanation for this is the fact that in the different included studies the biological sample collection differed, for example (Coskunoglu A et al., 2017) used serum in their evaluation instead of plasma (Xiong, H. et al 2016; Goto, F. et al, 2012).

2. Full blood count

Two included studies found significantly lower and higher MPV in tinnitus participants, with a third study fining no significant difference. There is therefore no indication what relationship, if any, exists between MPV and tinnitus. Differences across the three studies which may explain the varied findings included differences in sample size and inclusion criteria (hearing loss, tinnitus severity), and so further more consistent studies are needed if this factor is to be tested further.

3. Thyroid function, Glucose blood level, Sedimentation velocity, C – reactive Protein, Mineral-salts and unspecific Immunoglobulins

There was no evidence for an association between thyroid function, glucose blood level, sedimentation velocity, C - reactive Protein and unspecific Immunoglobulins, and tinnitus. In terms of inorganic ions, the two included studies provided contradictory findings concerning Zn concentration in blood. They used different tools for Zn measurement, this precludes direct comparison between the studies and indicates the need for larger scale studies and stronger methodological design.

4. Ion channels

The polymorphism of KCNE1 did not correlate with tinnitus severity. The potassium channel subunit KCNE3 and chloride channel ClC-Kb gene were not confirmed as biomarkers of risk or severity of tinnitus.

Limitations

Heterogeneity of included studies with regards to methodologies, measurement tools, and study populations precluded quantitative syntheses. The exclusion of studies not reported in English from this review introduces a geographical bias. Nevertheless, we assume that the wide use of English in the scientific community minimises the effect of this bias (Figure 5.3-2).

Conclusions

We conclude that metabolic or endocrine disorders such as hyperlipidemia and hypertension are factors contributing to the etiology of tinnitus, probably by means of microvascular alterations. Inflammation, oxidative stress and chronic stress are probably cellular mechanisms involved in tinnitus pathogenesis. Subjective chronic tinnitus is not a single gene disorder but rather a complex trait, controlled by complex interactions between multiple genes, environmental risk factors and personality traits.

Future studies should include larger populations, with stricter exclusion criteria regarding co-morbidities and methodological design that allows better control of possible confounding variables, namely noise exposure or associated hearing loss. Working definitions, e.g. of values taken as Vitamin B_{12} deficiency, also need to be strictly applied and consistent across studies.

Biological markers are an emerging field in the area of Otology. Once identified, they may provide a means of determining the time-course or most effective treatment for an individual with tinnitus, presbycusis or any other otologic disease or impairment.

5.4.Tinnitus in an older Portuguese population

In this subchapter are presented the four original articles originated from the PhD studied population in older adults to explore the contribution of audiological, inflammatory, genetic factors and psychologic or quality of life factors to tinnitus etiology.

5.4.1. Characteristics, psychological problems, quality of life, and tinnitus in a Portuguese Older Population

Submitted to Plos One

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Abstract

Subjective tinnitus is a symptom involving the perception of a sound without an external source, which is only heard by the affected person. It is associated with negative psychological and emotional effects leading to impaired quality of life. Explore these variables with clinical populations is essential to ensure assessment and management is adequately targeted. The aim of this study was to explore psychological symptoms and quality of life in a sample of older Portuguese with and without tinnitus. We performed a clinical evaluation including audiological, tinnitus assessment, a structured interview and ENT observation. The Brief Symptom Inventory (BSI) was used to evaluate psychological distress. The Portuguese version of the Medical Outcomes Study Short Form Health Survey (MOS-SF36) was used to assess quality of life. Our population included 114 participants, 92 of whom had tinnitus. Participants were aged from 55 to 75 years. In our analysis higher self-reported quality of life (as measured by the General Health Perceptions subscale of the MOS-SF36) was associated with lesser tinnitus (i.e. a protective effect), while psychological complaint (as measured by the General Severity Scale of the BSI) and hearing loss, were both significant risk factors for tinnitus. Tinnitus and hearing loss disorders can have a high negative impact on quality of life. With our aging population, it is likely that the problems identified here will be increasingly prevalent and add to the frailty of older adults. An adequate tinnitus treatment demands a multidisciplinary professional team to ensure coverage of all dimensions of the patient.

Keywords: Tinnitus; Quality of life; Psychological distress; Eldery

Introduction

Tinnitus is a symptom involving the subjective perception of sound deprived of an external source. It is common, affecting in general 10% to 15% of the population, and around 33% of people older than 65 (Nondahl et al., 2002; Tyler & Baker, 1983). Prevalence is also higher in patients with hypochondriacal disorder (27%) or with somatization disorders (42%) (Hiller, Janca, & Burke, 1997)(Scott & Lindberg, 2000). For about 10 to 15% of people, tinnitus results in significant handicap and psychological distress (Bartels, Middel, van der Laan, Staal, & Albers, 2008; Kennedy, Wilson, & Stephens, 2004) impairing everyday life, sleep, mood, concentration, and ability to work, ultimately impairing quality of life (Gopinath, McMahon, Rochtchina, Karpa, & Mitchell, 2010; Kennedy et al., 2004).

The World Health Organization (Group, 1993) defines quality of life as 'an individual's perception of their position in life in the context of the culture and value system in which they live and in relation to their goals, expectations, standards, and concerns. It is a broad ranging concept affected in a complex way by the person's physical health, psychological state, level of independence, social relationships, and their relationship to the salient features of their environment'. The concept of quality of life included the perception of the physical restrictions in relation to a condition, the ability of the individual to continue within a social and work environment, and the mental and physical capacity of the person to be able to handle all the difficulties inherent to a condition. Awareness of the impact and severity of a condition may not relate with the patient's perception. Accordingly it is important to consider the individual's perception of the condition and its effect on the treatment and rehabilitation of the disease (Grimby & Wiklund, 1994). Several studies have documented symptoms related to stress that have a strong association with severity of tinnitus and reduced quality of life (Carpenter-Thompson, McAuley, & Husain, 2015; Erlandsson & Hallberg, 2000; Nondahl et al., 2007). Associations have also been reported between severity of tinnitus and the occurrence of sleep disorders (Alster, Shemesh, Ornan, & Attias, 1993; Crönlein, Langguth, Geisler, & Hajak, 2007; Fioretti, Fusetti, & Eibenstein, 2013) and emotional exhaustion (Hebert, Canlon, & Hasson, 2012), bodily pain, vitality and mental health are associated with increased tinnitus severity (Nondahl et al., 2007).

Tinnitus is widely understood to be associated with psychological problems. Reynolds and colleagues (2004) found anxiety to be the principal psychological symptom in 28% of tinnitus patients. The same study found that although there was a reduction of the psychological symptoms after treatment, moderate to severe anxiety and depression remained significant. Anxiety has also been identified as a disorder with a higher predictive value for the development tinnitus related psychological distress (Andersson & Vretblad, 2000; Erlandsson & Hallberg, 2000; Langenbach, Olderog,

Michel, Albus, & Köhle, 2005; Reynolds, Gardner, & Lee, 2004). Depression has also been significantly associated with tinnitus. For example, in a longitudinal study of a Swedish working population Hebert et al., 2012 found a long-term co-variation between the severity or prevalence of tinnitus and depressive symptoms. The relationship was also evidenced in a cross-sectional correlation study, with 1274 patients from Tinnitus Research Initiative database(Zeman, Koller, Langguth, & Landgrebe, 2014).

Negative thinking has been a factor influenced by experiencing tinnitus and has been associated with more problematic tinnitus. On the other hand, positive thinking has an important role in reducing negative thinking about tinnitus and in focusing about the positive thinking, and this reflets that positive thinking is not related to unproblematic tinnitus. (Handscomb, Hall, Shorter, & Hoare, 2017). Negative thinking is central to the cognitive model of tinnitus distress (McKenna, Handscomb, Hoare, & Hall, 2014). This model suggests that when the patient engages in negative thoughts about their tinnitus and its meaning, this triggers a cycle of emotional distress, selective attention and avoidance behaviors, which guarantees that tinnitus remains a distressing and negative experience. Catastrophic thinking also appears to be associated with tinnitus distress in clinical populations (Cima, Crombez, & Vlaeyen, 2011; Conrad et al., 2015; Weise et al., 2013).

A common approach to tinnitus management is therefore Cognitive behavior therapy (CBT). Several studies have shown CBT to be beneficial in reducing tinnitus distress, improving mood, and reducing stress (Hesser, Weise, Westin, & Andersson, 2011; Hoare, Kowalkowski, Kang, & Hall, 2011). Some studies have however questioned the efficacy of the CBT in reducing the severity of tinnitus, mainly because studies using visual analogue scale (VAS) did not demonstrate improvements (Langguth et al., 2007; Martinez-Devesa, Perera, Theodoulou, & Waddell, 2010). In any case, treatment needs to be individualized to address the problem set of the individual patient.

To date, only one study has explored quality of life and psychological problems in a Portuguese tinnitus patient population; Oliveira and Meneses (2009) found that social function, general health, mental health, and vitality were the dimensions of the SF-36 most affected. According to a recent survey Portugal as other Southern European countries usually do not have specialised tinnitus clinics or multidisciplinary health professionals organised for tinnitus care and many people seem to refer to themselves for their tinnitus complaints. Generally ENT's and audiologists are responsible for the management of tinnitus patients but there is a demand for the enrolment of other health professionals namely for mental health care.

The purpose of this study was to explore our patient population and consider whether their needs are currently met by the standard of care. We evaluated hearing, tinnitus, psychological symptoms, and quality of life, in a sample of older Portuguese patients presenting with and without tinnitus.

Methods

Participants

This study included a sample with 114 participants (n=60 women, n=54 men). Inclusion criteria were: individuals aged from 55 to 75 years, any gender recruited from a Portuguese population. In this study, the sample was divided into two groups, according to presence or absence of tinnitus. The first group consisted in a control group with 22 participants, while the second group is composed by 92 participants with tinnitus.

Our exclusion criteria, consisted of participants incapacity to comprehend and sign the informed consent owing to cognitive impairment. Also, an uncompensated medical disorder or a serious psychiatric disorder are considerate as exclusion criteria. Patients with tinnitus from disease of the outer ear (occlusive exostosis, outer otitis), Ménière's disease, chronic otitis media, otosclerosis; history of ototoxic drugs use; exposure to massive noise; history of previous malignancy with chemotherapy; history of autoimmune disorders; neurodegenerative or demyelinating disease are excluded for this study.

The present study was approved by the Ethical Committees from Hospital Cuf Infante Santo (in 26th November, 2014), by the Nova Medical School (nº65/2014/CEFCM) and the National Department of Personal Data Protection (authorization number:1637/2016). The study was conducted in accordance with the Declaration of Helsinki.

Clinical Evaluation

Data were collected from all participants concerning their personal past and present and family history, and audiological assessment as part of the clinical evaluation. All participants signed written informed consent.

Epidemiologic data (demographic, previous and present diseases, toxicological habits, and exposure to noise) was collected using a structured interview.

All participants were submitted to immittance to rule out middle ear pathology (Model: Madsen Zodiac 901, Serial No.:389122).

Audiological assessment:

Pure tone audiometry (air and bone) to evaluate the hearing thresholds according to ISO 8253 and 389. The exam was accomplished in a soundproof booth employing an Interacoustics®, Assens, Denmark audiometer (Model: AC40, Serial No.: 98 019 046) and TDH39/HDA300 headphones fitted with noise-excluding headset ME70 and bone conductor B-71. Audiometry was performed at frequencies from 0.25 kHz to

16 kHz (standard tonal audiometry and extended high frequency). The category of Hearing Loss (HL) was defined according to the recommendations of Bureau International d'Audiophonologie (BIAP): normal or subnormal hearing (below 20 dB), mild hearing loss (21-40), moderate hearing loss (41-70), severe hearing loss (71-90), very severe hearing loss (91-119) or total hearing loss – cophosis (over 120). Puretone average hearing loss was calculated as the average of thresholds at 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz. Frequencies not heard were given a value of 120 dB. Retrieved May 15, 2018 from:http://www.biap.org/en/recommandations/recommendations/tc-02-classification/213-rec-02-1-en-audiometric-classification-of-hearing-impairments/file.

Speech audiometry was conducted with headphones (using mp3 player), or open field, where the evaluator was hiding his lips to prevent lip-reading. The number of disyllables that patient repeated correctly was recorded. This intelligibility threshold for two-syllable words intends to measure hearing sensitivity threshold through the intensity level identification in which the patient can correctly identify 50% or more of a disyllables list. Moreover, the speech discrimination evaluates the lowest intensity level at which a listener can understand speech.

Tinnitus assessment:

Psychoacoustic assessment in tinnitus collects information about loudness match, pitch match, minimum masking level (MML) or Feldmann masking curves, residual inhibition, and loudness discomfort levels (LDL). Through the Portuguese validated version of Tinnitus Handicap Inventory (THI; Newman, Jacobson, & Spitzer, 1996; Oliveira & Meneses, 2008) was evaluated the tinnitus severity. This inventory consistes of 25 questions related to tinnitus, with "Yes", "Sometimes" and "No" as possible responses, corresponding to scores of 4, 2 and 0 respectively. In a total, it's obtained a punctuation between 0 and 100 that reflects the severity of tinnitus. This questionnaire consists of three different sub-scales: Functional scales (11 items - contributing 0-44 for the finalresult), Emotional scale (9 items - contributing 0-36 for the final result) and Catastrophic scale (5 items - contributing 0-20 for the final result). These scales allow us to understand the most affected aspects of tinnitus in daily life, in order to delineate a more adequate intervention. Depending on the final result, it is possible to establish a classification of tinnitus according to severity or daily impact: - 0-16: Slight or no handicap (Grade 1), 18-36: Mild handicap (Grade 2), 38-56: Moderate handicap (Grade 3), 58-76: Severe handicap (Grade 4), 78-100: Catastrophic handicap (Grade 5). In addition, , THI is a self-administered instrument, with good psychometrics properties and easy to complete and to interpret (McCombe et al., 2001).

Medical Outcomes Study Short Form Health Survey:

Medical Outcomes Study Short Form Health Survey (MOS SF-36), Portuguese validated version, evaluates health-related quality of life (Ferreira, 2000), administered as an interview. The MOS SF-36 questions allows to evaluate the physical and mental health through the assessment of aspects related to function, well-being, disability, and personal evaluation. The questions are classified in order to group into eight domains to measure the health constructs: Physical Functioning, Role-Physical, Bodily Pain, General Health Perceptions, Vitality, Social Functioning, Role-Emotional, and Mental Health. These domain can be classified with a punctuation between 0 and 100, being that a higher score is more predictive of better quality of life. A combination of the eight domains results in a two summary scales: Physical Component Summary scale (PCS) and Mental Component Summary scale (MCS).

Brief Symptom Inventory:

Psychological symptoms were evaluated using the Portuguese version of Brief Symptom Inventory (BSI) (CANAVARRO, 1999). The BSI is a self-reported inventory, composed by 53 items, with punctuation from 0 (not at all) to 4 (extremely), with higher scores indicating more severe psychopathology or psychological distress. Participants' responses correspond to the psychological symptoms experienced in the last seven days. The BSI involves nine subscales: somatization (SOM), obsessive–compulsive (O-C), interpersonal sensitivity (I-S), depression (DEP), anxiety (ANX), hostility (HOS), phobic anxiety (PHOB), paranoid ideation (PAR) and psychoticism (PSY). In addition, BSI inventory provides three global indices of distress: the General Severity Scale (GSI), the Positive Symptom Distress Index (PSDI), and the Positive Symptom Total (PST). The GSI scales allows asses the level of present distress. The PSDI scales indicates if the way a person responds increases their level of suffering. The PST scale corresponds to the set of all symptoms that the participant reports, even if it is at low levels (Derogatis & Melisaratos, 1983). The BSI includes four additional items, which do not belong to any subscale, but are considered in the three indices. These items assess the presence of sleep disturbances, thoughts of death, feelings of guilt and loss of appetite.

Statistical analysis

Descriptive analyses were performed for the sample in study and for the results of psychological evaluation and quality of life. Subsequently, association analyses were performed between the presence of tinnitus and the parameters evaluated for health-related quality of life and psychological symptoms. These analyzes were performed with non-parametric tests because the variables did not present a standard distribution and homogeneity in the variances Fisher Exact Test was used for the general association between two variables. Mann-Whitney or Kruskal-Wallis (for more than two groups) tests were employed to compare the presence and severity of tinnitus. Taking into account that non-parametric tests were used in this study, and these evaluate the associations between the variables through the medians and the quartiles, the

descriptive results are presented as median and quartiles. The level of significance considered was p=0.05. All the results were analyzed through logistic regression model, where age and gender where considered as control variables. In the cases where we had missing data we have considered n= total of entries.

Results

Sample Distribution:

Our study population is composed by 114 people aged 64.0 ± 5.6 years old (range= 55-75). Most participants were female (n=60, 52.6%) and 54 were men (47.4%).The mean age for the subgroup without tinnitus was 64.6 ± 3.8 years old, while the mean age for tinnitus group was 64.0 ± 5.6 years old. Interestingly, a high number of participants belonged to the sector 'specialists of scientific and intellectual activities', followed in number by 'technicians and professionals of intermediate level' and 'administrative staff'. The majority of participants with previous history of psychiatric medication and current psychiatric medication belong to the group of participants with tinnitus. Table 5.4-1 presents the characteristics of participants in each subgroup (i.e. without and with tinnitus).

Table 5.4-1 - Participant characteristics.

		Subgroup		Total
		Without Tinnitus	With Tinnitus	
Gender	Male	8	46	54
	Female	14	46	60
N		22 (19.3%)	92 (80.7%)	114
Age Mean and Std. Deviation		64.6 ± 3.8	64.0 ± 5.6	64.0 ± 5.6
Marital status (n=98)	Single	1	0	98
	Married	18	62	
	Divorced	2	7	
	Widower	0	8	
Professions (N=112)	A	0	0	0
	B	2	3	5
	C	15	43	58
	D	2	14	16
	E	2	13	15
	F	0	2	2
	G	0	2	2
	H	0	5	5
	I	0	0	0
	J	1	8	9
	Total	24	88	112
Previous medication	Without medication	19	73	114
	Antidepressants only	1	10	
	Anxiolytic only	2	7	

	Antidepressants + Anxiolytic	0	1	
	Other	0	1	
	Without medication	21	73	
	Antidepressants only	0	2	
Current medication	Anxiolytic only	0	12	114
	Antidepressants + Anxiolytic	0	4	
	Other	1	1	

Legend: A – Military professional; B – Representant of executive bodies or legislative power, leaders, directors and executive managers; C – Specialists of scientific and intellectual activities; D – Technicians and professionals of intermediate level; E – Administrative staff; F – Workers of personal, safety and security services and sellers; G – Farmers and qualified agriculture, fisheries and forestry workers; H – Qualified industry, construction and e artwork workers; I – Installation operators and machinery and assembly workers; J – Non qualified workers. According to Portuguese classification of professions (2010).

Audiological assessment:

Average hearing thresholds by group are shown in Figure 5.4-1 (tonal audiometry) and Figure 5.4-2 (speech audiometry). Tinnitus participants had higher hearing thresholds above 1.5 kHz.

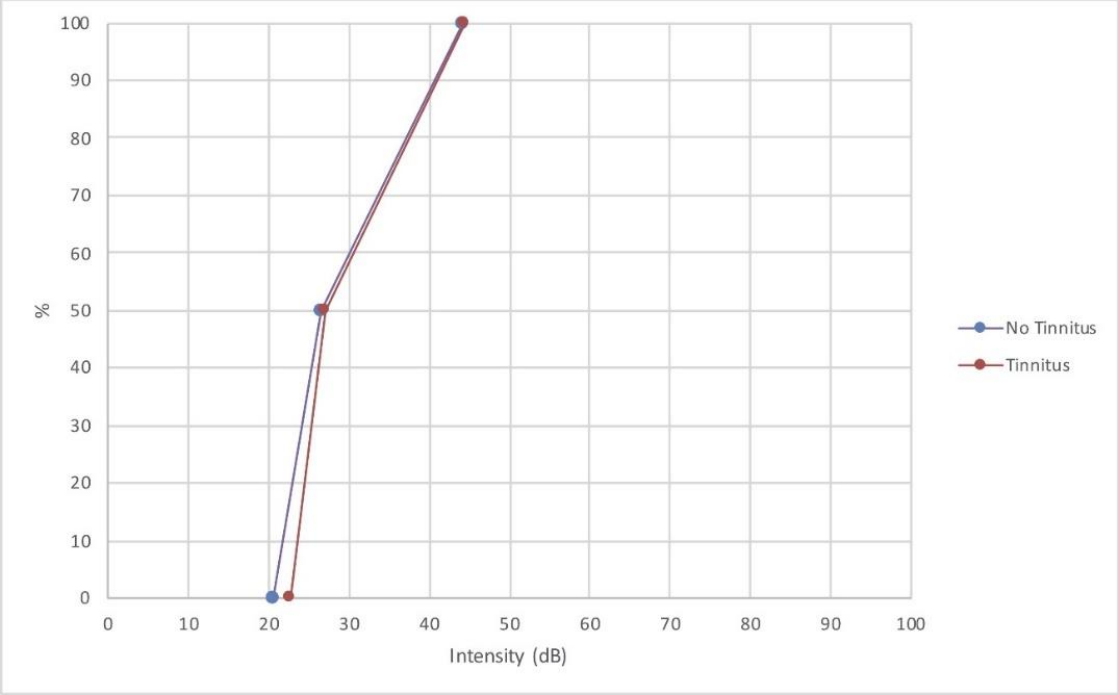

Figure 5.4-1 - Tonal Audiometry in the subgroups with and without tinnitus.

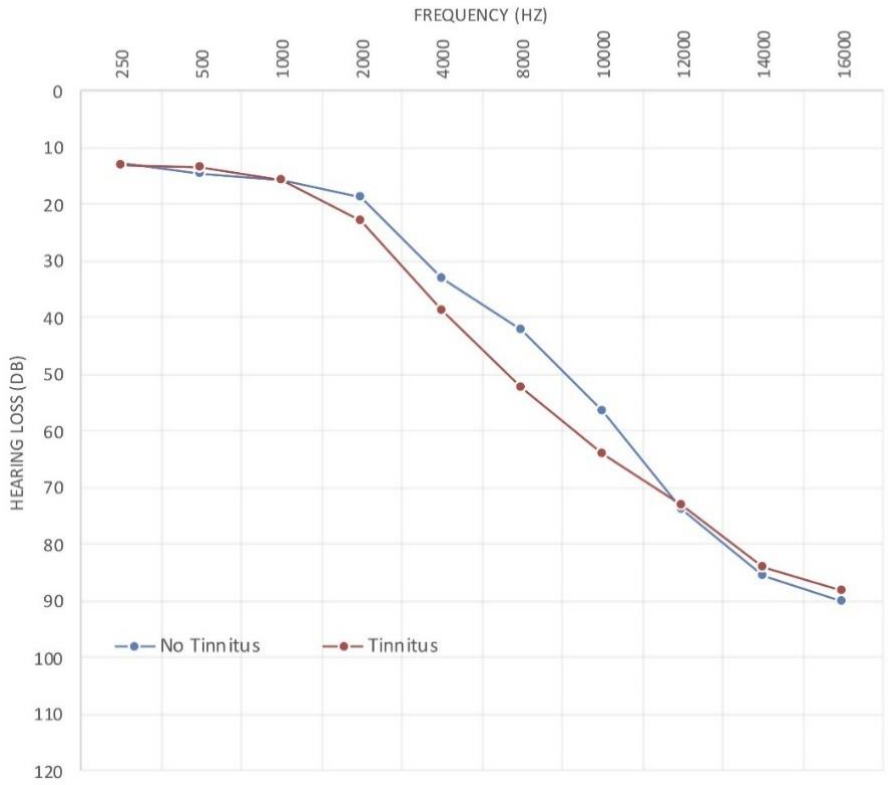

Figure 5.4-2 - Speech Audiometry in the subgroups with and without tinnitus.

Tinnitus Evaluation:

The mean tinnitus duration was 7.8 ± 8.6 years, with the mean tinnitus intensity of 3.3 ± 1.6, on a visual analogue scale (VAS) of 1-10 (Table 5.4-2). For most participants tinnitus was central (i.e. perceived in the head) (47.8%) and tonal (53.2%).In the majority of participants tinnitus was constant (87%). Tinnitus onset was gradual for 49% of them and abrupt for 19.5% of participants. Dizziness, often associated with tinnitus, was reported by 38% of participants with tinnitus, while 54.4% reported not having dizziness symptoms. In most participants, tinnitus worsened in situations in which they are nervous (58.7%). Finally, 48.9% of the participants reported reduced sound tolerance, 33.7% of the participants with tinnitus had unprotected exposure to noise, while only four participants used protection when exposed to noise.

Table 5.4-2 - Clinical characterization of tinnitus sample.

Clinical variables	Participants with tinnitus (n=92)
Tinnitus Duration (mean in years)	7.8 ± 8.6
Intensity of tinnitus (scale 1-10)	3.3 ± 1.6
Manifestation of tinnitus	
Constant	80 (87%)
Intermittent	7 (7.6%)
Pulsatile	4 (4.3%)
Omitted	1 (1.1%)
How did tinnitus begin?	

Gradual	45 (49%)
Abrupt	18 (19.5%)
Omitted	29 (31.5%)
Dizziness	
Yes	35 (38%)
No	50 (54.4%)
Omitted	7 (7.6%)
Does tinnitus gets worse when you're nervous?	
Yes	54 (58.7%)
No	37 (40.2%)
Omitted	1 (1.1.%)
Lower noise tolerance	
Yes	45 (48.9%)
No	47 (51.1%)
Noise exposure	
Yes, with protection	4 (4.3%)
Yes, without protection	31 (33.7%)
No	57 (62%)

Tinnitus severity was determined according THI scores (Figure 5.4-3). In our tinnitus subgroup, 17 had a slight or no handicap 38 had a mild handicap, 22 had a moderate handicap, 14 had a severe handicap, and just one participant scored as having catastrophic tinnitus.

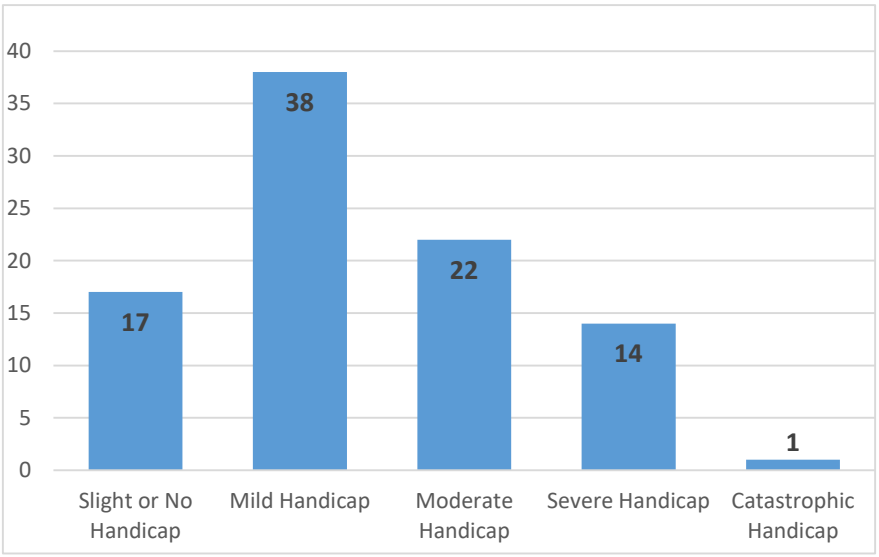

Figure 5.4-3 - THI scores for participants in the tinnitus subgroup.

Table 5.4-3 shows the psychoacoustic characteristics of tinnitus in the tinnitus subgroup (n = 92). In terms of Feldmann's curve, the most frequent types were convergent (43.4%) or distant (27.1%). Most participants experienced no (39.1%) or partial (32.6%) residual inhibition, although some (14.1%) also experienced complete residual inhibition.

Table 5.4-3 - Psychoacoustic tinnitus assessment.

Audiological measurements	Participants with tinnitus (n=92)
Pitch (n=83)	4000Hz (2000Hz; 8000Hz)
Loudness (n=83)	0 dB (0 dB; 5.0 dB)
Laterality	
Central	44 (47.8%)
Right	15 (16.3%)
Left	25 (27.2%)
Omitted	8 (8.7%)
Type	
Pure Tone	49 (53.2%)
Narrow Band Noise	34 (37%)
Omitted	9 (9.7%)
Feldmann's Curve	
Congruent	17 (18.4%)
Convergent	40 (43.4%)
Divergent	1 (1.1%)
Distant	25 (27.1%)
Persistent	1 (1.1%)
Omitted	8 (8.7%)
Residual inhibition	
Negative	36 (39.1%)
Partial	30 (32.6%)
Complete	13 (14.1%)
Rebound Effect	3 (3.3%)
Omitted	10 (10.9%)

Psychological symptoms

Table 5.4-4 presents the subgroup medians and quartiles of the nine scales and three indices of BSI. The results were very similar for each subgroup. Nevertheless, in the subgroup without tinnitus the median was higher for the Interpersonal Sensitivity and Paranoid Ideation subscales, while in the group with tinnitus the subscales with the highest scores were the Somatization and Paranoid Ideation. There was a significant difference between the groups without and with tinnitus in the Obsessive-compulsive scale, Interpersonal sensitivity scale, General severity scale and Positive symptom total scales.

The median of the nine sub-scales and for the three indices were not above the criterion of significance (≥ 63) in the two groups. This means that, through median analysis, none of the scales and indexes are clinically significant in characterizing the psychological symptoms of the two subgroups.

Concerning scores obtained at clinical significance level (≥63) at the 3 BSI Indexes, for General Severity Scale, 18 tinnitus participants have a significant level score

while 4 participants without tinnitus also have this level. In the tinnitus subgroup (18 participants), 3 have irrelevant THI score, 5 mild, 4 moderate and 6 severe or catastrophic THI score. Concerning Positive Symptom Distress Index we found 5 participants with tinnitus and 4 participants without tinnitus reaching the clinical significance level. Among the 5 tinnitus participants their THI score is: 2 irrelevant, 2 severe or catastrophic and 1 moderate. In the third index, Positive Symptom a total of 18 tinnitus participants and 6 participants without tinnitus reach scores ≥63. Tinnitus severity in the 18 participants is such: 7 severe or catastrophic, while 3 have irrelevant THI score, 4 mild and 4 moderate.

Table 5.4-4 - Brief Symptoms Inventory: Median and quartiles distributed by the population with and without tinnitus.

BSI Scales	Subgroup					
	Without Tinnitus			With Tinnitus		
	P25	P50	P75	P25	P50	P75
SOM	34.5	53.0	62.0	41.0	49.5	56.0
OC	48.0	53.0	64.0	40.0	46.0	59.5
p-value			0.009*			
I-S	41.5	62.0	64.0	37.0	45.0	59.0
p-value			0.026*			
DEP	0.0	43.0	60.0	33.0	42.0	57.0
ANX	37.8	51.0	59.0	35.0	43.5	59.0
HOS	0.0	49.0	55.0	38.5	44.0	57.3
PHOB	0.0	48.5	59.0	0.0	45.0	58.3
PAR	32.3	56.0	62.0	40.0	48.0	58.0
PSY	0.0	40.0	61.0	0.0	19.0	56.0
GSI	45.0	50.0	61.3	35.0	43.5	59.8
p-value			0.020*			
PSDI	42.0	49.5	56.5	34.0	42.0	55.5
PST	46.8	51.0	63.3	37.0	46.5	60.8
p-value			0.023*			

Legend: * p-value<0.05; SOM – Somatization: OC – Obsessive-compulsive; I-S – Interpersonal sensitivity; DEP – Depression; ANX – Anxiety; HOS – Hostility; PHOB – Phobic Anxiety; PAR – Paranoide Ideation; PST – Psychoticism; GSI – General Severity Scale; PSDI – Positive Symptom Distress Index; PST – Positive Symptom Total.

