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EAR FUNCTIONALITY ASSESSMENT AND
MODELLING THROUGH A NOVEL PRESSURELESS
ACOUSTIC IMMITTANCE MEASUREMENT METHOD

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All models are wrong, but some are useful [1]

G.E.P. Box

Abstract

Otologic disorders pose a major global health challenge, often leading to hearing impairment that significantly affects communication, cognitive development, and quality of life. Early diagnosis and timely intervention are essential to prevent permanent auditory deficits, particularly in pediatric and elderly populations. However, conventional diagnostic tools such as tympanometry and Wideband Acoustic Immittance (WAI) require the application of external pressure and specialized expertise, limiting their use in large-scale or primary-care screening. To address these limitations, this thesis investigates and characterizes a novel diagnostic technology called Pressure-Less Acoustic Immittance (PLAI)TM, which allows middle ear assessment under ambient pressure conditions, providing a fully non-invasive alternative to traditional methods.

The first phase of the study, aimed at the characterization of the device, was articulated into three main components: (i) the comparison between PLAITM and conventional tympanometric parameters, (ii) the investigation of age-related variability in the parameters derived from the novel pressure-less technique, and (iii) the definition of age groups and preliminary normative reference values for both healthy and pathological populations. The comparison between tympanometry and the resonance frequency (F_{res}) obtained from PLAITM measurements was performed on a cohort of 57 adult subjects, including both healthy and pathological ears. This analysis identified a significant linear relationship between F_{res} and the tympanometric Ear Canal Volume (ECV), enabling the estimation of this parameter without the application of an external pressure gradient. A slight correlation with tympanometric compliance was also observed; however, it was not sufficiently robust to support a reliable estimation of this parameter based on F_{res} alone. A subsequent analysis, conducted on 134 healthy individuals, focused on evaluating the age dependency of PLAITM parameters and on further comparing pressure-less and tympanometric measurements. This study revealed logarithmic relationships between several parameters and age, and confirmed the

feasibility of estimating tympanometric metrics such as ECV, Tympanic Width (TW), and compliance using pressure-less parameters. Nonetheless, the accuracy of these estimations was found to be limited in the presence of pathological conditions, highlighting the need for further investigation and refinement.

The final step of the first phase consisted in the definition of normative reference ranges for parameters extracted from the PLAITM admittance curves in healthy subjects, and in their comparison with pathological cases, primarily Otitis Media with Effusion (OME). A multi-center clinical study involving 218 subjects aged from four months to eighty years demonstrated clear logarithmic, age-dependent trends across all PLAITM parameters, allowing the identification of three physiologically distinct age groups: 0–3 years, 3–12 years, and over 12 years. These results established the first normative database for the PLAITM device and confirmed its strong association with traditional tympanometric measures. Moreover, statistically significant differences between healthy and OME-affected ears were observed in key parameters, including resonance frequency, band limits, and equivalent ear canal volume, thereby confirming the sensitivity of the method to functional alterations of the middle ear.

Building on these findings, the diagnostic potential of PLAITM is then explored through machine learning–based classification models. Random Forest algorithms trained within each age group achieved macro F1-scores above 0.78, with the best performance (0.84) in children aged 3–12 years. Feature relevance analysis using SHapley Additive exPlanations (SHAP) identified resonance frequency, peak admittance, and canal volume as the most influential predictors, aligning with physiological expectations.

Extending beyond diagnostic classification, a lumped element ear model derived from PLAITM data and a physical phantom are then developed. These models approximate the mechanical and acoustic behavior of the ear, enabling a first simulation of both healthy and pathological conditions, including experimentally induced variations. The modeling framework provides an introductory quantitative bridge between measured admittance parameters and underlying biomechanical properties, offering a foundation for advanced diagnostic interpretation and personalized assessment.

Overall, the results demonstrate the potential of PLAITM as a reliable, pressure-free, and age-adaptable technique for middle ear evaluation, even though additional analyses are still needed to prove the diagnostic reliability of this new technique in multi-measurement

specialistic evaluation of otologic illnesses. Its ability to detect functional and pathological changes without external pressure or specialized operators positions it as a valuable tool for early screening and monitoring of otologic disorders. Moreover, the integration of machine learning and physical ear modeling establishes the groundwork for automated, data-driven diagnostic support systems, enhancing accessibility to accurate and non-invasive hearing assessments in both clinical and community healthcare settings.

Sommario

Le patologie otologiche rappresentano una rilevante sfida sanitaria a livello globale, poiché spesso conducono a deficit uditivi che compromettono in modo significativo la comunicazione, lo sviluppo cognitivo e la qualità della vita. La diagnosi precoce e un intervento tempestivo sono fondamentali per prevenire danni uditivi permanenti, in particolare nelle popolazioni pediatriche e geriatriche. Tuttavia, gli strumenti diagnostici convenzionali, quali la timpanometria e la WAI, richiedono l'applicazione di una pressione esterna e competenze specialistiche, limitandone l'utilizzo in programmi di screening su larga scala o in contesti di assistenza primaria. Per superare tali limitazioni, questa tesi analizza e caratterizza una nuova tecnologia diagnostica denominata PLAITM, che consente la valutazione dell'orecchio medio in condizioni di pressione ambientale, offrendo un'alternativa completamente non invasiva ai metodi tradizionali.

La prima fase dello studio, finalizzata alla caratterizzazione del dispositivo, è stata articolata in tre componenti principali: (i) il confronto tra i parametri ottenuti mediante PLAITM e quelli timpanometrici convenzionali, (ii) l'analisi della variabilità dei parametri della nuova tecnica pressure-less in funzione dell'età e (iii) la definizione di gruppi d'età e di valori di riferimento normativi preliminari per popolazioni sane e patologiche. Il confronto tra la timpanometria e la frequenza di risonanza (F_{res}) ricavata dalle misure PLAITM è stato condotto su una coorte di 57 soggetti adulti, comprendente orecchie sane e patologiche. Tale analisi ha evidenziato una relazione lineare significativa tra F_{res} e l'ECV timpanometrico, consentendo la stima di questo parametro senza l'applicazione di un gradiente di pressione esterna. È stata inoltre osservata una correlazione con la compliance timpanometrica; tuttavia, essa non è risultata sufficientemente robusta da permettere una stima affidabile di tale parametro basandosi esclusivamente su F_{res} . Una successiva analisi, condotta su 134 individui sani, è stata finalizzata alla valutazione della dipendenza dall'età dei parametri PLAITM e a un ulteriore confronto tra misure pressure-less e timpanometriche. Questo studio ha

messo in evidenza relazioni di tipo logaritmico tra diversi parametri e l'età, confermando la possibilità di stimare parametri timpanometrici quali ECV, TW e la compliance a partire da misure pressure-less. Tuttavia, l'accuratezza di tali stime risulta limitata in presenza di condizioni patologiche, sottolineando la necessità di ulteriori studi e approfondimenti.

L'ultima fase del primo stadio dello studio ha riguardato la definizione di intervalli di riferimento normativi per i parametri estratti dalle curve di ammettenza PLAI™ in soggetti sani e il loro confronto con casi patologici, principalmente OME. Uno studio clinico multicentrico che ha coinvolto 218 soggetti di età compresa tra quattro mesi e ottant'anni ha evidenziato chiare tendenze logaritmiche dipendenti dall'età per tutti i parametri PLAI™, permettendo l'identificazione di tre gruppi d'età fisiologicamente distinti: 0–3 anni, 3–12 anni e oltre i 12 anni. Questi risultati hanno consentito la definizione del primo database normativo per il dispositivo PLAI™ e hanno confermato una forte associazione con le misure timpanometriche tradizionali. Inoltre, sono state osservate differenze statisticamente significative tra orecchie sane e affette da OME in diversi parametri chiave, tra cui la frequenza di risonanza, i limiti di banda e il volume equivalente del condotto uditivo, confermando la sensibilità del metodo alle alterazioni funzionali dell'orecchio medio.

Sulla base di questi risultati, il potenziale diagnostico di PLAI™ è stato successivamente esplorato mediante modelli di classificazione basati su tecniche di machine learning. Algoritmi Random Forest, addestrati separatamente per ciascun gruppo d'età, hanno raggiunto valori di macro F1-score superiori a 0,78, con le migliori prestazioni (0,84) nella popolazione pediatrica di età compresa tra 3 e 12 anni. L'analisi di rilevanza delle caratteristiche condotta tramite SHAP ha identificato la frequenza di risonanza, il picco di ammettenza e il volume del condotto uditivo come i predittori più influenti, in accordo con le aspettative fisiologiche.

Estendendo l'analisi oltre la classificazione diagnostica, sono stati infine sviluppati un modello dell'orecchio a elementi concentrati, derivato dai dati PLAI™, e un fantoccio fisico. Tali modelli approssimano il comportamento meccanico e acustico dell'orecchio, consentendo una prima simulazione di condizioni sia fisiologiche sia patologiche, incluse variazioni indotte sperimentalmente. Il framework di modellazione fornisce un collegamento quantitativo tra i parametri di ammettenza misurati e le proprietà biomeccaniche sottostanti, offrendo una base solida per un'interpretazione diagnostica avanzata e una valutazione personalizzata.

Nel complesso, i risultati dimostrano il potenziale di PLAI™ come tecnica affidabile, priva di pressurizzazione e adattabile all'età per la valutazione dell'orecchio medio, seppur con una necessità di dati e modelli ulteriori per validare l'affidabilità diagnostica all'interno di una valutazione multi strumentale specialistica. La capacità di rilevare variazioni funzionali e patologiche senza l'applicazione di pressioni esterne né la necessità di operatori altamente specializzati ne fa uno strumento promettente per lo screening precoce e il monitoraggio delle patologie otologiche. Inoltre, l'integrazione di tecniche di machine learning e di modelli fisici dell'orecchio pone le basi per lo sviluppo di sistemi di supporto diagnostico automatici e data-driven, migliorando l'accessibilità a valutazioni uditive accurate e non invasive sia in ambito clinico sia in contesti di assistenza territoriale.

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List of Acronyms

AOM Acute Otitis Media

ART Acoustic Reflex Testing

COM Chronic Otitis Media

CSOM Chronic Suppurative Otitis Media

CT Computed Tomography

EAC External Auditory Canal

ECV Ear Canal Volume

ETD Eustachian Tube Dysfunction

IRCCS Istituto di Ricovero e Cura a Carattere Scientifico

LEM Lumped Element Model

MEMS Micro Electro-Mechanical Systems

OME Otitis Media with Effusion

PLAI Pressure-Less Acoustic Immittance

PPE Personal Protective Equipment

PTA Pure Tone Audiometry

QoL Quality of Life

RF Random Forest

RMSE Root Mean Square Error

ROC Receiver Operating Characteristic

SD Standard Deviation

SG-AR Spectral Gradient - Acoustic Reflectometry

SHAP SHapley Additive exPlanations

SMOTE Synthetic minority oversampling technique

TM Tympanic Membrane

TMP Tympanic Membrane Perforation

TMR Tympanic Membrane Retraction

TPP Tympanometry Peak Pressure

TW Tympanic Width

WAI Wideband Acoustic Immittance

Introduction

Hearing is one of the most essential human senses, fundamental to communication, learning and social interaction. It allows individuals to perceive and interpret the acoustic environment, supporting not only spoken language and emotional exchange but also spatial awareness and personal safety. Through hearing, humans acquire information about the world, detect potential dangers, and engage in the social and cultural activities that shape personal identity. When hearing is impaired, the consequences extend far beyond sensory loss, often affecting cognitive development, emotional well-being, and overall quality of life.

The auditory system is a remarkable example in the evolution of sense apparatuses. Its function depends on a precise sequence of mechanical, hydraulic, and neural processes that convert sound waves into electrical signals interpretable by the brain. Sound collected by the external ear travels through the auditory canal to reach the tympanic membrane, which transmits its vibrations to the ossicular chain in the middle ear. These small bones amplify and transfer the vibrations to the inner ear, where the cochlea transforms them into neural impulses. This highly coordinated mechanism operates within a delicate equilibrium of pressure, elasticity, and impedance, so even minor alterations to these components can significantly reduce auditory sensitivity or distort sound perception.

Because of this complexity, the ear is susceptible to a variety of pathological conditions. Among these, disorders of the middle ear represent one of the leading causes of hearing impairment across all age groups. In children, middle ear diseases are particularly common, often following upper respiratory infections. These conditions, such as otitis media with effusion (OME), can interfere with language development and learning, leading to social challenges and difficulties in the educational path. In adults, middle ear dysfunction may result from chronic inflammation, trauma, or degenerative changes, frequently causing hearing loss or tinnitus. In the elderly, hearing decline compounds the risk of cognitive

deterioration and social isolation, further underlining the societal relevance of auditory health.

Despite significant advances in medical imaging and audiological testing, the diagnosis of middle ear pathologies continues to pose challenges. The structures involved are small, enclosed, and functionally interconnected, making it difficult to obtain direct measurements of their mechanical properties. Current clinical practice relies primarily on non-invasive acoustic techniques, among which tympanometry and wideband acoustic immittance are the most widely used. These methods provide valuable information on the compliance and pressure behavior of the tympanic system. However, they require the controlled application of pressure within the ear canal to assess how the tympanic membrane and ossicular chain respond to different loading conditions. While effective in experienced hands, these procedures have limitations as they can be uncomfortable for patients, are unsuitable in cases of tympanic membrane perforation, and often depend heavily on operator skill for correct interpretation.

The need for reliable, accessible, and non-invasive diagnostic tools has therefore become a central issue in modern otology. In clinical settings, such instruments could enable earlier detection of disease and more accurate monitoring of therapeutic outcomes. In public health, they could support large-scale screening programs aimed at identifying hearing disorders in early childhood or preventing age-related auditory decline. To achieve this, new diagnostic approaches must combine precision and simplicity, allowing accurate assessment of middle ear function even outside specialized hospital environments.

Recent technological advances have created promising opportunities for innovation in auditory diagnostics. Developments in acoustics, Micro Electro-Mechanical Systems (MEMS), and signal processing have enabled the design of a system capable of analyzing how sound waves interact with the ear under natural, pressure-free conditions. By capturing both the reflected and transmitted components of an acoustic stimulus, this technique can infer the functional state of the middle ear. This approach not only minimizes patient discomfort but also expands accessibility to groups traditionally difficult to assess, such as infants, elderly individuals, and patients with tympanic membrane perforations. In addition, the extensive quantitative data generated by this system can be effectively leveraged through computational and statistical analysis, allowing the identification of pathological patterns and the creation of decision-support algorithms.

Nevertheless, since this emerging technique relies on a measurement principle fundamentally different from those traditionally employed, it is essential to investigate how ear parameters and their variations are represented within its measurements and to pave the way towards an effective clinical use. This process, together with the definition of normative ranges, is crucial to ensure that healthcare professionals can interpret results accurately, formulate diagnoses, and monitor therapeutic outcomes. Moreover, the high dimensionality of the acquired parameters opens the possibility of developing analytical models of the ear that simulate its mechanical and acoustic behavior based on measurable parameters. Such models could provide rapid and reliable diagnostic guidance, making large-scale screening feasible even in primary care or community settings with non-specialized personnel. As an intermediate step toward this goal, statistical analysis of data collected from both healthy and pathological ears can be used to develop machine learning-based decision support systems that operate directly on measured features, offering a practical and efficient solution while also paving the way toward comprehensive equivalent ear modeling.

Thesis Objectives

The main aim of the doctoral work was the characterization of the Pressure-Less Acoustic Immittance (PLAITM) methodology in order to allow for a safe, effective and broad use of the new technology and, possibly, to develop analytical models and decision support systems useful in a clinical setting for the diagnosis of common otological diseases.

This objective was divided in a first characterization on healthy volunteers, which led to the comprehension of the relations between tympanometric and pressureless parameters followed by a more comprehensive clinical study on a large number of healthy and pathologic subjects of all the different ages from infancy to elderly age enrolled in six different hospitals, which allowed to determine the age dependency of pressureless parameters with age and the definition of normative age groups. Consequently, the dataset from the largest cohort was used again to develop a decision aid system for the categorization of four different pathologies. Ultimately, a first model of ear functionality was introduced and briefly tested on physical phantoms and healthy subjects both in normal condition and during Valsalva maneuver, simulating the tympanic distension present in OME-affected ears while

maintaining External Auditory Canal (EAC) volume. This allowed to test the calibration of the model and verify the correctness of the equivalent circuit used.

The thesis is divided in one literature review and three research phases, each described in one of the Thesis' chapters:

1. **Ear and Hearing:** Anatomy, physiology and pathology of ear and hearing are discussed. The chapter then describes the common methods used in the clinical setting to formulate diagnoses and the technologies available, detailing strengths and weaknesses of each technique. The PLAITM methodology is then introduced.
2. **PLAITM Characterization:** In this research chapter the characterization of the device is described, finding at first the main age groups for the healthy and pathologic population and observing the relations occurring between PLAITM and tympanometric parameters. This was obtained through small scale studies with healthy population and with a major study involving patients from six hospitals throughout Italy.
3. **Decision Support Systems:** The chapter describes the development of a decision support system based on machine learning methods capable of discriminating between different common otologic pathologies.
4. **Ear models based on PLAITM:** In order to further describe the differences between healthy and pathologic ears and to provide an analytical framework, the chapter illustrates a simple equivalent ear model obtained from the available data, the test on healthy patients and a first comparison between healthy and induced pathologic condition.

Chapter 1

Ear and Hearing

Hearing, the ability to detect and understand sound, is a fundamental human sense. Since ancient times, it has allowed humans to detect and avoid threats quickly and later became the basis for communication and social interaction. This sense is primarily located in the ear, a complex and delicate organ whose anatomy and function are the result of various evolutionary steps[2]. In fact, recent studies showed the large amount of genetic information required to correctly define the various structures [3] and the complexity of the processes that lead to the development of ear structures during fetal and postpartum growth of humans[4, 5]. Furthermore, studies in general population reveal that hearing and balance, both located in the ear, are considered among the three most important senses along with sight [6].

The significance of hearing is particularly evident when evaluating the impact of hearing loss. Among children, deafness can significantly impair the development of speech and language capabilities, which in turn can lead to educational, behavioural and emotional challenges, and ultimately to difficulties in integrating into society [7, 8, 9]. In adults, hearing loss is associated with reduced communication abilities, leading to social isolation, poorer mental health, and an increased risk of earlier onset dementia or cognitive decline [10, 11, 12]. Moreover, hard-of-hearing workers tend to be more isolated and less productive, representing a hidden societal cost of otologic disorders [13].

Hearing loss is a major issue in the modern world, with an estimated 1.57 billion people affected globally [14]. Analysis of prevalence data reveals that 40% of affected individuals are under 50 years of age, highlighting not only the impact of this condition on Quality of Life (QoL) but also the importance of prevention and mitigation of risk factors [15, 16].

Preventive measures against otologic pathologies cover a broad range of applications, population age groups, and implementation costs. The main ones include:

- Childhood and adolescence
 - Maternal and neonatal care [17, 18]
 - Screening for middle ear diseases (such as Otitis Media) [19]
 - Vaccination against rubella and meningitis [20]
 - Hygiene [21, 22]
- Adulthood and elderly life
 - Occupational health screenings for at-risk workers [23]
 - Incentives for Personal Protective Equipment (PPE) use in occupational and recreational settings [23, 24]
 - Education on hearing protection [25]
 - Management of chronic diseases [26, 27]
 - Use of non-ototoxic medications [28]

In this framework, it is essential not only to strengthen measures for hearing protection but also to improve the means for screening and early diagnosis of otologic illnesses and, more broadly, to further research the ear and its fundamental inner workings. These efforts aim to reduce the incidence of hearing loss and improve the quality of life for hard-of-hearing patients [29].

The present chapter presents key information about ear anatomy and physiology, some of the most common pathologies affecting the middle ear, and the main technologies used for their diagnosis commonly available in outpatient setting. This knowledge will support a better understanding of the Pressure Less Acoustic Immittance (PLAITM) methodology, a novel measurement technique analyzed in the following chapters.

1.1 Ear anatomy and physiology

The ear is a complex organ essential for both hearing and balance. The first function involves converting sound waves into electrical signals that are transmitted to the brain

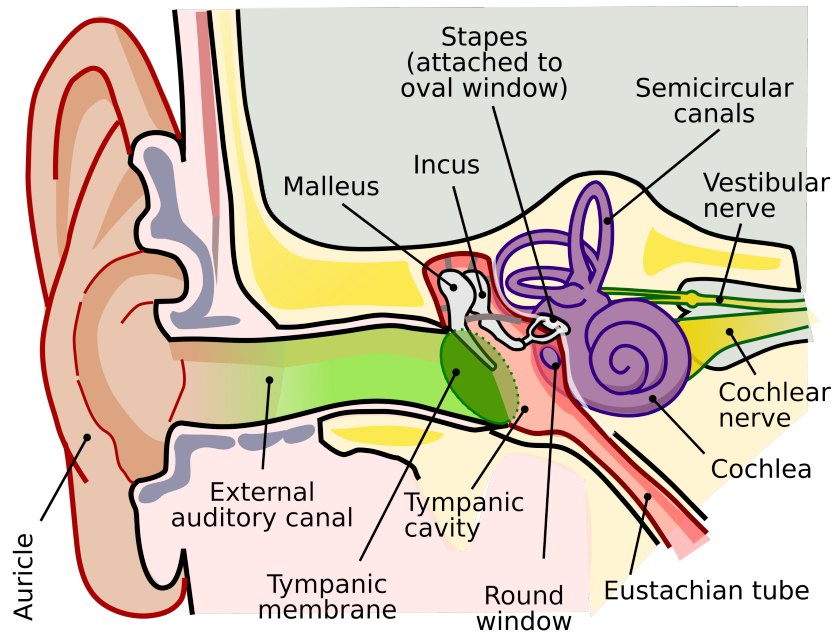


Figure 1.1: Ear anatomy [30]

through a series of ducts, membranes, and mechanical structures. Balance, on the other hand, relies on the movement of fluid within the semicircular canals.

The auditory system becomes even more sophisticated when considering that humans possess two ears, allowing the brain to integrate information from both sides to determine additional details, such as the direction and distance of sound sources.

For engineers, understanding ear anatomy and physiology is fundamental to interpreting the measurable phenomena associated with auditory function, also used to detect diseases, and to replicating the natural mechanisms refined through millennia of evolution in technologies for sound acquisition and hearing prostheses.

Given the high level of complexity of the auditory and vestibular systems and the large number of components involved, it is practical, for our purposes, to focus specifically on hearing and on the parts of the ear responsible for this function. From this perspective, the ear can be divided into three main sections (as illustrated in Figure 1.1): the outer ear, the middle ear, and the inner ear, each responsible for a distinct role in transforming acoustic waves into electrical signals.

As a general overview, the auditory process starts from the pinna (or auricle), which collects and directs sound waves into the external auditory canal (EAC), guiding them toward the Tympanic Membrane (TM), or eardrum. The resulting vibration of the TM sets the ossicular chain, a series of three small bones within the air-filled middle ear into motion.

These ossicles form a mechanical linkage between the tympanic membrane and the oval window of the inner ear. Through this finely tuned leverage system, the middle ear compensates for the impedance mismatch between air and the fluid of the inner ear, efficiently transmitting acoustic energy. As a result, sound-induced vibrations are conveyed to the cochlea, where they stimulate sensory hair cells within the fluid-filled chambers. The mechanical motion is then converted into neural signals that travel along the cochlear nerve to the auditory cortex, where they are interpreted as sound[31].

1.1.1 External Ear

The external ear comprises the auricle (pinna) and the EAC, ending at the tympanic membrane. Its primary function is to collect and direct sound waves toward the tympanic membrane for subsequent transmission through the auditory system.

As the only portion of the ear exposed to the external environment, the external ear is structurally designed to protect the inner components from potential hazards while minimizing any loss of acoustic information. Consequently, its structures are highly responsive to external stimuli yet resilient enough to prevent damage.

The auricle is the visible part of the ear and serves to funnel sound waves into the EAC while aiding in sound localization [32, 33, 34]. It becomes visible around the sixth week of gestation [35] and reaches full development by approximately 12 years of age [36]. In adults, the auricle typically measures 5.5–6.5 cm in vertical length, while the horizontal dimension corresponds to 50–60% of that value [37].

The external auditory canal (EAC) is the conduit connecting the auricle to the tympanic membrane. It functions not only to channel sound waves but also to protect the eardrum by trapping debris and foreign particles through the combined action of hair and cerumen (earwax). Recent studies suggest that the EAC's soft sigmoid shape and variable cross-sectional geometry can modify high-frequency sound transmission, thereby enhancing sound localization initiated by the auricle [31].

The EAC reaches its final dimensions by around nine years of age [38], typically measuring 25–30 mm in length with a cross-sectional area of 25–30 mm² [39], resulting in a total volume ranging from 0.65 ml to 1.75 ml (and between 0.3 ml and 1.0 ml in children) [40]. In addition to dimensional changes, structural composition evolves significantly with age: in children, the EAC walls are predominantly cartilaginous, whereas in adults, bone replaces

most of the canal, leaving only the outer one-third cartilaginous [31].

Lastly, the tympanic membrane (TM, also known as the eardrum) is a thin, semitransparent structure suspended by the walls of the EAC and connected at its central region to the ossicular chain, which separates the external ear from the middle ear. The primary functions of the TM are the transmission of sound waves to the ossicles—converting airborne vibrations into mechanical motion—and the protection of the middle ear from external agents. The TM measures approximately 1 cm in diameter and 100 μm in thickness, and it is composed of three distinct layers: an outer epithelial layer, a middle fibrous connective tissue layer, and an inner mucosal layer [41, 42]. During growth the TM becomes thinner, and with age it becomes less vascularized, less elastic and more rigid[43].

1.1.2 Middle Ear

The middle ear is responsible for transmitting sound from the tympanic membrane to the inner ear. It is structured as an air-filled cavity containing three ossicles that connect the TM to the oval window of the cochlea. To maintain pressure equilibrium within this cavity, the Eustachian tube connects the middle ear to the nasopharynx [44, 45].

The volume of the middle ear cavity in healthy adults typically ranges from 0.5 to 1.5 ml, though the literature reports considerable variation depending on individual anatomy and measurement techniques [46, 47]. The mastoid air cells, which are pneumatically connected to the middle ear, also vary greatly in size and contribute to the regulation of pressure within the system.

The ossicular chain, composed of the malleus, incus, and stapes, serves as a mechanical bridge transmitting vibrations from the tympanic membrane to the cochlea. Anatomically, the malleus is attached to the tympanic membrane and articulates with the incus, which in turn connects to the stapes. Variations in ossicular morphology, particularly in the stapes, have been documented and may influence the efficiency of sound conduction [48]. Functionally, the ossicles act as a mechanical amplifier, enhancing sound pressure by approximately 20-fold, a mechanism essential for perceiving faint sounds [49]. Ossicular development is largely complete by the age of two years [50].

The Eustachian tube is a narrow conduit approximately 36 mm long and 3 mm in diameter, connecting the nasopharynx to the middle ear. Normally closed at rest, it opens during swallowing or chewing to equalize middle ear pressure with atmospheric pressure and to

facilitate mucus drainage [51]. During growth, structural changes in the temporal bone alter the Eustachian tube's orientation, making it longer and more oblique compared to the nearly horizontal position typical of infancy [52].

1.1.3 Inner Ear

The inner ear comprises the cochlea (responsible for hearing) and the vestibular system (responsible for balance, including the semicircular canals, utricle, and saccule). It is encased within the temporal bone and consists of both bony and membranous labyrinths. The cochlea converts mechanical sound vibrations into electrical signals through specialized hair cells, which transmit information to the brain via the auditory nerve. The organ of Corti, located within the cochlea, houses inner and outer hair cells that perform distinct functions in auditory processing.

Structurally, the cochlea measures approximately 35 mm in length when uncoiled and is structured like an helix with 2.5 to 2.75 turns. It develops early during gestation, with coiling and differentiation of the sensory epithelia occurring prenatally [53]. After birth, the primary changes observed in the cochlea are age-related degenerative processes affecting hair cells and neurons, which contribute to hearing loss in later life [54].

1.2 Pathology

The ear, being a complex and highly specialized organ, is susceptible to a wide range of pathological conditions that can affect its structure and function. These disorders may involve anatomical alterations (e.g., congenital malformations, genetic syndromes, or neoplastic growths), inflammatory processes (e.g., otitis media), mechanical damage or structural impairment (e.g., trauma or tympanic membrane rupture secondary to infection), or sensorineural dysfunction (e.g., Ménière's disease[55]). Each of these conditions can compromise auditory and/or vestibular function, often resulting in partial or total hearing loss, balance disturbances, and a marked reduction in QoL.