Quality of Life:

Descriptive of MOS scores for each subgroup are presented in Table 5.4-5. Compared to the group without tinnitus, patients with tinnitus had lower median scores on the scales of Physical Functioning, Bodily Pain, General Health Perception, Vitality and Mental Health. Although, only the General Health Perception scale have significant difference between the two groups. The tinnitus subgroup also had lower summary

scores on the Physical Component Summary scale, Mental Component Summary scale and Final mean. Scores on these scales indicating a lower quality of life for those with tinnitus.

Table 5.4-5 -Medical Outcomes Study: Median and quartiles distributed by the population with and without tinnitus.

MOS Scales	Subgroup					
	Without Tinnitus			With Tinnitus		
	P25	P50	P75	P25	P50	P75
PF (%)	68.9	90.0	95.0	71.3	85.0	95.0
RP (%)	50.0	100.0	100.0	50.0	100.0	100.0
BP (%)	51.8	74.0	100.0	51.0	72.0	84.0
GHP (%)	52.0	69.5	83.3	45.0	62.0	72.0
p-value				0.042*		
VIT (%)	50.0	72.5	90.0	50.05	65.0	78.8
SF (%)	75.0	87.5	100.0	75.0	87.5	100.0
RE (%)	58.4	100.0	100.0	66.7	100.0	100.0
MH (%)	68.0	78.0	89.0	57.0	72.0	84.8
HC	3.0	3.0	3.0	3.0	3.0	4.0
PCS (%)	61.6	79.9	89.3	60.2	75.7	85.3
MCS (%)	61.2	85.7	92.3	60.2	81.2	89.2
Final Mean (%)	60.2	81.2	89.2	61.8	78.2	83.9

Legend: * p-value<0.05; PF – Physical Functioning; RP – Role-Physical; BP – Bodily Pain; GHP – General Health Perceptions; VIT – Vitally; SF – Social Functioning; RE – Role-Emotional; MH – Mental Health; HC – Health Change; PCS – Physical Component Summary scale; MCS – Mental Component Summary scale.

BSI additional Items and Tinnitus:

In order to enable statistical analysis of BSI additional items we grouped the answers such that the responses 'nothing' 'little' or 'moderately' scored 1 and 'very' and 'extremely' scored 2.

In our tinnitus subgroup, 79 participants reported little, moderate or no sleep difficulty, while 13 participants had very and extreme disturbances at sleep level. On the item 'thoughts of death', 90 participants had no, little or moderate thoughts, and two of participants mentioned having many thoughts of death. Eighty-nine participants reported no, little or moderately frequent feelings of guilt, while 3 reported very or extreme frequency of such feelings. Finally, on the item 'loss of appetite', all participants with tinnitus report having not appetite loss, or having low to moderate levels of this complaint (Table 5.4-6).

The Fisher's exact test revealed significant statistical differences when comparing the loss of appetite between the two subgroups with or without tinnitus (p= 0.001).

Table 5.4-6 - Analysis of BSI Additional Items - Sleep disturbance, Thoughts of death, Feelings of guilt and Lack of appetite - for the population with and without tinnitus.

BSI Additional Items		Subgroup		Total n
		Without Tinnitus – total score	With Tinnitus – total score	
Sleep Disturbance	Nothing, little and moderately	19	79	98
	Very and extremely	3	13	16
p-value Fisher's Exact Test		0.629		
Thoughts of death	Nothing, little and moderately	21	90	111
	Very and extremely	1	2	3
p-value Fisher's Exact Test		0.478		
Feelings of Guilt	Nothing, little and moderately	21	68	110
	Very and extremely	1	3	4
p-value Fisher's Exact Test		0.581		
Loss of appetite	Nothing, little and moderately	18	92	110
	Very and extremely	4	0	4
p-value Fisher's Exact Test		0,001*		

Legend: * p-value<0.05

Quality of life and Tinnitus:

Only scores on General Health Perceptions scale were significantly different between subgroups (Table 5.4-6, *U*= 721.5, *p*= 0.042).

Modelling the data – BSI and MOS

In a logistic regression modelling quality of life and psychological complaint, age, gender and hearing loss where considered as confounding variables. The independent variable in the model was presence of tinnitus. The results for the MOS scales and hearing loss are presented in Table 5.4-7.

Table 5.4-7 - Logistic regression model of quality of life and hearing loss applied to severe tinnitus.

Variable*	**OR**	**p- value (Wald test)**	**(95% IC)**
General Health Perceptions – MOS	0.956	0.007*	(0.926, 0.988)
Hearing Loss	1.111	0.010*	(1.026, 1.203)

Legend: * p-value<0.05

For the MOS score the Odds Ratio (OR) was below 1 and significant so is considered a 'protector variable' for developing tinnitus. On the other hand, for hearing

loss OR is above 1 and significant, so 'hearing loss' is a risk variable and the odds to have tinnitus is 1.1 higher for those with hearing loss.

Regarding tinnitus severity, we applied the logistic regression model with the aim of verifying which variables are statistically significant at higher levels of tinnitus severity, controlling for gender, age and noise exposure. The independent variable in the model was severe tinnitus (n=15), (the assemble of severe and catastrophic grades from THI, see F Figure 5.4-3). Results for BSI and MOS scales are presented in Table 5.4-8.

Table 5.4-8 - Logistic regression model in the BSI and MOS applied to severe tinnitus.

Variable*	OR	p- value (Wald test)	(95% IC)
General Severity Scale - BSI	1.049	0.037*	(1.003, 1.097)
General Health Perceptions – MOS	0.962	0.037*	(0.928, 0.998)

Legend: * p-value<0.05

It was found that quality of life and psychological complaint were statistically significant relative to the severity of tinnitus. Because the OR of MOS was below 1, the variable General Health Perceptions is considered as a "protector factor" for the risk of developing severe tinnitus. Thus, if the general health perceptions (MOS) scale increases, which means that the quality of life increases on that scale, it decreases the risk of developing severe tinnitus. On the other hand, the variable General Severity Scales was above 1, so is considered as a "risk factor". i.e. the odds of developing severe tinnitus in the presence of psychological complaint was 1.049 higher.

Table 5.4-9 - Logistic regression model in the BSI and MOS applied to tinnitus presence.

Variable	OR	p- value (Wald test)	(95% IC)
BSI T≥ 63	16.528	0.035*	(1.215, 224.831)
General Health Perceptions – MOS	0.946	0.004*	(0.911, 0.982)
Hearing Loss	1.118	0.012*	(1.024, 1.221)

* p-value<0.05

We explore which variables are statistically significant to the presence of tinnitus, through the logistic regression model (Table 5.4-9). The results showed that General Health Perceptions has a statistical significant level and because OR is below 1 it is considered "protector factor" for developing tinnitus. Thus, in the presence of a higher score in general health perceptions (MOS) that means more quality of life, and decreases the risk of developing tinnitus. Also presenting statistical significant level the variables hearing loss and the variable BSI scales equally or above 63 (the criterion of clinical significance) are considered as "risk factors" because OR is above 1. This means that the odds of developing tinnitus is higher in the presence of BSI scales above the criterion of significance or in the presence of hearing loss.

Discussion

Here we explored presenting characteristics, psychological symptoms, and quality of life in a sample of participants aged between 55 and 75 years. We aimed to identify aspects that can contribute to the diagnosis and guide therapeutic interventions.

We found higher scores in tinnitus patients on the psychological complaint scales of somatization and paranoia. Overall, it seems that patients with tinnitus suffer for more distress arising from awareness of bodily representations and present paranoid behavior related to a disordered mode of thinking (Derogatis & Spencer, 1982). The somatization scale focuses on cardiovascular, gastrointestinal and respiratory complaints, pain and discomfort of the gross musculature, and anxiety symptoms derived from somatic complaints. Our results are congruent with other studies that found higher prevalence of somatization symptoms in tinnitus patients (Hiller et al., 1997; Scott & Lindberg, 2000).

Although it was expected to find more psychological symptomatology in the group with tinnitus, particularly depressive and anxious symptomatology (Holgers, Zöger, & Svedlund, 2005; Krog, Engdahl, & Tambs, 2010; Shargorodsky, Curhan, & Farwell, 2010), this was not observed; there were no statistically significant difference on any psychological complaint scale. This may have been because there was no psychological symptomatology control related to conditions other than tinnitus and also because the questionnaire used (the BSI) only assesses psychological complaints experienced in the week before its completion. Of note, 22 of our participants have a previous history of psychiatric medication, while 20 participants currently take psychiatric medication. Concerning psychiatric medication, 20.7% of tinnitus patients had a history of psychiatric medication in the whole sample of tinnitus participants, those corresponding to 86.3% of the 22 participants who have a previous history of medication. Regarding current psychiatric medication, 20.6% of tinnitus sample are currently medicated, which corresponds to 95% of the 20 participants that currently take psychiatric medication (Table 5.4-1). Those results strongly suggest that the most severely affected tinnitus patients will benefit from the care of mental health professionals.

Considering tinnitus severity, only 15 participants had severe or catastrophic tinnitus. Since the majority of our tinnitus participants weren´t severely affected (they have a mild handicap) it is likely that our BSI scores did not reach clinical significance at the group level. The diagnosis of depressive and anxious symptoms is more frequent in tinnitus patients with severity and catastrophic handicap (Holgers et al., 2005).

Significant differences were found in the Obsessive-compulsive and Interpersonal sensitivity scales concerning the presence of tinnitus. These findings

reveal that these scales differ in the tinnitus and non-tinnitus groups. The interpersonal sensitivity dimensions focuses on inferiority and inadequacy feelings when compared with others, and with depreciation and restlessness during interpersonal interactions. In turn, the Obsessive-Compulsive scale focuses on the thoughts, impulses and actions of the individual that are considerate as irresistible, but are of undesired nature (Derrogatis & Spencer, 1982). This supports the use of CBT for tinnitus to address negative automatic thoughts, safety behaviors, inaccurate beliefs (McKenna et al., 2014). Those results strongly proposes including psychologists to the multidisciplinary approach with different professionals involved at tinnitus treatment.

Concerning to MOS scales, only the General Health Perceptions scales showed significant results between subgroups with and without tinnitus. This scale measures the concept of holistic health perception, including not only current health but also disease resistance and healthy appearance (Ferreira, 2000). Although it was verified that the MOS scales are generally lower in the population with tinnitus, on both physical and emotional dimensions suggesting a lower quality of life in this population. Our results showed that the physical component of MOS was lower than the emotional component, which indicates physical limitations, more impact of the intensity and discomfort caused by the pain, and the person's own perception of his overall health. On the other hand, the vitality scale referring to energy and fatigue levels, and that qualifies the differences in well-being, is the lowest scale referring to the emotional component.

Interestingly, significant differences in psychological complaint and quality of life were found when comparing the more severe grades of tinnitus participants (severe and catastrophic from THI) with the other grades. Most BSI scale scores were increased (showing a clinically relevant score ≥63) revealing the psychological impact of the participants that were severely affected by tinnitus. Higher grades on the scales such as somatization, depression and anxiety were related to the severity of tinnitus. This finding is in accordance with other published studies showing that depressive and anxiety symptoms are more prevalent in patients with higher scores of tinnitus severity (Erlandsson & Hallberg, 2000; Holgers et al., 2005).

Frailty refers to a condition in the elderly where several accelerated inter-related diminishment in physiological systems occur, causing high vulnerability to the individual (Clegg, Young, Iliffe, Rikkert, & Rockwood, 2013). In this disorder small insults (e. g. new medication or a minor infection) can have a disproportionate effect in the person's health status (e. g. to become dependent on others).

Using regression statistical models, we have proven that the scales with more relevant statistical association to tinnitus are the General Severity Index (the more sensitive BSI scale to distress symptoms) and the General Health Perception. These findings bring to discussion the issue of frailty, particularly relevant for the age range

focused in our study. Clearly, an individual will be more prone to have tinnitus if he has high General Severity Index (t≥63) and low values at General Health Perception.

Study limitations

We have to recognize that the sample size of population is a limitation of this study, a larger sample size would permit more statistic power. Participants in this study did not have a psychologist evaluation so we relied entirely on self-report to categorize participants as having psychological complaint or not. Furthermore, the questionnaire used, the BSI, evaluates the emotional status of the previous seven days only so does not capture general longer term psychological complaints which may fluctuate in the same way that tinnitus is known to (Hallam et al., 1984; Gopinath et al., 2010).

Conclusions

With an increasingly ageing population worldwide raises concerns about quality of life with augmented years lived with disability. Taken together, tinnitus and hearing loss disorders have a high negative impact on the quality of life of the affected persons especially if the grade of tinnitus severity is high. On the other hand, elevated stress levels may not only damage an auditory system that is inefficiently protected to undesirable stress reactions, but also, prolong a state of emotional distress that by itself may exacerbate the tinnitus perception (Mazurek, Haupt, Olze, & Szczepek, 2012; Szczepek & Mazurek, 2017). Epidemiologic data confirmed that hearing loss is a tinnitus major risk factor. Nevertheless, tinnitus is not always present in every case of hearing loss, and often occurs later in time, such as during psychological stress (Han, Lee, Kim, Lim, & Shin, 2009).

Our study brings and reinforces new insights concerning the importance of the holistic assessment and management of the individual relevant to tinnitus as a multidimensional symptom (Hall et al., 2018). To our knowledge this is the first study to use the BSI General Severity Index and identify scoring that represents a risk factor for tinnitus and impaired quality of life, and what scoring on the General Health Perception scale of MOS represents a protector factor for tinnitus.

Accordingly, the assessment of the individual with tinnitus and therapy strategies should be multidisciplinary to ensure coverage of all dimensions of the patient. Moreover, therapeutic strategies should be tailored to the individual, after proper information and with respect for patient choice and individual needs.

5.4.2. Audiological markers of tinnitus in an older Portuguese population

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Abstract

Tinnitus is a phantom sound perceived in the absence of external acoustic stimulation. It is described in a variety of ways (e.g., buzzing, ringing, roaring) and can be a single sound or combination of different sounds. Our study aims to evaluate associations between audiological parameters and the presence or severity of tinnitus in order to allow us to improve tinnitus diagnosis, treatment and prognosis. Our sample included 114 older individuals (60 women and 54 men), aged between 55 to 75 years from the Portuguese population, with or without sensory presbycusis and/or tinnitus. All participants were subjected to a clinical evaluation through a structured interview, complete ENT observation, audiological evaluation (standard and extended audiometry, psychoacoustic tinnitus evaluation, ABR and DPOEs) and tinnitus scoring through Tinnitus Handicap Inventory (THI). Our data confirm that the odds of developing tinnitus was significantly higher in the presence of noise exposure and hearing loss. Participants with abrupt tinnitus onset were 5.13 times more likely to develop severe or catastrophic tinnitus (p=0.024). Participants who present a negative or rebound effect at residual inhibition have 82.2% lower odds of developing a severe or catastrophic tinnitus as compared to those having a complete or partial effect. Higher values of ABR_mean_RE_LE_Ratio are associated with higher odds for developing severe or catastrophic tinnitus (p=0.046). We also found reduction of the amplitude in the ABR wave I in patients with tinnitus. Advancing knowledge concerning specific tinnitus subtypes origin and maintenance mechanisms is of paramount importance for adequate treatment.

Keywords: Tinnitus; Audiological markers; Pure Tone Average; Auditory Brainstem Response; Distortion Product Otoacoustic Emissions.

Introduction

Tinnitus is a phantom sound perceived in the absence of external acoustic stimulation being described in a variety of ways (e.g., buzzing, ringing, roaring) and can be a single sound or combination of different sounds (Coles, Vernon, & Moller, 1995; Stouffer & Tyler, 1990). It can also be perceived in one ear, both ears, or in the head, as a constant sound or fluctuating in intensity (loudness) or pitch. Tinnitus is frequently perceived as extremely loud, but when matched with calibrated acoustic signals, is typically within 10 dB of audiometric threshold (Hall III & Haynes, 2001). Tinnitus is categorized as objective or subjective, being the objective tinnitus described as a real sound produced by the body that can be heard by an examiner. In contrast, an examiner cannot hear subjective tinnitus. Subjective tinnitus is thought to be caused by abnormal neural activity in the peripheral or central auditory system (Aage R. Møller, 2006).

Tinnitus has a variety of etiological factors and may be associated with other diseases. Often accompanies hearing loss or hyperacusis, but neither is necessary for its presence (Eggermont, 2013, 2015; Eggermont & Roberts, 2004). Most studies have found that patients with high-pitched tinnitus have hearing loss in high frequencies and the patients with low-pitched tinnitus (below 1,500 Hz) more frequently have low frequency hearing loss (König, Schaette, Kempter, & Gross, 2006; Moore, Vinay, & Sandhya, 2010; Sereda et al., 2011). In a recent study involving patients with presbyacusis, the authors found an average pitch in men of 4781,3Hz and women of 3869,8Hz, considering individuals with a mean age of 69,75 (+-6,53) (Seimetz et al., 2016).

The causes and pathogenesis of tinnitus remains unclear and there are no objective audiological or non-audiological tests for the diagnosis of tinnitus. Currently, the presence and impact of tinnitus is established using subjective measures such as questionnaires (e.g., Tinnitus Handicap Inventory – THI (C. W. Newman, Jacobson, & Spitzer, 1996); or the Tinnitus Reaction Questionnaire – TRQ (Wilson, Henry, Bowen, & Haralambous, 1991) (Henry et al., 2013; Szczepek, Haupt, Klapp, Olze, & Mazurek, 2014). Psychoacoustic assessment of tinnitus can also be performed. Although, patients with similar psychoacoustic measurements can report it has very different impacts on their lives, it may be useful to interpret neurophysiological mechanisms of tinnitus perception. It is observed, that when tinnitus is accompanied by hearing loss, particularly sloping configurations, the tinnitus pitch is either localized in the “edge” frequencies or within the lowest regions of the hearing loss (Langers, de Kleine, & van Dijk, 2012). Diminished peripheral input caused by hearing loss is thought to lead to reorganizations of the tonotopic framework of the Auditory Cortex (AC) (Eggermont, 2003; Pinkl, Wilson, Billingsly, & Munguia-Vazquez, 2017). Theoretically, when the neural activity within a selected region of the cortical tonotopic map is diminished, if the tinnitus pitch corresponds to a region of hearing loss, synaptic connections of the

affected regions merge with stronger neighboring neural areas which lead to this over-representation of “edge” frequencies (Rajan & Irvine, 1998). The over-representation of cortical activity within a specific region of the tonotopic map naturally promotes neural synchronization potentially causing a phantom perception. Furthermore, it is suspected that adaptive changes in the AC can alter its connections with the Inferior Colliculus making it more susceptible to incoming afferent spontaneous signals, enhancing tinnitus perception (Wang et al., 2013). On the other hand, if the tinnitus percept exists at the “edge” frequency, alterations of the tonotopic map would be implicated as the primary source of tinnitus percept, possibly supplemented by affected IC tuning curves (Langers et al., 2012).

Abnormal synchronous neural activity can be detected by specialized clinical tests, namely auditory-evoked potentials (AEPs). Previous studies have used AEPs measures to study the abnormal neuronal activity in tinnitus patients (Gopal, Bishop, & Carney, 2004; Gopal, Thomas, Nandy, Mao, & Lu, 2017; Gu, Herrmann, Levine, & Melcher, 2012; Hsu et al., 2013; Kehrle et al., 2008). The most widely used AEP is the Auditory Brainstem Response (ABR). The ABR is a series of vertex-positive waves that occur within 15ms of the onset of a click stimulus in human adults. The most commonly evaluated ABR peaks include I, III and V and much is known about the generators of the ABR waves. Moller et al. concluded that wave I arises from the distal portion of the auditory nerve (within the inner ear), wave III from the cochlear nucleus, and wave V from the termination of the lateral lemniscus within the IC of the contralateral side (A R Møller, Jho, Yokota, & Jannetta, 1995). This technique is especially important in tinnitus evaluation because it is an objective electrophysiological measure of cochlear and brainstem auditory pathway functioning. Moreover, it may allow the differentiation between central or peripheral tinnitus (Shulman & Seitz, 1981).

Differences in the traces of the ABR can be seen depending on the type of stimulus used to evoke the response, type of hearing loss, the degree of hearing loss, the presence of tinnitus among others. Regarding the degree of hearing loss and the type of the stimulus used, elevated hearing thresholds reduce the amplitude of wave V using click stimuli, so using tone burst ABR when the tone burst characteristic frequency falls within the frequency region of the hearing loss may provide higher sensitivity (Lewis, Kopun, Neely, Schmid, & Gorga, 2015). According to Serpanos (2004), listeners with sloping configurations of cochlear hearing loss can benefit if more frequency-specific stimuli are used, such as brief tones, in order to provide more precise information on the nature of the relation between loudness growth and ABR wave V latency. Considering the models of pathologic enhanced neural synchronization and the potential cortical influence on subcortical tuning functions, it is hypothesized that unique ABR readings if any, will become more pronounced in tinnitus subjects if the ABR parameters are adjusted from click stimuli to tone burst stimuli matched to the tinnitus pitch (Pinkl et al., 2017).

Although there is a lack of consensus regarding the use of AEPs as diagnostic tool of tinnitus, most probably due to the lack of homogeneity in the participant's groups and methodologies, AEP measures may contribute to the clarification of the origin of tinnitus and provide potentially objective diagnostic indicators (Gopal et al., 2017). Furthermore, identifying potential correlations between ABR readings and tinnitus pitch can help formalize tinnitus diagnostic procedures (Pinkl et al., 2017).

Regarding the cochlear function as an important role in the generation of tinnitus perception, it is essential the assessment of the inner ear for the evaluation of tinnitus. The otoacoustic emissions (OAEs) are sound signals produced by the cochlea and reflects some activity of the outer hair cells (OHC). Trough OAEs, the cochlear function can be tested in an objective and non-invasive way (Fabijańska et al., 2012; Lapsley Miller & Marshall, 2007). Studies on OAEs in tinnitus patients used distortion product otoacoustic emissions (DPOAEs) measure a wide range of primary frequencies (f1 and f2) and their levels (L1 and L2) (Fabijańska et al., 2012). There are two cochlear processes that explain the generation mechanisms of DPOAS: 1) a nonlinear interaction of the primary tones induced by the traveling wave, and mainly at the cochlear site in and around the region basal to the f2 location, and 2) a linear coherent reflection of the traveling wave from the location corresponding to the distortion product frequency of 2f1−f2 (Fabijańska et al., 2012; Kalluri & Shera, 2001). In the literature, there are controversial results regarding the levels of DPOAEs in tinnitus patients. On the one hand, we can see decrease levels in the DPOAEs in tinnitus patients when compared with patients without tinnitus (Ozimek, Wicher, Szyfter, & Szymiec, 2006; Shiomi, Tsuji, Naito, Fujiki, & Yamamoto, 1997). On the other hand, an increase in the DPOAEs levels are seen in tinnitus patients (Gouveris, Maurer, & Mann, 2005; Janssen, Kummer, & Arnold, 1998). If we consider hearing loss the results become even more contradictory. Ami et al., 2008, found in patients with normal hearing with tinnitus a significantly reduction in the mean baseline DPOAEs levels when compared with patients with normal hearing without tinnitus, suggesting that reduced outer hair cell activity would result in tinnitus even before there is a shift in hearing threshold. On the other hand, in patients with reduced hearing loss, they found the opposite, in the group without tinnitus there was a markedly reduced mean DPOAEs when compared with the group with tinnitus, also postulate that markedly low levels of cochlear hair cell activity may actually terminate the source of aberrant peripheral neural activity in tinnitus (Ami, Abdullah, Awang, Liyab, & Saim, 2008). Opposite results were found by Sztuka et al., 2010, patients with normal hearing with tinnitus have a markedly higher DPOAE amplitudes when compared with patients with normal hearing and without tinnitus, suggest that tinnitus may be caused by increased motility of the OHC induced by decreasing efferent fiber activity, and not by OHC failure (Sztuka, Pospiech, Gawron, & Dudek, 2010).

Depending on where the tonotopic reorganization (cortical plasticity) takes place we can speculate on the chronicity of tinnitus, taking into account, as described above, that patients with tinnitus can have neural dysfunction at the level of the cochlea, auditory nerve and/or brainstem (Sindhusake et al., 2003).

Biomarker is any substance or biological structure that can be measured in the human body and may influence, explain or predict the incidence or outcome of disease (Gallo et al., 2011). The identification of these biological markers in individuals with tinnitus allows us to improve the diagnosis, treatment and prognostic of tinnitus (Ami et al., 2008).

Our study aims to identify associations between audiological parameters and the presence or severity of tinnitus in order to allow us to improve tinnitus diagnosis, treatment and prognosis.

Methods

The present cross-sectional study was approved by the Ethical Committees from Hospital CUF Infante Santo (26th November 2014), Nova Medical School (nº65/2014/CEFCM) and the National Department of Personal Data Protection (authorization number:1637/2016). The study was conducted in accordance with the Declaration of Helsinki.

Participants

Our sample included 114 older individuals (60 women and 54 men). Inclusion criteria was adults of any gender, aged between 55 to 75 years from the Portuguese population. The presence and absence of presbyacusis and the presence of tinnitus were recorded. Presbycusis was defined as bilateral sensorineural deafness in down slope audiometric pattern, above 1000 Hz with poor speech discrimination (Speech Recognition Threshold (SRT) > 40 dB SPL and 100% discrimination to 60 dB or worse).

Our exclusion criteria were: tinnitus from disease of the outer ear (occlusive exostosis, outer otitis), Ménière's disease, chronic otitis media, otosclerosis, history of ototoxic drugs use, exposure to massive noise, history of previous malignancy with chemotherapy, history of autoimmune disorders, neurodegenerative or demyelinating disease, uncompensated medical disorder or a serious psychiatric disorder. Additionally, patients unable to comprehend and sign the informed consent or with cognitive impairment were also excluded.

All participants signed a written informed consent and all the data were blind coded.

Clinical Evaluation

Data were collected from all participants concerning their personal clinical history (past and present), family history, and audiological assessment, including the rating of tinnitus intensity in a scale from 0 to 10 (being 10 the loudest possible). As part of the clinical evaluation was included a complete ENT observation.

Epidemiologic data (demographic, previous and present diseases, toxicological habits, and exposure to noise) was collected using a structured interview.

All participants were subjected to immittance to rule out middle ear pathology (Model: Madsen Zodiac 901, Serial No.:389122).

Audiological assessment

Tonal Audiometry:

Hearing thresholds were determined by pure tone audiometry (air and bone) according to ISO 8253 and 389. The exam was performed in a soundproof booth, (Model: IAC), using an Interacoustics®, Assens, Denmark audiometer (Model: AC40, Serial No.: 98 019 046) and TDH39/HDA300 headphones fitted with noise-excluding headset ME70 and bone conductor B-71. Audiometry was performed at frequencies from 0.25 kHz to 16 kHz (standard tonal audiometry and extended high frequency). The category of Hearing Loss (HL) was defined according to the recommendations of Bureau International d'Audiophonologie (BIAP): normal or subnormal hearing (below 20dB), mild hearing loss (21-40), moderate hearing loss (41-70), severe hearing loss (71-90), very severe hearing loss (91-119) or total hearing loss – cophosis (over 120). Pure tone average (PTA) was taken as the average threshold across 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz. Frequencies not heard were recorded as 120 dB threshold. Retrieved May 15, 2018 from: http://www.biap.org/en/recommandations/recommendations/tc-02-classification/213-rec-02-1-en-audiometric-classification-of-hearing-impairments/file.

"High frequency" pure-tone average (HF_PTA) was calculated as the average thresholds across 2, 4, and 8 kHz (D. L. Newman et al., 2012).

Tinnitus assessment

Several tests for measurement and evaluation of tinnitus were performed to all the participants.

Psychoacoustic tinnitus evaluation:

Tinnitus evaluation was performed after audiometric testing in a soundproof booth using an Interacoustics®, Assens, Denmark audiometer (Model: AC40, Serial No.: 98 019 046) and TDH39/HDA300 headphones fitted with noise-excluding headset ME70. First, it was established whether the tinnitus percept was more similar to a pure tone or a noise. Both sounds were presented to the participant who was asked which of the two

had most resemblance to their tinnitus. Estimation of tinnitus frequency was then performed using frequencies from 125 to 16000Hz (pure tones or narrow-band noise centered on the same frequencies). The procedure for determining tinnitus pitch was a forced choice between two presented stimuli. Stimuli were presented to the participant who identified which most closely resembled their tinnitus. The test continued until a correspondence between the tinnitus and the presented stimulus was found.

For the estimation of tinnitus loudness (intensity) the elected frequency (from previous step) was offered at an intensity similar to the individuals hearing threshold and gradually increased (5dB steps) until is reached the closest matching to participants tinnitus percept.

Loudness discomfort levels (LDL):

The collection of the discomfort thresholds is performed for each ear individually on the frequencies tested in the tonal audiogram as well as for the frequency at which the tinnitus was identified using pure tones. The patient is instructed to signal when the sound becomes uncomfortable, not only loud but uncomfortable. The method used to collect the discomfort thresholds is ascending and three tests should be carried out to investigate the thresholds to ensure the reliability of the test (Goldstein & Shulman, 1996).

The difference between the auditory threshold and the discomfort thresholds gives us the auditory dynamic field (Goldstein & Shulman, 1996). Once this is determined, the presence or absence of hyperacusis is evaluated.

Feldmann masking curves or Minimum Masking Levels (MML):

This test was performed at the frequencies where tonal audiometry was tested, using narrow band noises or pure tones (where tinnitus was not masked by narrow band noises). Sound was presented in 5dB steps (1-2 seconds stimulation), from hearing thresholds, until the participant reported that they could no longer hear their tinnitus. According to the spatial relationship of the resulting curves from hearing thresholds and tinnitus masking, MMLs were categorized: 1 - Convergent; 2 – Divergent; 3 – Congruent; 4 – Distant; 5 – Persistent (Lokenberg R et al, 2000).

Residual inhibition:

Residual inhibition (RI) was tested by presenting participants with a narrow band noise centered at their tinnitus pitch, at 10 dB above the tinnitus loudness, for 1 minute. RI was categorized: 1 – complete (tinnitus is not audible); 2 – partial (tinnitus became quiter); 3 – negative (no change at tinnitus percept); and 4 – “rebound” effect (tinnitus became louder). At categories 1 and 2 we measured the duration of time that tinnitus was abolished or diminished in seconds or minutes, the time that takes for the tinnitus

percept to come back to basal characteristics, in terms of loudness (Coles & Hallam, 1987; Goldstein & Shulman, 1997).

THI

Self-reported tinnitus severity was measured using the Portuguese validated version of Tinnitus Handicap Inventory (THI (Newman et al., 1996; Oliveira & Menezes, 2008). This inventory consists of 25 questions related to tinnitus, with "Yes", "Sometimes" and "No" as possible responses, corresponding to scores of 4, 2 and 0 respectively, giving a total score between 0 and 100. This questionnaire consists of three different sub-scales: Functional (11 items - contributing 0-44 for the final result), Emotional (9 items - contributing 0-36 for the final result) and Catastrophic (5 items - contributing 0-20 for the final result). Severity is interpreted according to the total score where 0-16 = Slight or no handicap (Grade 1), 18-36 - Mild handicap (Grade 2), 38-56 = Moderate handicap (Grade 3), 58-76 = Severe handicap (Grade 4), and 78-100 = Catastrophic handicap (Grade 5).

Auditory Brainstem Response

ABR examination was performed in a sound proofed electrically insulated room Participant were placed in a comfortable position in order to have good relaxation of cervical muscles. The Vivosonic audiometer system (Model: Integrity^{TM} V500, Serial No. IP0960) was used to collect ABR and find electrophysiological thresholds. The earphones used were the ER-3A, calibrated according to ANSI S3.6-1996, and a 4000 Hz tone burst was used to evoke ABR, calibrated in decibel peak-equivalent to the sound pressure level (dBpeSPL) (Jiang, 1998). We used an alternating split polarity with a stimulus rate of 27.7 stimuli/s, high pass filter cutoff frequency at 30 Hz and low pass filter cutoff frequency at 1500 Hz, high pass filter rollof at 12dB/Octave and low pass filter rollof at 24dB/Octave, notch filter off, a Blackman windowing and a Rise-Plateau-Fall of 2-0-2. The non-inverting electrode was placed according to the 10-20 system at Frontal Upper Forehead (Fz) and the inverting at the mastoid ($M_{1,2}$) at the examining side (Jasper, 1958). The neutral electrode is placed at Frontal Lower Forehead (Fzd) region. The parameters evaluated monoaural were the absolute latencies for waves I, III and V, interwave (interpeak) latency interval (IWI) for waves I-III. III-V, and I-V, V/I amplitude ratio.

Distortion Product Otoacoustic Emissions

DPOAEs were performed with a Vivosonic audiometer system in a sound proofed room. We tested the DPOAEs for the frequencies of 500, 750, 1000, 1500, 2000, 2500, 3000, 3200, 3500, 4000, 4500, 5000, 5500, 6000, 7000 and 8000 Hz, with a 1.22 F2/F1 ratio and with an intensity of 65 dB SPL and 55 dB SPL for L1 and L2, respectively. The presence of OAEs was considered when the signal-to-noise ratio (SNR) was equal to or above 6 dB.

Statistical analysis

All the variables considered were analyzed and when appropriated a data modelling phase was performed.

An exploratory analysis of all registered variables was carried out initially, followed by a data modeling phase. Categorical data were presented as frequencies and percentages, and continuous variables as median and inter-quartile range (25^{th} percentile; 75^{th} percentile), as they presented asymmetric distributions and deviations from normality. When appropriate or clinically relevant, some of the variables were categorized.

In the univariable study, to access the association between the presence of tinnitus and the demographic and audiological variables, nonparametric tests were used. The qualitative variables were analyzed by the chi-square test or Fisher's exact test, and the continuous variables by the Mann-Whitney U or Kruskal-Wallis tests.

Additionally, a univariable logistic regression analysis was used to obtain odds ratios estimates ($\widehat{OR}$) and corresponding 95% confidence intervals (95%CI). Logistic regression logit linearity assumption was assessed using the Box-Tidwell test (Box & Tidwell, 1962).

With the purpose of evaluating the discriminative capacity of certain audiological parameters the area under the ROC (receiver operating characteristic) curve (AUC), was reported.

The level of significance α=0.05 was considered. Statistical analysis was performed using the SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

With the purpose of evaluating the discriminative capacity of certain audiological parameters the (AUC), area under the ROC (receiver operating characteristic) curve was reported. The bilateral statistical tests were considered significant when the respective p values were lower than the significance level of 0.05. Statistical analysis was performed using the SPSS program Statistics® version 22.0. (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

Results

Participant’s demographics and comorbidities

Participants in our study were 114 elderly with median age of 63.0 (59.8; 68.3) years old. Most participants were female (n=60, 52.6%), presenting a median age of 63.5 (59.0; 68.3) years old. For men (n=54, 47.4%), the median age was 63.0 (60.0; 68.5) years old.

Concerning to comorbidities, mumps are present in 56% in the tinnitus sample and 31.8% for the sample without tinnitus. Also, 53.3% the population with tinnitus and 22.7% without tinnitus have hearing loss. We have only found statistically significant differences between the two subgroups for mumps (p=0.041**)** and hearing loss (p=0,010).

Audiological assessment

Two levels of statistical analysis were done. The first considered the sample divided in two groups, individuals with tinnitus and individuals without tinnitus. The second level of analysis was considered dividing our sample into four subgroups (Table 5.4-10), the subgroup without hearing loss and without tinnitus considered as control group (subgroup 1), subgroup 2 formed by individuals without hearing loss but presenting tinnitus, subgroup 3 composed by individuals with hearing loss but without tinnitus and the subgroup 4 with individuals with hearing loss and with tinnitus. These groups allow the comparison of the presence of tinnitus (subgroup 2 + subgroup 4) with its absence (subgroup 1 + subgroup 3).

Table 5.4-10 - Distribution of the individuals of the sample by 4 subgroups.

Subgroup	Audiological Characteristic	Gender (n)		n (%)	Median Age (years)
		Male	Female		
1	PTA≤20 without Tinnitus	5	12	17 (14.9%)	63.0 (59.8; 68.3)
2	PTA≤20 with Tinnitus	15	27	42 (36.8%)	
3	PTA >20 without Tinnitus	3	2	5 (4.4%)	
4	PTA >20 with Tinnitus	31	19	50 (43.9%)	
Total		54	60	114	

Legend: PTA= Pure Tone Average.

The described subgroups can be distinguished at tonal audiometry (Figure 5.4-4) where the best hearing thresholds are in the control group (subgroup 1- individuals with no hearing loss and no tinnitus).

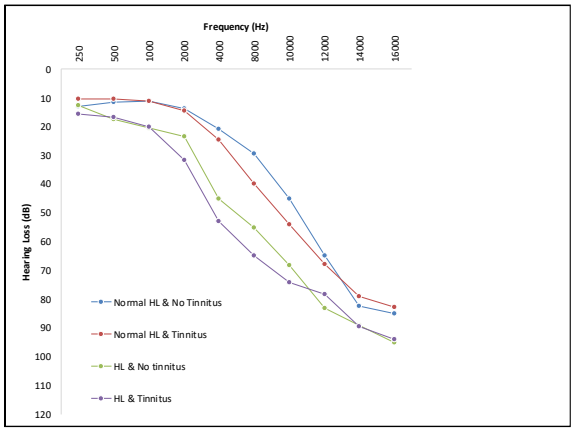

Figure 5.4-4 - Tonal Audiometry (average curves) in each of the 4 subgroup.

In the comparison of tinnitus patients versus non-tinnitus patients (Figure 5.4-5), PTA and HF_PTA were statistically higher in those individuals with tinnitus (Table 5.4-11).

Table 5.4-11 - PTA and HF_PTA according tinnitus presence.

Variables	All patients (n=114)	With Tinnitus (n=92;80.7%)	Without Tinnitus (n=22;19.3%)	*p* value
PTA_mean_RE_LE	20.0 (15.0; 28.3)	21.6 (16.4; 29.4)	15.9 (11.7; 20.3)	0.003
HF_PTA_mean_LE	36.7 (23.3;48.3)	40 (28.8; 50.0)	22.5 (18.3; 31.7)	< 0.001
HF_PTA_mean_RE	33.3 (23.3;46.7)	36.7 (27.1; 48.3)	22.5 (16.7; 28.3)	< 0.001
HF_PTA_mean_RE	35.4 (23.1; 46.9)	37.9 (28.8; 48.3)	22.8 (17.9; 29.8)	< 0.001*

Legend: Data are summarized as median (25th percentile; 75th percentile); PTA= Pure Tone Average, RE= Right Ear, LE= Left Ear, HF_PTA="High frequency" pure-tone average

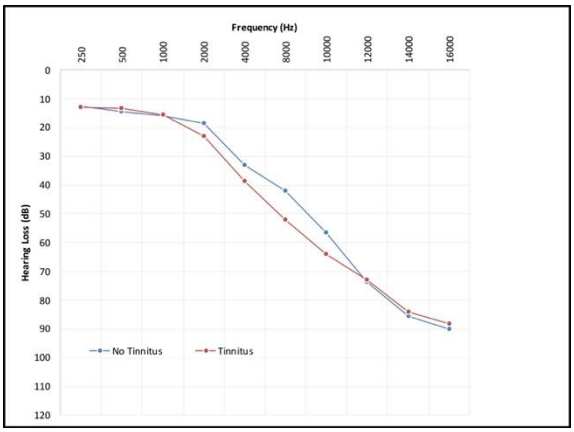

Figure 5.4-5 - Tonal Audiometry according to the presence of tinnitus.

Tinnitus Evaluation

Clinical characteristics of tinnitus in the individuals of the sample presenting it (n = 92) are presented in Table 5.4-12. The median duration of tinnitus was 5.0 years (2.0; 10.0), with a median intensity of 3.0 (2.0; 4.0) on a scale of 1-10. Tinnitus was constant in the majority of participants (88.9%), while onset was gradual for 71.4% and abrupt for 28.6%. In most participants tinnitus worsened in situations where they were nervous (59.3%). Finally, 50.6% of the participants with tinnitus reported reduced noise tolerance.

Table 5.4-12 - Clinical characterization of tinnitus sample.

Clinical variables	Participants with tinnitus (n=92)
Tinnitus Duration (in years)	5.0 (2.0; 10.0)

Intensity of tinnitus (scale 1-10)	3.0 (2.0; 4.0)
Manifestation of tinnitus (n=90)	
Constant	80 (88.9%)
Intermittent	6 (6.7%)
Pulsatile	4 (4.4%)
How did tinnitus begin? (n=63)	
Gradual	45 (71.4%)
Abrupt	18 (28.6%)
Does tinnitus gets worse when you're nervous? (n=90)	
Yes	54 (59.3%)
No	37 (40.7%)
Lower noise tolerance (n=89)	
Yes	45 (50.6%)
No	44 (49.4%)
Familiar history with Tinnitus	
Yes	25 (29.4%)
Dizziness	
Yes	35 (41.2%)
With deafness	
Yes	49 (53.3%)
Noise exposition n=113	
Non exposed	56 (61.5%)
Exposed without protection	31 (34.1%)
Exposed with protection	4 (4.4%)

Psychoacoustic estimates of tinnitus are given in Table 5.4-13. Frequencies matched to tinnitus pitch ranged from 2000 Hz to 8000 Hz, with 4000 Hz being the most frequently found, while the loudness is 0 dB (with a variation of + or – 5dB considering hearing threshold). Our sample is characterized by a majority in central laterality (52.4%) and pure tone type (59.0%) of tinnitus. Concerning to Feldmann's curve, the convergent (47.6%) and distant type (29.8%) are the most frequent, while in the residual inhibition, the negative (43.9%) and partial (36.6%) characterize a large part of our sample.

Table 5.4-13 - Psychoacoustic tinnitus assessment.

Audiological measurements	Participants with tinnitus (n=92)
Pitch (n=83)	4000Hz (2000Hz; 8000Hz)
Loudness (n=83)	0 dB (0 dB; 5.0 dB)
Laterality (n=84)	
Central	44 (52.4%)
Right	15 (17.9%)
Left	25 (29.8%)
Type (n=83)	
Pure Tone	49 (59.0%)
Narrow Band Noise	34 (41.0%)
Feldmann's Curve (n=84)	

Congruent	17 (20.2%)
Convergent	40 (47.6%)
Divergent	1 (1.2%)
Distant	25 (29.8%)
Persistent	1 (1.2%)
Residual inhibition (n= 82)	
Negative	36 (43.9%)
Partial	30 (36.6%)
Complete	13 (15.9%)
Rebound Effect	3 (3.7%)

Tinnitus severity was categorized by means of the THI scores. The majority of the individuals had a mild handicap (38 participants), followed by moderate handicap (22), slight or no handicap (17), severe handicap (14) and finally, one participant had a catastrophic handicap score (Figure 5.4-6).

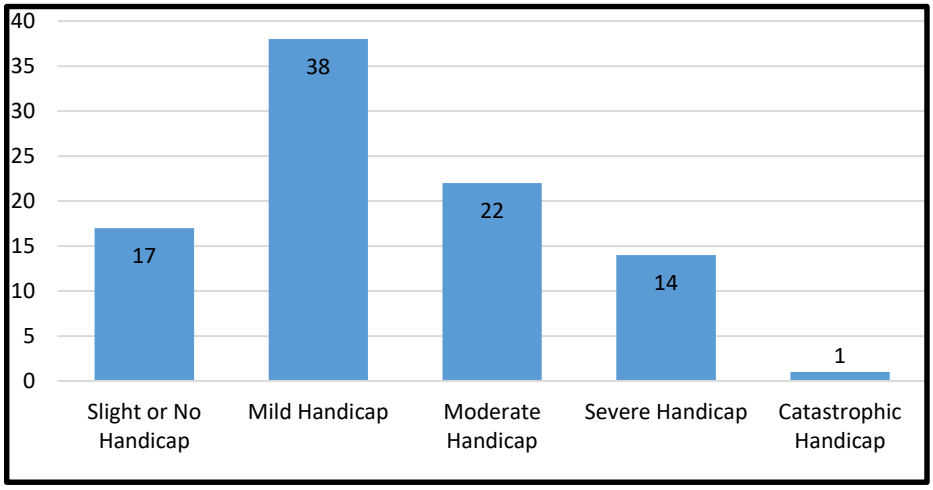

Figure 5.4-6 - THI scores for the tinnitus individuals of the sample.

Auditory Brainstem Response

Table 5 gives ABR absolute latencies for waves I, III and V, interwave (interpeak) latency interval (IWI) for waves I-III, III-V, and I-V, amplitude wave I and V, and V/I amplitude ratio. When comparing the patients with tinnitus and no tinnitus (Table 5.4-14) we can see a statistically significant difference in the amplitude of wave I (p=0.006) (Figure 5.4-7).

Table 5.4-14 - Comparison of Auditory Brainstem Response between patients with and without tinnitus.

Auditory Brainstem Response	All patients (n=114)	With Tinnitus (n=92;80.7%)	Without Tinnitus (n=22;19.3%)	p-value
ABR_mean_LE_RE_Lat_I	2.0 (1.9; 2.1)	2.0 (1.9; 2.2)	2.0 (1.9; 2.1)	0.191
ABR_mean_LE_RE_Lat_III	4.2 (4.1; 4.4)	4.2 (4.1; 4.4)	4.2 (4.1; 4.4)	0.928
ABR_mean_LE_RE_Lat_V	6.2 (6.0; 6.3)	6.2 (6.0; 6.3)	6.1 (6.0; 6.3)	0.974
ABR_mean_LE_RE_Lat_I-III	2.2 (2.1; 2.3)	2.2 (2.1; 2.3)	2.2 (2.1; 2.4)	0.202
ABR_mean_LE_RE_Lat_III-V	1.9 (1.8; 1.9)	1.9 (1.8; 2.0)	2.0 (1.9; 2.0)	0.941
ABR_mean_LE_RE_Lat_I-V	4.1 (4.0; 4.3)	4.1 (4.0; 4.3)	4.2 (4.1; 4.3)	0.255
ABR_mean_LE_RE_Amp_I	0.7 (0.4; 1.1)	0.7 (0.4; 1.0)	0.9 (0.6; 1.5)	0.006
ABR_mean_LE_RE_Amp_V	0.2 (0.1; 0.3)	0.2 (0.1; 0.2)	0.2 (0.1; 0.3)	0.180
ABR_mean_LE_RE_Ratio_V/I	2.9 (1.8; 6.6)	3.2 (1.9; 7.7)	2.2 (1.6; 3.6)	0.118

Legend: Data are summarized as median (25th percentile; 75th percentile); ABR= Auditory Brain Responses; RE=Right Ear; LE= Left Ear; Lat_I= Latency of wave I; Lat_III=Latency of wave III; Lat_V=Latency of wave V; Amp_I=Amplitude of wave I; Amp_V=Amplitude of wave V; ABR_RE_LE_Ratio=of wave V/I amplitudes.

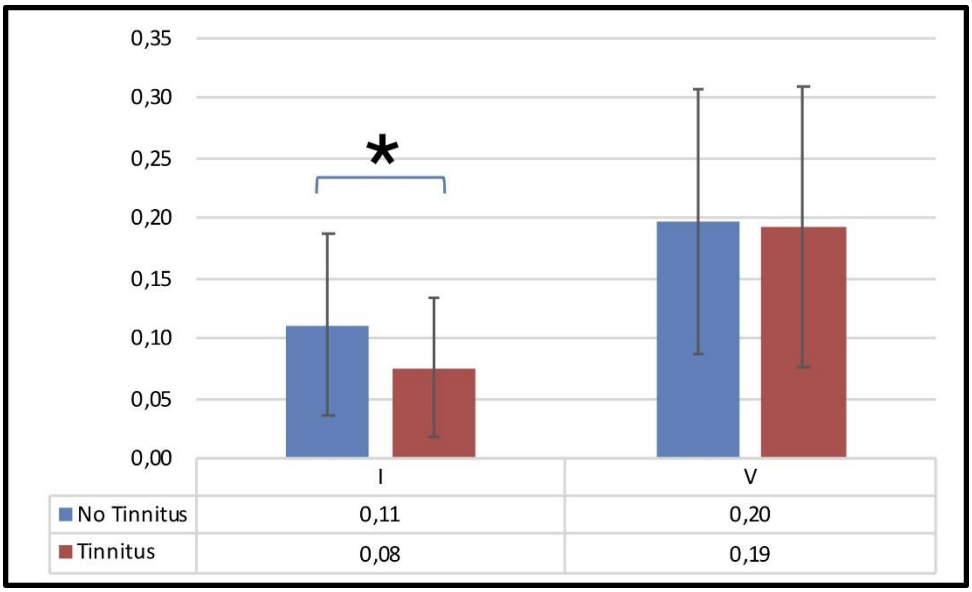

Figure 5.4-7 - Amplitude of wave I and V in patients with tinnitus and no tinnitus.

When comparing the 4 subgroups in relation to ABR variables, statistically significant differences were found with respect to the I-III interval (Figure 5.4-8) when comparing the subgroup 1 (individuals without hearing loss and with no tinnitus) with the subgroup without hearing loss and tinnitus (p=0.050) (Figure 5.4-8).

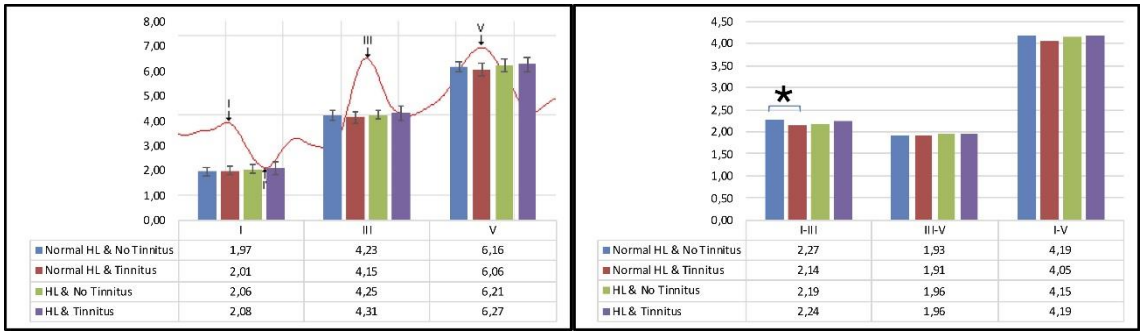

Figure 5.4-8 - Absolute latencies for waves I, III and V, and interpeak latency intervals I-III, III-V and I-V for the four subgroups.

Distortion Product Otoacoustic Emissions

In the evaluation of DPOAEs we found that the subgroup with tinnitus presents lower values (Table 5.4-15 and Figure 5.4-9).

Table 5.4-15 - Comparison of Distortion Product Otoacoustic Emissions in patients with and without tinnitus.

Variables	All patients (n=114)	With Tinnitus (n=92;80.7%)	Without Tinnitus (n=22;19.3%)	*p*-value
Mean_500_8000_ DPOAE _RE	13.7 (11.7; 16.5)	13.2 (11.5; 16.2)	14.9 (12.5; 18.5)	0.041
Mean_500_8000_ DPOAE _LE	13.0 (11.1; 16.1)	12.5 (11.1; 15.5)	14.9 (12.1; 17.9)	0.028

Legend. Data are summarized as median (25th percentile; 75th percentile); DPOAE = Distortion Product Otoacoustic Emissions; RE=Right Ear; LE= Left Ear.

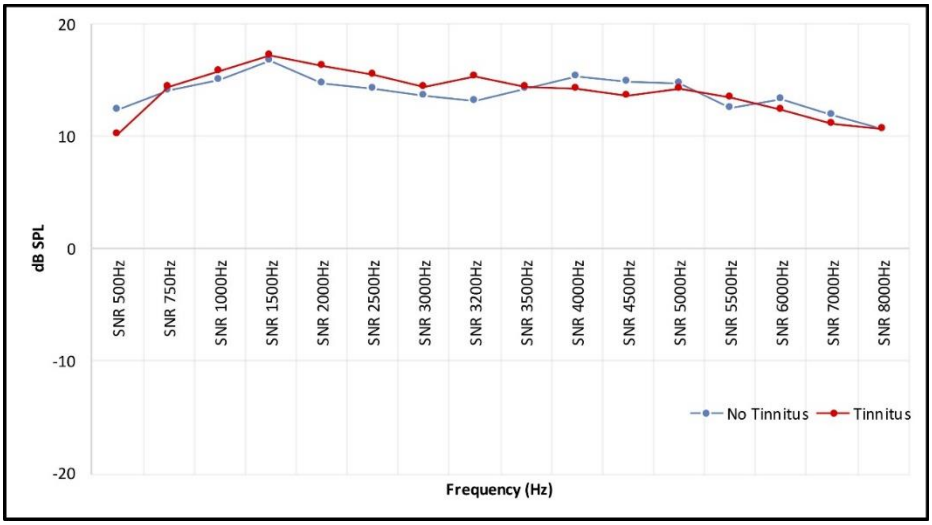

Figure 5.4-9 - Distortion Product Otoacoustic Emissions according to the presence of tinnitus.

Modelling the data according to presence of tinnitus

Considering the presence of tinnitus, a model phase was performed in order to identify the effects of the presence of tinnitus. Results of logistic regression at univariable analysis are shown in Table 5.4-16.

Table 5.4-16 - Univariable analysis: logistic regression model for the presence of tinnitus.

Variable	$\widehat{OR}$	*p*-value	(95%CI)
Noise exposure	3.96	0.036	(1.09, 14.36)
PTA_mean_RE_LE	1.09	0.009	(1.02, 1.17)
Hearing Loss	3.87	0.014	(1.32, 11.39)
Mumps	2.73	0.046	(1.02, 7.34)
HF_PTA_ mean_RE	1.07	0.001	(1.03, 1.11)
HF_PTA_ mean_LE	1.06	0.002	(1.02, 1.10)
HF_PTA_mean_RE_LE	1.07	0.001	(1.03, 1.11)
Mean_DPOEA_500_8000_LE	0.88	0.043	(0.77, 1.00)
ABR _RE_Amp_I	0.338[(1)]	0.004	(0.163, 0.701)
ABR _LE_Amp_I	0.479[(2)]	0.048	(0.231, 0.993)

Legend: [(1)] odds of having tinnitus for each ten units of increase of ABR _RE_Amp_I; [(2)] odds of having tinnitus for each ten units of increase of ABR _LE_Amp_I; $\widehat{OR}$ odds ratio estimate; CI confidence interval; PTA=Pure Tone Average; RE=Right Ear; LE= Left Ear; HF_PTA="High frequency" pure-tone average; DPOAE = Distortion Product Otoacoustic Emissions; ABR= Auditory Brain Responses.

According to the statistical model developed (Table 5.4-16) , several variables are associated with higher odds for tinnitus: "noise exposure" (p=0.036), "PTA mean for both ears" (p=0.009), "Hearing loss" (p=0.014), "Mumps" (p=0.046), "HFPTA_ mean for right ear (p=0.001), and for left ear" (p=0.002) and "HFPTA mean for both ears" (p=0.001). According to this model some variables are considerate with lower odds for developing tinnitus, namely "Mean OEA for left ear" (p=0.043) and "ABR wave 1 amplitude for right (p=0.004) and left ear" (p=0.048).

Modelling the data – OAE

Table 8 presents the results according to noise exposure in patients with tinnitus. Two different groups have been identified according whether or not have noise exposure. The comparison between this two groups only revealed significant differences for left ear (Table 5.4-17).

Table 5.4-17 - OEA results in patients with tinnitus, according to noise exposure condition (n=91).

Variables n (%)	Submitted to noise exposure (n=35;38.5%)	Non submitted to noise exposure (n=56;61.5%)	*p*-value
Mean_500_8000_DPOAE_RE	12.1 (11.5; 15.6)	14.7 (11.1; 16.7)	0.094
Mean_500_8000_DPOAE_LE	11.8 (10.1;13.7)	13.2 (11.2; 16.7)	0.038

Legend: Data are summarized as median (25th percentile; 75th percentile); DPOAE = Distortion Product Otoacoustic Emissions; RE=Right Ear; LE= Left Ear.

Modelling the data – severity of tinnitus

Table 5.4-18 presents the statistically significant results according to the severity at THI. Two different subgroups are identified, lower THI (slight or no handicap, light handicap and moderate handicap) and high THI scores (severe or catastrophic handicap). A significant difference concerning tinnitus onset (p=0.026) between slight or no handicap, light handicap and moderate handicap compared to patients with severe or catastrophic tinnitus. In the hyperacusis group there is a significant difference between lower and high tinnitus severity scores (p=0.007) and the same is verified for the residual inhibition (p=0.038).

Table 5.4-18 - Univariable analysis: Patient Characteristics by group (High versus lower THI score).

Variables	All patients (n=92)	Lower Score THI (n=77; 83.7%)	High Score THI (n=15;16.3%)	*p*-value
Tinnitus appearance (n=63)				
Gradual	45 (71.4%)	41 (77.4%)	4 (40.0%)	0.026(1)
Abrupt	18 (28.6%)	12 (22.6%)	6 (60.0%)	
Hyperacusis (n=85)				
Negative	66 (77.6%)	58 (80.6%)	8 (61.5%)	
Moderate	5 (5.9%)	4 (5.6%)	1 (7.7%)	0.007(2)
Light	11 (12.9%)	10 (13.9%)	1 (7.7%)	
Severe	3 (3.5%)	0 (0%)	3 (23.1%)	
Residual inhibition				
Negative	36 (43.9%)	34 (48.6%)	2 (16.7%)	
Partial	30 (36.6%)	21 (30.0%)	9 (75.0%)	0.038(2)
Complete	13 (15.9%)	12 (17.1%)	1 (8.3%)	
Rebound Effect	3 (3.7%)	3 (4.3%)	0 (0%)	

Legend: (1) Chi-square Test p-value; (2) Fisher Exact Test p-values.

Table 5.4-19 - Univariable analysis logistic regression model: Tinnitus Characteristics by group (Higher versus lower THI score).

Variables	$\widehat{OR}$ (95% CI)	*p*-value
Tinnitus appearance Gradual	5.125 (1.240, 21.188)	p=0.024

Abrupt		
Hyperacusis	7.556[(1)] (1.603, 35.616)	
Negative + Light		p=0.011
Moderate + Severe		
Residual inhibition	0.178[(2)] (0.036, 0.874)	
Complete + Partial		p=0.033
Negative + Rebound Effect		

Legend: $\widehat{OR}$ odds ratio estimate; [(1)] reference category is light or negative hyperacusis; [(2)] reference category is partial or complete

Considering patient characteristics (Table 5.4-19), individuals with abrupt tinnitus onset were 5.13 times more likely to develop severe or catastrophic tinnitus($\widehat{OR} = 5.125, p = 0.024, CI = 1.240 - 21.188$). Regarding sound tolerance, the participants with moderate or severe sensitivity to sound had 7.56 times greater odds of developing severe or catastrophic tinnitus($\widehat{OR} = 7.556, p = 0.011, CI = 1.603 - 35.616$). In respect to the residual inhibition, the presence of a negative or rebound effect was associated with lower odds of catastrophic tinnitus $\left(\widehat{OR} = 0.178, p = 0.033, CI = 0.036 - 0.874\right)$. In fact, those patients had an 82.2% lower odds of developing a severe or catastrophic tinnitus compared to those who had a partial or complete residual inhibition. In relation to Feldmann masking curves, the patients with divergent, distant and persistent curve have 85.6% less chance of having severe or catastrophic tinnitus in comparison to participants with congruent or convergent curve. However, the association is not statistically significant (p-value = 0.07).

Concerning auditory brain response evaluation, the logistic regression model showed that the mean ratio of PEAP for both ears the odds are 1.1 times higher for developing severe or catastrophic tinnitus($\widehat{OR} = 1.1, p = 0.046, CI = 1.002 - 1.208$).

Although several variables have been identified in the univariable analysis as potential candidates to the multivariable analysis, no multiple model was obtained.

Discussion

In this study, we have explored and presented an exhaustive audiological evaluation of older patients, presenting with or without hearing loss and/or tinnitus.

Our data confirm that the odds of developing tinnitus were significantly higher in the presence of noise exposure and hearing loss. Our data evidence that participants having an abrupt tinnitus onset have more chance to develop severe or catastrophic tinnitus. The immediate interpretation of this result is that people having a gradual tinnitus onset, develop easily a natural habituation processes (Hallam et al., 1984).

We found statistically significant differences for both the mean PTA and HFPTA when we compared patients with and without tinnitus, thus there is a possible correlation between the development of hearing loss and the appearance of tinnitus. These results are in agreement with the literature where has been hypothesized that tinnitus is an epiphenomenon of a neuronal process to attempt normalizing impaired hearing thresholds (Gollnast et al., 2017). One of the possible causes for tinnitus patients to present tonal thresholds at higher frequencies, higher than those without tinnitus, is probably due to our population, patients aged between 55 and 75 who present with presbycusis (sloping configurations). Studies in individuals with tinnitus on average revealed that they suffer from a stronger hearing loss in high frequencies compared with individuals with no tinnitus (Gollnast et al., 2017; Moore et al., 2010).

In the individuals of our sample, the pitch tinnitus frequency ranges from 2000Hz to 8000Hz, being 4000Hz the most frequently found. This could be explained by the expected localization of the tinnitus pitch in the “edge” frequencies or within the lowest regions in individuals presenting both hearing loss and tinnitus (Langers et al., 2012). Diminished peripheral input caused by hearing loss, particularly sloping configurations, as observed in our sample, can lead to reorganizations of the tonotopic framework of the AC (Eggermont, 2003; Pinkl et al., 2017). Such tonotopic reorganizations over-represent neural activity near tonotopic regions of the “edge” frequency or midpoint of the hearing loss slope (Eggermont, 2003; König et al., 2006).

Residual inhibition, transient modulation in tinnitus loudness (or abolishment) after a presentation of a sound, can be achieved in the vast majority of tinnitus patients and is thought to suppress tinnitus by temporarily reducing the underlying hyperactivity in the ascending auditory pathway (Roberts, Moffat, Baumann, Ward, & Bosnyak, 2008). Our data have shown that the presence of a complete (tinnitus abolishment) or rebound effect (tinnitus exacerbation) in residual inhibition was found at a statistical regression model as a “protector factor”. One possible way of viewing this result is that patients with a complete residual inhibition easily mask their tinnitus with environmental sounds, so it is expectable that tinnitus will not become very disturbing because is only audible in very silent places (e. g. bedroom at sleeping time).