The following sections present four common otologic pathologies primarily affecting the middle ear, which are prevalent in the general population and, if left untreated, may result in significant and potentially irreversible hearing impairment.

Other conditions not addressed in this thesis, as they primarily involve structures outside

the middle ear, include otitis externa (inflammation of the external auditory canal, commonly known as swimmer's ear)[56], cerumen impaction[57], tinnitus[58], and labyrinthitis[59].

1.2.1 Otitis Media

Otitis media is characterized by inflammation of the middle ear. This condition can manifest in several forms, including Acute Otitis Media (AOM), Chronic Otitis Media (COM), and Otitis Media with Effusion (OME), each presenting distinct clinical features and implications for hearing and overall health.

Acute otitis media (AOM) often develops as a complication of upper respiratory tract infections and is characterized by the rapid onset of symptoms such as ear pain, fever, and irritability, particularly in children [60]. If inadequately treated, AOM may progress to COM, which involves persistent inflammation and otorrhea lasting for more than three months. Chronic Suppurative Otitis Media (CSOM), a subtype of COM, is of particular concern due to its potential complications, such as cholesteatoma, which can lead to structural damage and progressive hearing loss [61, 62, 63].

The epidemiology of otitis media is influenced by multiple factors, including environmental, anatomical, and immunological conditions. Children are particularly susceptible due to the anatomical characteristics of their Eustachian tubes, which are shorter and more horizontally oriented, facilitating the entry of pathogens into the middle ear [64]. Additional risk factors include allergic rhinitis, exposure to tobacco smoke, and low socioeconomic status, all of which are associated with increased incidence rates [65].

Otitis Media with Effusion (OME) is characterized by the accumulation of non-purulent fluid (serous or mucoid) in the middle ear in the absence of acute infection. The condition is particularly common in children and may present with hearing difficulties, ear fullness or balance disturbances, but often without the pain or fever typical of acute otitis media [66]. Although transient in most cases, chronic or recurrent OME can cause significant conductive hearing loss and affect speech and language development [67].

The primary underlying mechanism of OME is Eustachian Tube Dysfunction (ETD), which prevents normal aeration of the middle ear and leads to negative pressure that draws serous or mucoid fluid into the middle-ear cavity[68]. ETD may result from viral upper respiratory infections, allergic rhinitis, or anatomical abnormalities that compromise tube patency.

OME represents the most common cause of acquired hearing loss in children[69] and epi-

demographic data indicate that by the age of four, nearly 80% of children experience at least one episode of OME [70].

The principal manifestation of OME is conductive hearing loss, which may cause speech delay, impaired language acquisition, and difficulties in school in children. Adults often report muffled hearing or ear fullness. Otoscopic examination typically reveals a retracted tympanic membrane with visible air–fluid levels or bubbles [71].

Diagnosis relies on a combination of clinical examination and objective tests [72]:

- Pneumatic otoscopy: decreased tympanic membrane mobility or visible effusion;
- Tympanometry: a flat (“Type B”) curve indicates fluid in the middle ear;
- Audiometry: confirms conductive hearing loss, typically between 20–40 dB [73];
- Nasopharyngoscopy: indicated in adults or recurrent unilateral cases to rule out nasopharyngeal mass.

The initial management of OME typically involves watchful waiting for up to three months, as most cases resolve spontaneously. During this period, addressing modifiable risk factors, such as managing allergic rhinitis or avoiding smoke exposure, can improve outcomes. Surgical intervention is indicated for persistent effusions associated with hearing loss, developmental delay, or recurrent infections. The preferred procedure is tympanostomy tube insertion, which equalizes middle-ear pressure and facilitates drainage. Adenoidectomy may be performed concurrently when adenoidal hypertrophy contributes to ETD [74].

The prognosis for OME is generally favorable. The majority of cases resolve without permanent hearing impairment, particularly with appropriate follow-up and management. Chronic or untreated OME can lead to structural changes in the tympanic membrane, ossicular erosion, or cholesteatoma formation. Prolonged effusion may also predispose to recurrent acute otitis media and, rarely, long-term sensorineural hearing loss [71].

1.2.2 Tympanic Perforation

Tympanic Membrane Perforation (TMP) is a common condition in otology, impacting auditory function and potentially leading to complications such as recurrent infections and cholesteatoma. The causes of TMP are varied, including traumatic events, chronic otitis media and surgical interventions. The consequences of tympanic membrane perforation

extend beyond initial auditory disruption, as it can result in significant morbidity, especially in vulnerable populations such as children and those suffering from chronic ear conditions [75].

The pathophysiology of TMP relates to its etiologies, which may involve trauma from foreign objects, barometric pressure changes, or complications from infections such as chronic suppurative otitis media. Research indicates that the size and location of the perforation significantly influence the degree of conductive hearing loss experienced, emphasizing the importance of thorough assessment and tailored management strategies for tympanic membrane defects. For instance, tympanic perforations located in the posterior quadrant typically result in greater auditory impairment compared to those situated anteriorly [76]. Additionally, advances in imaging technologies have improved the precision in assessing perforation size, aiding treatment decisions and prognostications regarding audiometric outcomes [77].

After diagnosis, normally done employing otoscopy, management approaches for tympanic membrane perforations generally encompass either conservative or surgical interventions, with tympanoplasty being the most frequently employed surgical procedure. Recent studies have shown varying success rates based on the surgical techniques used, detailing differences in closure rates and postoperative hearing outcomes between endoscopic and microscopic approaches [78]. The utilization of various graft materials such as temporalis fascia, cartilage, and innovative bioengineering scaffolds is being explored to enhance tympanic membrane healing and improve functional recovery [79].

1.2.3 Otosclerosis

Otosclerosis is a disease characterized by abnormal bone remodeling in the otic capsule of the temporal bone, most commonly resulting in conductive hearing loss due to the fixation of the stapes in the oval window. This condition typically manifests with a distinct audiometric finding known as the Carhart notch, which denotes an increase in bone conduction thresholds around 2000 Hz. This frequency is associated with the mechanical effects of stapes fixation, leading to a notable dip in the bone conduction threshold without a corresponding change in air conduction thresholds [80].

The etiology of otosclerosis is still under investigation, with hypotheses suggesting the involvement of genetic factors, viral infections (notably the measles virus), and immuno-

logical responses [81]. Comprehensive evaluations, including imaging techniques such as Computed Tomography (CT), aid in assessing the extent of bony involvement and assisting in surgical planning[82].

Surgical intervention via stapedectomy or stapedotomy serves as the primary treatment for otosclerosis when conservative management fails. These procedures aim to restore mobility to the stapes and subsequently improve auditory function. The successful elimination of the Carhart notch post-surgery has been documented, demonstrating that patients often exhibit improved bone conduction thresholds at 2000 Hz following the operation, thus resolving the notch and reflecting enhanced sound transmission capabilities[83].

Recent studies also emphasize the importance of evaluating preoperative audiograms to effectively predict post-surgical outcomes. Evidence suggests that the presence of a distinct Carhart notch may serve as a prognostic marker for auditory rehabilitation following stapes surgery, providing insights into expected functional outcomes [83]. Nonetheless, residual or recurrent conductive hearing loss can occur, necessitating further evaluation of the middle ear dynamics to adapt appropriate surgical strategies [81].

1.2.4 Tympanic Retraction

Tympanic Membrane Retraction (TMR) is a significant condition in otology, characterized by the inward displacement of the tympanic membrane (TM) into the middle ear space. This retraction can often occur due to negative pressure in the middle ear, which results from ETD or COM. Retraction pockets can lead to various complications, including hearing loss and the potential development of cholesteatoma [84].

Clinicians are faced with numerous challenges when managing TMR. The absence of a consensus around management approaches signifies a need for deeper understanding and standardized treatment guidelines . Current treatment methodologies range from conservative monitoring to surgical interventions such as tympanoplasty or ventilation tube placement. The latter has been shown to improve Eustachian tube function, thereby relieving the negative pressure within the middle ear and promoting TM return to its normal position[85].

1.3 Instrumental semiotics for otologic diseases

As introduced in the previous sections, accurate otologic diagnosis is often dependent on the use of specific measurement devices, which can vary considerably depending on the structure being examined and the characteristics of the patient. Consequently, performing a reliable otologic examination requires both access to multiple specialized instruments and the technical expertise to operate them effectively [86]. However, screening is often challenging due to the limited availability of trained specialists. General practitioners may lack the equipment or clinical training necessary for precise otologic assessment, leading to potential misdiagnoses or delayed treatment. While telemedicine can help address the shortage of personnel, diagnostic equipment must also be adapted and made accessible in resource-limited and rural settings [87].

Ideally, a screening device should be affordable, easy to operate by non-specialized personnel, and capable of assisting in the diagnosis of a broad spectrum of otologic disorders. Although such an all-encompassing tool is not yet available, current technologies already enable accurate diagnosis of several common conditions with minimal resources and training. The following chapters describe the most commonly used ear-screening technologies and subsequently introduce the Pressure-Less Acoustic Immittance (PLAITM) method, a novel diagnostic approach analyzed in this thesis.

1.3.1 Otoscopy

Otoscopy, consisting in the insertion of an otoscope in the ear and the visualization at room pressure or with external pressurization of all the external ear structures and the middle ear through the tympanic membrane, is a critical procedure for diagnosing various ear conditions, particularly middle ear diseases [88]. Its significance is underscored by studies indicating that effective training and technological advancements can dramatically improve diagnostic accuracy. Medical professionals, especially primary care physicians and pediatricians, often face challenges in accurately diagnosing otologic diseases due to insufficient training in otoscopy. Research highlights low diagnostic accuracy rates, indicating a pressing need for enhanced educational frameworks for medical students and more comprehensive training programs for healthcare providers [89].

Advancements in technology, particularly through the integration of virtual reality (VR)

and digital otoscopy systems, have been explored as methods to enhance training effectiveness. Virtual reality otoscopy simulators have demonstrated substantial promise in terms of face and construct validity, enabling medical trainees to practice otoscopy skills in a controlled environment [90]. Furthermore, the application of smartphone-enabled otoscopy in telemedicine settings suggests that non-specialists could be trained to perform otoscopic examinations with similar efficacy to traditional otoscopy tools. This approach could also reduce barriers to access for marginalized communities lacking dedicated healthcare facilities [91, 92].

1.3.2 Tympanometry

Tympanometry is a fundamental diagnostic test used to assess middle ear function, specifically designed to evaluate the impedance of the tympanic membrane and the middle ear system. The process primarily measures the compliance and mobility of the tympanic membrane by observing its response to changes in air pressure. This technique is crucial for diagnosing conditions such as otitis media with effusion (OME), Eustachian tube dysfunction (ETD), and other middle ear pathologies.

The fundamentals of tympanometry are based on the principle of acoustic immittance. During testing, a probe is inserted into the ear canal, delivering sound stimuli while varying the air pressure, typically from +200 to -400 daPa. This approach evaluates the tympanic membrane's response and provides insights into middle ear compliance and acoustic reflexes, generating a tympanogram. The tympanogram (as shown in Figure 1.2) is a plot of compliance (in ml or cm³) versus pressure (in daPa), representing external and middle ear function graphically by indicating the compliance of the acoustic pathway at different pressure levels. This curve, which normally exhibits a central peak (as shown in Figure 1.2), can vary substantially. The most commonly identified tympanogram types include[93, 94]:

- **Type A** tympanogram represents normal middle ear function, with a distinct peak that signifies proper pressure regulation and mobility of the tympanic membrane. This pattern indicates a well-aerated middle ear and typically correlates with normal auditory function. Subtypes include Type As, reflecting reduced compliance suggestive of stiffness (e.g., otosclerosis or scarring), and Type Ad, indicating excessive compliance, often associated with a flaccid tympanic membrane or ossicular chain discontinuity;

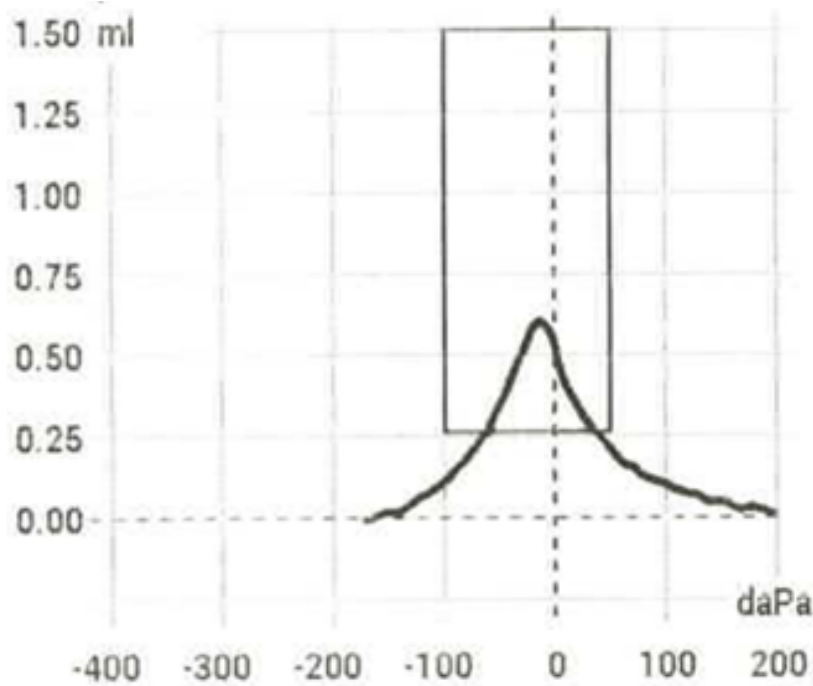


Figure 1.2: Example of single-tone tympanometry curve.

- **Type B** tympanogram is characterized by a flat trace with no identifiable peak, indicating poor mobility of the tympanic membrane. It is commonly associated with fluid in the middle ear, as seen in Otitis Media with Effusion (OME). Approximately 85–100% of confirmed middle ear effusion cases present with a Type B tympanogram. The presence of this pattern often correlates with significant hearing impairment, necessitating close monitoring and possible surgical intervention;
- **Type C** tympanogram demonstrates negative middle ear pressure, reflecting Eustachian tube dysfunction (ETD) or the early stages of middle ear effusion. Subtypes include C1 (pressure ranging from -100 to -199 daPa) and C2 (pressure < -200 daPa), which may indicate varying degrees of ETD severity and associated conductive hearing loss. However, caution is warranted in interpreting Type C tympanograms, as this pattern does not universally indicate pathology and can occur in otherwise healthy individuals.

Four main parameters can be extracted from the tympanogram and, together with the tympanometric type, provide a concise description of ear functionality[93, 94]:

- **Peak Compensated Static Admittance (Y_a):** The peak compliance of the middle ear system (i.e., the height of the tympanogram), indicating the mobility of the tym-

panic membrane and ossicles;

- **Tympanometric Peak Pressure (Tympanometry Peak Pressure (TPP)):** The pressure at which peak compliance occurs; in healthy ears, this is typically near 0 daPa (ambient pressure);
- **Tympanometric Width (TW):** The width of the tympanogram at half its peak height; in cases of OME, this parameter tends to increase;
- **Ear Canal Volume (ECV):** The estimated volume of the ear canal, measured at high positive pressure.

Despite its clinical utility, tympanometry has inherent limitations. Numerous studies have shown that its sensitivity and specificity vary with age and clinical context. For example, conventional 226 Hz tympanometry may be less reliable in infants under six months due to the anatomical and physiological characteristics of their middle ear, potentially leading to misdiagnosis of middle ear effusion [95, 96]. Recent advancements have proposed the use of high-frequency tympanometry (1,000 Hz), which significantly enhances diagnostic accuracy, particularly in infants [97].

An additional limitation of tympanometry is its restricted accuracy in estimating EAC volume. Although the values obtained with this technique provide a convenient and cost-effective first approximation, measurement errors tend to increase in the presence of pathological conditions or at larger volumes [98, 99]. Overall, tympanometry is adequate for rapid and economical assessments of EAC volume; however, when high precision is required, alternative techniques such as CT imaging are more appropriate, despite their higher cost.

Wideband Acoustic Immittance (WAI) represents a major advancement in middle ear assessment, offering a more comprehensive analysis than traditional single-frequency tympanometry. Unlike standard tympanometry, WAI measures the acoustical properties of the ear across a broad frequency range (typically 0.25–8 kHz), producing results that encompass both pressure and frequency domains (as shown in Figure 1.3). This method evaluates the energy reflectance and absorbance of the tympanic membrane and middle ear, enabling a detailed, non-invasive assessment of sound transmission mechanics [100, 101, 102].

One of the primary applications of WAI is the differentiation of various types of hearing loss, particularly between conductive and sensorineural impairments. Studies have

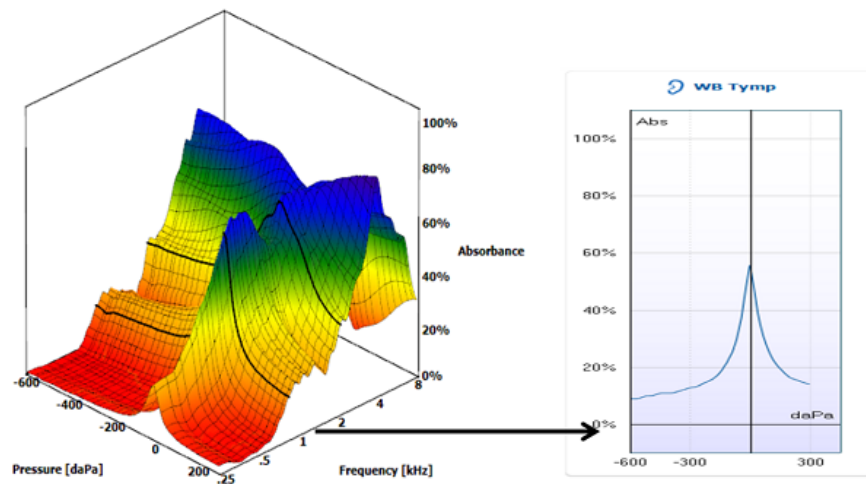


Figure 1.3: Example of WAI curve.

demonstrated that WAI can effectively identify middle ear pathologies such as otosclerosis or ossicular chain dysfunction by analyzing how sound energy is transmitted through the middle ear. The high sensitivity of WAI to even subtle changes in middle ear compliance makes it advantageous in detecting conditions that may elude traditional low-frequency tympanometry.

The clinical applicability of WAI also extends to specific populations, including children and individuals with atypical auditory anatomy. Research indicates that in pediatric audiology, WAI provides valuable insights into middle ear status, especially in infants and neonates, where conventional methods may yield inaccurate results due to anatomical factors [103]. In adults, WAI has been applied to assess age-related auditory changes, supporting longitudinal tracking of middle ear function across the lifespan [86].

Nonetheless, WAI faces certain limitations. The complexity of data interpretation, which arises from variability in ear canal acoustics and individual anatomical differences, complicates clinical integration. Moreover, although WAI demonstrates improved sensitivity compared to traditional measures, its adoption in clinical practice has been slower than anticipated. Another challenge lies in the requirement for specialized equipment and trained personnel, which may limit its accessibility in some clinical settings [100, 102, 104].

Finally, another important drawback of tympanometry is its reliance on external pressure application, which may cause discomfort or even harm in at-risk individuals, such as newborns or patients with tympanic membrane perforations [93, 97, 100, 101].

1.3.3 Acoustic reflectometry and SG-AR

Acoustic Reflectometry (AR), first introduced in 1984 [105], estimates the probability of middle ear effusion by analyzing the acoustic response of the tympanic membrane to a tone emitted by the device. The reflected sound is quantified as reflectivity, defined as the extent to which the reflected sound waves interfere with and cancel the incident waves produced by the instrument. This degree of cancellation depends on both the phase and frequency characteristics of the emitted and reflected signals. A subsequent advancement, known as Spectral Gradient - Acoustic Reflectometry (SG-AR), measures the tympanic membrane's response to a broadband acoustic stimulus in the 1.8–4.4 kHz range. It graphically represents the relationship between incident and reflected sound as a reflectivity curve (shown in Figure 1.4), from which the spectral gradient angle can be derived and analyzed[106].

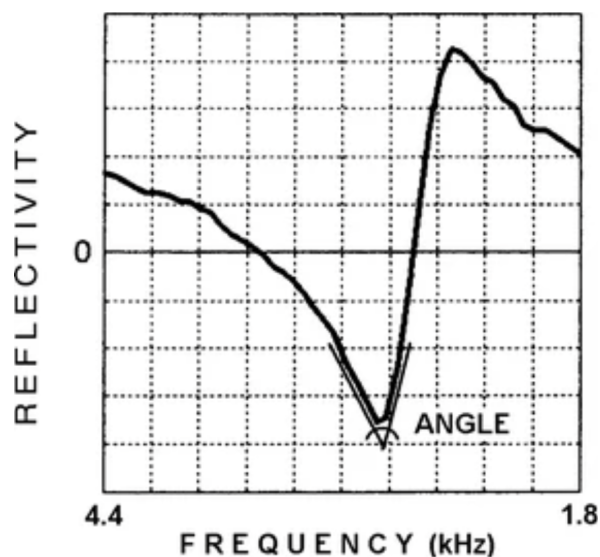


Figure 1.4: Example of Spectral Gradient Acoustic Reflectance curve [107]

This second technology has emerged as a notable diagnostic tool in the field of otology, particularly in diagnosing conditions such as otitis media with effusion (OME). Compared to traditional tympanometry, SG-AR has the advantage of not requiring an airtight seal in the ear canal, making it a more feasible option for examining children, who may be difficult to manage in clinical settings. Its ability to produce pleasant sounds makes it less intimidating for young patients, which is crucial for obtaining reliable diagnostic result [108].

Recent studies have shown that SG-AR can successfully detect middle ear effusions, thereby improving the diagnostic capability of primary healthcare providers [109]. Furthermore,

SG-AR is especially effective in contexts where tympanometry might fail due to lack of cooperation from the patient, particularly in children under three years of age [110]. This means that SG-AR can serve effectively as an adjunct to otoscopy, providing additional data to clinicians about the status of the middle ear [109].

Nevertheless, the wider adoption of SG-AR in clinical practice has been slower than anticipated. This can be attributed to several factors. Firstly, the prevalence of more established methods, such as pneumatic otoscopy and tympanometry, continues to dominate the diagnostic landscape due to their established efficacy and familiarity among practitioners [111]. Often, these methods achieve a high diagnostic success rate (up to 83% for tympanometry), which reinforces their continued use in primary care settings [112]. Furthermore, SG-AR and other emerging techniques have not yet been universally validated across diverse populations, creating uncertainty regarding their reliability compared to traditional methods. Additionally, economic considerations play a role in the slow adoption of SG-AR. The cost of implementing and training staff to use new technologies can be substantial, especially for smaller clinics or those in resource-limited settings. Thus, despite its clinical promise, SG-AR remains an adjunct rather than a primary diagnostic tool in many healthcare environments.

1.3.4 Audiometry

Pure Tone Audiometry (PTA) serves as a fundamental tool in audiology, widely utilized to evaluate hearing sensitivity across various audiometric frequencies. The technique uses pure tones at discrete frequencies, typically administered between 250 Hz and 8 kHz, and is instrumental in identifying both the type and degree of hearing loss [113]. The procedure is categorized as a subjective test wherein patients respond to stimuli, thus establishing threshold levels that inform clinicians about auditory capabilities, which can subsequently guide rehabilitation strategies such as hearing aid fittings [114].

The utilization of PTA encompasses a broad spectrum of applications in clinical settings. Moreover, the assessment of high-frequency thresholds beyond the conventional PTA range (up to 8 kHz) is gaining significance, particularly in identifying cochlear damage often overlooked by traditional testing methods. This underscores the evolving nature of audiometric assessments, where extended high-frequency audiometry (EHF) becomes crucial in providing a more comprehensive auditory profile, especially in scenarios involv-

ing noise exposure and cochlear toxicity[115].

Despite its foundational role, PTA faces several limitations that can impede its effectiveness. Subjects may present normal thresholds within the PTA frequency range but still exhibit audiometric abnormalities at higher frequencies or specific frequency notches indicative of noise-induced hearing loss. Furthermore, PTA's reliance on patient cooperation and subjective responses introduces variability and potential biases, particularly in populations unable to provide reliable feedback, such as young children or cognitively impaired individuals. Operational limitations further characterize PTA as a relatively time-consuming process, requiring a sound-treated environment and trained audiologists for precise threshold determination. This poses logistical challenges in large-scale screenings, where the practical application of PTA may not always be feasible without adequate resources or trained personnel. Moreover, emerging methods, such as automated audiometry and smartphone applications, are being explored for their efficiency and accessibility compared to traditional PTA methods. These new modalities may enhance the reliability and speed of hearing assessments while addressing issues related to patient engagement and environmental constraints [116].

1.3.5 Acoustic Reflex Testing

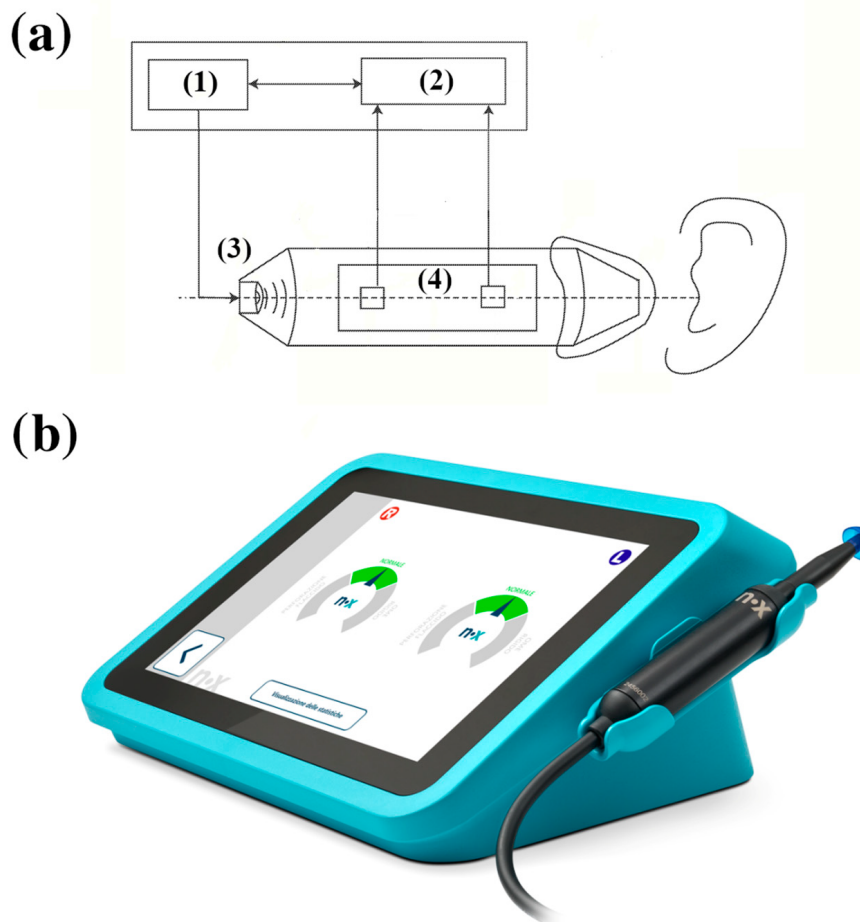
Acoustic Reflex Testing (ART) represents an important component of audiological assessment, aiding in the identification of auditory disorders and middle ear dysfunctions. The fundamentals of ART are grounded in the anatomical and physiological mechanisms of the auditory system, specifically the contraction of the stapedius muscle in response to high-intensity sounds. This reflex acts as a protective mechanism that attenuates loud acoustic stimuli, thereby safeguarding the delicate structures of the inner ear from potential damage [117]. When properly performed, ART provides valuable insights not only into the functional status of the middle ear but also into the integrity of neural pathways involved in auditory processing. Absent or abnormal reflex responses may signal underlying neurological pathologies, such as auditory neuropathy or disorders affecting cranial nerve pathways [118].

Measurement of the Acoustic Reflex Threshold, typically conducted via immittance audiometry, involves presenting a tonal or broadband stimulus to the ear and monitoring the reflexive contraction of the stapedius muscle [117]. Recent developments have extended

the scope of ART through the adoption of wideband acoustic measures, which enhance sensitivity in detecting subtle pathologies that may be missed by traditional testing methods [119]. In particular, wideband reflectance techniques provide a more comprehensive evaluation of middle ear function across a broad frequency range, thereby improving diagnostic accuracy [120]. This approach is especially advantageous when conventional testing yields absent reflex responses, as it helps reduce the likelihood of misdiagnosing hearing loss in clinical practice [121].