We also defend that the rebound effect can be correlated with hyperacusis phenomena which are easily treatable with desensitization techniques using sound enrichment. In our tinnitus population, three participants have a rebound effect on residual inhibition and from those 2 have mild hyperacusis and 1 has moderade level. One relevant demonstration of hyperacusis treatment comes from Noreña and Chery-Croze (2007) who showed that passive exposure to a low-level, complex background sound covering the hearing loss region for a few hours a day for 15 weeks rescaled abnormal loudness tolerance by as much as 15 dB in hyperacusis patients (Noreña & Chery-Croze, 2007).

In analogy with the sensation of pain and phantom limb perception, tinnitus emerges from damages in the cochlea (e.g. hair cell loss or synaptic damages) that leads to a frequency-specific decrease in electric output towards the brain. Our clinical data show that participants with tinnitus have higher hearing thresholds and interestingly that participants with moderate, light and severe hyperacusis have more risk to develop a severe or catastrophic tinnitus (THI scores). Data from the literature points out that there are common pathways for the pathophysiology of tinnitus and hyperacusis resulting as a central compensatory gain due to reduced neural activity from a damaged cochlea.

ABR measures in tinnitus patients with normal PTA revealed lower Wave I amplitudes pointing out the existence of an auditory nerve damage (Schaette & McAlpine, 2011). Lower wave I amplitude correlates with partial synaptic uncoupling (or de-afferentation) of the inner hair cells (IHC) and the afferent neurons at specific frequency-regions where tinnitus is thought to be perceived (Bauer, Brozoski, & Myers, 2007; Rüttiger et al., 2013; Singer et al., 2013; Tan, Lecluyse, McFerran, & Meddis, 2013; Weisz, Hartmann, Dohrmann, Schlee, & Norena, 2006).

Regarding the ABR our main findings were in waves I and III, namely in the reduction of the amplitude in the wave I in patients with tinnitus, and an increase of the interval I-III when compared the group without hearing loss with tinnitus, and the group with hearing and tinnitus. The reduction of the wave I amplitude is in accordance to the published studies in tinnitus patients (Attias, Urbach, Gold, & Shemesh, 1993; Gu et al., 2012; Schaette & McAlpine, 2011). As mentioned before, wave I arise from the distal portion of the auditory nerve (within the inner ear) (A R Møller et al., 1995). There are several possibilities for this reduced amplitude in wave V, particularly changes in the IHC and or Auditory Nerve Fibers (ANFs). In relation to IHC, there may be a diffuse loss of them compared with patients with no tinnitus that results in a lowered wave I amplitude (Gu et al., 2012). In another model the IHC are equally intact in tinnitus patients compared to patients without tinnitus but in one of them, there is a diffuse loss of the ANFs while in the other the ANFs remains equally intact when the two groups are compared (Gu et al., 2012; Le Prell, Halsey, Hughes, Dolan, & Bledsoe, 2005; Le Prell, Shore, Hughes, & Bledsoe, 2003). Other possibilities are that ANFs are equally intact, and the reduction of the amplitude wave I is due to the reduced excitability of ANFs via lateral olivocochlear efferents which terminate on their endings, or there is a diffuse loss of ANFs sufficient to manifest a reduction in mean wave I amplitude.

Another finding regarding the ABR is the statistical difference with respect to the interpeak latency I-III when comparing the subgroup without hearing loss and no tinnitus, and the subgroup without hearing loss and tinnitus. When we compared both subgroups we can see a diminished interval interpeak I-III in the group with normal hearing with tinnitus. According to a literature review, we didn't find similar results. However, although we did not find significant differences in absolute latency of wave I

latency in our sample when we compared both subgroups, in the subgroup with normal hearing and tinnitus, wave I started later when compared to the subgroup with normal hearing and without tinnitus. This could be the reason for the difference in the interpeak latencies I-III when we compared both groups since the wave I, according to several authors, in tinnitus patients had a significant prolongation (Ikner & Hassen, 1990; Kehrle et al., 2008; Lemaire & Beutter, 1995; Rosenhall & Axelsson, 1995). This prolongation in wave I in tinnitus patients signaling a peripheral lesion in the auditory system (Kehrle et al., 2008; Rosenhall & Axelsson, 1995). Lemaire & Beutter, 1995 found a significantly lengthened in wave I too in tinnitus patients and suggests that this modification is due to a dysfunction of the nucleus tegmenti which is part of the efferent system (Lemaire & Beutter, 1995). Future research should be performed in this direction in order to clarify this finding.

Our findings were more relevant for the ABR amplitude wave I as a protective factor to the odds to have tinnitus and Ratio RE_LE as a risk factor to the odds of having a severe or catastrophic grade of tinnitus. This parameter is also related to the amplitude of waves I and V.

As we can see above, the literature regarding the levels of the DPOAEs in tinnitus patients can be controversial. From our data, we have to conclude that DPOAEs results are lower when we compared patients with and without tinnitus (Table 6 and Figure 6). However, in this case, we don't consider the presence or not of hearing loss. Our results are in agreement with the results reported by Ozimek et al. and Shiomi et al., which points us to conclude that these observed changes are specific to the functions of OHC instead of nonspecific non-linearity of the basilar membrane system (Ozimek et al., 2006; Shiomi et al., 1997).

Distortion product OAEs are statistically significant lower in participants that had noise exposure. In fact, this is a protective variable, and when it's higher the odds to have tinnitus diminishes. These results are in accord with the results of the work developed by Sindhusake et al (Sindhusake et al., 2003). To conclude, noise exposure is a risk factor for tinnitus.

Conclusions

Our study confirms that in older people tinnitus is highly correlated with hearing loss.

Current view on tinnitus is that it is a symptom encompassing a distributed network of peripheral and central pathways of the nervous system. Due to its complex nature, tinnitus should be approached in a multidisciplinary fashion involving different health professionals that are specialized to deal with each of the dimensions encompassed within this symptom (Hall et al., 2018a).

Our study puts in evidence some interesting findings especially concerning audiological tinnitus characteristics or its natural history. Our data may contribute to defining the odds of a patient to develop severe or catastrophic grade of tinnitus. And objectively the ABR Ratio OD_OE as a risk factor for having a severe or catastrophic grade of tinnitus, if confirmed in larger populations studies is a potential candidate as an audiological marker of tinnitus severity. These are certainly the most original contributions of this study since we have documented the relevant audiological tinnitus severity markers.

Important highlights of our findings go to the necessity to have appropriate tinnitus subtyping to understand the most probable underlying mechanisms and consequently the most appropriate management in terms of diagnosis and treatment strategies.

Future research should be designed to improve the sensitivity of non-invasive electrophysiological measures of cochlear synaptopathy in humans and to examine the broader neurophysiological impacts of noise exposure. In order also to have a clear distinction of mechanisms more specific for tinnitus or for hearing loss. Advancing knowledge concerning specific tinnitus subtypes origin and maintenance mechanisms is of paramount importance for adequate treatment.

5.4.3. Tinnitus, hearing loss and inflammatory processes in an older Portuguese population

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Abstract

Tinnitus is the perception of sound in the absence of acoustic stimulation and is frequently a consequence of hearing loss or activation of the somatosensory system. Tinnitus is associated with various conditions such as presbycusis, infectious and autoimmune diseases, and many other diseases.

Our study aims to identify an association between the inflammatory markers and the presence of tinnitus or HL.

Our sample included 60 women and 54 men (55 to 75 years) from the Portuguese population, with or without sensory presbycusis and/or tinnitus. All participants were subjected to a clinical evaluation through a structured interview, complete ENT observation, audiological evaluation, tinnitus scoring through Tinnitus Handicap Inventory and an evaluation of inflammatory markers.

There was a significant difference in IL10 between the group with and without tinnitus (p=.025). In the group with tinnitus, we found a significant difference in TGF-β (p=,034), in IL1α (p=.033) and in IL2 (p=.019) for the age, type of tinnitus and residual inhibition, respectively. Additionally, we observed a negative correlation between tinnitus duration and IL10 (r=-.281). In relation to HSP70 and loudness, the correlation coefficient was negative (r=-.377). Concerning the blood sample collection time, we had found a significant difference in the inflammatory parameters between the morning and afternoon period (p=.012). Lastly, we found significant results for the afternoon subgroup and the presence of tinnitus for IL10 (p=.032) and IFN-γ (p=.045).

The results of our study clearly demonstrate that inflammatory mechanisms are involved not only in hearing loss pathogenesis but also in tinnitus.

Keywords: Tinnitus; Inflammation; ARHL (Age-Related hearing loss); elderly.

Introduction

Tinnitus is the perception of sound in the absence of acoustic stimulation and is frequently a consequence of hearing loss (HL) or activation of the somatosensory system (Mazurek et al. 2010; Shore 2011). Tinnitus is frequently associated with various conditions such as presbycusis, ototoxicity, a number of infectious and autoimmune diseases and sleep disturbances, cognitive problems, psychological disorders and many other diseases (Seydel et al. 2013; Heller 2003; Hoffmann and Reed 2004).

Evidence suggests that frailty is due to a low-grade inflammatory response that persists for prolonged time, even in the absence of inflammatory stimulus (Hubbard et al. 2009; Leng et al. 2007; Qu et al. 2009a; 2009b). Thus, the mechanisms leading to frailty involve inflammation affecting the immune and neuroendocrine systems among others (Walston et al. 2006; Ferrari and Magri 2008; Poeggeler 2005) with inflammatory cytokines such as Interleukin (IL) 6, C-reactive protein and Tumor necrosis factor-α (TNF-α), playing an important role (Collerton et al. 2012; Leng et al. 2007; Qu et al. 2009a; 2009b).

Persistent tinnitus can have a significant negative impact on quality of life and frequently causes major psychological distress (Bartels et al. 2007). The relationship between tinnitus and distress is complex and manifests itself as the auditory attention focused on the tinnitus sound with consequent increased irritability, anxiety, depressive mood or somatic complains (Tyler et al. 2014, 2007; Hiller and Goebel 1992). More recently, research has focused on the link between stress or other psychosocial factors and inflammation markers. In fact, circulating levels of C-reactive protein, IL6 and TNF-α have been associated with psychological components of many disorders (Steptoe et al. 2007).

Tinnitus can also be regarded as a chronic stressor that interferes with cytokine production. The pro-inflammatory cytokines comprise interleukin 1 alfa and beta (IL1 α and IL1 β), IL 6 and TNF-α. Besides being associated with inflammatory or infectious diseases, changes in circulating levels of IL1 β, IL6 and TNF-α have also been associated with the aging process, exposure to stress, and some neurological disorders (Zhang et al. 2013). In addition, serum concentrations of IL1 β (Szczepek et al. 2014) and TNF-α are biological markers that have been correlated to tinnitus-related distress (Szczepek et al. 2014; Weber et al. 2002). However, IL 6 doesn't seem to associate with tinnitus-induced distress (Szczepek et al. 2014). Moreover, the neutrophil/lymphocyte ratio is a marker of stress and a parameter of systemic inflammation. In tinnitus, which is highly correlated with stress, the neutrophil/ lymphocyte ratio can be considered as a potential biomarker of tinnitus progression or degree (Ozbay et al. 2015).

The cochlear resident cells in the organ of Corti have immune competences and participate in the cochlear immune response to acoustic overstimulation (Cai Q et al. 2014). Disruption of gene expression related to pain and inflammation has been described as involved in noise-induced tinnitus and spontaneous hyperactivity in the

Cochlear Nucleus (CN) (Manohar et al. 2016). Also, the inputs from CN has been suggested as a mechanism of tinnitus by leading to the disruption of auditory-somatosensory pathway. This disruption results from maladaptive auditory-somatosensory plasticity, which is a form of axonal sprouting that is promoted by transforming growth factor (TGF-β) signaling, which can be inhibited by losartan (Mum et al. 2018).

Cochlear and auditory nerve degeneration may elicit a chronic neuroimmune response (activation of microglia) and the up-regulation of proinflammatory cytokines such as IL1β (Fuentes-Santamaria et al. 2013) through the up-regulation of the glutamate transporter Slc17a6.

Animal studies explored the association between HSP-70 protein and the auditory system (Trune et al. 1998; Gong and Yan 2002), being HSP – 70 associated to the increase of autoimmune response in the inner ear (Gong and Yan, 2002). Controversial results are published in the association of HSP-70 protein with HL, from no association (Trune et al.,1998) to its assumption as a prognosis marker of idiopathic sudden sensorineural hearing loss (ISSHL) (Düzer et al. 2014).

A relation between the decrease of TNF-α following a relaxation training program applied to patients with chronic tinnitus was described (Weber et al, 2002). This training program is described as responsible for a significant decrease in stress, anxiety depression, anger and other tinnitus associated disorders.

Although the association between proinflammatory cytokines and tinnitus has been rarely reported some authors have tried to study it and our study aims to identify association between the inflammatory markers and the presence or severity of tinnitus or HL. We expect that our work will contribute for advancing knowledge about tinnitus underlying mechanisms and consequently allow improving tinnitus diagnosis, treatment and prognosis.

Methods:

Participants

Our sample included 114 elderly individuals (n=60 women, n=54 men). Inclusion criteria were adults originating from the Portuguese population persons of both genders, aged between 55 to 75 years, presenting or not with sensory presbycusis and tinnitus.

For the purposes of inclusion, presbycusis was defined as bilateral sensorineural deafness in downslope audiometric pattern, above 1000 Hz with poor speech discrimination (discrimination threshold > 40 dB SPL and 100% discrimination to 60 dB or worse).

Exclusion criteria comprised inability to understand and sign the informed consent due to a significant cognitive impairment, an uncompensated medical disorder

that requires urgent evaluation or a presence of serious psychiatric disorder. Moreover, we have excluded individuals with one of the following conditions: Ménière's disease, chronic otitis media, otosclerosis, tinnitus induced by occlusive exostosis, otitis externa, or a history of ototoxic drugs use, noise exposure, chemotherapy.

This study had the approval of the Ethical Committees from Hospital Cuf Infante Santo (26 the November, 2014), Nova Medical School (nº65/2014/CEFCM) and the National Department of Personal Data Protection (authorization number:1637/2016). The study was conducted in accordance with the Declaration of Helsinki.

Clinical Evaluation

Data were collected from all participants concerning their personal clinical history (past and present), family history, and audiological assessment, including the rating of tinnitus intensity in a scale from 0 to 10 (being 10 the loudest possible). As part of the clinical evaluation, a complete ENT (Ear Nose and Throat) evaluation was performed.

Epidemiologic data (demographic, previous and present diseases, toxicological habits, and exposure to noise) were collected using a structured interview.

Audiological assessment:

Pure Tone Audiometry:

Hearing thresholds were determined by pure tone audiometry (air and bone) according to ISO 8253 and 389. The exam was performed in a soundproof booth, (Model: IAC), using an Interacoustics®, (Assens, Denmark) audiometer (Model: AC40, Serial No.: 98 019 046) and TDH39/HDA300 headphones fitted with noise-excluding headset ME70 and bone conductor B-71. Audiometry was performed at frequencies from 0.25 kHz to 16 kHz (standard tonal audiometry and extended high frequency). The category of hearing loss (HL) was defined according to the recommendations of the Bureau International d'Audiophonologie (BIAP): normal or subnormal hearing (below 20dB), mild hearing loss (21-40), moderate hearing loss (41-70), severe hearing loss (71-90), very severe hearing loss (91-119) or total hearing loss – cophosis (over 120). Pure tone average (PTA) was taken as the average threshold across 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz. Frequencies not heard were recorded as 120 dB threshold. Retrieved May 15, 2018 from: http://www.biap.org/en/recommandations/recommendations/tc-02-classification/213-rec-02-1-en-audiometric-classification-of-hearing-impairments/file.

"High frequency" pure-tone average (HF_PTA) was calculated as the average thresholds across 2, 4, and 8 kHz (Newman et al. 2012).

All participants were submitted to immittance to rule out middle ear pathology (Model: Madsen Zodiac 901, Serial No.:389122).

Tinnitus assessment

Psychoacoustic tinnitus evaluation:

This step was performed after audiometric testing in a soundproof booth using an Interacoustics®, Assens, Denmark audiometer (Model: AC40, Serial No.: 98 019 046) and TDH39 headphones fitted with noise-excluding headset ME70. At the beginning, we checked if the tinnitus percept was more similar to a tone or a noise, and the evaluation of tinnitus frequency was performed by offering frequencies from 125 to 16000Hz, two stimuli each time, asking the participants to choose, which was the sound more similar to its own precept. For the identification of tinnitus loudness (intensity), the elected frequency (from the previous step) was offered in an intensity similar to the hearing threshold, and loudness was gradually increased (5dB each step) until it reached the closest match to tinnitus percept.

Loudness discomfort levels (LDL):

This evaluation was performed for each ear individually, using pure tones (those from tonal audiometry) in an ascending method. The participant should state when the sound was uncomfortable (Goldstein and Shulman 1996).

Feldmann masking curves or Minimum Masking Levels (MML):

This test was performed at the frequencies where tonal audiometry was tested, using narrow band noises or pure tones (in case tinnitus was not masked by narrow band noises), using an ascending method, 5dB each step during 1-2 seconds, from hearing thresholds until the participant noticed he/she can't hear tinnitus. According to the spacial relation of the curves from hearing thresholds and tinnitus masking, this one was designated as: 1 - Convergent; 2 – Divergent; 3 – Congruent; 4 – Distant; 5 – Persistent, (Lokenberg R et al, 2000).

Residual inhibition:

Procedure: at the identified tinnitus pitch (frequency), the participant was stimulated with a narrow band noise, 10dB above the tinnitus loudness for 1 minute. According to participants responses, 4 categories were possible: 1) complete (tinnitus is not audible); 2) partial (tinnitus became quitter); 3) negative (no change at tinnitus percept); and 4) "rebound" effect (tinnitus became louder). At categories 1 and 2 we measured the time that tinnitus was abolished or diminished (Coles and Hallam 1987; Goldstein B, et al, 2007).

The severity of tinnitus was evaluated using the Tinnitus Handicap Inventory (THI; Newman et al. 1996). THI is a self-administered instrument, easy to quote, to interpret and has good psychometrics properties (McCombe et al. 2001). THI comprises 25 questions concerning tinnitus, and the response options were "Yes", "Sometimes" and "No", respectively corresponding to 4, 2 and 0, accounting for a total score that may vary between 0 and 100. The questionnaire comprehends 3 sub-scales or dimensions: Functional (11 items - contributing 0-44 for the final score), Emotional (9 items - contributing 0-36 for the final score) and Catastrophic (5 items - contributing 0-20 for the final score). This allowed to verify which were the most affected aspects and accordingly to choose the therapeutic interventions. The total score of the responses allowed tinnitus classification according to its severity or impact in daily life - 0-16: Slight or no handicap (Grade 1), 18-36: Mild handicap (Grade 2), 38-56: Moderate handicap (Grade 3), 58-76: Severe handicap (Grade 4), 78-100: Catastrophic handicap (Grade 5).

Evaluation of inflammatory markers

Venous blood samples were collected into tubes without anticoagulant agents. Samples were allowed to coagulate for 30 min at room temperature and were centrifuged afterwards. After the separation from cells, sera were further divided in labeled aliquots of about 500 µL, which were frozen at -80º C until analysis. Each aliquot was used only once.

For the evaluation of IL1α, IL1β, IL2, IL6, IL10, IFN (Interferon)-γ and TNF-α, a BD CBA Flex Set (BD Biosciences, San Jose, CA, USA) bead based multiplex assay was used. The protocol was performed following strictly the instructions of the manuracturer. In brief, after the preparation of standards and other ancillary reagents, serum samples were incubated with specific capture beads for 1 hour at room temperature in cytometry tubes. The detection reagent was then added to the samples and incubated for 2 hours at room temperature in the dark. After a washing step, beads were resuspended and analyzed using BD FACS Canto II flow cytometer, previously set up according to the BD CBA Flex Set recommendations. A minimum of 300 beads were acquired for each cytokine in each sample. The FCAP Array Software (BD Biosciences) was used for data analysis. Standard curves covering a 0–2,500 pg/mL concentration range were generated after serially diluting reconstituted standards. To be accepted, all 10-point standard curves should present at least r^2 >99.90. Minimum detection levels were: 1.0 pg/mL for IL1α; 2.3 pg/mL for IL1β; 11.2 pg/mL for IL2; 1.6 pg/mL for IL6; 0.13 pg/mL for IL10; 1.8 pg/mL for IFN-gama; and 0.7 pg/mL for TNF-alfa.

A similar BD CBA Flex Set protocol was performed for TGF-beta, using the Human TGF-β1 Single Plex Flex Set (BD Biosciences). The difference between this the the previous tests was that it requires activation of the latent TGF-β1 to its immunoreactive form. Therefore, Sample Activation Kit 1 (R&D, Minneapolis, MN, USA) was used with

samples being acidified for 10 minutes with 1N HCL and then neutralized using 1.2N NaOH/0.5M HEPES, according to the kit's procedure. After activation, samples were incubated with capture beads for 2 hours, washed and incubated with detection reagent. Acquisition and analysis were performed as described above. For TGF-beta, standard curves covered a 0–10,000 pg/mL concentration range, and minimum detection level was 14.9 pg/mL.

Finally, Heat Shock Protein 70 (HSP70/HSPA4) was assayed using the HSPA4 (HSP70) Human ELISA Kit (ThermoFisher, Frederick, MD, USA), a classical ELISA plate-based assay. Samples were assayed in duplicates, following the steps described in the manufacturer's instructions, including sample incubation with capture antibodies adsorbed in the plate, Biotinylated Antibody, Streptavidin-HRP Reagent, TMB Substrate and finally, Stop solution. After all washing and incubation steps, absorbances were assessed at 450nm in an ELISA plate reader (Stat Fax® 2100, Fisher Bioblock Scientific, France). Data were analyzed using Logit regression V21042005 free-software, available at www.xs4all.nl/~ednieuw. The Range for HSP70/HSPA4 was 2-600 ng/mL, and all mean values below the detection limit were evaluated as zero.

Results

Sample Distribution

We include in our sample 114 adults with median age of 63.0 (P_{25}=59.8, P_{75}=68.3) years old. Most of the individuals were female (n=60, 52.6%), presenting a median age of 63.5 (P_{25}=59.0, P_{75}=68.3) years old. For men (n=54, 47.4%), the median age was 63.0 (P_{25}=60.0, P_{75}=68.5) years old.

Participants were grouped primarily as 'tinnitus' versus 'no tinnitus', and secondarily, as 'with hearing loss' versus 'without hearing loss'. For some analyses we further subdivided patients' subgroups (1) without hearing loss and without tinnitus (control group), (2) without hearing loss but with tinnitus, (3) with hearing loss but no tinnitus, and (4) with hearing loss and tinnitus (Table 5.4-20). As such we could compare tinnitus (subgroup 2 + subgroup 4) with no tinnitus (subgroup 1 + subgroup 3).

Table 5.4-20 - Distribution of the individuals by subgroups.

Subgroup	Audiological Characteristic	Gender		n (%)	Median Age (years, percentile 25 and 75)
		Male	Female		
1	PTA≤20 without Tinnitus	5	12	17 (14.9%)	63.0 (59.8, 68.3)
2	PTA≤20 with Tinnitus	15	27	42 (36.8%)	
3	PTA >20 without Tinnitus	3	2	5 (4.4%)	
4	PTA >20 with Tinnitus	31	19	50 (43.9%)	
	Total	54	60	114	

Audiological assessment

The described subgroups can be distinguished at tonal audiometry (Figure 5.4-10) where the best hearing thresholds are in the control group (subgroup 1- individuals with no hearing loss and no tinnitus).

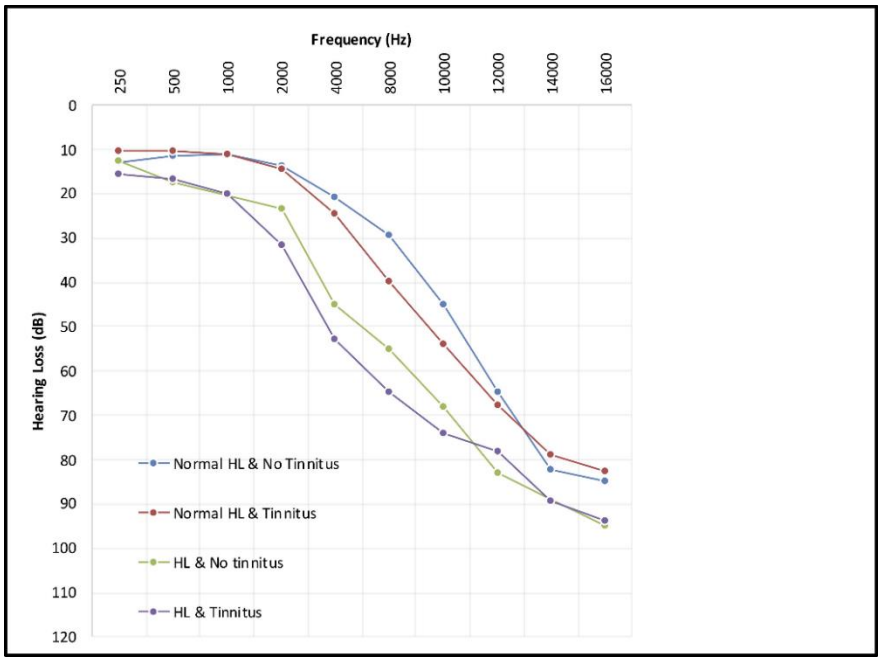

Figure 5.4-10 - Pure Tone Audiometry (average curves) in each of the 4 subgroups.

PTA and HF_PTA were higher in those individuals with tinnitus than those who did not have tinnitus (Figure 5.4-11).

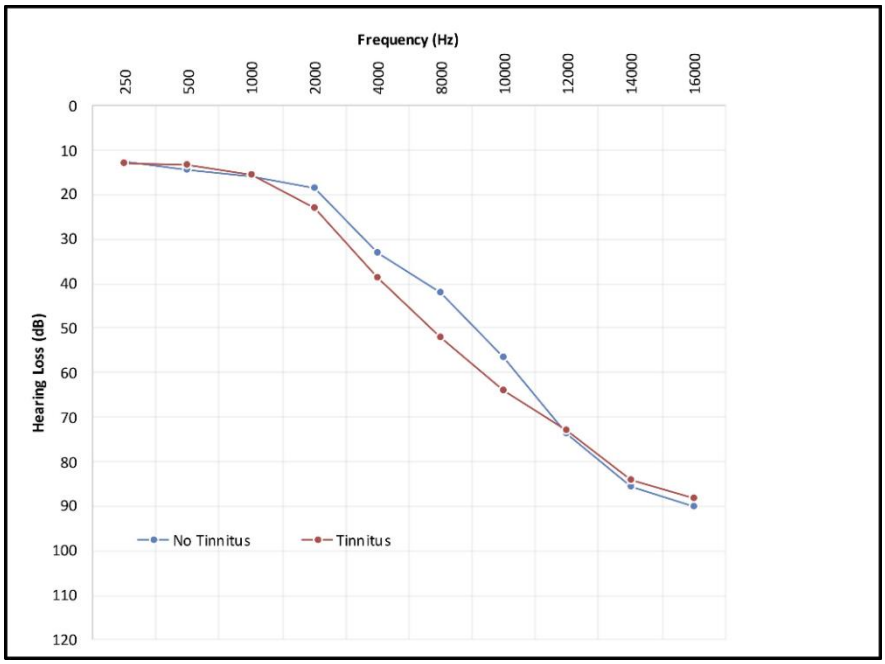

Figure 5.4-11 - Pure tone audiometry profiles based on the presence of tinnitus.

Tinnitus characteristics

The mean tinnitus duration was 7.8 ± 8.6 years. Mean tinnitus intensity was 3.3 ± 1.6, on a visual analogue scale (VAS) of 1-10 (Table 5.4-21). For most participants tinnitus was central (i.e. perceived in the head) (47.8%) and tonal (53.2%). In the majority of participants tinnitus was constant (87%). Tinnitus onset was gradual for 49% and abrupt for 19.5% of participants. Dizziness, often associated with tinnitus, was reported by 38% of participants with tinnitus, while 54.4% reported not having dizziness symptoms. In most participants, tinnitus worsened in situations where they were nervous (58.7%). Reduced sound tolerance was reported by 48.9% of participants. 33.7% of participants with tinnitus had unprotected exposure to noise, while only four participants used protection when exposed to noise.

Table 5.4-21 - Clinical characterization of tinnitus sample.

Clinical variables	Participants with tinnitus (n=92)
Tinnitus Duration (mean in years)	7.8 ± 8.6
Intensity of tinnitus (scale 1-10)	3.3 ± 1.6
Manifestation of tinnitus	
Constant	80 (87%)
Intermittent	7 (7.6%)
Pulsatile	4 (4.3%)
Omitted	1 (1.1%)
How did tinnitus begin?	
Gradual	45 (49%)
Abrupt	18 (19.5%)
Omitted	29 (31.5%)
Dizziness	
Yes	35 (38%)
No	50 (54.4%)
Omitted	7 (7.6%)
Does tinnitus gets worse when you're nervous?	
Yes	54 (58.7%)
No	37 (40.2%)
Omitted	1 (1.1.%)
Reduced sound tolerance	
Yes	45 (48.9%)
No	47 (51.1%)
Noise exposure	
Yes, with protection	4 (4.3%)
Yes, without protection	31 (33.7%)
No	57 (62%)

Psychoacoustic estimates of tinnitus are presented in Table 5.4-22. Frequencies matched to tinnitus pitch ranged from 2000 Hz to 8000 Hz, with 4000 Hz being the most frequently matched. Loudness was matched to 0 dB (with a variation of + or – 5dB according to hearing threshold). Most participants reported central (52.4%) and pure tone (59.0%) tinnitus. Convergent (47.6%) and distant (29.8%) Feldmann's curve types were the most frequent. Residual inhibition was negative in 43.9% of participants and partial in 36.6%.

Table 5.4-22 - Psychoacoustic tinnitus assessment.

Audiological measurements	Participants with tinnitus (n=92)
Pitch (n=83)	4000Hz (2000Hz; 8000Hz)
Loudness (n=83)	0 dB (0 dB; 5.0 dB)
Location (n=84)	
Central	44 (52.4%)
Right	15 (17.9%)
Left	25 (29.8%)
Type (n=83)	
Pure Tone	49 (59.0%)
Narrow Band Noise	34 (41.0%)
Feldmann's Curve (n=84)	
Congruent	17 (20.2%)
Convergent	40 (47.6%)
Divergent	1 (1.2%)
Distant	25 (29.8%)
Persistent	1 (1.2%)
Residual inhibition (n= 82)	
Negative	36 (43.9%)
Partial	30 (36.6%)
Complete	13 (15.9%)
Rebound Effect	3 (3.7%)

Tinnitus severity was categorized by means of the THI scores. The majority of the individuals had a mild handicap (38 participants), followed by moderate handicap (22), slight or no handicap (17), severe handicap (14) and one participant had catastrophic handicap (Figure 5.4-12).