However, even if very useful in clinical practice, ART is subject to several limitations that affect both its diagnostic precision and its clinical usefulness. The test does not allow a clear distinction between damage to the auditory nerve and lesions in the lower brainstem, since the reflex arc involves several interconnected structures such as the auditory nerve, cochlear nucleus, facial nerve and stapedius muscle. As a result, it cannot precisely identify the location of a possible dysfunction [122]. The method is also sensitive to the presence of conductive hearing loss, as middle ear conditions like otitis media, tympanic membrane perforations, or ossicular discontinuities can interfere with the contraction of the stapedius muscle, producing absent or distorted reflexes that may lead to misleading results [123]. Interpretation can be further complicated because the acoustic reflex may be absent in individuals with severe sensorineural hearing loss or even in people with normal auditory function, thus reducing diagnostic reliability. In addition, the test outcomes are influenced by patient-related variables such as age, gender, and overall hearing condition. Reflex responses tend to weaken with aging, and conditions such as tinnitus can mask or alter the reflex signal, making detection difficult [124]. Cognitive and attentional factors may also influence the amplitude of the response, introducing variability in both clinical and research settings. Finally, performing acoustic reflex testing in newborns and infants is particularly challenging because of the small size of their ear canals and the difficulty of keeping them still during the procedure, which can significantly affect the accuracy and reproducibility of the results [125].

The clinical utility of ART extends beyond diagnosis. It is instrumental in monitoring therapeutic interventions and evaluating auditory pathway function over time, particularly in patients undergoing treatments such as cochlear implantation [126]. In pediatric audiology, the implementation of wideband ART has shown promise in improving neonatal hearing screening outcomes, thereby enhancing early detection of auditory dysfunctions



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Figure 1.5: (a) Schematic of the Pressure-Less Acoustic Immittance (PLAI™) device, showing key components: (1) Sound source, (3) Speaker, (4) Sensors to capture reflected sound, and (2) Processing unit for data analysis. (b) Image of the PLAI™ device.

and enabling timely intervention [120]. Furthermore, understanding the role of efferent auditory pathways, which modulate the acoustic reflex response, adds depth to ART interpretation—especially in conditions that affect auditory processing and perception [100].

1.3.6 Pressure-Less Acoustic Immittance

Given the limitations of standard and wideband tympanometry (WAI), both of which require external pressure and may cause discomfort, early identification of middle ear pathologies, particularly in infants and in individuals with tympanic membrane perforations, remains a clinical challenge. To address this need and enable more effective and timely diagnosis, a novel non-invasive technology, the Pressure-Less Acoustic Immittance (PLAI™, Neuranix Srl, Naples, Italy), has been developed (Figure 1.5).

The Pressure-Less Acoustic Immittance (PLAITM) system is a MEMS-based, CE marked diagnostic device designed to assess middle ear function without the need for external pressurization. Unlike conventional tympanometry, which relies on controlled pressure variations, PLAITM operates under ambient pressure using a dual-sensor configuration that simultaneously measures acoustic pressure and air velocity at the same point. The instrument emits a wideband acoustic signal ranging from 100 to 2000 Hz, which interacts with the ear canal and tympanic membrane. The reflected sound waves, containing information on the mechanical and acoustic properties of the outer and middle ear, are then recorded by two microphones that measure both pressure and the axial velocity of air particles within the canal.

From these measurements, the system derives an acoustic admittance curve (Figure 1.6) as a function of frequency. The curve represents how the ear transmits and reflects sound energy and provides insight into middle ear mechanics. From the admittance curve, the device extracts several quantitative parameters, including:

- Resonance frequency (F_{res});
- Peak admittance (Peak);
- Minimum and maximum frequency bounds (minBnd, maxBnd);
- Bandwidth mean amplitude (AltBnd);
- Bandwidth length (LBnd);
- EAC volume (Vol).

Additional variables, such as age and diagnostic class labels, are included in the dataset for subsequent statistical or machine learning analysis. The resonance frequency depends primarily on the air volume within the ear canal and the mechanical properties (mass, viscosity, and elasticity) of the outer and middle ear [127]. Because measurements are performed entirely at atmospheric pressure, the method is non-invasive and suitable for application even in infants or in subjects with tympanic membrane perforations.

Since PLAITM is based on different physical principles than traditional tympanometry, its derived parameters are not directly comparable to those of the gold standard procedure. This makes it necessary to establish reference data for both healthy subjects and at least one

pathological population, in order to define diagnostic thresholds and enable potential clinical implementation. Furthermore, as with conventional tympanometry, anatomical differences in the external and middle ear that vary with patient age imply that age-dependent effects may also be present in PLAITM measurements [128].

Currently, the diffusion of this innovative technique is still limited, with only one other research group actively working on its clinical characterization. Their studies confirm that PLAITM provides a reliable, non-pressurized method for assessing middle ear mechanics through the evaluation of resonance frequency and peak admittance. Their results show that PLAITM can effectively distinguish between normal and pathological ears: higher F_{res} and admittance values are associated with increased stiffness (as in otosclerosis or ossicular fixation), whereas lower values correspond to greater compliance (as observed in tympanic membrane atrophy or effusion) [129]. In patients with chronic ventilation disorders, PLAITM enabled early detection of ossicular chain fixation, and in otosclerosis it demonstrated a strong correlation between resonance frequency and the air–bone gap [130]. Pediatric investigations further confirmed its potential in identifying otitis media with effusion [131].

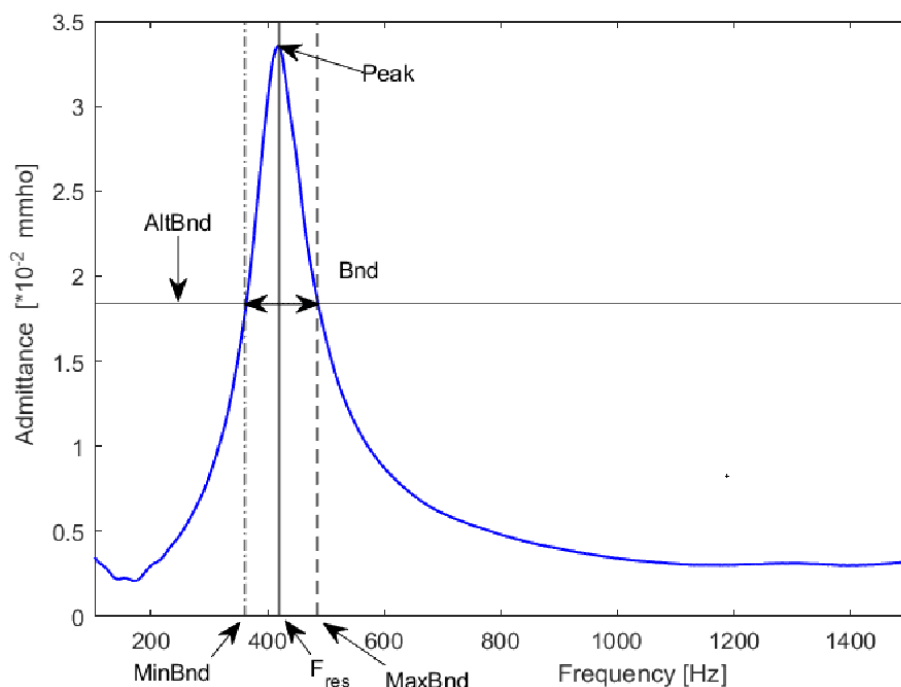


Figure 1.6: Example of an Admittance curve from PLAITM with main parameters extracted.

Chapter 2

PLAITM Characterization

The present chapter presents research aimed at characterizing measurements obtained using the PLAITM methodology. The initial phase of the research focused on establishing relationships between PLAITM and gold-standard parameters, and was conducted in two steps:

1. Extraction of relationships between equivalent volume and compliance from tympanometry and resonance frequency from PLAITM in a population of 57 subjects, both healthy and pathologic [FB1].
2. Estimation of the age dependency of tympanometric and PLAITM parameters, and relationships between them, in a population of 134 healthy subjects aged between 0 and 80 years [FB2].

Having established in the previous phase that tympanometric and PLAITM parameters are correlated and identified their age dependencies, the subsequent step was to isolate age groups and develop age-specific reference ranges for pressure-less parameters. This study was conducted on 318 healthy ears and 68 OME-affected ears, covering an age range from 4 months to 80 years[FB3].

The second and third studies were conducted through a multicenter data collection process involving healthy and pathologic subjects aged 0 to 80 years, recruited from the outpatient departments of six hospitals: Gemelli University Hospital Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) (Rome), “Guglielmo da Saliceto” Hospital (Piacenza), “Federico II” University Hospital (Napoli), “Giovanni XXIII” Children’s Hospital (Bari), Fondazione IRCCS Policlinico “S. Matteo” (Pavia), and IRCCS Maternal and Child Health In-

stitute “Burlo Garofolo” (Trieste). The research protocol was approved by the Ethics Committees of all six participating institutions (Protocol No. 5389, January 19, 2023, IRCCS “A. Gemelli” Hospital, Rome). In accordance with Italian regulations, additional authorization was obtained from the Italian Ministry of Health (MEDWAVE-2, IT-23-03-042692, May 9, 2023). Informed consent was obtained from all adult participants, and consent for minors was provided by a parent or legal guardian.

These three studies allowed to obtain a clear framework for retrospective indicative comparison with literature data and provided initial reference values for healthy and OME patients using the new technology, but also highlighted the considerable variability of data, both in PLAI™ and in tympanometric measurements.

2.1 Comparison between PLAI™ parameters and tympanometry

The objective of this study[FB1] was to assess the feasibility of estimating one or more parameters that are usually measured through tympanometry, the current gold standard technique, by using parameters obtained from PLAI™.

This study focused on investigating the relationships between the resonance frequency (F_{res}) measured by PLAI™ and two tympanometric parameters: Compliance and ECV. The analysis was carried out on a general population that included both healthy individuals and patients affected by OME.

2.1.1 Materials and methods

To evaluate the relationships between measurements obtained with standard tympanometry and those from PLAI™ technology, both ears of 57 subjects (27 males and 30 females) aged between 25 and 93 years were examined at “S. Jacopo” Hospital in Pistoia. Compliance and Equivalent Volume were extracted from tympanometry, while the resonance frequency was obtained from PLAI™. All participants provided informed consent before testing.

In the analysis of the relationship between F_{res} and ECV, 9 out of 114 ears were excluded because of missing tympanometric or PLAI™ data. The final dataset therefore included

105 ears, 35 affected by OME and 70 without ear pathologies.

In the evaluation of the relationship between F_{res} and Compliance, 32 ears were excluded due to missing data, mainly caused by type B tympanograms where no visible admittance peak was present. As a result, 82 ears were considered for analysis, 17 affected by OME and 65 from healthy subjects.

Since the relationship between F_{res} and ECV showed an approximately linear trend, a linear regression model was applied to evaluate the significance of the relationship and to calculate the correlation coefficient (R). The coefficients obtained from the regression were then used to estimate the equivalent volume measured by tympanometry from the F_{res} parameter of PLAITM. The estimated and measured ECV values were compared using a Bland-Altman plot[132].

In contrast, because the relationship between F_{res} and Compliance was nonlinear, a hyperbolic model was fitted to the data using the Nonlinear Least Squares method to minimize the Root Mean Square Error (RMSE). All analyses were performed in MATLAB[®] version R2023b using proprietary scripts.

2.1.2 Results

Figure 2.1 illustrates the relationship between the equivalent volume (ECV) measured by tympanometry and the resonance frequency measured by PLAITM, along with the corresponding linear regression curve and the 95% prediction bounds. The equation describing this linear relationship is:

$$ECV_{tym} = -0.0020 * F_{res} + 1.91 \quad (2.1)$$

The linear regression is statistically significant ($p < 0.001$) and shows a correlation coefficient (R) of 0.65, which indicates a moderate variability among the observations, as visible in Figure 2.1.

To assess the agreement between the ECV estimated through the regression model and that measured directly by tympanometry, Figure 2.2 presents the Bland-Altman plot obtained from the two datasets. The plot shows a low bias and good agreement between the two measurements, as 95 percent of the values have an absolute error smaller than 0.4 milliliters.

Regarding the relationship between the Compliance measured by tympanometry and the

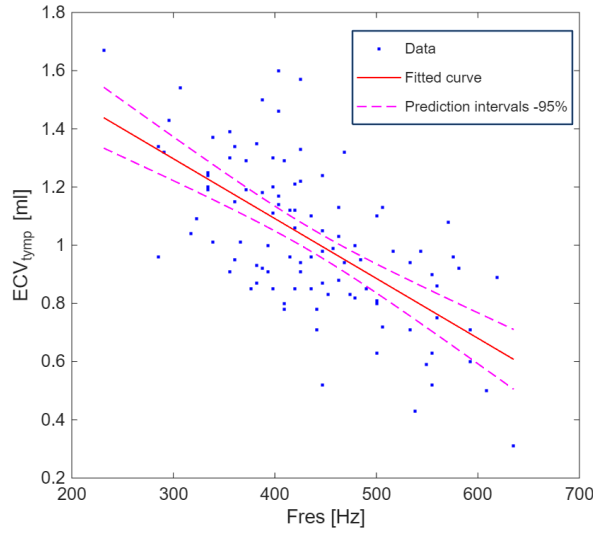


Figure 2.1: Relationship between the resonance frequency (F_{res}) from PLAITM and the equivalent volume measured by tympanometry, including the linear regression curve (red line) and the 95% prediction bounds (dashed lines).

resonance frequency measured by PLAITM, Figure 2.3 displays a hyperbolic trend fitted by the following equation:

$$Compliance_{tymp} = \frac{140}{F_{res} - 217} \quad (2.2)$$

This model was estimated using data from 82 ears by minimizing the root mean square error (RMSE), which resulted in an RMSE of 0.17 mmho. For comparison, the RMSE obtained with a linear fit was 0.20 mmho.

2.1.3 Discussion

The preliminary results obtained from both healthy and pathological ears, which displayed a wide range of ECV and Compliance values, indicate a statistically significant relationship between PLAITM resonance frequency and the two tympanometric parameters. In particular, ECV shows a linear correlation with the resonance frequency (F_{res}), and the Bland-Altman analysis demonstrates good agreement between the two Volume estimations. These findings support the feasibility of using F_{res} to estimate ECV in a noninvasive manner, without the need to apply positive or negative pressure on the eardrum.