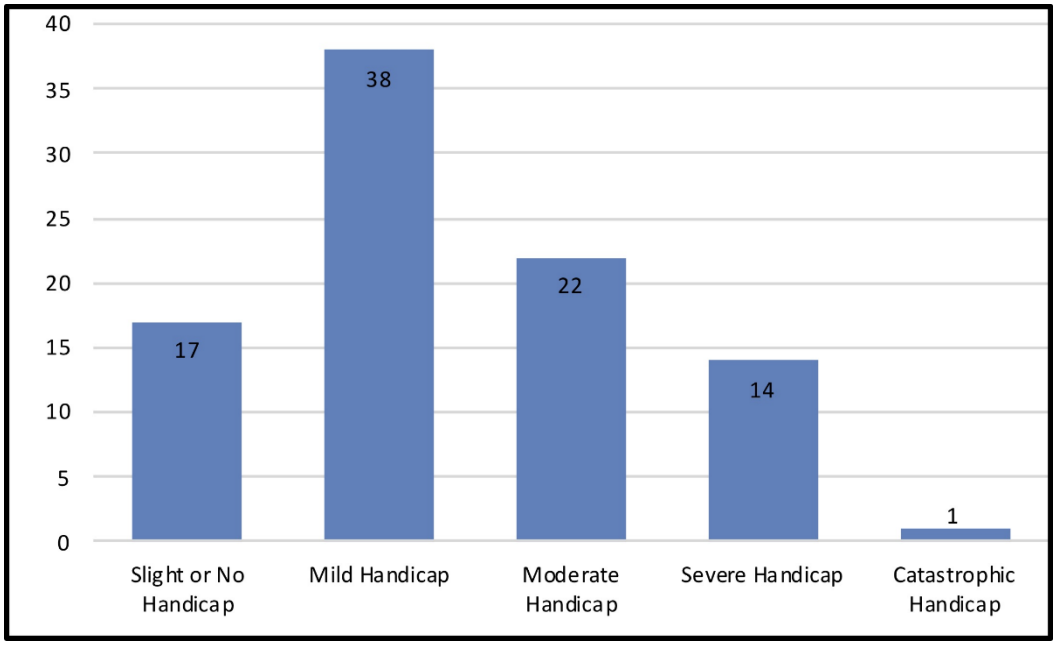

Figure 5.4-12 - THI scores of tinnitus participants.

Inflammatory characteristics

Table 5.4-23 demonstrates the mean values and standard deviation of each inflammatory parameter in the groups with and without tinnitus. This study included 112 participants (two participants were excluded) however, in the analyses of HSP-70 were included 80 participants. The only significant difference between groups was for IL10 (p =.025) and for IL6 and TGFβ we had a marginally significant result (p = .052) and (p = .064) respectively.

Table 5.4-23 - Descriptive analyses of inflammatory parameters for tinnitus.

Inflammatory parameters	With Tinnitus	Without Tinnitus	p value
IL1α (pg/mL)	0.698±2.51	0.362±0.68	.300
IL1β (pg/mL)	1.424±5.40	0.810±1.85	1.000
IL2 (pg/mL)	0.464±1.62	0.227±0.70	.980
IL6 (pg/mL)	2.023±3.00	2.164±1.48	.052
IL10 (pg/mL)	1.175±1.30	1.843±2.51	**.025***
IFN-γ (pg/mL)	3.321±9.88	6.483±16.57	.116
TNF-α (pg/mL)	2.563±10.24	1.829±4.96	.841
HSP70 (ng/mL)	0.496±1.24	0.391±0.69	.827
TGF-β (pg/mL)	1450.609±775.71	1339.357±865.55	.064

Legend: * $p \leq 0,05$

Table 5.4-24 shows mean values and standard deviations with reference to presence of deafness in relation to the inflammatory parameters evaluated.

Table 5.4-24 - Descriptive analyses of inflammatory parameters for hearing loss.

Inflammatory parameters	With hearing loss	Without hearing loss	p value
IL1α (pg/mL)	0.736±2.64	2.741±17.22	.433
IL1β (pg/mL)	1.535±5.68	4.637±28.02	.461
IL2 (pg/mL)	0.454±1.63	0.311±1.24	.171
IL6 (pg/mL)	5.339±19.45	1.937±3.49	.582
IL10 (pg/mL)	1.184±1.18	1.849±4.83	.470
IFN-γ (pg/mL)	3.985±12.29	7.461±17.60	.181
TNF-α (pg/mL)	2.573±10.46	5.424±26.93	.691
HSP70 (ng/mL)	0.396±0.96	0.531±1.24	.544
TGF-β (pg/mL)	1827.441±1254.80	1807.449±1102.73	.801

Legend: * $p \leq 0,05$

Table 5.4-25 shows the mean values and standard deviation of inflammatory parameters with reference to the different degrees of deafness - normal, slight and moderate. With the exception of IL2 and IFN-γ, the values of the inflammatory parameters were lower in the moderately hearing impaired group compared to the normal group and mild hearing impairment. It is interesting to note that the mean value of several inflammatory parameters (IL1α, IL1β, IL10, IFN-γ, TNFα, and HSP70) decreased progressively as the degree of hearing loss increased. However, differences were not statistically significant.

Table 5.4-25 - Descriptive analyses of inflammatory parameters for deafness grade.

Inflammatory parameters	Hearing impairment grade			p-value
	Normal <20dB	Slight 21-40dB	Moderate 41-70dB	
IL1α (pg/mL)	2.693±17.07	0.828±2.88	0.296±0.24	.768
IL1β (pg/mL)	4.557±27.78	1.772±6.20	0.365±0.31	.539
IL2 (pg/mL)	0.306±1.23	0.428±1.74	0.657±1.01	.089
IL6 (pg/mL)	1.904±3.46	6.111±21.21	1.571±1.80	.647
IL10 (pg/mL)	1.827±4.79	1.273±1.24	0.747±0.66	.239
IFN-γ (pg/mL)	7.381±17.46	2.738±5.85	11.298±29.57	.302
TNF-α (pg/mL)	5.331±26.71	3.052±11.40	0.138±0.37	.391
HSP70 (ng/mL)	0.531±1.24	0.473±1.04	0.000	.333
TGF-β (pg/mL)	1819.252±1096.71	1861.179±1330.40	1550.385±780.86	.699

Legend: * $p \leq 0,05$

Association tests concerning to inflammatory parameters:

Tinnitus and comorbidities

Concerning the comorbidities, the presence of smoking habit was significantly associated to levels of IFN-**γ** (p=.041).

Clinical characterization and psychoacoustic assessment in tinnitus group:

We explored the significant results related to the clinical characterization and the psychoacoustic assessment in tinnitus group for all inflammatory parameters. Concerning the age, we have divided the patients into two groups: 55-64 and 65-75 years old. The data showed statistically significant differences between these subgroups regarding to TGF-β (*U*= 721.5, *p*= .034), being significantly lower in the older group (Table 7). There were also significant differences in IL1α (*U*= 577.000, *p*= .033) levels according to tinnitus type: IL1α values were statistically higher in patients with tonal tinnitus compared to narrow band tinnitus. Concerning residual inhibition, we found statistically significant differences for IL2 (*H* = 9,948, p = .019). Additionally, we observed a negative correlation between tinnitus duration and IL10 (r = -.281, p = .007).

Correlations between loudness and the inflammation factors, are shown in Table 5.4-26.

Table 5.4-26 - Correlations: inflammatory parameters and tinnitus loudness.

Inflammatory parameters	r- value
IL1α	-.018
IL1β	-.023
IL2	-.015
IL6	-.143
IL10	-.004
IFN-γ	.028
TNF-α	-.026
HSP70	-.397**
TGF-β	.115

Concerning to the correlation coefficient there was a significant negative weak correlation between HSP70 and tinnitus loudness (*r* = -.397). Since the coefficient is negative this means that higher tinnitus loudness values were associated to lower levels of HSP70.

Presence of tinnitus and sample collection time

In a further exploration of the data the study population was divided according to the time of collection (morning and afternoon) and the presence of tinnitus and the inflammatory parameters. For 36 participants blood samples were collected in the morning at 11:30h and for 78 participants collection was performed in the afternoon at 16:30h (Table 5.4-27). For the 'morning' period, with the exception ofHSP70, inflammatory factors had a higher mean value in the group with tinnitus. In the 'afternoon' group, tinnitus was associated with higher mean levels of IL1α, IL1β, IL2, TNF-α, HSP70 and TGF-β, and lower mean levels of IL6, IL10 and IFN-**γ**. Levels differed

significantly between time points (p = .012), which means that there were significant differences in the levels of the evaluated parameters according to the time of sample collection (morning or afternoon period).

Table 5.4-27 - Mean and standard deviation of the inflammatory markers in the morning and afternoon.

Inflammatory marker	Morning period		Afternoon period	
	Without tinnitus (n=2)	With tinnitus (n=33)	Without tinnitus (n=20)	With tinnitus (n=57)
IL1α (pg/mL)	0.745±0.96	5.131±22.73	0.307±0.65	0.346±0.54
IL1β (pg/mL)	3.155±4.02	8.984±37.17	0.560±1.45	0.610±1.37
IL2 (pg/mL)	0.000	0.556±2.03	0.239±0.72	0.343±1.24
IL6 (pg/mL)	2.940±2.75	8.064±25.14	2.038±1.37	1.602±2.03
IL10 (pg/mL)	6.300±8.72	2.186±6.11	1.347±0.60	1.032±0.87
IFN-γ (pg/mL)	5.090±4.69	7.293±17.30	6.442±16.98	4.645±13.77
TNF-α (pg/mL)	8.705±12.31	10.227±36.78	1.061±3.54	1.308±4.57
HSP70 (ng/mL)	1.115±.95 (n=2)	0.682±1.12 (n=14)	0.315±0.65 (n=19)	0.438±1.28 (n=45)
TGF-β (pg/mL)	694.370±315.22	2095.511±1402.92	1640.260±1349.39	1757.686±940.64

We found significant results for the subgroup who had collection time in the afternoon and the presence of tinnitus. The results were significant for IL10 and IFN-γ (Table 5.4-28). Hearing loss in high frequency also reached significant results (p = .012).

Table 5.4-28 - Inflammatory parameters for the presence of tinnitus in the afternoon time.

Variable	p- value
IL10	.032*
IFN-γ	.045*

Legend: * $p \leq 0,05$

Modelling the data

Presence of tinnitus and inflammatory factors

Table 5.4-29 presents a logistic regression modelling inflammatory factors, age, gender, high frequency, IFN-γ and exposure to noise as confounding variables. This analysis was first performed for all participants, and then just for the 'afternoon' group. The dependent variable in the model was presence of tinnitus.

Table 5.4-29 - Logistic regression model applied to presence of tinnitus.

Variable*	B	Wald	OR	p- value	(95% IC)
Sex	.015	.001	1.015	.978	(.345, 2.988)
Age	-.034	.481	.967	.488	(.878, 1.064)
High_frequency_PTA_OD_OE[a]	.092	6.502	1.096	.011*	(1.021, 1.176)
IFNg	.004	.051	1.004	.822	(.972, 1.036)
Exposure to noise	1.228	3.095	3.414	.079	(.869, 13.405)
Constant	1.416	.202	4.120	.653	
Sex	.109	.032	1.115	.858	(.337, 3.695)
Age	-.030	.310	.971	.577	(.875, 1.077)
High_frequency_PTA_OD_OE[b]	.079	4.099	1.082	.043*	(1.003, 1.168)
IFNg	.001	.002	1.001	.961	(.968, 1.035)
Exposure to noise	1.242	2.129	3.461	.144	(.653, 18.339)
Constant	1.080	.103	2.944	.749	

Legend: [a] whole group, [b] afternoon group ** p ≤ 0,05*

High frequency hearing loss in both ears represented a significant risk of tinnitus in all participants and in the 'afternoon' group, 1.096 and 1.082 respectively.

Severity of tinnitus and inflammatory factors

In a logistic regression modelling inflammatory factors, age, gender, IL2 and residual inhibition were considered as confounding variables. The dependent variable in the model was severity of tinnitus (Table 5.4-30).

Table 5.4-30 - Logistic regression model applied to severity of tinnitus and residual inhibition.

	B	Wald	OR	Sig.	*(95% IC)*
Sex (Female)	.813	.693	.535	0.367	(.138, 2.082)
Negative/rebound (1)	6.475	.728	6.381	0.011*	(1.531,26.599)
Age	.176	.060	1.026	0.674	(.911, 1.154)
IL2	.110	.205	.934	0.740	(.625, 1.397)
Constant	1.084	3.889	.017	0.298	

Legend: ** p ≤ 0,05*

The logistic regression revealed that the residual inhibition (β = -1.853; χ2 Wald (1) = 6.475; p = .011) has a statistically significant effect on the logit of the probability of patients having severe or catastrophic tinnitus. Thus, the odds for a patient to have severe or catastrophic tinnitus grade is higher in subjects having residual negative/rebound residual inhibition when compared to subjects having partial/complete residual inhibition. The IL2 mean value is 0.62 pg/mL for individuals with Negative or Rebound effect on residual inhibition and is 0.36 pg/mL for those

having a Complete or Partial effect on residual inhibition. Nevertheless the difference is not statistically significant (p = .504).

Discussion

In this study, we conducted an exhaustive audiological and inflammatory evaluation of older persons, with or without hearing loss and/or tinnitus.

Recent studies have shown that inflammatory responses occur in the inner ear under various damaging conditions, including overstimulation with noise (Fujioka et al. 2006) and cisplatin-induced ototoxicity (Park et al. 2009). Several other studies demonstrated possible relationship between inflammation and inflammatory mediators in the cochlea and the development of ear diseases such as deafness (Fujioka et al. 2014). Our results corroborate this evidence, showing that the mean value of several systemic inflammatory markers – in detail IL1α, IL1β, IL10, IFN-γ, TNF-α, and HSP70 - become progressively lower with the progression of the degree of hearing loss, which implies the importance of inflammation during the progress of hearing loss. Moreover, the concentration of evaluated inflammation markers correlated significantly with hearing loss, especially with the high frequency hearing loss, which is a statistically significant risk factor for tinnitus. Supporting this notion, in a study involving an older population, Doi and colleagues found an association between polymorphisms in the *IL6* gene at region – 174G/C and susceptibility to tinnitus (Doi et al. 2015). Our data has shown a nearly statistical significant result for IL6 when comparing tinnitus to non-tinnitus subgroups, but we found a clearly statistical relevant difference for IL10.

Animal models suggest that the presence of proinflammatory cytokines such as IL1β and TNF-α are contributing factors to the prolongation of inflammation processes implicated in the development of noise-induced hearing loss (Fujioka et al. 2006, Tan et al. 2013; Fuentes-Santamaria et al. 2017) being also described an association between TNF-α levels and the susceptibility to tinnitus in elderly individuals with history of exposure to occupational noise (Marchiori et al. 2018).

Circadian rhythms control essential functions such as sleep, renal function, hormone secretion, metabolism, inflammation, as well as auditory functions. Only recently it was demonstrated that cochlea possesses its own circadian cycle. Studies in mice have shown that noise trauma with same duration and intensity (6–12 kHz, 1 h, 100 dB SPL) had worse consequences if exposure was during night (9pm), causing permanent hearing loss (measured by ABR 15 days after noise trauma) while the morning (9 am) exposure lead to total recovery (Meltser et al. 2014).

We have divided our study sample according to the day time period of blood collection (morning and afternoon groups) and we have found significant differences (p = .012) between the morning and afternoon groups, which means that the time of blood sample collection can affect the results of inflammatory factors. In particular, we have found statistically relevant results for IL10 in regards to tinnitus presence in the whole group. And in the afternoon group for IL10 and INF-□. Interestingly, we have found a

correlation between tinnitus duration and IL10 levels, since these levels are progressively lower according to longer tinnitus duration. Regarding tinnitus intensity (loudness) we found a negative correlation with HSP70, as well as between IL10 and the duration of tinnitus. To our best knowledge it is the first time that are stablished those correlations of tinnitus characteristics with inflammatory markers.

Salicylate-induced tinnitus in rats has been a popular animal model for the study of human tinnitus (Jastreboff et al. 1988; Paul et al. 2009; Yu et al. 2008; Hwang et al. 2011a,b; Hu et al. 2014). The mechanism of salicylate-induced tinnitus includes an accumulation of arachidonic acid caused by inhibition of cyclooxygenase (COX). This could potentiate the N-methyl D-aspartate receptor (NMDAR) that currents synapses between inner hair cells and dendrites of the cochlear spiral ganglion neuron. Some studies suggest that tinnitus arises from an increase in excitatory neurotransmission and is associated with the NMDAR activity (Ruel et al. 2008; Guitton et al. 2003), previously linked to tinnitus in animal models (Guitton et al. 2003; Hwang et al. 2011a). Recent animal studies on tinnitus-related genes expression highlights the role of neural inflammation and oxidative metabolism in tinnitus pathophysiology. Studies of salicylate-induced tinnitus in mice revealed that the expression levels of NMDA receptors type2B (NR2B), TNF-α and IL1β genes increased significantly, whereas cyclooxygenase type 2 (COX-2) gene expression decreased in the cochlea and Inferior Colliculus (IC) (Hwang et al., 2011a, 2011b).

Our results open new therapeutic options regarding prevention or retardation of the mechanisms involved in age-related hearing loss and tinnitus that, although complex, are surely associated to inflammatory mechanisms. Nakamoto *and colleagues* suggested that the suppression of the proinflammatory cytokine HSF-1 in the cochlea by the administration of geranylgeranylacetone (GGA) may be an important way of protecting the inner ear (Nakamoto et al. 2012). Epidemiologic prospective studies also confirm the association between inflammation and hearing loss (long-term serum C-reactive protein levels) in ARHL (Nash et al. 2013).

Study limitations

That blood was collected at two different time points reduced the power of the sample. Nevertheless, it is interesting to note that the statistically relevant results were found for those whose samples were collected in the afternoon, implying that inflammatory parameters have a circadian effect. Future studies should consider the sample collection time.

Conclusions

Due to progressive aging of population it is estimated that in 2050 there will be 2 billion people older than 65. Results from the most recent World Health Organization

(WHO) Global Burden of Diseases (2015) reports hearing loss as the fourth leading cause of years lived with disability. Given the strong links between hearing loss and tinnitus, then tinnitus surely follows this trend. In order to improve quality of life of people with those disabilities it is imperative to have investment in studies to clarify the underlying causal mechanism in order to enable a more efficient prevention or treatment and avoid the progression to frailty and related mental health disabilities.

The results of our study clearly demonstrate that inflammatory mechanisms are involved not only in hearing loss pathogenesis but also in tinnitus. In addition, we have shown for the first time that the systemic concentration of IL10 and INF-γ are statistically associated with the presence of tinnitus. Another interesting finding is that higher IL1α levels are associated with tonal type of tinnitus and HSP70 and HL10 are negatively correlated to tinnitus loudness and tinnitus duration respectively. This suggest the need for further research, not only for confirmation in larger samples, but also regarding pathophysiological mechanisms underlying this phenomenon. Because of the trend for negative correlations between the inflammatory markers and tinnitus characteristics it's reasonable to think that inflammatory mechanism are probably involved in the acute phase of tinnitus emergence. Future studies should involve higher samples with a rigorous methodological design for controlling of confounding factors.

5.4.4. Biomarkers of Presbycusis and Tinnitus in a Portuguese Older Population

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Biomarkers of Presbycusis and Tinnitus in a Portuguese Older Population

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Introduction: Presbycusis or age-related hearing loss (ARHL) is a ubiquitous health problem. It is estimated that it will affect up to 1.5 billion people by 2025. In addition, tinnitus occurs in a large majority of cases with presbycusis. Glutamate metabotropic receptor 7 (*GRM7*) and *N*-acetyltransferase 2 (*NAT2*) are some of the genetic markers for presbycusis.

Objectives: To explore patterns of hearing loss and the role of *GRM7* and *NAT2* as possible markers of presbycusis and tinnitus in a Portuguese population sample.

Materials and Methods: Tonal and speech audiometry, tinnitus assessment, clinical interview, and DNA samples were obtained from patients aged from 55 to 75 with or without tinnitus. *GRM7* analysis was performed by qPCR. Genotyping of single nucleotide polymorphisms (SNPs) in *NAT2* was performed by PCR amplification followed by Sanger sequencing or by qPCR.

Results: We screened samples from 78 individuals (33 men and 45 women). T allele at *GRM7* gene was the most observed (60.3% T/T and 33.3% A/T). Individuals with a T/T genotype have a higher risk for ARHL and 33% lower risk for tinnitus, compared to individuals with A/A and A/T genotype, respectively. Being a slow acetylator (53%) was the most common *NAT2* phenotype, more common in men (55.8%). Intermediate acetylator was the second most common phenotype (35.9%) also more frequent in men (82.6%). Noise exposed individuals and individuals with 'high frequency' hearing loss seem to have a higher risk for tinnitus. Our data suggests that allele AT of *GRM7* can have a statistically significant influence toward the *severity* of tinnitus.

Conclusion: For each increasing year of age the chance of HL increases by 9%. The risk for ARHL was not significantly associated with *GRM7* neither *NAT2*. However, we cannot conclude from our data whether the presence of T allele at *GRM7* increases the odds for ARHL or whether the A allele has a protective effect. Genotype A/T at *GRM7* could potentially be considered a biomarker of tinnitus severity. This is the first study evaluating the effect of *GRM7* and *NAT2* gene in tinnitus.

Keywords: presbycusis, *GRM7*, *NAT2*, tinnitus, markers, comorbidities

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INTRODUCTION

Presbycusis [age-related hearing loss (ARHL)] is a universal feature of mammalian aging in which the auditory function is compromised, hearing thresholds increase, and frequency resolution gets poorer. As a result, in noisy environments speech-understanding deteriorates and temporal processing deficits in gap detection measures increase (Lee,2013). In humans, this condition affects tens of millions of people world-wide (Yamasoba et al.,2013). Many people with hearing loss also experience tinnitus, which is the perception of a sound in one or both ears or in the head in the absence of an external sound source (Jastreboff and Hazell,1993).

Presbycusis is complex in that it has repercussions at a physical, cognitive, emotional, and social level; quality of life can deteriorate, and for some people presbycusis could lead to depression, social isolation and lower self-esteem (Lee, 2013;Ciorba et al.,2015). Environmental factors such as diet, physical exercise, smoking, and intake of medications are some of the extrinsic factors predisposing to presbycusis. There are several auditory structures affected by presbycusis, such as hair cells, *stria vascularis*, afferent spiral ganglion neurons and the central auditory pathways (Fuentes-Santamaría et al.,2013). Based on results of audiometric tests and temporal bone pathology,Schuknecht and Gacek(1993)and later modified byNelson and Hinojosa(2003), classified presbycusis as either sensory (downslope audiometry and cochlear degeneration), neural (downslope audiometry and very poor speech discrimination, spiral ganglion and nerve fibers degeneration), metabolic (audiometry in a platform and strial atrophy), cochlear conductive (downslope audiometry and thickening and stiffening of basilar membrane), mixed (mixture of the above), or undetermined (none of the above) types.

Depending on the type and severity of the hearing loss, several options are available to reduce the hearing difficulties and consequently improve quality of life. When patients are appropriately fitted and motivated, hearing aids and cochlear implants (CIs) are the most commonly used devices for treating mild-severe presbycusis. Electric-acoustic stimulation and active middle ear implants may also be suitable solutions for treating presbycusis (Sprinzl and Riechelmann,2010).

Biological markers are widely used in oncology, hematology and in other medical disciplines to diagnose or to monitor various diseases. In otology, biological markers are not yet widely used, but once identified, they could provide a means of determining the time-course or most effective treatment for an individual with presbycusis or tinnitus. Potential biomarkers include mutations in mitochondrial DNA, chromosomal mutations, state of chronic inflammation, presence of certain diseases associated with earlier onset or progression of presbycusis (e.g., diabetes, hypertension) and metabolic diseases (Van Eyken et al., 2007;Verschuur et al.,2014). It was recently estimated that 35–55% of auditory aging could have a genetic background (Ruan et al.,2014). Of interest are genes coding for glutamate receptors as glutamate is the main excitatory neurotransmitter in the peripheral and central auditory pathways. It has been suggested that increased release of glutamate may be involved in the auditory aging and the generation and maintenance of tinnitus by causing "excitotoxicity." There are many types of glutamate receptors, such as *N*-methyl-D-aspartate (NMDA) and alfa-amine propionic acid (AMPA), the latter being the most relevant receptor in physiological neurotransmission at auditory pathways. NMDA receptors are not essential for the auditory transmission, but they have been shown to be expressed in the cochlea after induction of tinnitus. Moreover, it has been demonstrated that the application of NMDA antagonists directly into the cochlear fluid can block salicylate-induced tinnitus in animals (Figueiredo et al.,2008).

GRM7 encodes a metabotropic glutamate receptor subtype 7 (mGluR7), a G protein-coupled receptor regulating auditory nerve excitability. When bound by L-glutamate, mGluR7changes the configuration of adenylyl cyclase, which has implications in the metabolism of AMPc, control of cellular cycle, and normal functioning of central nervous pathways. mGluR7 plays a general role in glutamate synaptic transmission (Voytenko and Galazyuk,2011). In the auditory periphery, mGluR7 is thought to mediate glutamate excitotoxicity (Pujol et al.,1993) and in the cochlea mGluR7 maintains the glutamate-dependent equilibrium between the inner hair cells and the spiral ganglion neurons (Newman et al.,2012). Its role in the higher auditory pathways remains unclear (Lu,2014). Single nucleotide polymorphisms (SNPs) of *GRM7* have been demonstrated to be associated with auditory aging in European (Friedman et al.,2009) and American populations (Newman et al.,2012) but not of a Chinese population (Luo et al.,2013). Interestingly,Newman et al.(2012) have reported that certain SNP variants of *GRM7* associate with poorer speech recognition in the elderly. The importance of *GRM7* in the auditory system is supported by the detection of mGluR7 in the inner and outer hair cells and in the spiral ganglion nerve (Friedman et al.,2009).

Highly concentrated glutamate may affect membrane permeability in the hair cells, causing an increase in Cl^- influx, and consequently an osmotic imbalance and membrane disruption (Puel et al.,1998). In addition, glutamate excitotoxicity induces apoptotic cell death and inflammation (Sahley et al.,2013). This was demonstrated in an animal model to be directly responsible for the loss of inner hair cells in a time-, dose- and tonotopy-dependent manner (Hu et al.,2015). Interestingly, neonatal exposure to monosodium glutamate has been shown to induce neuronal atrophy and dysmorphia in the cochlear nucleus and in the superior olivary complex (Foran et al.,2017). The physiological effects of glutamate excitotoxicity therefore are concluded to include ARHL (Pujol et al.,1993) and tinnitus (Brozoski et al.,2012;Sahley et al.,2013;Yu et al.,2016).

Oxidative stress represents an imbalance between the production of reactive oxygen species (ROS) and their detoxification and has been postulated to play a major role in the overall aging process and to significantly contribute to the ARHL. Oxidative stress in the inner ear, secondary to impairments in defense mechanisms caused by certain polymorphisms related to a battery of antioxidant systems, could make individuals more susceptible to ARHL (Seidman et al., 2002;Fujimoto and Yamasoba,2014).

In the adult inner ear, presence of several detoxification and antioxidant enzymes including catalase, superoxide dismutase,

glutathione peroxidase, and glutathione *S*-transferases (GST) has been demonstrated.

One of the sources leading to accumulation of ROS are insufficiently acetylated drugs which accumulate and may be converted into reactive drug metabolites by oxidative enzymes. *N*-acetyltransferase (*NAT*) are enzymes responsible for the detoxification of exogenic substrates via *N*-acetylation or *O*-acetylation. In humans, the catalytic activity by *NAT* isoenzymes *NAT1* and *NAT2* may be regulate by these substrate concentration. Both isoenzymes are highly polymorphic and catalyze many aromatic amines and hydrazine substances important for the balance of the oxidative status. In addition, *NATs* are known to be involved in the detoxification of harmful xenobiotics (Vatsis and Weber,1993;Hein,2002;Ünal et al., 2005b).

Variation in *NAT2* alleles or haplotypes resulting from combination of SNPs is responsible for the *N*-acetylation polymorphism. Regarding the latter, rapid, intermediate, and slow acetylator phenotypes have been demonstrated. These phenotypes are associated with the rate of catalytic activity and accordingly predispose toward drug toxicity (Rajasekaran et al., 2011).

Because the individuals with the null genotype for *NAT2* may be more susceptible to effects of environmental toxins and oxidative free radical cellular damage, the presbycusis becomes an ideal model for evaluation of gene-environmental interaction (Ünal et al.,2005a,b). Although many individuals have been exposed to several environmental risk factors, the ARHL develops to a different degree in various age groups. This suggests genetic host factor(s) contributing to the degenerative mechanisms (Ünal et al.,2005b).

Previous studies demonstrated the association between the common human *NAT2* alleles and ARHL. Independent studies have showed a significant association between *NAT2* polymorphisms and presbycusis, namely *NAT2* 6A* in the Turkish population (Ünal et al.,2005b) and in the European population (Van Eyken et al.,2007) with Caucasian subjects carrying a *NAT* 6A* mutant allele having an increased risk to Presbycusis (Bared et al.,2010). Other studies considering different *NAT2* alleles reported negative associations with ARHL (Dawes et al.,2015) and with the shape of the audiograms (Angeli et al.,2012), when considering audiometric patterns of presbycusis in older individuals. However, most authors suggested that *NAT2* gene is a susceptibility factor for development of hearing impairment (Ünal et al.,2005b;Dawes et al.,2015).

Here we explore the relationships between presbycusis, tinnitus, co-morbidities, and the genotypes of *GRM7* and *NAT2*, in a sample of older Portuguese adults.

PATIENTS AND METHODS

Subjects

Inclusion criteria was the presence of sensory presbycusis, with or without tinnitus, in adults of any gender, aged between 55 and 75 years, from the Portuguese population.

Our sample included 78 older individuals (n = 45 women, n = 33 men).

For the purposes of inclusion presbycusis was defined as bilateral sensorineural deafness in downslope audiometric pattern, above 1000 Hz with poor speech discrimination (discrimination threshold > 40 dB SPL and 100% discrimination to 60 dB or worse). Although all included participants have presbycusis we will consider a subgroup with normal hearing because the adopted classification uses conversational frequencies.

Exclusion criteria were considered: inability to understand and sign the informed consent due to a significant cognitive impairment, an uncompensated medical disorder that requires urgent evaluation or if the individual has a serious psychiatric disorder. Also individuals over 55 years who presented possible factors that may overlap the variables under study were excluded [e.g., Ménière's disease, chronic otitis media, otosclerosis, tinnitus from disease of the outer ear (occlusive exostosis, outer otitis)], history of ototoxic drugs use, massive noise exposure, a history of previous malignancy with chemotherapy, history of autoimmune disorders and neurodegenerative and demyelinating diseases.

This study had the approval of the Ethical Committees from Hospital Cuf Infante Santo (November 26th, 2014), Nova Medical School (n°65/2014/CEFCM) and the National Department of Personal Data Protection (authorization number:1637/2016).

Accordingly we obtained the Institutional Scientific Review Board approval of the process for taking informed consent and overall study design. The study was conducted in accordance with the Declaration of Helsinki.

Clinical Evaluation

Written informed consent, clinical and familial history, audiological evaluation and a blood sample, using Whatman℠ FTA℠ card technology, was obtained from every subject.

A questionnaire concerning epidemiologic data (demographic, previous and present diseases, toxicological habits and noise exposure) was completed by the researcher through participant interview.

Audiological Assessment

Hearing thresholds were determined by pure tone audiometry (air and bone) according to ISO 8253 and 389. The exam was performed in a soundproof booth using an Interacoustics℠, Assens, Denmark audiometer (Model: AC40, Serial No.: 98 019 046) and TDH39 headphones fitted with noise-excluding headset ME70 and bone conductor B-71. Audiometry was performed at frequencies from 0.25 to 16 kHz (standard tonal audiometry and extended high frequency). The category of Hearing Loss (HL) was defined according to the average threshold across 500, 1000, 2000, and 4000 Hz in the better ear as mild (21–40 dB), moderate (41–70 dB), severe (71–95 dB) or profound (>95 dB), from an average of thresholds at 500, 1000, 2000, and 4000 Hz in the better ear, according to the European Working Group Genetics of Hearing Impairment (AfterLiu and Xu,1994;Parving and Newton,1995).