In contrast, although a hyperbolic relationship was observed between the Compliance measured by tympanometry and the F_{res} extracted from PLAITM, a considerable number of outliers were found outside the 95 percent prediction bounds of the fitted curve, indicating a

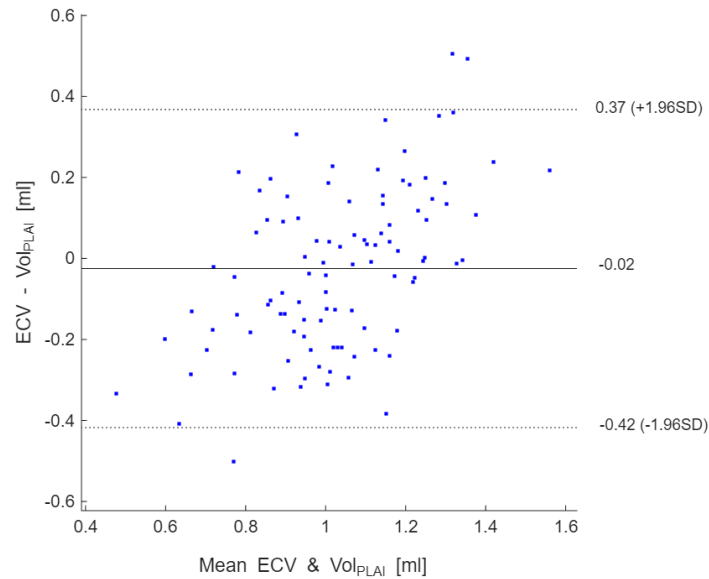


Figure 2.2: Bland-Altman plot comparing the equivalent volume measured by tympanometry with that estimated using the PLAITM system.

high variability of values. This may be explained by the fact that, while subjects with OME generally exhibit higher F_{res} values than healthy individuals, the Compliance values of the two groups remain relatively similar. Consequently, our findings suggest that F_{res} alone is not a suitable parameter for estimating Compliance.

Furthermore, because tympanometry cannot be performed on ears with tympanic membrane perforations, the results reported here refer only to measurements obtained from ears with an intact eardrum. Nevertheless, we are confident that the PLAITM technique could also be applied in cases of tympanic membrane perforation, enabling accurate volume estimations in situations where conventional devices fail. However, validation of PLAITM volume measurements in such cases will require comparison with established reference methods, such as CT imaging. In addition, the use of higher-accuracy techniques for volume estimation, including CT imaging, would allow for a more reliable benchmarking of the PLAITM method, given the known limitations of tympanometry in volume assessment [98, 99].

In conclusion, this preliminary study identified specific relationships between parameters measured by tympanometry and those obtained using PLAITM. In particular, the linear relationship between ECV and resonance frequency suggests that volume can be estimated even when tympanometry cannot be performed. In this last case, the measured volume will

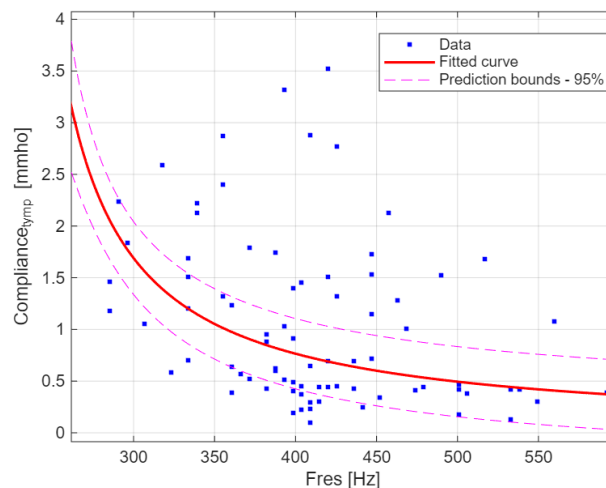


Figure 2.3: Relationship between the Compliance measured by tympanometry and the resonance frequency from PLAI™, including the fitted hyperbolic curve and the 95% prediction bounds.

be the sum of the external auditory canal and the middle ear, so in some cases the resonance frequency could be too low to be measured.

2.2 Age dependency of parameters

The aims of the present study [FB2] were to provide a comprehensive review of age-related reference values for tympanometric parameters, to model the age-dependent variation of tympanometric and PLAI™ parameters in a population of healthy subjects, and to identify the main relationships between tympanometric measurements and PLAI™ parameters across different age groups.

2.2.1 Materials and methods

Subjects aged between 0 and 80 years were recruited from the outpatient departments of six hospitals: Gemelli University Hospital IRCCS (Rome), “Guglielmo da Saliceto” Hospital (Piacenza), “Federico II” University Hospital (Napoli), “Giovanni XXIII” Children’s Hospital (Bari), Fondazione IRCCS Policlinico “S. Matteo” Hospital (Pavia), and IRCCS Maternal and Child Health Institute “Burlo Garofolo” (Trieste).

The inclusion criteria required ears that were currently healthy, with no history of auditory disorders, and belonging to individuals without significant disabilities or behavioral

conditions that might interfere with standard hearing assessments.

All participants first underwent otoscopic examination to exclude any diseases or anatomical abnormalities affecting the ear. Subsequently, both standard single-tone tympanometry and pressureless acoustic immittance measurements were performed. Tympanometry provided the following parameters: tympanogram type, Equivalent Canal Volume (ECV), Peak-compensated static admittance (Y_a), and tympanogram width (TW). Because all participants were healthy, tympanometric peak pressure (TPP) was recorded but not included in the analysis, as its value was consistently close to zero across the entire sample.

For this analysis, only ears with complete tympanometry and PLAITM data were included. The normality of the measurements was verified using a Lilliefors test [133]. Means and standard deviations were calculated with a one-sample t-test, and outliers, defined as values deviating by more than 2.5 standard deviations from the mean, were excluded from subsequent analyses.

Age-related trends for each parameter were evaluated using several fitting models, including linear, logarithmic, and third-order polynomial functions.

The tympanometric results were compared with reference data available in the literature, selected to include various age groups and to reflect the morphological characteristics of subjects from North American and European populations [134, 135, 136, 137, 138, 139, 140]. The comparison was based on the means and standard deviations reported for corresponding age groups.

Finally, correlation coefficients were calculated between tympanometric and PLAITM parameters. All analyses were performed using proprietary MATLAB[®] scripts (version R2024a).

2.2.2 Results

A total of 134 subjects participated in the study and were categorized by age as follows: 32 individuals were under 24 months, 47 were between 25 months and 18 years, and 55 were over 18 years. After data selection and cleaning, the initial dataset of 268 individual ears was reduced to 153 ears. The final dataset was distributed as 29 ears from subjects under 24 months, 61 ears from those aged 25 months to 18 years, and 63 ears from individuals over 18 years, with the oldest participant being 76 years old.

The means and standard deviations of the tympanometric and PLAITM parameters for each age group are reported in Table 2.1.

Table 2.1: Healthy subjects reference values

| Parameter | 0-24 months (29 subjects) | 2-18 years (61 subjects) | 18 + years (63 subjects) |
|----------------------------|------------------------------|-----------------------------|-----------------------------|
| ECV [ml] | 0.55 ± 0.14 | 0.85 ± 0.27 | 1.20 ± 0.33 |
| TW [daPa] | 133 ± 73 | 97 ± 31 | 64 ± 25 |
| Ya [mmho] | 0.47 ± 0.32 | 0.58 ± 0.47 | 0.87 ± 0.54 |
| Volume [ml] | 0.78 ± 0.22 | 1.18 ± 0.44 | 1.70 ± 0.49 |
| F_{res} [Hz] | 534 ± 56 | 457 ± 62 | 388 ± 49 |
| maxBnd [Hz] | 653 ± 55 | 550 ± 73 | 483 ± 48 |
| minBnd [Hz] | 341 ± 101 | 315 ± 75 | 289 ± 55 |
| LBnd [Hz] | 311 ± 80 | 235 ± 64 | 194 ± 42 |
| Peak [$* 10^{-2}$ mmho] | 1.13 ± 0.32 | 1.48 ± 0.35 | 1.59 ± 0.43 |
| AltBnd [$* 10^{-2}$ mmho] | 0.68 ± 0.17 | 0.87 ± 0.19 | 0.89 ± 0.23 |

The optimal fitting functions for the obtained data, corresponding to the lowest root mean square error (RMSE), were of the form $y = a + b * \log_{10}x$, with the coefficient values reported in Table 2.2.

The tympanometric values for the examined population, along with the corresponding fitted curves, are shown in Figure 2.4. A graphical comparison between the fitted curves obtained in this study and the values reported in the literature is presented in Figure 2.5.

Table 2.3 shows the correlation coefficients of the significant (p -value < 0.0001) relations between tympanometric and PLAI™ parameters.

2.2.3 Discussion

The tympanometric parameters measured in healthy ears of subjects aged 0 to 76 years were consistent with findings reported in the literature, which mostly focused on narrower age ranges. This consistency made it possible to identify a logarithmic relationship between these parameters and age. Both the tympanometric ECV and the PLAI™-derived volume estimates showed the expected increase with age during growth, followed by stabilization in adulthood. The growing variability observed with age is likely attributable to individual anatomical differences [137, 138].

Table 2.2: Logarithmic fitting curves

| Parameter | a | b | RMSE |
|--------------------------------|------|------|------|
| ECV [ml] | 0.59 | 0.35 | 0.25 |
| TW [daPa] | 128 | 39.4 | 38.9 |
| Ya [mmho] | 0.45 | 0.24 | 0.47 |
| Volume [ml] | 0.83 | 0.51 | 0.42 |
| F _{res} [Hz] | 520 | -81 | 53 |
| maxBnd [Hz] | 632 | -96 | 54 |
| minBnd [Hz] | 338 | -30 | 72 |
| LBnd [Hz] | 295 | -66 | 57 |
| Peak [$\cdot 10^{-2}$ mmho] | 1.20 | 0.27 | 0.37 |
| AltBnd [$\cdot 10^{-2}$ mmho] | 0.72 | 0.12 | 0.20 |

Table 2.3: Tympanometry - PLAITM correlation coefficients

| Pair of parameters | Correlation coefficient R |
|------------------------|---------------------------|
| ECV - Volume | 0.69 |
| ECV - Peak | 0.43 |
| ECV - F _{res} | -0.71 |
| ECV - maxBnd | -0.74 |
| ECV - LBnd | -0.54 |
| TW - Volume | -0.42 |
| TW - F _{res} | 0.46 |
| TW - maxBnd | 0.45 |
| TW - LBnd | 0.45 |
| Ya - Volume | 0.59 |

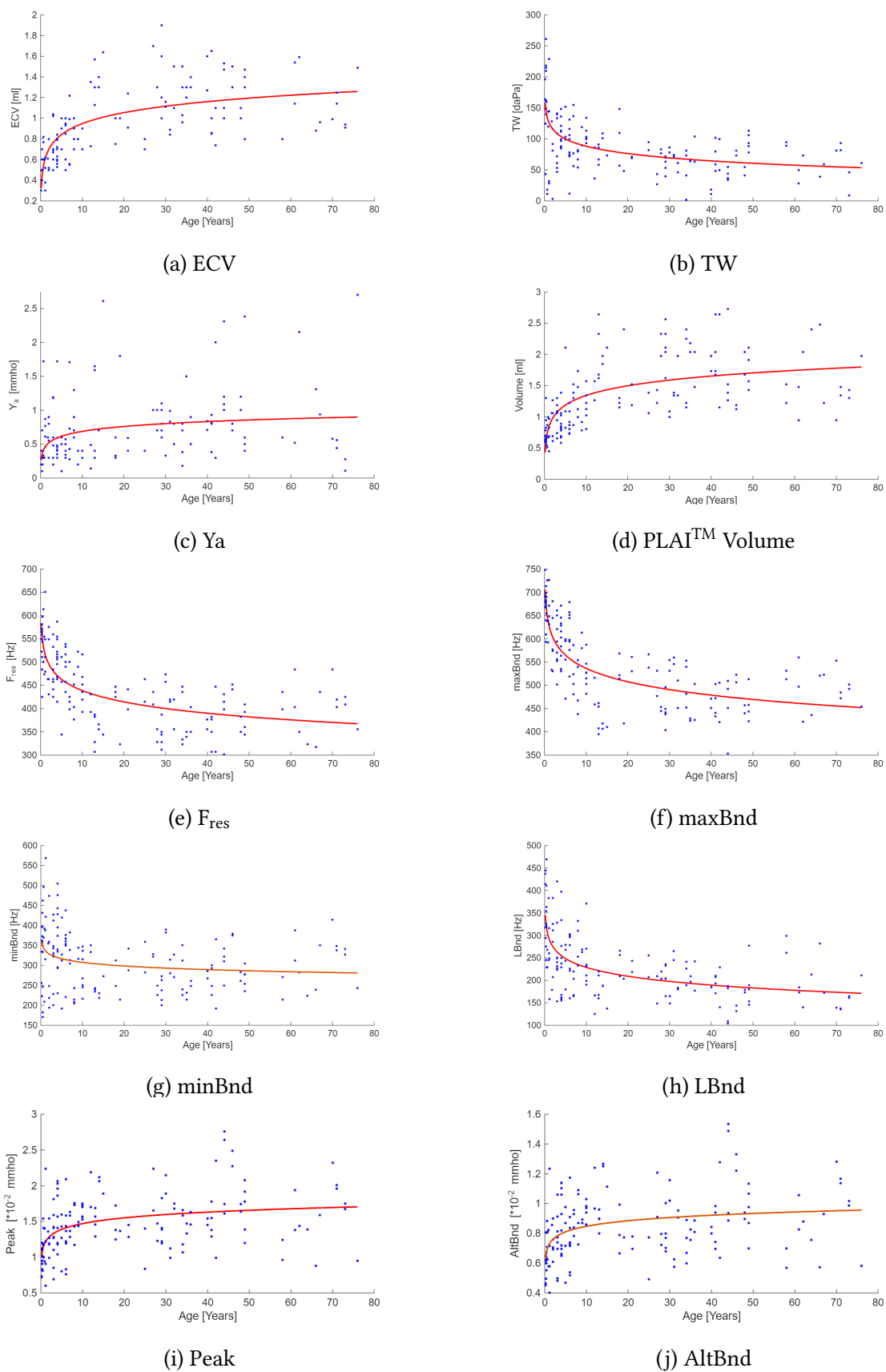
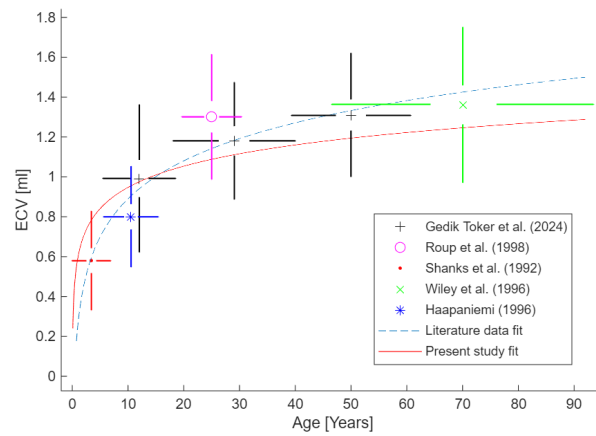
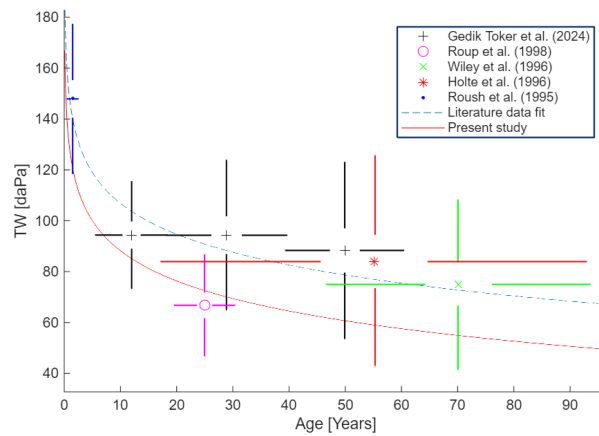