Speech audiometry evaluation was obtained with headphones (using mp3 player), or in open field, where the evaluator was

hiding his lips to prevent lip-reading. The number of disyllables that patient repeats correctly was recorded. This intelligibility threshold for two-syllable words intends to measure hearing sensitivity threshold through the intensity level identification in which the patient can correctly identify 50% or more of a disyllables list. On the other hand, the speech discrimination evaluates the lowest intensity level at which a listener can understand speech.

Tinnitus Assessment

Psychoacoustic assessment consisted of loudness match, pitch match, minimum masking level (MML) or Feldmann masking curves, residual inhibition, and loudness discomfort levels (LDL). The severity of tinnitus was evaluated using the Tinnitus Handicap Inventory (THI;Newman et al.,1996). THI comprises 25 questions concerning tinnitus, and the response options are "Yes," "Sometimes," and "No," respectively, corresponds to 4, 2, and 0, accounting for a total score that may vary between 0 and 100. The questionnaire comprehends three sub-scales or dimensions: Functional (11 items – contributing 0–44 for the final score), Emotional (9 items – contributing 0–36 for the final score) and Catastrophic (5 items – contributing 0–20 for the final score). This allow to verify which are the most affected aspects and accordingly choose the therapeutic interventions. The total score of the responses allows tinnitus classification according to its severity or impact in daily life – 0–16: Slight or no handicap (Grade 1), 18–36: Mild handicap (Grade 2), 38–56: Moderate handicap (Grade 3), 58–76: Severe handicap (Grade 4), 78–100: Catastrophic handicap (Grade 5).

Additionally THI is a self-administered instrument, easy to quote, to interpret and has good psychometrics properties (McCombe et al.,2001).

Genetic Analysis

Total genomic DNA was extracted from a blood sample on FTA cards using a commercial NZY Tissue gDNA Isolation Kit (NZYTech, Lisbon, Portugal), strictly according to the manufacturer's instructions. Molecular analysis of *GRM7* gene was assessed by qPCR for A/A, A/T, and T/T genotypes, at rs11928865 SNP. Concerning *NAT2* gene, rs1041983, rs1801280, rs1799929, rs1799930, rs108 and s1799931 were assessed by qPCR or by bidirectional sequencing of the target region in order to identify all the SNPs.

Statistical Analysis

We conducted a descriptive analysis for variables such as gender and age. The audiograms were analyzed considering the best ear (estimated based on the lowest average of frequencies of 0.5–4 kHz). We also evaluated the "high frequency" pure-tone

square Test or Fisher Exact Test for general association between two variables were used. Mann–Whitney or Kruskal–Wallis (for more than two groups) tests were employed to compare hearing thresholds. A Dunn's test with a Bonferroni correction was applied for multiple pairwise comparisons. The level of significance considered was $p = 0.05$.

All the results were analyzed through logistic regression model, where age and gender where considered as control for all other variables.

RESULTS

Participants in our study were 78 older adults aged 64.6 ± 5.58 years old (range = 55–75 years old). Most participants were female ($n = 45$, 57.7%), presenting an average age of 64.1 ± 5.35 years old. For men ($n = 33$, 42.3%), the mean age was 65.3 ± 5.89 years old (**Table 1**).

Hearing Thresholds

The average hearing threshold values (by gender and age) are shown in **Figure 1**. There were significant differences between the gender groups regarding average and median values of hearing thresholds at the frequencies of 4 kHz (p-value = 0.007) and 8 kHz ($p = 0.031$) when comparing male and female (**Figure 1**). There were significant differences between age groups regarding the average and median values of hearing thresholds at frequencies of 4 kHz ($p = 0.003$), 8 kHz ($p < 0.001$), 10 kHz ($p < 0.001$) and 12 kHz ($p < 0.001$) when comparing the different age groups (**Figure 1**).

When comparing hearing thresholds between the different age groups, we found significant differences in females at 4 kHz ($p = 0.009$), 8 kHz ($p = 0.011$), 10 kHz ($p = 0.018$) and 12 kHz ($p = 0.002$) (**Figure 2**). For males statistically significant differences were observed between age groups at 8 kHz ($p = 0.009$), 10 kHz ($p = 0.003$) and 12 kHz ($p = 0.004$) (**Figure 2**).

According to age and gender grouping and comparing males to females we found significant differences for hearing thresholds for the age group 55–60 years old for 1 kHz frequency ($p = 0.022$) and 4 kHz frequency ($p = 0.028$) (**Figure 2**).

Distribution of the individuals according to the hearing loss and tinnitus presence (**Table 1**) shows that in subgroup 1, 18 (23.1%) individuals who had normal hearing thresholds at speech frequencies (0.5–4 kHz) but not tinnitus; subgroup 2, 23 (29.5%) individuals who had normal hearing thresholds at speech frequencies and tinnitus; subgroup 3, 10 (12.8%) individuals who had hearing loss but not tinnitus; and subgroup 4, 27 (34.6%) individuals who had hearing loss and tinnitus (see also **Figure 3**). There are no statistical differences in age or gender between those four subgroups.

TABLE 1 | Distribution of the individuals by subgroups according to hearing loss, tinnitus presence (PTA = Pure Tone Average) and gender.

Subgroup	Audiological characteristic	Gender		*n*
1	PTA ≤ 20 without Tinnitus	5	13	18 (28%) 2
	PTA ≤ 20 with Tinnitus	8	15	23 (29.5%)
3	PTA ≥ 20 without Tinnitus	6	4	10 (12.8%)
4	PTA ≥ 20 with Tinnitus	14	13	27 (34.6%)
Total		33	45	78

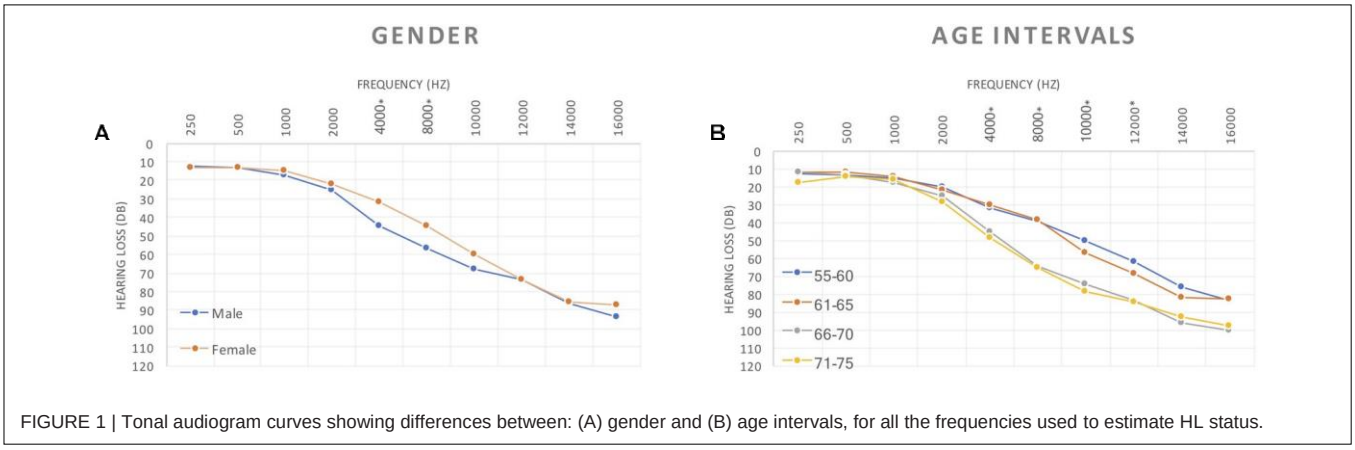

FIGURE 1 | Tonal audiogram curves showing differences between: (A) gender and (B) age intervals, for all the frequencies used to estimate HL status.

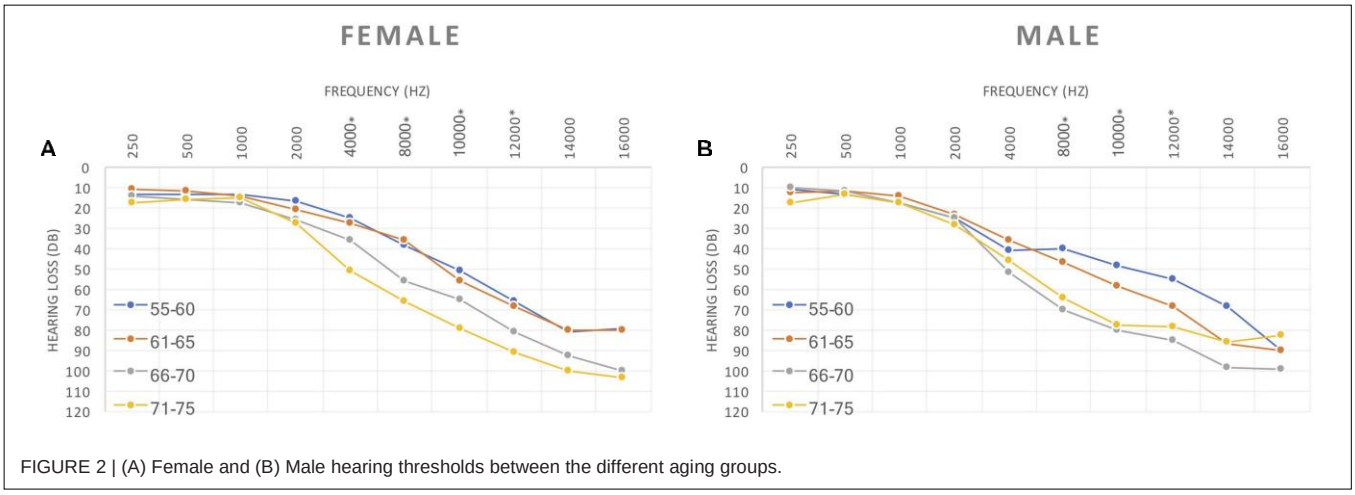

FIGURE 2 | (A) Female and (B) Male hearing thresholds between the different aging groups.

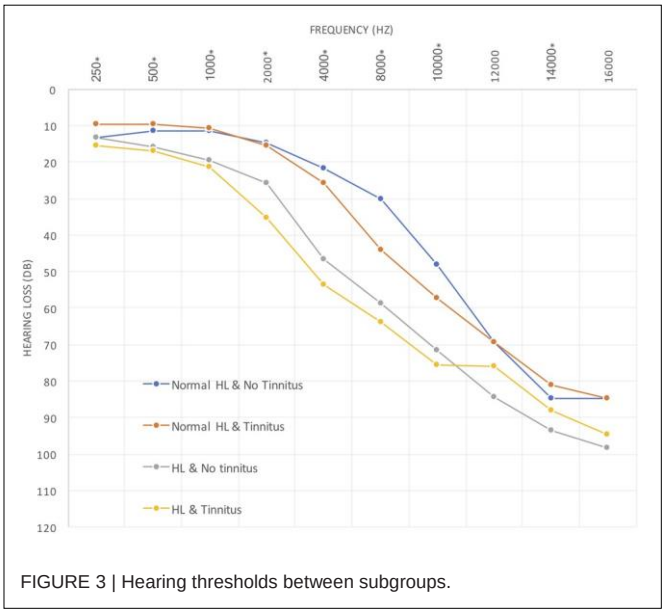

FIGURE 3 | Hearing thresholds between subgroups.

We found statistically relevant differences between the four described groups which corroborates the logical of having chosen this subdivision of our study population. ($p < 0.001$ at the majority of frequencies).

There were significant differences in speech audiograms (PTA, speech recognition threshold [SRT], 100%; p-value= <0.001; <0.001; <0.001, respectively) between subgroups, either for the right ear or for the left ear. The differences were found between subgroups 4 or 3 and the subgroups 1 and 2 for PTA (0%), SRT (50%) and (100%) (**Figure 4**).

Because our study population represents older adult individuals with sensory presbycusis we evaluated the "high frequency" pure-tone average (PTA) at 2, 4, and 8 kHz. We compared the groups of individuals with and without tinnitus and the four subgroups (**Table 1**). In respect to having or not tinnitus we found statistical differences between those groups (p 0.003) (for more details see Appendix 1). We found statistically significant differences ($p < 0.001$) when comparing the four subgroups described in **Table 1** (for more details see Appendix 2).

Characterization of the considered comorbidities in our sample are presented in **Table 2**. Concerning hearing thresholds according to presence or not of the studied comorbidities, we found the following relevant significant differences: from 0.5 to 4 kHz for cholesterol; at 4 kHz for measles.

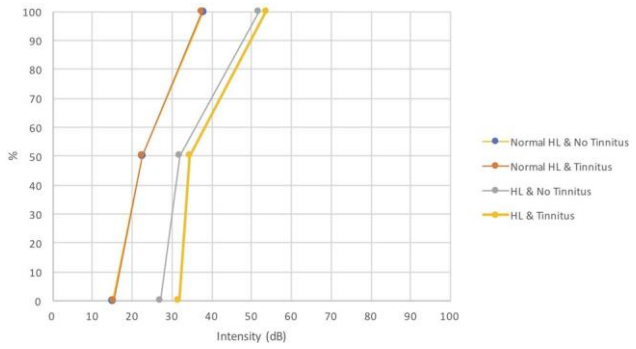

FIGURE 4 | Speech audiograms between subgroups.

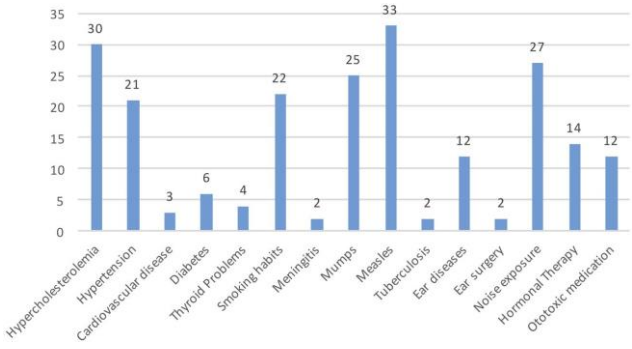

FIGURE 5 | Distribution of the most common comorbidities in the individuals with tinnitus.

When comparing the group of participants with and without tinnitus the most statistical relevant results were concerning 'high frequency' hearing loss and noise exposure. In our study population, 50 individuals (64.1%) had tinnitus.

We have determined the distribution of studied comorbidities in our tinnitus population (**Figure 5**). The most prevalent were measles, hypercholesterolemia, noise exposure, mumps, smoking and hypertension, in a descendent order of frequency.

In our sample, 49 participants (62.8%) reported to have high blood values of cholesterol. Of those, 27 individuals (55.1%) were taking medication (statins) (Appendix 3). There was a significant association between tinnitus and statins intake in those individuals reporting hypercholesterolemia ($\widehat{OR}$ 0.28, p 0.045, CI 0.08–0.99) (**Table 3**). We found no relevant association between statins intake and hearing loss.

TABLE 2 | Distribution of the most common comorbidities in the individual of the sample.

Comorbidities	*n*	
	Absent	Present
Cholesterol	29 (37.2%)	49 (62.8%)
Hypertension	43 (55.1%)	35 (44.9%)
Cardiovascular disease	73 (93.6%)	5 (6.4%)
Tinnitus	28 (35.8%)	50 (64.1%)
Diabetes	65 (83.3%)	13 (16.7%)
Thyroid problems	70 (89.7%)	8 (10.3%)
Smoking habits	44 (56.4%)	34 (43.6%)
Meningitis	77 (98.7%)	1 (1.3%)
Mumps	44 (56.4%)	34 (43.6%)
Measles	21 (26.9%)	57 (73.1%)
Tuberculosis	75 (96.2%)	3 (3.8%)
Ear diseases	62 (79.5%)	16 (20.5%)
Ear surgery	76 (97.4%)	2 (2.6%)
Noise exposure	51 (65.4%)	27 (34.6%)
Hormonal therapy	55 (70.5%)	23 (29.5%)
Ototoxic medication	58 (74.4%)	20 (25.6%)

Tinnitus Evaluation

Subgroups 2 and 4 included participants with tinnitus. Concerning tinnitus laterality, 33 of them reported to have a unilateral tinnitus (12 on the right ear and 21 on the left ear) and 17 participants have a bilateral tinnitus. According to THI score (**Figure 6**) for most participants tinnitus was bothersome, only 10 subjects had a slight handicap.

Modeling the data

All the results were analyzed through a logistic regression model age and gender were considered in all the models with the objective of controlling eventual confounding since these two factors are known to be related to hearing loss.

The regression logistic model was applied to HL considering female as reference (for more details see Appendix 4). The odds of developing presbycusis was significantly higher for males than for females ($\widehat{OR}$ 2.9, p 0.032). When considering age as a covariate, the effect was slight but significant, being the odds of having hearing loss 9% higher for each increasing year ($\widehat{OR}$ = 1.09, p = 0.03).

Using this statistical model for all the comorbidities considered and controlled for age the odds of having hearing loss was significantly lower for subjects with high cholesterol ($\widehat{OR}$ 0.33, p 0.034).

We found no association between HL and high blood pressure or noise exposure.

In addition, using the regression logistic model for tinnitus considering men and the absence of tinnitus as reference, we found that noise exposure seems to influence the occurrence of tinnitus ($\widehat{OR}$ 3.65, p 0.026, CI 1.2 – 11.4), when considered isolated. This result is statistically very relevant.

There were no other statistically significant results concerning other comorbidities in this study (for more details see Appendix 5).

GRM7 and NAT2 genes

Results for *GRM7* gene at rs11928865 SNP refers to A or T alleles and contribute for three possible genotypes: A/A, A/T, or T/T. *GRM7* data are presented comparatively (**Table 4**) with data for Iberian Peninsula and Europe in order to compare our population with others.

TABLE 3 | Association between tinnitus and statins intake in individuals reporting hypercholesterolemia.

	Statins intake	Without Statins intake	$\widehat{OR}$	*p*-value (fisher test) test)	95% IC
Tinnitus	13	17	0.28	$p = 0.045$	[0.08 – 0.99]
Without tinnitus	14	5			

* *p-value < 0.05.*

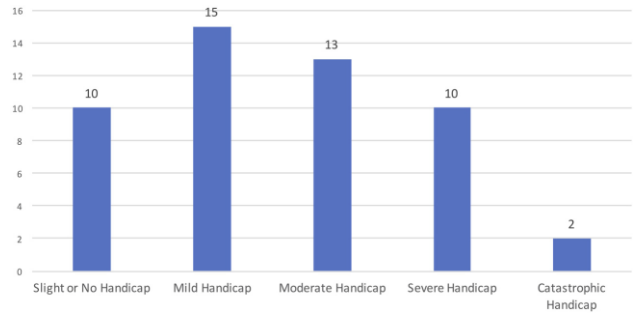

FIGURE 6 | Distribution of the individuals according to THI.

Some genetic specificity has been reported for different populations regarding deafness genes, and, interestingly, genotypes representativeness for the individuals of our sample were in accordance with values described in the European population as well as in the Iberian population.

Analyzing these results and considering the hearing thresholds, no significant differences were found in males or females when the three genotypes were compared, however, some differences in the pattern of the curves on the audiogram can be seen.

Considering the Tinnitus Handicap Inventory scores (please see **Figure 6**) the variable severe tinnitus ($n = 12$) joins the severe and catastrophic grades. We found relevant statistical association between the presence of *GRM7* and severe tinnitus (individuals having scored severe or catastrophic grade in THI). The results are present in the **Table5**.

We found no relevant statistical differences considering *GRM7* when comparing the four sub groups already described in **Table 1**, evidencing no relation with this SNP and the presence of presbycusis with or without tinnitus.

When considering genetic data, *GRM7* genotype was found not to be associated with the risk of developing presbycusis ($p = 0.889$). However, the odds of HL is

TABLE 4 | Comparative results for rs11928865 SNP on *GRM7* gene and comparison with other populations.

Genotypes	*N*	Frequency	Europe	United Kingdom	Iberian peninsula
A/A	5	0.064	0.087	0.055	0.065
A/T	26	0.333	0.382	0.473	0.393
T/T	47	0.603	0.531	0.473	0.542
Total	78	1	1	1	1

TABLE 5 | Association between THI score (tinnitus severity) and *GRM7* gene.

GRM7	Severe tinnitus	*p*-value	Fisher test
A/A	1		
A/T	7	0.0233*	0.0175
T/T	4		

* *p-value < 0.05.*

higher in individuals presenting A/T (29%) or T/T (2%) genotype, than in A/A genotype. The same results were observed when controlling for age and gender, however, in this case the odds of HL in A/T genotype individuals was nearly 39% higher than for A/A genotype individuals. The odds of HL in T/T genotype was 15% higher than in A/A genotype.

The relation between tinnitus and GRM7 gene was evaluated considering two groups, one defined as "having an A allele" (AA + AT) other defined as "not having an A allele" (TT). Results were not significant $(\widehat{OR} = 0.96)$ however, since the estimated $\widehat{OR} < 1$, a decrease in the risk for tinnitus could be thought. The GRM7 genotype was not identified as a risk factor for tinnitus, neither when controlling for age $(\widehat{OR} = 0.94)$ ($\widehat{\text{OR}} = 0.94$) for gender $(OR = 0.93)$ or both simultaneously $(\widehat{OR} = 0.93)$. Similar analysis was performed considering also two groups but defined as "having a T allele" (TT and AT genotypes). However, no significant association with tinnitus was found.

Genetic analysis of *NAT2* gene was performed in 65 individuals, 39 females (60%), and 26 males (40%). Rapid (R) phenotype was least common (12.3%, $n = 8$), followed by Intermediate (I) phenotype (35.4%, $n = 23$) and Slow (S) phenotype (52.3%, $n = 34$) (please see **Table 6**).

The genotype 4/4 (considered as wild type) was observed in 9.1% ($n = 6$) of the individuals being the allele 4 present in 56.9% ($n = 37$) of the genotypes. The genotype 6A/6A previously associated with presbycusis was found in 6.2% ($n = 4$) of the individuals being the allele 6A present in 23.1% ($n = 15$) of the individuals. The most common genotype is 5B/5B accounting for 50% ($n = 11$) of all the homozygous genotypes (33.9%, $n = 22$) the sample (Kuznetsov et al.,2009).

We found no statistical differences in *NAT2* gene expression across our four subgroups described in **Table 1**, evidencing no relation with the presence of presbycusis with or

TABLE 6 | Genotypes observed in the sample and their corresponding phenotypes.

Genotype	Phenotype
NAT2 4/NAT2* 5U; NAT2* 6A/NAT2* 6A; NAT2* 5B/NAT2* 5D; NAT2* 6J/NAT2* 13A; NAT2* 5A/NAT2* 5B; NAT2* 6N/NAT2* 6N;*	S
NAT2 6A/NAT2* 6B; NAT2* 5D/NAT2* 5G; NAT2* 5B/NAT2* 5B; NAT2* 5R/NAT2* 12A; NAT2* 4/NAT2* 5J*	
NAT2 4/NAT2* 4; NAT2* 12A/NAT2* 12C; NAT2* 4/NAT2* 12C*	R
NAT2 4/NAT2* 5B; NAT2* 4/NAT2* 6A; NAT2* 4/NAT2* 5B; NAT2* 4/NAT2* 5A; NAT2* 5B/NAT2* 12A; NAT2* 4/NAT2* 13A; NAT2* 4/NAT2* 5V; NAT2* 6A/NAT2* 13A; NAT2* 5B/NAT2* 13A*	I

without tinnitus. No significant association with ARHL was found, for the in the right and left ear or best or worst ear.

Considering the Tinnitus Handicap Inventory scores, we found significant association between severity of tinnitus (grades severe and catastrophic from THI) and the presence of *NAT2* gene (please see more details in the next sub-heading).

Modeling the Data – *GRM7* and *NAT2*

All the results were analyzed through logistic regression model (**Tables 7**, **8**) where age, gender and noise exposure were considered in the models with the purpose of controlling for confounding. The independent variable in the model was severe tinnitus (n ‡2), (the sum of severe and catastrophic grades from THI) (**Figure 6**).

We have considered the genotype T/T because after crossing the *GRM7* gene with the tinnitus population, we found that the T/T genotype is more frequent and it is the most representative so it was chosen as the reference category.

The odds of developing severe tinnitus was significantly higher in the presence of genotype A/T when compared to genotype T/T $(\widehat{OR} = 14.2,\ p = 0.009,\ CI = 2.0 - 97.8)$. When considering the genotype A/A, no statistically significant difference was found $(\widehat{OR} = 2.9,\ p = 0.443,\ CI = 0.2 - 42.2)$. The probability of severe tinnitus among individuals with genotype A/T is significantly higher when compared with individuals with the genotype T/T (for more details see Appendix 6).

When analyzing the presence of severe tinnitus through a logistic regression model considering *NAT2* as the independent variable and controlling for age, gender and noise exposure, the odds of developing severe tinnitus was significantly higher in the presence of slow acetylator phenotype when compared to intermediate acetylator $(\widehat{OR} = 5.7,\ p = 0.095,\ CI = 1.5 - 21.9)$. No statistically significant difference was found with respect to rapid acetylator $(\widehat{OR} = 2.8,\ p = 0.504,\ CI = 0.4 - 20.8)$(for more details see Appendix 7).

TABLE 7 | Logistic regression model in the *GRM7* applied to severe tinnitus considering the genotype T/T as reference.

Variable*	$\widehat{OR}$	*p*-value (Wald test)	(95% IC)
GRM7			
A/A	2.9	0.443	(0.2, 42.2)
A/T	14.2	0.009**	(2.0, 97.8)

* * *p-value* < *0.05.*

TABLE 8 | Logistic regression model in the *NAT2* applied to severe tinnitus considering intermediate acetylator as reference.

Variable*	$\widehat{OR}$	*p*-value (Wald test)	(90% IC)
NAT2			
Rapid acetylator	2.8	0.504	(0.4, 20.8)
Slow acetylator	5.7	0.095	(1.5, 21.9)

DISCUSSION

In the present research, we conducted a case history questionnaire, hearing evaluation and gene screening analysis for *GRM7* and *NAT2* in a sample of patients aged between 55 and 75 years, in an attempt to find factors that might contribute to the diagnosis of presbycusis and tinnitus, which could be useful for diagnosis and future therapeutic interventions.

Comorbidities Effect

Although in previous literature was described that individuals with thyroid problems present increased hearing thresholds, suggesting that thyroid hormones may act as regulators of the auditory system (Forrest et al.,1996) our results do not show any statistical relevance concerning this, one possible explanation is the sample size. Only 10% of our participants report thyroid problems which precludes statistical analysis.

Possibly for a similar reason our data doesn't show that individuals with high blood pressure may be at greater risk of presbycusis than the normotensive. Hypertension has previously been associated with increasing of the hearing threshold (Agarwal et al.,2013, p. 614). Since both presbycusis and hypertension are common and widespread disorders, the fact that hypertension may influence presbycusis strongly suggests adding cardiologists to the multidisciplinary team of professionals screening for presbycusis and improving the quality of life of positively identified individuals (Agarwal et al.,2013).

Our results found that hypercholesterolemic individuals had a lower risk of HL, probably this is due to the fact that the majority of them (67%) were having medication (statins) to control cholesterol levels. These results are in accordance with previous publications (Gopinath et al.,2011). In individuals with hypercholesterolemia the chance of occurring tinnitus is 72% lower in those who have statins intake. According to our results It seems like the statins have a protector effect.

Noise exposure and "high frequency" hearing loss seems to influence the occurrence of tinnitus, those were two of the most statistical relevant findings in our study population, which is in accordance with previous literature (Hoffman and Reed,2004).

Gender and Age Effect

Significant differences on the HL degree were observed in different frequencies for different age groups (**Figure 2**). Our results show a significant age-dependent increase of hearing loss in about 13% for both genders, although the risk of developing presbycusis is about three times higher for men. This finding is consistent with a previous reports (Pearson et al.,1995) but contradicts another (Homans et al.,2016) where women were found to have more hearing loss.

According to our data, the risk of presbycusis increases 9% per year of life. Considering the increase in life expectancy of the population in industrialized countries, our result presents obvious consequences and must be considered for future clinical management guidelines.

In our sample tinnitus was present in 60.7% of the participants and men showed 53% more likelihood of developing tinnitus than women. This contradicts other results (Vielsmeier et al.,

2012) who reported higher tinnitus prevalence in women but in a much younger population.

According to our data, and in agreement with previous literature (Hoffman and Reed,2004;Shargorodsky et al.,2010) age is not associated with the risk of developing tinnitus.

GRM7 and *NAT2* Effect

We did not find a significant relationship between *GRM7* genotype and either presbycusis or tinnitus. Especially for men, some differences concerning the pattern in the audiogram curves were observed in relation to *GRM7* phenotypes. For both genders, the T allele in *GRM7* gene is the most common allele in our sample of older adults with presbycusis and tinnitus, where genotypes A/T and T/T present higher level of hearing loss compared to A/A genotype. Perhaps in a larger population it could be demonstrated that the allele A of *GRM7* plays a protective role in presbycusis.

Hence, according to our results, *GRM7* genotype does not seem to be predictive of presbycusis since the odds to have ARHL is not significant ($p = 0.78$). Corroborating our results, Luo et al.(2013)studying an all-male population found that the T-allele frequency was significantly different from the genotype A/A &A/T comparing ARHL patients and healthy controls and that the *GRM7* SNP A > T was significantly different between the two groups (Luo et al.,2013). On the other hand, our findings differ fromFriedman et al.(2009) most likely due to sample size (Luo et al.,2013). Moreover, the impact of the other variables – environmental, lifestyle, noise exposure, cholesterol levels and stochastic element – perhaps has prevailed over the genetic factor, declining the importance between *GRM7* gene and ARHL. Certainly multicenter studies with higher sample sizes would overcome these aspects.

Concerning *NAT2* gene, Rapid (R) phenotype was the least common, followed by the Intermediate (I) and Slow (S) phenotypes.

We found relevant statistical association between the presence of the allele A/T of *GRM7* and severe tinnitus. The chance for having a severe grade of tinnitus (severe or catastrophic grades in THI) is 14,2 higher for those carrying the allele A/T compared to T/T. Probably in larger scale studies could be demonstrated the role of allele A/A that is the less frequent in our sample.

The odds of developing severe tinnitus was relatively higher in the presence of slow acetylator phenotype of *NAT2* when compared to intermediate acetylator.

Our data suggests that allele A/T of *GRM7* can have a statistically significant influence toward the *severity* of tinnitus. As well slow acetylator phenotype of *NAT2* seems to have a similar influence (not statistically relevant in our results). Nevertheless, those results should be interpreted with caution and future studies in larger scale are necessary to confirm this correlation.

However, present data shows that genotype A/T and T/T present, respectively, a 70 and 33.3% lower risk of developing tinnitus, when compared to A/A genotype. No other studies were found relating *GRM7, NAT2* and tinnitus.

CONCLUSION

To the best of our knowledge, this is the first study on the association between *GRM7* and *NAT2* gene and the presbycusis and tinnitus in a population of Portuguese older adults.

Tinnitus was present in the majority of the presbycusis individuals.

Age and gender significantly influence the risk for presbycusis but not for tinnitus. Overall hearing thresholds rates increase exponentially with age (9% per year), and the increment rate and speed were gender-specific, but this increasing rate and velocity are different for women and men.

High blood pressure, thyroid diseases and hypercholesterolemia seem to have an effect on the hearing thresholds but no significant associations were found.

Our findings agree with previously observed correlations between tinnitus, noise exposure, and “high frequency” hearing loss.