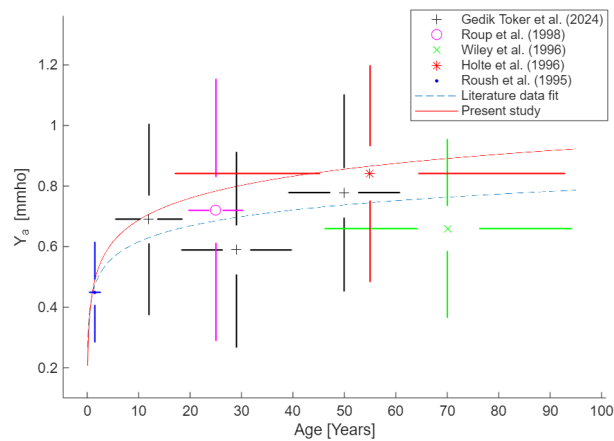
Figure 2.4: Measured values and corresponding logarithmic fit curves. The high variability in both tympanometric and PLAITM measurements can be attributed to individual anatomical differences.



(a) ECV [134, 135, 136, 137, 138]



(b) TW [134, 135, 137, 139, 140]



(c) Ya [134, 135, 137, 139, 140]

Figure 2.5: Relations between literature tympanometric values and the fitting curves calculated in this study. Each point represents a cohort of subjects, with the horizontal lines depicting the overall age range and the vertical lines indicating the standard deviation for that cohort within the study.

Similarly, the peak-compensated static admittance (Y_a) exhibited an age-related increase, likely associated with changes in eardrum stiffness, surface area, and the compliance of ear canal walls [134, 43]. The variability of Y_a also increased with age, reflecting interindividual anatomical diversity. In contrast, tympanometric width (TW) decreased with age, possibly due to progressive stiffening of the ear canal, eardrum, and middle ear structures involved in sound transmission [134]. In this case, variability tended to diminish over time, with measurements becoming more homogeneous in healthy ears. The parameters Peak and AltBnd followed patterns similar to Y_a and volume, while F_{res} , minBnd, maxBnd, and LBnd showed trends comparable to TW, likely influenced by the same physiological mechanisms. The substantial variability observed across the six participating hospitals may be partially explained by differences in tympanometer models and operator expertise.

Notably, ECV demonstrated strong correlations ($R = 0.54\text{--}0.74$) with resonance-related parameters such as F_{res} , maxBnd, LBnd, and volume. This relationship likely reflects the resonance behavior of sound waves within a confined space [141]. Furthermore, the correlation between Y_a and volume may result from developmental changes: as ear volume increases, modifications in eardrum properties enhance sound wave reflection and transmission through the ossicular chain [43].

Despite the considerable variability observed both in the tympanometric and PLAI™ parameters, this preliminary study revealed a consistent set of logarithmic relationships between age and both tympanometric and PLAI™ parameters, corroborating previous reports limited to specific age ranges. Additionally, several meaningful correlations were identified between standard tympanometric and pressureless acoustic immittance parameters. These findings suggest that PLAI™ measurements can be interpreted in physiologically meaningful ways, potentially enabling this fully noninvasive technique to serve as a practical screening tool for middle ear assessment.

While a full characterization of the physiological significance of PLAI™ parameters is undergoing, the pressureless method may still be used to estimate tympanometric parameters, thus allowing for specialists to diagnose pathologies without using external pressure gradients that may damage fragile or already unstable ear structures. This possibility needs to be validated with specific studies, validating the diagnoses on single pathologies using PLAI™ as a substitute for tympanometry in multiple instrument evaluations for patients where tympanometry is not an appropriate measurement method.

2.3 Age groups definition and reference values

Aim of this study [FB3] was to establish reference value ranges for each parameter derived from PLAITM curves in healthy ears, as well as in one of the most common middle ear pathologies, Otitis Media with Effusion (OME), across all age groups. These reference ranges are intended to provide a foundation for distinguishing healthy individuals from those affected by middle ear disorders in future clinical applications.

2.3.1 Materials and methods

In this multicenter study, subjects were enrolled from six Italian clinical institutions: Gemelli University Hospital IRCCS (Rome), “Guglielmo da Saliceto” Hospital (Piacenza), “Federico II” University Hospital (Napoli), “Giovanni XXIII” Children’s Hospital (Bari), Fondazione IRCCS Policlinico “S. Matteo” Hospital (Pavia), and IRCCS Maternal and Child Health Institute “Burlo Garofolo” (Trieste). Participants included individuals with healthy ears as well as those affected by Otitis Media with Effusion (OME).

Subjects were recruited between May 2023 and April 2024 from outpatient clinics, without age restrictions. Exclusion criteria included a history of otologic surgery, significant comorbidities, or cognitive impairments that could interfere with measurements. All participants underwent otoscopy and tympanometry to confirm the status of their auditory organs. Only confirmed cases of OME and healthy ears were included in subsequent analyses. Ears diagnosed with OME and without additional otologic comorbidities comprised the pathological group, while ears without any otologic pathology were assigned to the healthy group. A total of 360 healthy ears and 76 pathological ears were collected from 218 subjects aged 4 months to 80 years.

All measurements were manually reviewed for errors, including motion artifacts and probe misplacement, by two independent experts. Following this quality control, 318 healthy and 68 pathological measurements were retained for analysis.

To evaluate potential age dependency of the parameters in healthy ears, several analytical models were tested to fit the data and approximate age-related trends. The models included: (1) a logarithmic curve, (2) a ramp superimposed on a logarithmic curve, (3) a polynomial curve up to third order, (4) an exponential curve, and (5) an inverse exponential curve. Goodness of fit was assessed using the RMSE between measured values and fitted curves,

alongside the number of model coefficients. The model with the lowest overall RMSE and the fewest coefficients was selected as optimal.

To account for age effects, the data were divided into discrete age classes, with class sizes determined by the rate of parameter variation with age. For each age class and parameter, the mean and standard deviation were calculated using a custom MATLAB® script. Pair-wise comparisons between age classes were performed using the Wilcoxon rank-sum test with Bonferroni correction.

Subsequently, the group of subjects with OME-affected ears was analyzed to assess differences between healthy and pathological cases. The same age-based stratification applied to the healthy cohort was used for pathological cases to examine potential age-related trends. This approach not only validates the relevance of age-based grouping in a clinical context but also demonstrates the potential diagnostic utility of the method in distinguishing between healthy and OME-affected ears.

2.3.2 Results

Selection of Fit Functions Based on RMSE Performance

The analysis of the fitting functions revealed distinct grouping patterns based on RMSE values, allowing the functions to be classified into two main clusters according to their performance. The first cluster, which included second- and third-order polynomial curves as well as logarithmic and logarithmic-plus-linear approximations, exhibited lower RMSE values. In contrast, the second cluster, comprising linear, exponential, and inverse exponential functions, showed slightly higher RMSE values for the Peak, AltBnd, and Volume parameters, and substantially higher values for F_{res} , MinBnd, MaxBnd, and Bnd (Table 2.4). Within the best-performing cluster, the logarithmic function was chosen as the optimal model due to its combination of the lowest RMSE values and minimal complexity in terms of the number of coefficients. Table 2.4 presents the mean RMSE values for each parameter within the two clusters, while Table 2.5 reports the median coefficient values of the logarithmic approximation curves, along with their 95% confidence intervals and corresponding RMSE.

Table 2.4: Mean values of RMSE of each group of the fit functions for each parameter

| | First Group | Second Group |
|----------------------------------|-------------|--------------|
| Peak(* 10 ⁻² mmho) | 0.38 | 0.40 |
| AltBnd (* 10 ⁻² mmho) | 0.20 | 0.21 |
| Volume (ml) | 0.49 | 0.52 |
| Fres (Hz) | 73 | 112 |
| MinBnd (Hz) | 85 | 97 |
| MaxBnd (Hz) | 101 | 126 |
| LBnd (Hz) | 100 | 111 |

Table 2.5: Median values and 95% confidence intervals of the coefficients of the logarithmic fit ($a * \log_{10}(\text{age}) + b$) for each parameter, together with the RMSEs.

| | a | b | RMSE |
|----------------------------------|--------------------|-------------------|------|
| Pk (* 10 ⁻² mmho) | 0.31 (0.25 , 0.36) | 1.08 (1.01, 1.14) | 0.38 |
| AltBnd (* 10 ⁻² mmho) | 0.16 (0.13, 0.19) | 0.64 (0.61, 0.68) | 0.20 |
| Vol (ml) | 0.60 (0.53, 0.68) | 0.77 (0.68, 0.86) | 0.50 |
| Fres (Hz) | -99 (-110, -88) | 543 (530, 556) | 72.7 |
| MinBnd (Hz) | -48 (-61, -36) | 365 (350, 380) | 85.7 |
| MaxBnd (Hz) | -137 (-151, -122) | 695 (678, 713) | 98.9 |
| LBnd (Hz) | -88 (-103, -74) | 330 (313, 348) | 98.1 |

Age-Related Trends and Variability in Acoustic Parameters

Figure 2.6 presents the seven parameters as a function of age, along with their corresponding logarithmic fitting curves. The scatter plots reveal substantial variability across the entire age range. A clear age-related trend is observed, with parameters such as Peak, AltBnd, and Volume increasing rapidly, while F_{res} , MinBnd, MaxBnd, and Bnd decrease, up to approximately 10–14 years. Notably, for all parameters, the logarithmic fit reaches 85% of the final value before the age of 12. Beyond this point, the parameters stabilize and remain relatively constant.

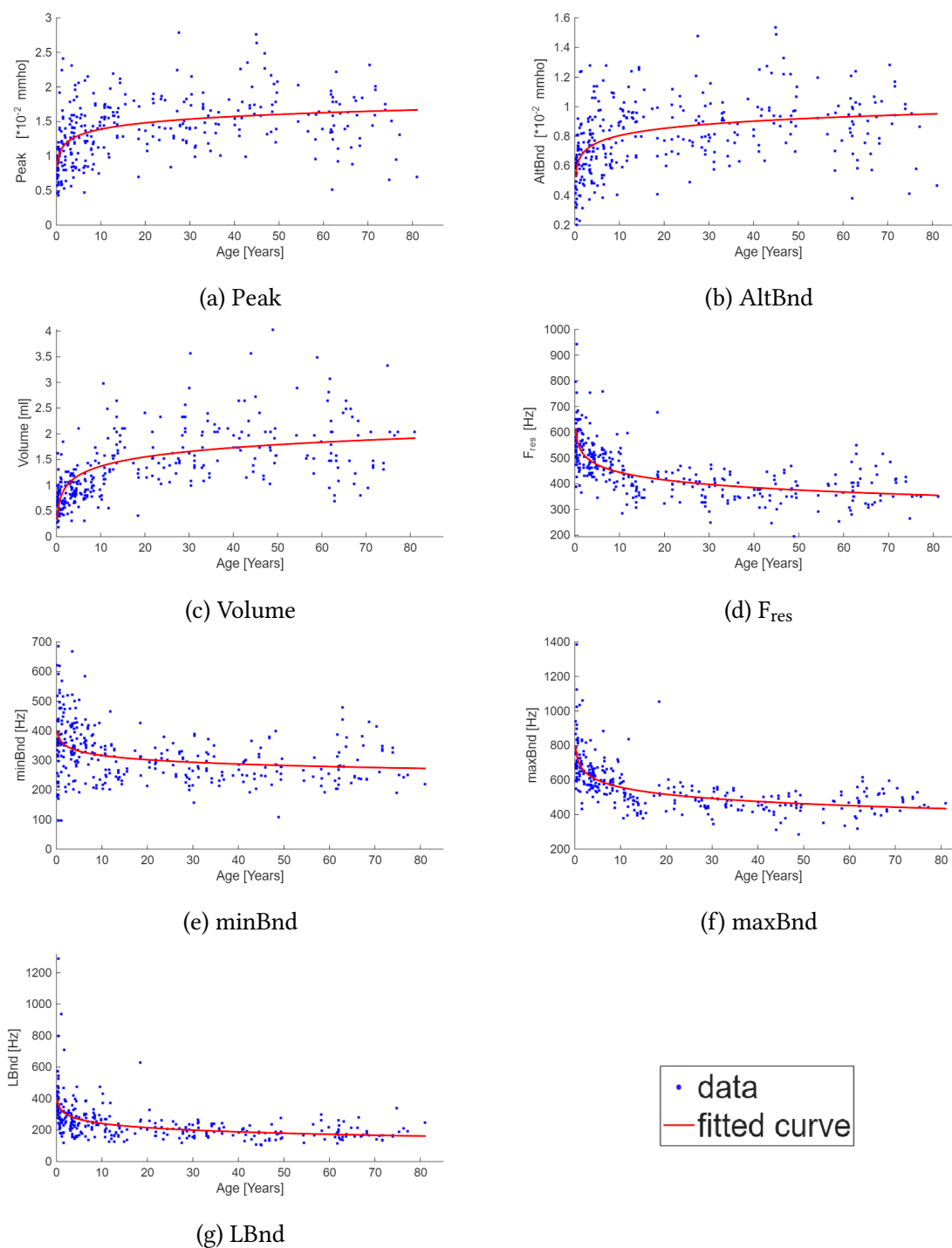


Figure 2.6: Age distribution of the seven considered PLAI™ parameters, with the logarithm-fitted curves