No significant associations between presbycusis, tinnitus, and *GRM7* or *NAT2* were found in our sample. Our results precludes a definitive clarification about the role of *GRM7* as a possible genetic biomarkers for ARHL, although since the genotypes A/T and T/T have higher odds for HL than A/A genotypes, thus A allele could be pointed as protective biomarker for HL Nevertheless, the current state of knowledge regarding *GRM7* impact in presbycusis is insufficient to make conclusions, and so, further large-scale studies are necessary to clarify this relation.

Considering tinnitus severity (according to THI), our results bring-up very innovative conclusions.

Our data suggests the tracks that can lead to the pathway of a tinnitus severity biomarker. Potentially individuals carrying the allele A/T of *GRM7* and slow acetylator phenotype of *NAT2* (the later one with smaller statistic relevance) are prone to develop a more severe form of tinnitus, that requires specific therapeutic interventions and ideally personally tailored.

The occurrence of presbycusis is thought to be determined by genetic factors but can also be influenced by environmental or comorbidities effects, with a huge impact on quality of life and general health (Huang and Tang,2010;Ciorba et al.,2015). However, there is still much research to explore and elucidate which risk factors contribute more to presbycusis and tinnitus, so this could help on therapeutic or preventive interventions (Huang and Tang,2010).

Information on family history and clinical epidemiological data may help the design and development of future clinical management plans for an increasing presbycusis population.

AUTHOR CONTRIBUTIONS

HH conceived and designed this study and had contributions to all its stages. MAp and MA performed the statistical analysis. HH, MF, and HC contributed equally to all other stages of the manuscript development, drafted and revised the manuscript. DR worked with HH on interpretation of results and created appendices. DR created all audiometric figures. JP, MA, AS, DH, and GF provided consultative advice and revised the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at:https://www.frontiersin.org/articles/10.3389/fnagi.2017.00346/full#supplementary-material

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6. Discussion and Conclusions

The present study aimed to identify audiological, immunological or genetic factors associated to tinnitus, in a way to promote a better understanding of tinnitus pathophysiology and improve diagnosis and treatment of tinnitus. Additionally, this study allowed us to characterize the most relevant comorbidities associated to tinnitus.

Characteristics of the population in study:

We explored different aspects in a sample of participants aged between 55 and 75 years, with or without hearing loss and/or tinnitus. In the 114 participants aged 64.0 ± 5.6 years old, most of them were female (52.6%). The mean age for the subgroup without tinnitus was 64.6 ± 3.8 years old, while the mean age for tinnitus group was 64.0 ± 5.6 years old. Our tinnitus population included a high number of individuals belonging to the sector 'specialists of scientific and intellectual activities', followed in number by 'technicians and professionals of intermediate level' and 'administrative staff'.

Tinnitus and comorbidities

Using a structured interview, we have explored the following comorbidities in our sample: cholesterol, hypertension, cardiovascular disease, diabetes, thyroid problems, smoking and alcohol habits, meningitis, mumps, measles, tuberculosis, ear diseases, ear surgery, and noise exposure.

From our sample, 68 participants (59.6%) had high blood values of cholesterol. Of those, 27 individuals (55.1%) were taking medication (statins). There was a significant association between tinnitus and statins intake in those individuals reporting hypercholesterolemia (OR =0.28, p=0.045, CI=0.08-0.99). This finding is in agreement with those reported in our systematic review of tinnitus biomarkers. There is good evidence therefore for an association between the presence or severity of tinnitus and hyperlipidemia.

We found no relevant association between statins intake and hearing loss.

Concerning the effect of history of noise exposure and hearing loss, the results showed that the odds of developing tinnitus were significantly higher ($p=0.036$ and p=0.009) respectively in the presence of these factors. These results are in agreement with the literature where it is hypothesized that tinnitus is an epiphenomenon of a neuronal process to attempt normalizing impaired hearing thresholds (Gollnast et al., 2017). Similarly, some epidemiological studies have confirmed correlations between hearing loss, noise exposure, and tinnitus (Chung et al., 1984; Hoffman & Reed,2004; Roberts et al., 2010; Sindhusake et al., 2003, 2004;).

High frequency hearing loss in both ears represents a significant risk of tinnitus (p=0.001).

Regarding comorbidities in our sample, mumps are present on 56% of the individuals with tinnitus and 31.8% for the individuals without tinnitus. Also, 53.3% the individuals with tinnitus and 22.7% without tinnitus had hearing loss. We only found statistically significant differences between the two subgroups for mumps (p=0.041) and hearing loss (p=0.010).

According to statistical analysis, the odds of having tinnitus was higher for those participants having noise exposure (p=0.036), high frequency hearing loss or presbyacusis (p=0.001), hearing loss (p=0.014) and mumps (p=0.046).

Clinical characterization of tinnitus sample

Through the Portuguese validated version of Tinnitus Handicap Inventory (THI) (Newman et al., 1996; Oliveira & Menezes, 2008), we analyzed the THI scores for categorization of tinnitus severity in our sample. A mild handicap score was the most frequent grade in our sample being present in 38 participants (41.3%) followed by moderate handicap (22 participants, 23.9%), slight or no handicap (17 participants, 18.5%), severe handicap (14 participants, 15.2%). One participant had a catastrophic handicap score (1.1%). We analyzed the possible factors contributing to development of tinnitus or different grades of tinnitus severity and found some clinical aspects, described ahead, that may contribute more to developing severe or catastrophic tinnitus.

Our results showed that the onset of tinnitus is an important factor contributing to the severe or catastrophic tinnitus grade. In our sample, 49% of participants showed a gradual tinnitus onset followed by 19.5% of participants that had abrupt onset tinnitus. Our results evidence that an abrupt tinnitus onset is 5.13 times more likely to develop a severe or catastrophic tinnitus. This indicates that a gradual tinnitus onset permits a natural habituation process which is also mentioned in the bibliography (Hall, Rachman & Hinchcliffe, 1984).

Psychoacoustic tinnitus assessment

We performed several tests to characterize, measure and evaluate tinnitus percept in all the participants with this complaint. Tinnitus pitch ranged from 2000 Hz to 8000 Hz, with 4000 Hz being the most frequently found. Mean loudness was 0 dB. We have considerate the difference between tinnitus intensity at the frequency identified by psychoacoustic measurements and hearing threshold at that frequency as the tinnitus sensation.

The majority of our sample had a pure tone tinnitus (59%) and central location (52.4%). The Feldmann's curve showed that the convergent (47.6%) and distant type (29.8%) are the most frequent. Residual inhibition was generally negative (43.9%) or partial (36.6%). Patients with negative or rebound residual inhibition had an 82.2% higher odds of developing a severe or catastrophic tinnitus. Complementary, the presence of a complete (tinnitus abolishment) or rebound effect (tinnitus exacerbation) in residual inhibition was found as a "protector factor". This result seems to be related to the possibility that patients with complete residual inhibition can more easily mask their tinnitus with environmental sounds. Hence it is expected that tinnitus will not become very disturbing because is only audible in very silent places (e. g. bedroom at sleeping time). These results represent novel findings for the importance of residual inhibition in the diagnosis.

Audiological markers of tinnitus

The exploration of audiological markers in our participants, with or without tinnitus, allowed us to identify new relevant insights regarding tinnitus evaluation.

Hyperacusis (over sensitivity to sound) demonstrates a relationship with tinnitus severity. In our sample, participants with moderate or severe hyperacusis were 7.56 times more likely to develop severe or catastrophic tinnitus (OR=7.556, p=0.011, CI=1.603-35.616).This finding favors the central gain theories regarding common pathophysiology for tinnitus and hyperacusis.

Data from the literature suggest common pathways for the pathophysiology of tinnitus and hyperacusis resulting as a central compensatory gain due to reduced neural activity from a damaged cochlea (Auerbach et al., 2014; Knipper et al., 2013).

The study of possible Auditory Brainstem Response (ABR) markers in tinnitus patients identified a reduction of the amplitude in the wave I in patients with tinnitus, and an increase of the I-III interval when compared the group without hearing loss with tinnitus, and the group with hearing loss and tinnitus. The reduction of the wave I amplitude had already been reported in other studies (Attias, et al., 1993; Gu et al., 2012; Schaette & McAlpine, 2011). Also, the ABR amplitude wave I was considered as a protective factor in relation to developing tinnitus and Ratio RE_LE as a risk factor to the odds of having a severe or catastrophic grade of tinnitus. This parameter is also related to the amplitude of waves I and V. The reduction of amplitude I may be due to changes in the IHC and/or in Auditory Nerve Fibers (ANFs). Changes can be a diffuse loss of the IHC compared with patients with no tinnitus that results in a lowered wave I amplitude (Gu et al., 2012) or a diffuse loss of the ANFs while the IHC remains intact (Gu et al., 2012; Le Prell et al., 2003, 2005). Other possibilities are that ANFs are equally intact, and the reduction of the amplitude wave I is due to the reduced excitability of ANFs via lateral olivocochlear efferents which terminate on their endings, or there is a diffuse loss

of ANFs sufficient to manifest a reduction in mean wave I amplitude. The evaluation of ABR audiological markers also adds that the mean ratio of PEAP (Potential Evoked Auditory Potentials) for both ears are 1.1 times higher for developing severe or catastrophic tinnitus((OR)ˆ=1.1,p=0.046,CI=1.002-1.208).

Analysis of DPOAEs showed that are lower in patients with tinnitus (p=0.041 for RE and p=0.028 for LE) to those without. However, in this case, we do not consider the presence or not of hearing loss. Our results are in agreement with those reported by Ozimek et al. and Shiomi et al., which points us to conclude that these observed changes are specific to the functions of OHC instead of nonspecific non-linearity of the basilar membrane system (Ozimek et al., 2006; Shiomi et al., 1997). In addition, DPOAEs were statistically lower (p=0.038 in the LE) in participants that had noise exposure. In fact, this is a protective variable, and when it is higher the odds of having tinnitus diminish. These data are in accordance with others results (Sindhusake et al., 2003). Thus, noise exposure was a risk factor for tinnitus in our sample, which is in agreement to published literature.

In summary, according to the regression statistical model some variables represent lower odds for developing tinnitus, namely “Mean OEA for left ear” (p=0.043) and “ABR wave 1 amplitude for right (p=0.004) and left ear” (p=0.048).

Immunological markers of tinnitus

The majority of the participants (n=112) of the sample were evaluated for IL1α, IL1β, IL2, IL6, IL10, IFN-γ, TNF-α and TGF-β and a small group (n=80) for HSP70 in peripheral blood samples.

The comparison between subgroups of individuals with or without tinnitus revealed significant difference for the levels of IL10 (p = 0.025) and for IL6 and TGFβ we observe a marginally significant result (p =0.052 and p = 0.064, respectively). We had two-day timepoint for blood sample collection and our results showed that inflammatory markers have a circadian cycle which is accordance with others authors (Altara et al., 2015). Interestingly, we observe more statistical relevant results for the afternoon group (p =0.012). This finding is in accordance with the recent demonstration that the cochlea also has a circadian cycle. In animal studies of hearing loss secondary to noise trauma consequences on hearing were significantly worse when noise trauma was delivered at 9pm than at 9am (Meltser et al. 2014).

The IL1α values were statistically higher in patients with tonal tinnitus compared to narrow band tinnitus. Regarding residual inhibition, we found statistically significant differences for IL2 (p = 0.019). Additionally, we observed a negative correlation between tinnitus duration and the level of IL10 (r = -0.281, p =0.007) and also a significant negative weak correlation between HSP70 and tinnitus loudness (r = -0.397). To our best

this is the first report of those correlations between tinnitus characteristics and psychoacoustic measurements with inflammatory markers.

The concentration of evaluated inflammation markers correlated significantly with hearing loss, especially with the high frequency hearing loss (presbycusis), which is a statistically significant risk factor for tinnitus.

Genetic markers of tinnitus

Our study of *GRM7* and *NAT2* found factors that might contribute to the diagnosis of presbycusis and tinnitus, eventually lead to future therapeutic interventions.

Results were obtained from 78 individuals from the sample, most participants were female (n=45, 57.7%), presenting an average age of 64.1 ± 5.35 years old. Male participants (n=33, 42.3%) had a mean age of 65.3 ± 5.89 years. *GRM7* gene was studied for rs11928865 SNP, which refers to A or T alleles and contribute for three possible genotypes: A/A, A/T or T/T. When considering *GRM7* genotypes results in the patients with tinnitus, we identified the genotype T/T as the most frequent and representative, thus it was chosen as the reference category for further statistical analysis. The odds of developing severe tinnitus was significantly higher in the presence of genotype A/T when compared to genotype T/T (OR=14.2, p=0.009, CI=2.0-97.8).

Genetic analysis of *NAT2* gene was performed in 65 individuals, 39 females (60%), and 26 males (40%). Rapid (R) phenotype was least common (12.3%, n=8), followed by Intermediate (I) phenotype (35.4%, n=23) and Slow (S) phenotype (52.3%, n=34). When analyzing the presence of severe tinnitus through a logistic regression model considering NAT2 as the independent variable and controlling for age, gender and noise exposure, the odds of developing severe tinnitus was significantly higher in the presence of slow acetylator phenotype when compared to intermediate acetylator (OR=5.7, p=0.095, CI=1.5-21.9). No statistically significant difference was found with respect to rapid acetylator (OR=2.8, p=0.504, CI=0.4-20.8).

Our data suggests individuals carrying the allele A/T at *GRM7* and slow acetylator phenotype of *NAT2* (the later one with smaller statistic relevance) are prone to develop a more severe form of tinnitus that requires specific therapeutic interventions, and ideally, personally tailored treatment.

Quality of life and psychological aspects of tinnitus

We explored characteristics, psychological symptoms, and quality of life in the individuals of the sample. For the whole sample, 22 of the participants had a previous history of psychiatric medication, while 20 participants were, at the moment, taking psychiatric medication. From the 22 participants with previous history of psychiatric

medication, 86.3% participants were patients with tinnitus (n= 19) with a previous history of medication. Regarding those having psychiatric medication, 20 patients had tinnitus (corresponding to 20.6% of all individuals with tinnitus) were, at the moment of inclusion in the study medicated, which corresponds to 95% of the total of the participants that were taking psychiatric medication. Those results strongly suggest that the most severely affected tinnitus patients will benefit from the care of mental health professionals.

Analysis of the Portuguese validated version of Brief Symptom Inventory (BSI) Canavarro, 1999) observe higher scores in tinnitus patients on the psychological complaint scales of somatization and paranoia. Overall, is described that patients with tinnitus suffer more distress arising from awareness of bodily representations and present paranoid behavior related to a disordered mode of thinking (Derogatis & Spencer, 1982). Significant differences were found in the Obsessive-compulsive ($p=0.009$) and Interpersonal sensitivity ($p=0.026$) scales concerning the presence of tinnitus. The interpersonal sensitivity dimensions focus on inferiority and inadequacy feelings when compared with others, and with depreciation and restlessness during interpersonal interactions. In turn, the Obsessive-Compulsive scale focuses on thoughts, impulses and actions of the individual that are considered irresistible, but are of an undesired nature (Derogatis & Spencer, 1982). This supports the importance of CBT in tinnitus treatment, mainly in respect to negative automatic thoughts, safety behaviors and inaccurate beliefs in relation to tinnitus (McKenna et al., 2014). Those results strongly propose including psychologists to the multidisciplinary approach with different professionals involved at tinnitus treatment.

The Portuguese validated version of the Medical Outcomes Study Short Form Health Survey (MOS SF-36), evaluates health-related quality of life (Ferreira, 2000). Here the MOS scales were generally lower in the population with tinnitus, on both physical and emotional dimensions, suggesting a lower quality of life in this population. Our results showed that the physical component of MOS was lower than the emotional component, which indicates physical limitations, more impact of the intensity and discomfort caused by the pain, and the person's own perception of his overall health. Also, the lowest scale concerning emotion was the vitality scale which measures energy and fatigue levels, and that qualifies the differences in well-being. According to another study in a Portuguese population using the MOS SF-36 (Oliveira & Meneses, 2008), vitally was one of the most affected scales, together with the social function, general health, mental health dimensions.

Regarding the comparison of the more severe grades of tinnitus participants (severe and catastrophic according to THI) with other grades, significant differences in psychological complaint and quality of life were found. Through the logistic regression model, with severe or catastrophic tinnitus as an independent variable, it was found

significant differences in General Severity Scale (BSI) (p=0.037) and in General Health Perceptions (MOS) (p=0.037). For the odds of developing severe tinnitus, the variable General Severity Scale was considerated as a risk factor (OR was above 1) and General Heath Perceptions was considerated as a protector factor (OR was below 1). Most BSI scale scores were increased (showing a clinically relevant score ≥63) revealing the psychological impact of the participants that were severely affected by tinnitus. Higher grades on the scales such as somatization, depression and anxiety were related to the severity of tinnitus, as reported in previous studies (Erlandsson & Hallberg, 2000; Holgers et al., 2005)

Using regression statistical models, we have proven that the scales with more relevant statistical association to tinnitus are the General Severity Index (the more sensitive BSI scale to distress symptoms according to Derogatis & Spencer, 1982) and the General Health Perception (from MOS). These findings bring to discussion the issue of frailty, particularly relevant for the age range focused in our study. Clearly, an individual will be more prone to have tinnitus if they have higher General Severity Index (t≥63) and lower values at General Health Perception scores.

Our results bring and reinforces new insights concerning the importance of the holistic assessment and management of the individual relevant to tinnitus as a multidimensional symptom (Hall et al., 2018a). To our knowledge this is the first study to use the BSI General Severity Index and identify scoring that represents a risk factor for tinnitus and impaired quality of life, and what scoring on the General Health Perception scale of MOS represents a protector factor for tinnitus.

European Research Network

Towards a global standardization in tinnitus scientific field

Many people with tinnitus do not seek medical support. Even for those reporting tinnitus as a bothersome problem, a large American survey found that only 50% had visited a physician for a consultation appointment (Bhatt et al., 2016). Although there are multiple management options for tinnitus, the majority lack high quality scientific evidence to support strong claims for their benefit. From of all therapeutic options, Cognitive Behavioral Therapy (CBT) delivered by a qualified clinical psychologist has the most support for its effectiveness in reducing tinnitus symptom severity (Cima et al., 2012; Hesser et al., 2011; Martinez Devesa et al., 2010).

Given the heterogeneity of the tinnitus condition, one of the major challenges to advancing the field of tinnitus concerns the lack of standardization in approaches to research and clinical management. To address this, a European consortium gathered together in 2014 under the auspices of a four-year European Cost Action BM1306 “Tinnitus Research Network” (TINNET) (http://tinnet.tinnitusreserch.net/). The main

objective of this Action was the creation of an international network for identifying clinically meaningful subtypes of tinnitus and their neurobiological underpinnings in order to develop better strategies for diagnosis and management. TINNET comprised five working groups (WG) covering clinical, database, neuroimaging, genetics and measurement outcomes. These coordinated efforts from basic scientists, technicians and clinicians working in different medical disciplines working together in close collaboration. TINNET interdisciplinary objectives were to create standards for identifying i) meaningful clinical and demographic characteristics for tinnitus subtyping (WG1), ii) assessing tinnitus-related changes in brain activity associated with different subtypes of tinnitus (WG3), iii) intermediate genetic phenotypes and genetic factors in the pathogenesis of tinnitus (WG4), iv) standards for assessing outcomes after clinical intervention (WG5). Cross-cutting all of these themes WG2 was aimed at establishing an electronic data repository to promote scientific transparency and support data sharing.

The legacy from TINNET is wide ranging. WG1 planned a roadmap in which a systematic review of existing tinnitus guidelines as well as a European survey on tinnitus management were the foundations for their final goal (Fuller & Haider et al., 2017) They have gathered the opinions of clinical experts using consultation methods to generate an evidence-based good practice guideline for the assessment and management of people with bothersome tinnitus. This is now in the final stage of dissemination, but was launched at the 11th Tinnitus Research initiative and 2nd TINNET conference in Regensburg in March 2018.

WG5 have gathered the opinions of many different stakeholders including people with tinnitus as well as professionals using consensus methods to produce recommendations for what tinnitus-related complaints are critical and important to measure when assessing treatment-related benefit. Of interest, these guidelines separately consider sound-based, psychology-based and pharmacology-based interventions, since each of these approaches has a different therapeutic rationale and therefore aims to address different tinnitus symptoms. For WG5, a series of publications has already appeared in various peer-reviewed journals (Hall et al., 2016, 2018a,b; Smith et al., 2018) and the main findings are again in the final stage of dissemination. The next step is to identify how the different tinnitus-related complaints should be measured. This work will continue over the next few years beyond the initial TINNET funding.

Diagnostic criteria for Somatosensory tinnitus: A Delphi consensus

From the need on increasing knowledge about subtypes of tinnitus we underwent also a scoping review regarding somatosensorial tinnitus pathophysiology, diagnostic and treatment. And participated in a study aimed to reach an international consensus on diagnostic criteria for somatosensory tinnitus (ST) among experts, scientists and clinicians, using a Delphi survey and consensus meeting strategy. The

consensus meeting panel recognized that somatic modulation, especially by voluntary movements, specific tinnitus characteristics and symptoms that can accompany the tinnitus are suggestive of the presence of ST, but added that the absence of somatic modulation does not rule out ST. None of these criteria have to be present in every single patient with ST, but in case they are present, they strongly suggest the presence of ST.

It was also considered the possibility of the presence of specific type of constant pulsatile tinnitus. Although not an item included there is a strong recommendation for attention to keep in mind that in some cases a pulsatile tinnitus, synchronous with the heartbeat, can be affected by somatic maneuvers.

There was an agreement concerning the cases where the somatosensory system is the main cause of the tinnitus. This study also report that a large proportion of patients has a secondary somatosensory influence on the tinnitus clearly caused by auditory deafferentation, such as noise exposure (Michiells et al., 2018).

Conclusions

The world's population is aging and hearing loss is one of the most prevalent chronic diseases that causes of disability (Wilson, Tucci, Merson, & O'Donoghue, 2017). The consequences of hearing impairment for general health condition of the affected people are considerable including reduced physical and mental activity and secondary social isolation caused by hearing loss (Arlinger, 2003) increases the risk of cognitive decline and dementia (Lin et al., 2011), mental illness (Matthews, 2013), and depression (Davis, 2011; Matthews, 2013).

Given the strong links between hearing loss and tinnitus, then tinnitus surely follows this trend. Altogether this symptoms contribute for progression to frailty.

Our audiological study highlits some interesting findings especially concerning audiological tinnitus characteristics. Our data may contribute to foresee the evolution of the severity of the tinnitus, namely the odds of a patient develop severe or catastrophic grade of tinnitus. ABR Ratio OD_OE as a risk factor for having a severe or catastrophic grade of tinnitus, if confirmed in larger populations studies is a potential candidate as an audiological marker of tinnitus severity.

In our study of inflammatory parameters, we have shown for the first time that the systemic concentration of IL10 and INF-γ are statistically associated with the presence of tinnitus. Another interesting finding is that higher IL1α levels are associated with tonal type of tinnitus and HSP70 and HL10 are negatively correlated to tinnitus loudness and tinnitus duration respectively. Because of the trend for negative correlations between the inflammatory markers and tinnitus characteristics, it is

reasonable to think that inflammatory mechanisms are involved in the acute phase of tinnitus emergence.

On the other hand, our data suggests a potential genetic tinnitus severity biomarker, since individuals carrying the A/T genotype at rs11928865 SNP of *GRM7* gene and slow acetylator phenotype of *NAT2* gene (the later bordering significance) are prone to develop a more severe form of tinnitus. This data allow the identification of tinnitus that requires specific therapeutic interventions and is ideally personalized treatment.

Taken together, tinnitus and hearing loss disorders have a high negative impact on the quality of life of the affected persons especially if the grade of tinnitus severity is high.

To our knowledge this is the first study to use the BSI General Severity Index and identify scoring that represents a risk factor for tinnitus and impaired quality of life, and what scoring on the General Health Perception scale of MOS represents a protector factor for tinnitus.

Our study brings and reinforces new insights concerning the importance of the holistic assessment and management of the patient pointing for tinnitus as a multidimensional symptom.

Accordingly, the assessment of the individual with tinnitus and therapy strategies should be multidisciplinary to ensure coverage of all dimensions of the patient. Moreover, therapeutic strategies should be tailored to the individual, after proper information and with respect for patient choice and individual needs.

Due to the huge diversity of tinnitus etiologies it is highly relevant to appropriately identify clinical tinnitus subtyping, thus deal with potential treatable causes and obtain the appropriate tinnitus management for each patient. Multidisciplinary guidelines are essential.

The establishment of guidelines for clinical diagnosis, treatment, neuroimaging assessments and outcome assessment, through the identification of clinically meaningful tinnitus subtypes, provides an important basis for the standardization of clinical research and management of tinnitus.

7. Future Directions

Worldwide the population is ageing, World Health Organization (WHO) Global Burden of Diseases (2015) estimated that in 2050 there will be 2 billion people older than 65 and reports hearing loss as the fourth leading cause of years lived with disability. Given the strong links between hearing loss and tinnitus, then tinnitus surely follows this

trend. Taken together, tinnitus and hearing loss disorders have a high negative impact on the quality of life of the affected persons especially if the grade of tinnitus severity is high. In order to improve quality of life of people with those conditions it is imperative to have investment in studies to clarify the underlying causal mechanism in order to enable a more efficient prevention or treatment and avoid the progression to frailty and related mental health disabilities.

Tinnitus and hearing loss

The association between hearing loss and tinnitus is more complex than simply having both conditions. When hearing loss is present, the tinnitus frequency usually matches the region of greatest threshold losses, especially in the case of down-sloping sensorineural hearing loss profiles (Schecklmann et al., 2012).

In this perspective the procedures to prevent hearing loss will have a reflection on tinnitus such as vaccination, especially in infants, against the most frequent agents of acute otitis media (*Haemophilus influenza* type B, *Streptococcus pneumoniae* and *Moraxella catarrhalis*) and also promote vaccination for measles, mumps and, rubella. Other preventive measures concern the avoidance of ototoxic medication and environmental risk factors such as noise exposure by means of ear protection (in leisure or work activities) and education before adolescence. Other important preventive measures regards lifestyle optimization by avoiding high calorie diet, toxic consumption (alcohol, tobacco), and a generally harmful lifestyle (Wong & Ryan, 2015). In those people already presenting hearing loss hearing rehabilitation is mandatory.

Future research lines in treatment

Ahlf and colleagues have proposed, after their work in animal models of noise induced tinnitus, that animals who did not develop tinnitus after noise trauma had an overall higher neurologic activity. Hence he suggests that an inhibitor of neuronal activity (such as GabaA) could prevent tinnitus development (Ahlf, Tziridis, Korn, Strohmeyer, & Schulze, 2012). Unfortunately, this remains speculative, and one of the limiting factors for interpretation is that noise trauma not only induces tinnitus but also hearing loss.

Future research should be designed to improve the sensitivity of non-invasive electrophysiological measures of cochlear synaptopathy in humans and to examine the broader neurophysiological impact of noise exposure. Additionally, it is essential to have a clear distinction of the specific mechanisms for isolated tinnitus or for isolated hearing loss.

Genetics is one of the most promising research areas regarding hearing loss because we can anticipate the cloning of some isolated genes responsible for hearing

loss and procedures of gene replacement. Similarly, advances in the immunology and pharmacology fields in combination with advanced cochlear drug delivery systems will allow the molecular replacement or treatment to prevent the loss of damaged cochlear sensorial epithelium (anti-apoptotic, proliferative, nurturing and, anti-inflammatory molecules).

Also, genetics bioengineering will allow us to have cochlear damaged ciliated cells replacement and probably rehabilitate or prevent hearing loss and associated tinnitus.

Those genetic research lines in combination with advances in neuropharmacology molecules, in turn inspired on animal research finding and cells-culture research will certainly allow reaching more effective therapies. Recent novel findings may open perspectives for new therapeutic approaches on molecular level (e.g. intracochlear application of NMDA antagonists, modulation of microtubule associated proteins molecular pathway, GABA modulation); on a systemic level (behavioral strategies, transcranial magnetic stimulation); "hybrid" solutions that would involve synergistic action of pharmacotherapy and Vagal Nerve Stimulation (Bojic et al., 2017) and lastly the intracochlear pharmacological interventions supported by a nonspecific, mostly anxiolytic pharmacotherapy (Guitton, 2012). Factors that determine the phase of tinnitus pathophysiological evolution (initiation or maintenance), the level (molecular or systemic), the mechanism (neurotransmission or neuromodulation) (Guitton, 2012) will in the future determine the therapeutic approach.

Our finding regarding audiological, inflammatory, genetic and psychological parameters suggest the need for further research, not only for confirmation in larger samples, but also regarding pathophysiological mechanisms underlying this phenomenon. Ideally future research on this area should include animal research combined with cell culture studies in order to establish more conclusive translational findings.

Final Considerations

Although there are multiple treatment options for tinnitus management most of them lack scientific evidence-based confirmation. Cognitive behavioral treatment (CBT) is the modality with the highest level of evidence for clinical efficacy (Martinez-Devesa et al., 2010; Hesser et al., 2011; Cima et al., 2012).

Another relevant aspect that should be considered in the development of new therapies is the patient's valorization and acceptance of each treatment option. When developing guidelines for tinnitus diagnosis and treatment, the involvement of the patient's perspective is paramount (Tyler, 2012).

Subjective chronic tinnitus's etiology is still unknown. Current view on tinnitus is that it is a symptom encompassing a distributed network of peripheral and central pathways of the nervous system. Due to its complex nature tinnitus should be approached in a multidisciplinary fashion involving different health professionals that are specialized to deal with each of the dimensions encompassed within this symptom (Hall et al., 2018a).

Frequently, scientific results are impaired due to small and heterogenous sample sizes and also because of lack of standardization among scientific research methods (Vielsmeier et al., 2016). The low rate of therapeutic effectiveness reflects the general dissatisfaction of both patients and health professionals (Hall et al., 2011). It also supports the urgent need for standardization both for clinical practice and for research. Moreover, the real impact of subjective chronic tinnitus in scientific research, clinical practice and patient's life is still undervalued (Hall et al., 2011).

The most recent arguments point to the benefits of a multidisciplinary approach both for tinnitus diagnosis and treatment. The collaboration of professional from different fields (audiologist, psychologist, otorhinolaryngologist, maxillo-facial surgeon, dentist, phisiatricion etc.) will ultimately improve care and improve patient's quality of life (Cima, et al., 2014).

Advancing knowledge concerning specific tinnitus subtypes, origins, and maintenance mechanisms is of paramount importance for obtaining for adequate treatment.

TINNET has been an important pan-European project in bringing together a range of multi-disciplinary experts with an interest in tinnitus. The four years have succeeded in generating a tinnitus community. While the guidelines thus created for clinical diagnosis, management, neuroimaging assessments and outcome measurements provide an important basis for standardization, the future challenge will be in ensuring that these recommendations are adopted and implemented on an international scale. In terms of tinnitus standardization, the next step is to identify how the different tinnitus-related complaints should be measured. Only by working together as a community can we ultimately pave the way to help our patients more effectively manage their symptoms and ultimately to find a cure for this debilitating condition.

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Appendix

Appendix I

cuf
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hospital

O Projecto de Doutoramento da licenciada Haula Faruka Haider intitulado "Presbiacúsia, Acufenos e Qualidade de Vida" está de acordo com os preceitos éticos que devem ser respeitados em trabalhos de investigação clínica desta natureza.

Lisboa, 26 de Novembro de 2014.

O Presidente da Comissão de Ética do

Hospital Cuf Infante Santo

Professor Doutor João Lobo Antunes

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Appendix II

Decisão final sobre o projeto "Presbiacusia, acufenos e qualidade de vida"

A Comissão de Ética da NMS|FCM-UNL (CEFCM) decidiu, por unanimidade, aprovar o projeto de investigação intitulado "Presbiacusia, acufenos e qualidade de vida" (nº65/2014/CEFCM), submetido pela Dra. Haúla Haider.

Lisboa, 13 de Outubro de 2015

O Presidente da Comissão de Ética,

(Prof. Doutor Diogo Pais)

TO WHOM IT MAY CONCERN

The Ethics Research Committee NMS|FCM-UNL (CEFCM) has unanimously approved the Project entitled "Presbiacusia, acufenos e qualidade de vida" (nr.65/2014/CEFCM), submitted by Dr. Haúla Haider.

Lisbon, October 13th, 2015

The Chairman of the Ethics Research Committee,

(Diogo Pais, MD, PhD)

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Appendix III

Declaração de Consentimento Informado

Este estudo irá decorrer no Hospital Cuf Infante Santo, serviço de Otorrinolaringologia (ORL). Tem por objectivo compreender os mecanismos que levam à presbiacusia (surdez no idoso) e acufenos (zumbido nos ouvidos). Os participantes serão submetidos a uma avaliação ORL completa, preenchimento de inquéritos, exames de audiologia, colheita de sangue (para avaliação de parâmetros de inflamação e genética).

Declaro que, após ter tomado conhecimento dos objectivos do projecto, nada tenho a opôr à minha participação no grupo controlo, reservando-me o direito de a qualquer momento retirar esta autorização (sem qualquer prejuízo), nomeadamente se se pretender utilizar estes resultados para outros estudos.

Nome:_____

Data de nascimento: ___/___/_____

Naturalidade (freguesia, cidade, País):_____

Morada:_____

Localidade: _____Código-Postal:_____-_____

Telefone/telemóvel:_____

Data:____/_____/_____

Declaro autorizar o tratamento informático e o contacto para as várias vertentes do estudo de acordo com o preconizado pela Comissão de Protecção de Dados Sim ☐ Não ☐

Desejo ter conhecimento dos resultados obtidos: Sim ☐ Não ☐

_____

(Assinatura)

Código do participante:_____

Declaro ter esclarecido as dúvidas do participante (oralmente e por escrito) e fornecido contacto telefónico (914475542) para esclarecimentos adicionais.

_____

(Assinatura do investigador)

É fornecida uma cópia deste documento ao participante

Appendix IV

COMISSÃO NACIONAL
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AUTORIZAÇÃO N.º 1637/2016

I. Pedido

Haúla Faruk Haider, no âmbito de Doutoramento pela Universidade Nova de Lisboa, notificou à Comissão Nacional de Protecção de Dados (CNPD) um tratamento de dados pessoais com a finalidade de elaborar um estudo intitulado "Presbiacusia, acufenos e qualidade de vida ".

O estudo tem por objetivo analisar alguns dos mecanismos fisiopatológicos implicados na presbiacusia – que se define como surdez neurosensorial, simétrica, de início insidioso e progressiva ao longo da idade -, e por outro lado compreender porque uma parte destes indivíduos tem simultaneamente acufenos (perceção de um som ou ruído num ouvido ou em ambos ou generalizado a toda a cabeça, na ausência de estímulo sonoro externo).

Os participantes no estudo serão todos os indivíduos com idade compreendida entre os 55 e os 75 anos que recorram à consulta de otorrinolaringologia do Hospital CUF Infante Santo, até ser obtida uma amostra de pelo menos 140 doentes.
A participação no estudo consistirá na recolha de dados pelo médico assistente, na realização de exames específicos, na colheita de amostra de sangue periférico e no preenchimento de questionários pelos doentes.

Os dados serão recolhidos num "caderno de recolha de dados", no qual não há identificação nominal do titular, sendo aposto um código de doente. A chave desta codificação só pode ser conhecida do médico investigador.

O responsável pretende recolher os seguintes dados: código de participante; género, idade, etnia, história pregressa e atual de doenças (otológicas ou não), consumo de fármacos, hábitos toxicofílicos (tabaco, álcool ou outros), exposição a ruído. Exames

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de audiologia (audiograma tonal de altas frequência, estudo dos acufenos, potenciais evocados auditivos e otoemissões acústicas). observação ORL completa (otoscopia, rinoscopia anterior, observação da orofaringe e avaliação da cabeça e pescoço, com auscultação se o acufeno for pulsátil); *Tinnitus Handicap Inventory* (THI) - somente os subgrupos com acufenos), *Brief Symptom Inventory* (BSY); Questionário de qualidade de vida SF-36, Colheita de amostra de sangue periférico para avaliação de parâmetros inflamatórios e de imunidade (HSP-70, IL1, IL2, IL-6, IL10, TNFalfa, IFNgamma e TGFbeta) e para análise de genética (GJB2, GJB6, NAT2 e GRM7, del 4977bp e haplogrupos do mtDNA).

Os destinatários são ainda informados sobre a natureza facultativa da sua participação e garantia de confidencialidade no tratamento, caso decidam participar, recolhendo o médico assistente/investigador o seu consentimento informado para o efeito.

II. Análise

A CNPD já se pronunciou na sua Deliberação n.º 1704/2015 sobre o enquadramento legal, os fundamentos de legitimidade, os princípios orientadores para o correto cumprimento da Lei n.º 67/98, de 26 de outubro, alterada pela Lei n.º 103/2015, de 24 de agosto (Lei da Proteção de Dados Pessoais – LPDP), bem como as condições gerais aplicáveis ao tratamento de dados pessoais para a finalidade de estudos de investigação na área da saúde.
Porque em grande parte referentes à vida privada e também à saúde, os dados recolhidos pela requerente têm a natureza de sensíveis, nos termos do disposto no n.º 1 do artigo 7.º da LPDP.

Em regra, o tratamento de dados sensíveis é proibido, de acordo com o disposto no n.º 1 do artigo 7.º da LPD. Todavia, nos termos do n.º 2 do mesmo artigo, o tratamento de dados da vida privada e de saúde é permitido, quando haja uma disposição legal que consagre esse tratamento de dados, quando por motivos de interesse público

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importante o tratamento for indispensável ao exercício das atribuições legais ou estatutárias do seu responsável ou quando o titular dos dados tiver prestado o seu consentimento.

Não estando preenchidas as duas primeiras condições de legitimidade, o fundamento de legitimidade só pode basear-se no consentimento dos titulares dos dados ou dos representantes legais, quando os titulares dos dados sejam incapazes.
Assim, é necessário o «consentimento expresso do titular», entendendo-se por consentimento qualquer manifestação de vontade, livre, específica e informada, nos termos da qual o titular aceita que os seus dados sejam objeto de tratamento (cf. artigo 3.º, alínea h), da LPD), o qual deve ser obtido através de uma "declaração de consentimento informado" onde seja utilizada uma linguagem clara e acessível.

Nos termos do artigo 10.º da LPDP, a declaração de consentimento tem de conter a identificação do responsável pelo tratamento e a finalidade do tratamento, devendo ainda conter informação sobre a existência e as condições do direito de acesso e de retificação por parte do respetivo titular.

Nos termos da Lei n.º 12/2005, de 26 de janeiro, é legítima a criação de um biobanco com a finalidade de investigação básica ou aplicada à saúde (cf. n.º 3 do artigo 19.º).

Os destinatários deverão ser informados sobre a finalidade do biobanco e que a utilização para estudos futuros será sujeita a um consentimento informado específico, sendo facultativa a sua participação e garantida a confidencialidade no tratamento.

O consentimento para a conservação dos dados no biobanco não dispensa a solicitação de um consentimento específico para a participação num futuro estudo.

Para a constituição do biobanco terá de ser obtida a autorização prévia da entidade credenciada pelo departamento responsável pela tutela da saúde, de acordo com o disposto no n.º 2 do artigo 19.º da Lei n.º 12/2005.

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A par das amostras de sangue, os dados clínicos recolhidos devem ser igualmente objeto de codificação, ou seja, do caderno de recolha de dados não deve constar qualquer elemento identificador do doente participante, sendo antes aposto um código de doente.

Deverão ser adotadas medidas de segurança adequadas, devendo ser dada especial atenção à necessidade de assegurar:

a) O direito de informação e acesso aos titulares dos dados, nos termos do artigo 10.º e n.º 5 do artigo 11.º da LPD, assim como do artigo 9.º da Lei n.º 12/2005;
b) A separação lógica entre dados administrativos e dados de saúde (cf. n.º 3 do artigo 15.º da LPD);
c) A adoção de medidas de segurança que impeçam o acesso à informação a pessoas não autorizadas; a informação de saúde deverá ser de acesso restrito aos médicos ou, sob a sua direção e controlo, a outros profissionais de saúde obrigados a segredo profissional (cf. n.º 4 do artigo 7.º da LPD).

No que respeita a recolha do dado raça, o responsável apresentou a seguinte justificação:

"O ramo da genética que estuda a interação entre o ambiente e o genoma é conhecido como epigenética. Os gatilhos que ativam ou desativam os genes são acionados por fragmentos do genoma que até pouco tempo atrás os cientistas tinham por inúteis – o chamado DNA lixo. Agora sabe-se que eles servem de elemento de ligação entre os fatores ambientais e os genes.
O tipo de alimentação, o nível de atividade física, o tabagismo, o uso de medicamentos, as experiências emocionais – todos estes fatores contribuem para "ligar" ou "desligar" determinados genes, ou seja, torná-los ativos ou conservá-los adormecidos. Na presbiacusia em particular, sabe-se que fatores intrínsecos e extrínsecos afetam os domínios físico, cognitivo, emocional e social (Lee, 2013). E estudar estes fatores separadamente torna-se bastante difícil.

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Os fatores intrínsecos incluem mutações de DNA mitocondrial, surdez monogénica relacionada com o envelhecimento, diabetes, hipertensão, doenças metabólicas e sistémicas (Eyken, Camp, & Laer, 2007). Neste âmbito, foi recentemente estimado que 35-55% dos casos de envelhecimento do ouvido interno têm um backgroung genético (Ciorba & Hatzopoulos, 2015). A raça é um fator de risco não modificável que influencia a presbiacusia (ARHL) - perda auditiva relacionada com a idade (Yamasoba, Lin, Someya, & Kashio, 2013). Vários estudos demonstraram que a raça negra tem 60 a 70% de probabilidade de ter ARHL quando se comparado com os caucasianos (e.g.: Agrawal, Platz, & Niparko, 2008; Cooper, 1994; Lin, Maas, Chien, & Carey, 2012). Um estudo epidemiológico revelou que a cor da pele, e o funcionamento melanocítico da cóclea é o mecanismo de proteção subjacente associado à raça (Lin et al., 2012).

Fatores extrínsecos incluem a exposição a ruído (sonotraumatismo), dieta e medicação ototóxica entre outros (Eyken et al., 2007; Fransen & Lemkens, 2003). Uma vez que tanto os fatores internos como externos variam culturalmente e geograficamente, a recolha de informação sobre a etnia e origem geográfica é crucial para contextualizar e compreender especificamente os resultados dos estudos genéticos, bem como o estado de saúde do paciente no geral e as consequências na qualidade de vida observadas. A influência de alguns fatores ambientais (i.e. externos) nos limiares de audição encontra-se bem documentada, no entanto, não é clara a sua influência na audição numa fase tardia.

Uma análise sistemática de genes candidatos poderá ajudar a identificar os fatores genéticos envolvidos na ARHL. Amostras amplas de diversas étnias e distribuições geográficas, são necessárias para detetar efeitos subtis (Fransen & Lemkens, 2003). As mutações em alguns genes de surdez tem uma distribuição geográfica, por exemplo, no gene GJB2 há mutações tipicamente asiáticas e outras tipicamente caucasianas. O mesmo se verifica para outros genes e regiões do genoma (haplogrupos do DNA mitocondrial, mutações da fenilcetonúria, etc).

Deste modo, o conhecimento da origem étnica e geográfica permite que o background genético dos indivíduos possa ser, mais facilmente, analisado. Orientando assim para uma mais adequada interpretação dos resultados eventualmente obtidos.

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Tendo em conta o teor da justificação a, entende a CNPD que é legítima a recolha e tratamento do dado raça.

O acesso aos dados identificados dos participantes deve ser feito no estrito cumprimento do disposto na Lei n.º 21/2014, de 16 de abril, com as alterações introduzidas pela Lei n.º 73/2015, de 27 de junho (Lei da investigação clínica).

A informação tratada é recolhida de forma lícita (artigo 5.º, n.º1 alínea *a*) da LPDP), para finalidades determinadas, explícitas e legítimas (cf. alínea *b*) do mesmo artigo) e não é excessiva.

O fundamento de legitimidade é o consentimento expresso do titular dos dados.

III. Conclusão

Assim, nos termos das disposições conjugadas do n.º 2 do artigo 7.º, n.º 1 do artigo 27.º, alínea *a*) do n.º 1 do artigo 28.º e artigo 30.º da Lei de Proteção de Dados Pessoais, com as condições e limites fixados na referida Deliberação n.º 1704/2015, que se dão aqui por reproduzidos e que fundamentam esta decisão, autoriza-se o tratamento de dados *supra* referido, consignando-se o seguinte:

Responsável pelo tratamento: Haúla Faruk Haider;
Finalidade: estudo intitulado "Presbiacusia, acufenos e qualidade de vida ";
Categoria de Dados pessoais tratados: código de participante; género, idade, etnia, história pregressa e atual de doenças (otológicas ou não), consumo de fármacos, hábitos toxicofílicos (tabaco, álcool ou outros), exposição a ruído. Exames de audiologia (audiograma tonal de altas frequência, estudo dos acufenos, potenciais evocados auditivos e otoemissões acústicas). observação ORL completa (otoscopia, rinoscopia anterior, observação da orofaringe e avaliação da cabeça e pescoço, com auscultação se o acufeno for pulsátil); *Tinnitus Handicap Inventory* (THI) - somente os

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subgrupos com acufenos), *Brief Symptom Inventory* (BSY); Questionário de qualidade de vida SF-36, Colheita de amostra de sangue periférico para avaliação de parâmetros inflamatórios e de imunidade (HSP-70, IL1, IL2, IL-6, IL10, TNFalfa, IFNgamma e TGFbeta) e para análise de genética (GJB2, GJB6, NAT2 e GRM7, del 4977bp e haplogrupos do mtDNA);

Entidades a quem podem ser comunicados: Não há.

Formas de exercício do direito de acesso e retificação: Junto do médico assistente/investigador.

Interconexões de tratamentos: Não há.

Transferências de dados para países terceiros: Não há.

Prazo de conservação: a chave de codificação dos dados deve ser destruída no prazo de 5 anos após o fim do estudo.

Dos termos e condições fixados na Deliberação n.º 1704/2015 e na presente Autorização decorrem obrigações que o responsável deve cumprir. Deve, igualmente, dar conhecimento dessas condições a todos os intervenientes no circuito de informação.

Lisboa, 10 de fevereiro de 2016

Filipa Calvão (Presidente)

Rua de São Bento, 148-3º • 1200-821 LISBOA
Tel: 213 928 400 Fax: 213 976 832
www.cnpd.pt

21 393 00 39
LINHA PRIVACIDADE
Dias úteis das 10 às 13 h
duvidas@cnpd.pt

Appendix V

Caderno Recolha de Dados - QUESTIONÁRIO SOBRE ZUMBIDO

NOME:…… **DATA:**…/…../2015

DATA DE NASCIMENTO:……/……/19……

1. Sexo: ❒ Masculino ❒ Feminino

2. História familiar de zumbido: ☐SIM ☐ NÃO

se SIM: ☐ Pai ☐ Mãe ☐ Irmão ☐ Filhos ☐ Outros

3. Há quanto tempo sente o zumbido?……

4. Onde localiza o seu zumbido?

☐ Ouvido direito ☐ Ouvido esquerdo

☐ Ambos, pior à esquerda ☐ Ambos, pior à direirta

☐ Ambos iguais ☐ Na cabeça ☐ Outro lugar

5. Como surgiu o seu zumbido? ☐ Gradual ☐ Abrupto

6. Como se manifesta o seu zumbido?

☐ Intermitente ☐ Constante ☐ Pulsátil (como o batimento do coração?)

7. Descreva por palavras suas como é o seu zumbido:

……

8. Descreva a intensidade do seu zumbido numa escala de 0 a 10.

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

(quase não se ouve) (ruído motor carro) (ruído avião)

9. O seu zumbido piora quando está nervoso? ☐ SIM ☐ NÃO

10. Tem surdez? ☐ SIM ☐ NÃO Se Sim, há quanto tempo?……

11. Usa aparelho (prótese) auditivo? ☐ SIM ☐ NÃO

Se Sim, de que lado? ☐ Esquerdo ☐ Direito Há quanto tempo?……

12. Tem menor tolerância para o ruído? ☐ SIM ☐ NÃO

13. Costuma ter vertigens ou tonturas? ☐ SIM ☐ NÃO

14. Medicação diária: ……

15. Consumo prévio de fármacos (ototóxicos):……

……

……

16. Exposição a ruído:……

17.Traumatismos crânio-cervicais prévios (com perda de conhecimento):

……

……

18. Alergias:……

19. Cirurgias prévias:……

20. Outros antecedentes pessoais relevantes:

……

……

20. Antecedentes familiares:……

……

……

OBS:

OD:……

OE:……

Rinoscopia anterior:……

Orofaringe:……

Auscultação (se acufeno pulsátil):……

Articulação Temporo-mandibular:……

Grupos musculares cervicais:……

Outros:……

Apendix VI

QUESTIONÁRIO DE AVALIAÇÃO DOS ZUMBIDOS

(Tinnitus Handicap Inventory) Newman et al, (1996)
Versão Portuguesa de Vasco Oliveira e Rute Meneses (2005)

Instruções: Este questionário pretende ajudar a identificar o grau de problemas que os seus zumbidos lhe possam estar a causar. Assinale as suas respostas ao lado de cada questão.
Não deixe nenhuma por responder.

		Sempre	Por vezes	Nunca
F-01	Tem dificuldade em concentrar-se por causa dos seus zumbidos?			
F-02	A intensidade dos zumbidos torna-lhe difícil ouvir as outras pessoas?			
E-03	Os seus zumbidos fazem com que fique irritado(a)?			
F-04	Os zumbidos fazem com que se sinta confuso(a)?			
C-05	Sente-se desesperado(a) por causa dos seus zumbidos?			
E-06	Queixa-se muito dos seus zumbidos?			
F-07	Tem dificuldade em adormecer à noite por causa dos seus zumbidos?			
C-08	Sente que não pode escapar aos seus zumbidos?			
F-09	Os seus zumbidos perturbam o prazer que retira das actividades sociais (como jantar fora, ir ao cinema, ...)			
E-10	Sente-se frustrado(a) por causa dos seus zumbidos?			
C-11	Acha que tem uma doença muito grave por causa dos seus zumbidos?			
F-12	Os seus zumbidos impedem-no(a) de gozar a vida?			
F-13	Os zumbidos interferem com o trabalho e com as actividades domésticas?			
E-14	Acha que está muitas vezes irritável por causa dos seus zumbidos?			
F-15	Tem dificuldade em ler por causa dos seus zumbidos?			
E-16	Fica preocupado(a) por causa dos seus zumbidos?			
E-17	Acha que há stress nas suas relações com familiares e amigos por causa dos seus zumbidos?			
F-18	É difícil deixar de pensar nos seus zumbidos e centrar-se noutras coisas?			
C-19	Acha que não consegue controlar os seus zumbidos?			
F-20	Devido aos seus zumbidos sente-se muitas vezes cansado(a)?			
E-21	Sente-se deprimido(a) por causa dos seus zumbidos?			
E-22	Fica ansioso(a) por causa dos seus zumbidos?			
C-23	Acha que já não consegue lidar com os seus zumbidos?			
F-24	Os seus zumbidos pioram quando está sob stress?			
E-25	Sente-se inseguro por causa dos seus zumbidos?			

F — Componente "funcional" (funções mentais, sociais, ocupacionais e físicas)

E — Componente "emocional" (respostas afetivas: ansiedade, raiva, frustração…)

C — Componente "catastrófica" (quantifica o desespero e incapacidade para lidar com o sintoma)

Sempre = 4 pontos Por vezes = 2 pontos Nunca = 0 pontos

0-16% (ou pontos) — acufeno irrelevante; 18-36% — leve; 38-56% — moderado; 58-76% — severo; 78-100% — catastrófico

Apendix VII

BSI- Brief Symptom Inventory

Instruções: Em baixo encontra-se uma lista de problemas que, às vezes, as pessoas apresentam. Por favor, leia com atenção cada um e coloque um círculo à volta do número que melhor descreve EM QUE MEDIDA ESSE PROBLEMA O/A PERTURBOU OU ABORRECEU DURANTE OS PASSADOS 7 DIAS INCLUINDO HOJE. Coloque um círculo somente num número para cada problema e não salte nenhum item. Se mudar de ideia, apague com cuidado a sua primeira marca. Leia, por favor, o exemplo em baixo antes de começar, e se tiver algumas questões, faça o favor de as colocar.

EXEMPLO Em que medida ficou perturbado/a por:	Nada	Um pouco	Moderadamente	Muito	Extremamente
1. Dores no corpo.	0	1	2	3	4

EM QUE MEDIDA FICOU PERTURBADO/A POR:	Nada	Um pouco	Moderadamente	Muito	Extremamente
1. Nervosismo ou agitação interior.	0	1	2	3	4
2. Sensações de tonturas ou desmaios.	0	1	2	3	4
3. Ideia de que alguém pode controlar os seus pensamentos.	0	1	2	3	4
4. Sentir que os outros são culpados da maior parte dos seus problemas.	0	1	2	3	4
5. Dificuldade em lembrar-se das coisas.	0	1	2	3	4
6. Sentir-se facilmente aborrecido/a ou irritado/a.	0	1	2	3	4
7. Dores no coração ou no peito.	0	1	2	3	4
8. Sentir medo em espaços abertos.	0	1	2	3	4
9. Pensamentos de acabar com a vida.	0	1	2	3	4
10. Sentir que não pode confiar na maior parte das pessoas.	0	1	2	3	4
11. Pouco apetite.	0	1	2	3	4
12. Assustar-se subitamente sem razão.	0	1	2	3	4
13. Acesso de cólera/irritação que não consegue controlar.	0	1	2	3	4
14. Sentir-se sozinho/a mesmo quando está com pessoas.	0	1	2	3	4
15. Sentir-se bloqueado/a ao tentar fazer as coisas.	0	1	2	3	4
16. Sentir-se só.	0	1	2	3	4
17. Sentir-se triste/melancólico/a.	0	1	2	3	4
18. Não sentir interesse nas coisas.	0	1	2	3	4
19. Sentir-se amedrontado/a.	0	1	2	3	4
20. Sentir-se facilmente ferido/a nos seus sentimentos.	0	1	2	3	4
21. Sentir que as pessoas não são amigáveis ou que não gostam de si.	0	1	2	3	4
22. Sentir-se inferior aos outros.	0	1	2	3	4
23. Náuseas ou indisposição de estômago.	0	1	2	3	4

1 Derogatis, L. (1975)

24. Sentir que está a ser observado/a ou sentir que os outros falam de si.	0	1	2	3	4
EM QUE MEDIDA FICOU PERTURBADO/A POR:	**Nada**	**Um pouco**	**Moderadamente**	**Muito**	**Extremamente**
25. Dificuldade em adormecer.	0	1	2	3	4
26. Ter de verificar e tornar a verificar o que faz.	0	1	2	3	4
27. Dificuldade em tomar decisões.	0	1	2	3	4
28. Sentir medo de viajar de autocarro, metro ou comboio.	0	1	2	3	4
29. Dificuldade em respirar.	0	1	2	3	4
30. Arrepios frios ou quentes.	0	1	2	3	4
31. Ter de evitar certas coisas, lugares ou actividades porque eles o/a assustam.	0	1	2	3	4
32. Fazer-se um vazio no seu espírito.	0	1	2	3	4
33. Entorpecimento ou formigueiro em partes do corpo.	0	1	2	3	4
34. A ideia de que deveria de ser castigado/a pelos seus pecados.	0	1	2	3	4
35. Sentir-se sem esperança acerca do futuro.	0	1	2	3	4
36. Dificuldade de concentração.	0	1	2	3	4
37. Sentir fraqueza em várias partes do corpo.	0	1	2	3	4
38. Sentir-se tenso/a ou excitado/a.	0	1	2	3	4
39. Pensamentos de morte ou de morrer.	0	1	2	3	4
40. Sentir impulsos de bater/magoar ou provocar danos a alguém.	0	1	2	3	4
41. Ter impulsos/vontade de partir ou esmagar coisas.	0	1	2	3	4
42. Sentir-se muito consciente de si próprio/a na presença de outros.	0	1	2	3	4
43. Sentir-se pouco à vontade no meio da multidão ou muita gente.	0	1	2	3	4
44. Nunca sentir-se próximo de outra pessoa.	0	1	2	3	4
45. Acessos de terror e pânico.	0	1	2	3	4
46. Envolver-se em discussões frequentes.	0	1	2	3	4
47. Sentir-se nervoso/a quando o/a deixam sozinho/a.	0	1	2	3	4
48. Os outros não darem o apreço devido aquilo que faz.	0	1	2	3	4
49. Sentir-se tão inquieto/a que não consegue ficar parado/a.	0	1	2	3	4
50. Sentir-se sem valor.	0	1	2	3	4
51. Sentir que as pessoas se podem aproveitar de si se você permitir.	0	1	2	3	4
52. Sentimentos de culpa.	0	1	2	3	4
53. Ideias de que algo está mal no seu espírito.	0	1	2	3	4

Apendix VIII

Apêndice - Adaptação portuguesa do MOS SF-36 (versão 1)

QUESTIONÁRIO DE ESTADO DE SAÚDE (SF-36)

INSTRUÇÕES: As questões que se seguem pedem-lhe opinião sobre a sua saúde, a forma como se sente e sobre a sua capacidade de desempenhar as actividades habituais.
Pedimos que leia com atenção cada pergunta e que responda o mais honestamente possível. Se não tiver a certeza sobre a resposta a dar, dê-nos a que achar mais apropriada e, se quiser, escreva um comentário a seguir à pergunta.

Para as perguntas 1 e 2, por favor coloque um círculo no número que melhor descreve a sua saúde.

1. Em geral, diria que a sua saúde é:

Óptima	Muito boa	Boa	Razoável	Fraca
1	2	3	4	5

2. Comparando com o que acontecia há um ano, como descreve o seu estado geral actual:

Muito melhor	Com algumas melhoras	Aproximadamente igual	Um pouco pior	Muito pior
1	2	3	4	5

3. As perguntas que se seguem são sobre actividades que executa no seu dia-a-dia. Será que a sua saúde o/a limita nestas actividades? Se sim, quanto?

(Por favor assinale com um círculo um número em cada linha)

	Sim, muito limitado/a	Sim, um pouco limitado/a	Não, nada limitado/a
a. **Actividades violentas**, tais como correr, levantar pesos, participar em desportos extenuantes.	1	2	3
b. **Actividades moderadas**, tais como deslocar uma mesa ou aspirar a casa	1	2	3
c. Levantar ou pegar nas compras de mercearia . . .	1	2	3
d. Subir **vários** lanços de escada	1	2	3
e. Subir **um** lanço de escadas.	1	2	3
f. Inclinar-se, ajoelhar-se ou baixar-se	1	2	3
g. Andar **mais de 1 Km**.	1	2	3
h. Andar **vários** quarteirões ou grupos de casas . . .	1	2	3
i. Andar **um** quarteirão ou grupo de casas	1	2	3
j. Tomar banho ou vestir-se sozinho/a	1	2	3

4. Durante as últimas 4 semanas teve, no seu trabalho ou actividades diárias, algum dos problemas apresentados a seguir como consequência do seu estado de saúde físico?

Por favor, em cada linha, ponha um círculo à volta do número 1 se a resposta for Sim e à volta do número 2 se a resposta for Não

	Sim	Não
a. Diminuiu o **tempo gasto** a trabalhar ou em outras actividades	1	2
b. Fez **menos** do que queria?	1	2
c. Sentiu-se limitado/a no **tipo** de trabalho ou em outras actividades . .	1	2
d. Teve **dificuldade** em executar o seu trabalho ou outras actividades diárias (por exemplo, foi preciso esforçar-se mais).	1	2

5. Durante as últimas 4 semanas, teve com o seu trabalho ou com as suas actividades diárias, algum dos problemas apresentados a seguir devido a quaisquer problemas emocionais (tal como sentir-se deprimido/a ou ansioso/a)?

Por favor, em cada linha, ponha um círculo à volta do número 1 se a resposta for Sim e à volta do número 2 se a resposta for Não

	Sim	Não
a. Diminuiu o **tempo gasto** a trabalhar ou em outras actividades	1	2
b. Fez **menos** do que queria?	1	2
c. Não executou o trabalho ou outras actvidades **tão cuidadosamente** como era costume	1	2

Para cada uma das perguntas 6, 7 e 8, por favor ponha um círculo no número que melhor descreve a sua saúde.

6. Durante as últimas 4 semanas, em que medida é que a sua saúde física ou problemas emocionais interferiram no seu relacionamento social normal com a família, amigos, vizinhos ou outras pessoas?

Absolutamente nada	Pouco	Moderadamente	Bastante	Imenso
1	2	3	4	5

7. Durante as últimas 4 semanas teve dores?

Nenhumas	Muito fracas	Ligeiras	Moderadas	Fortes	Muito fortes
1	2	3	4	5	6

8. Durante as últimas 4 semanas, de que forma é que a dor interferiu com o seu trabalho normal (tanto o trabalho fora de casa como o trabalho doméstico)?

Absolutamente nada	Pouco	Moderadamente	Bastante	Imenso
1	2	3	4	5

9. As perguntas que se seguem pretendem avaliar a forma como se sentiu e como lhe correram as coisas nas últimas quatro semanas.
Para cada pergunta, coloque por favor um círculo à volta do número que melhor descreve a forma como se sentiu.
Certifique-se que coloca um círculo em cada linha.

Quanto tempo, nas últimas quatro semanas...	Sempre	A maior parte do tempo	Bastante tempo	Algum tempo	Pouco tempo	Nunca
a. Se sentiu cheio/a de vitalidade? ...	1	2	3	4	5	6
b. Se sentiu muito nervoso/a?	1	2	3	4	5	6
c. Se sentiu tão deprimido/a que nada o/a animava?	1	2	3	4	5	6
d. Se sentiu calmo/a e tranquilo/a? ...	1	2	3	4	5	6
e. Se sentiu com muita energia?	1	2	3	4	5	6
f. Se sentiu triste e em baixo?	1	2	3	4	5	6
g. Se sentiu estafado/a?	1	2	3	4	5	6
h. Se sentiu feliz?	1	2	3	4	5	6
i. Se sentiu cansado/a?	1	2	3	4	5	6

10. Durante as últimas quatro semanas, até que ponto é que a sua saúde física ou problemas emocionais limitaram a sua actividade social (tal como visitar amigos ou familiares próximos)?

Sempre	A maior parte do tempo	Algum tempo	Pouco tempo	Nunca
1	2	3	4	5

11. Por favor, diga em que medida são verdadeiras ou falsas as seguintes afirmações.
Ponha um círculo para cada linha.

	Absolutamente verdade	Verdade	Não sei	Falso	Absolutamente falso
a. Parece que adoeço mais facilmente do que os outros	1	2	3	4	5
b. Sou tão saudável como qualquer outra pessoa	1	2	3	4	5
c. Estou convencido/a que a minha saúde vai piorar.....	1	2	3	4	5
d. A minha saúde é óptima	1	2	3	4	5