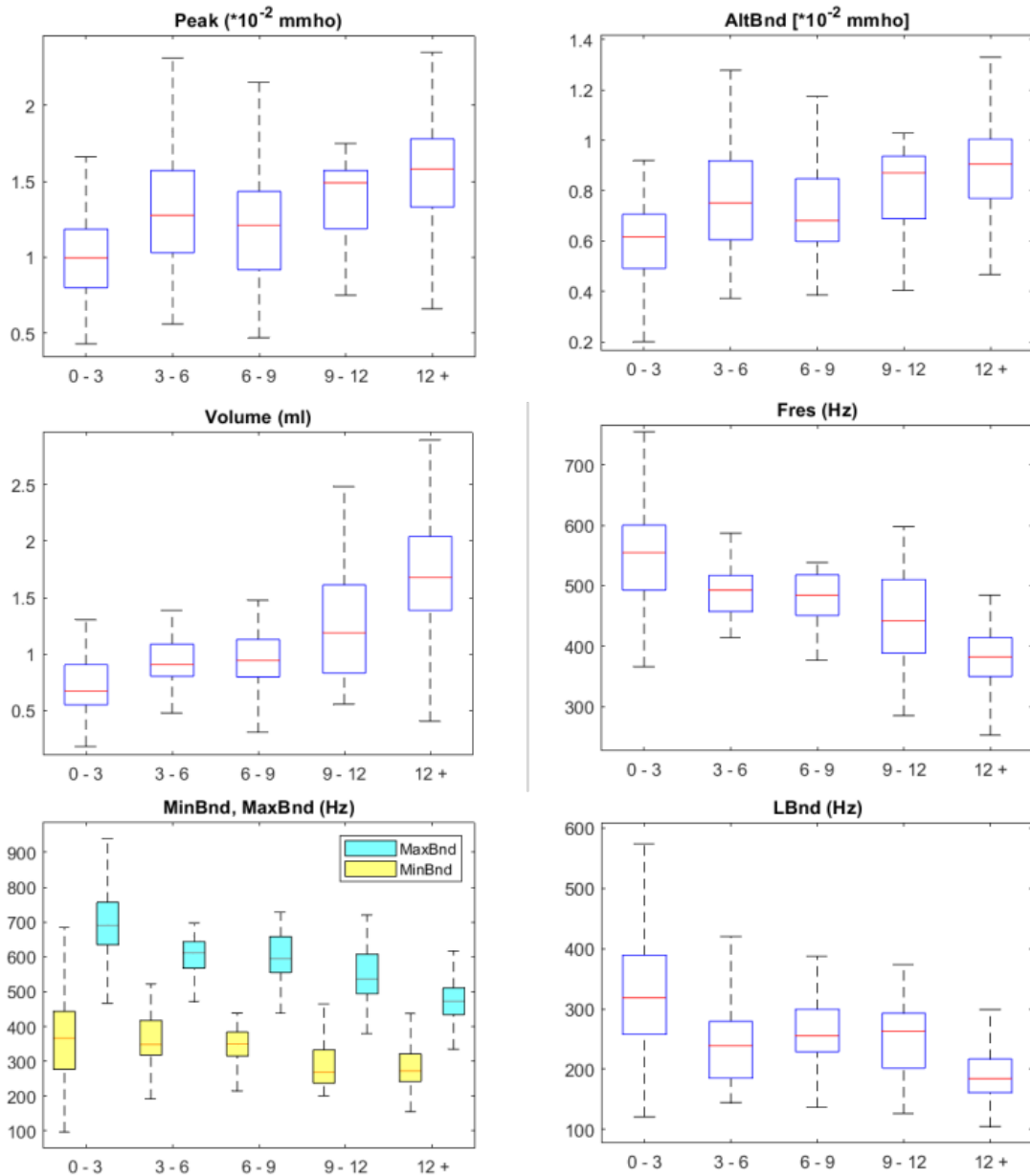


Figure 2.7: Box plots for the seven examined parameters using a five-class age subdivision

Statistical Comparison of Parameter Variability Across Age Groups

To identify the number of age groups exhibiting statistically significant differences while ensuring an adequate sample size per group, subjects were divided into four three-year age classes from birth to 12 years, with an additional group for participants older than 12 years. The distribution of ears across these classes was as follows: 76 in the 0–3 years group, 46 in the 3–6 years group, 29 in the 6–9 years group, 19 in the 9–12 years group, and 162 in the group older than 12 years.

Figure 2.7 shows the boxplots for each parameter, while Table 2.6 reports the mean values (± 1 SD) for each parameter and age class, along with the p-values for pairwise comparisons between the age groups.

Table 2.6: Mean values (± 1 SD) of each parameter in the five classes together with the p-values from the pairwise comparison between the age groups.

| | Peak * 10^{-2} mmho | AltBnd * 10^{-2} mmho | Vol ml | Fres Hz | MinBnd Hz | MaxBnd Hz | LBnd Hz |
|-------------|--------------------------|----------------------------|-----------------|--------------|---------------|---------------|---------------|
| 0-3 years | 1.02 \pm 0.35 | 0.61 \pm 0.19 | 0.78 \pm 0.33 | 548 \pm 94 | 357 \pm 118 | 711 \pm 151 | 354 \pm 177 |
| 3-6 years | 1.27 \pm 0.38 | 0.75 \pm 0.21 | 0.98 \pm 0.27 | 485 \pm 47 | 350 \pm 72 | 604 \pm 57 | 254 \pm 76 |
| 6-9 years | 1.25 \pm 0.40 | 0.74 \pm 0.22 | 0.99 \pm 0.28 | 487 \pm 68 | 345 \pm 80 | 607 \pm 87 | 262 \pm 58 |
| 9-12 years | 1.41 \pm 0.28 | 0.82 \pm 0.17 | 1.33 \pm 0.64 | 444 \pm 85 | 292 \pm 72 | 559 \pm 112 | 267 \pm 91 |
| 12+ years | 1.57 \pm 0.39 | 0.90 \pm 0.21 | 1.76 \pm 0.55 | 384 \pm 58 | 284 \pm 56 | 477 \pm 73 | 192 \pm 56 |
| 0-3 vs 3-6 | 0.00073 | 0.0009 | 0.00031 | 0.00029 | n.s. | < 0.0001 | < 0.0001 |
| 0-3 vs 6-9 | 0.042 | n.s. | 0.00046 | 0.00043 | n.s. | 0.00014 | 0.0077 |
| 0-3 vs 9-12 | 0.00015 | 0.00023 | 0.00030 | 0.00029 | 0.050 | < 0.0001 | n.s. |
| 0-3 vs 12+ | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| 3-6 vs 6-9 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| 3-6 vs 9-12 | n.s. | n.s. | n.s. | n.s. | 0.018 | n.s. | n.s. |
| 3-6 vs 12+ | 0.0011 | 0.0033 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| 6-9 vs 9-12 | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| 6-9 vs 12+ | 0.0005 | 0.002 | < 0.0001 | < 0.0001 | 0.00029 | < 0.0001 | < 0.0001 |
| 9-12 vs 12+ | n.s. | n.s. | 0.0091 | 0.0091 | n.s. | 0.0027 | 0.0004 |

n.s. : not significant ($p > 0.05$)

The patterns observed in Figure 2.7, along with the p-values reported in Table 2.6, indicate no significant differences among the 3–6, 6–9, and 9–12 year age classes, allowing them to be merged. As a result, three age groups were considered for further analysis: 0–3 years

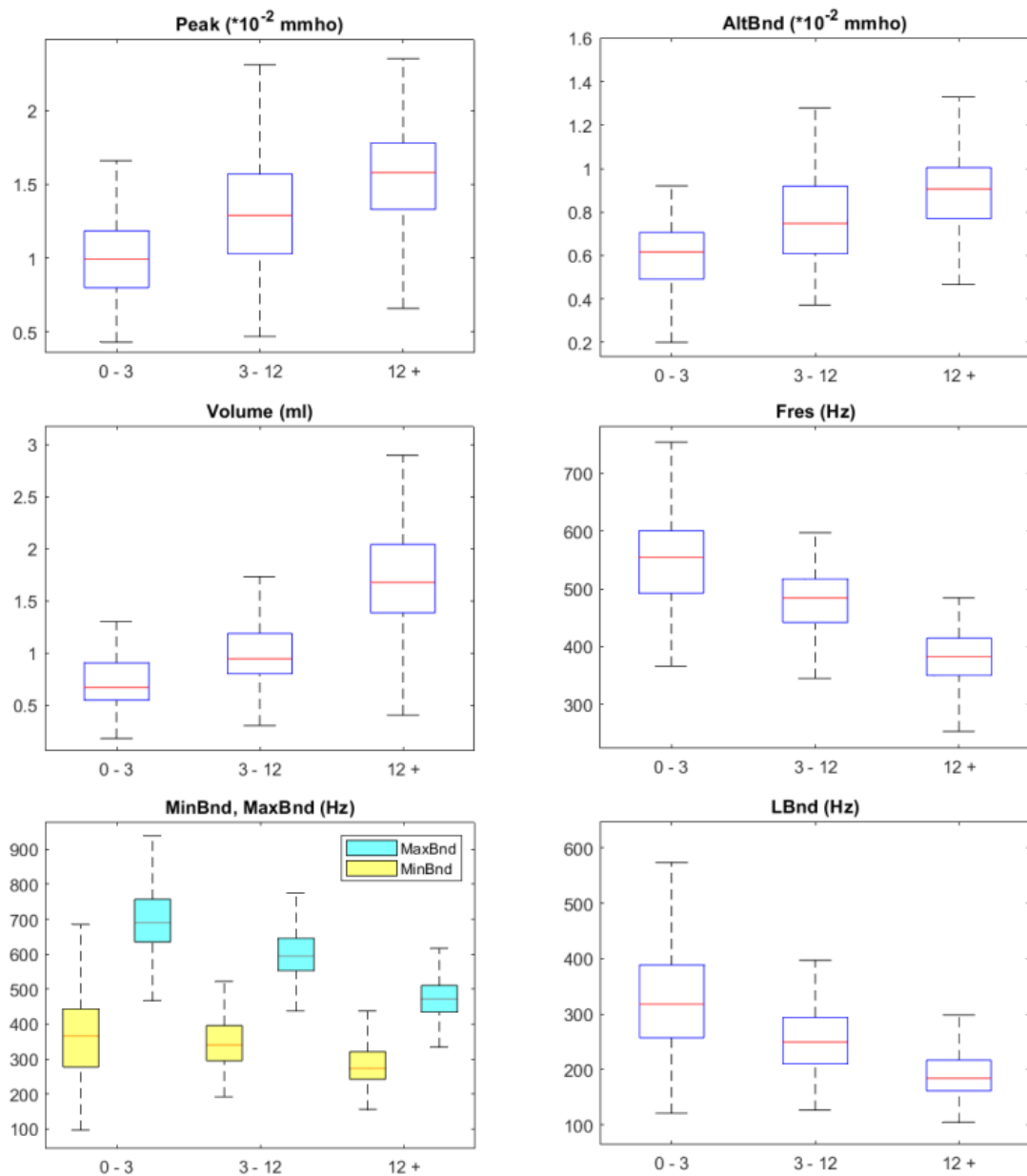


Figure 2.8: Box plots for the seven examined parameters using a three-class age subdivision.

(76 ears), 3–12 years (94 ears), and over 12 years (162 ears), as illustrated in the box plots in Figure 2.8. The mean values ± 1 Standard Deviation (SD) and the p-values for comparisons between class pairs are presented in Table 2.7.

Table 2.7: Mean values (± 1 SD) of each parameter in the three classes together with the p-values of each pair comparison test.

| | | Peak | AltBnd | Vol | Fres | MinBnd | MaxBnd | LBnd |
|---------|-------------|------------------|------------------|-----------------|--------------|---------------|---------------|---------------|
| | | * 10^{-2} mmho | * 10^{-2} mmho | ml | Hz | Hz | Hz | Hz |
| Healthy | 0-3 years | 1.02 \pm 0.35 | 0.61 \pm 0.19 | 0.78 \pm 0.33 | 548 \pm 94 | 357 \pm 118 | 711 \pm 151 | 354 \pm 177 |
| | 3-12 years | 1.29 \pm 0.37 | 0.76 \pm 0.20 | 1.06 \pm 0.40 | 477 \pm 65 | 336 \pm 77 | 595 \pm 82 | 259 \pm 74 |
| | 12+ years | 1.57 \pm 0.39 | 0.90 \pm 0.21 | 1.76 \pm 0.55 | 384 \pm 58 | 284 \pm 56 | 477 \pm 73 | 192 \pm 56 |
| p-val | 0-3 vs 3-12 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | n.s. | < 0.0001 | < 0.0001 |
| | 0-3 vs 12+ | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| | 3-12 vs 12+ | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

n.s. : not significant ($p > 0.05$)

Among all parameters, only minBnd did not show a significant difference between the 0–3 and 3–12 year age groups, mainly due to the high variability within the 0–3 year group and the relatively small difference in mean values between the two classes. In contrast, all other parameters exhibited statistically significant differences, despite the large standard deviations observed for MinBnd, MaxBnd, and Bnd.

Comparison between healthy and OME subjects

The pathological group consisted of 68 ears, distributed across three age classes as follows: 0–3 years (22 ears), 3–12 years (28 ears), and over 12 years (18 ears). Table 2.8 and Figure 2.9 summarize the parameters that exhibited statistically significant differences between healthy and OME-affected ears within these age groups. Significant differences were observed in ear canal volume (Vol), resonance frequency (F_{res}), minimum frequency (MinBnd), and maximum frequency (MaxBnd) across all age groups. In the 3–12 year and over 12 year groups, bandwidth (LBnd) also showed a significant difference. In contrast, peak at resonance frequency (Peak) and peak height (AltBnd) did not differ significantly in any age group.

Table 2.8: Mean values (± 1 SD) of each parameter in the three classes for healthy and OME subjects with the p-values of each pair comparison test

| | Age group | Peak | AltBnd | Vol | F _{res} | MinBnd | MaxBnd | LBnd |
|---------|-----------|-------------------------|-------------------------|-----------------|------------------|---------------|---------------|---------------|
| | Years | * 10 ⁻² mmho | * 10 ⁻² mmho | ml | Hz | Hz | Hz | Hz |
| Healthy | 0-3 | 1.02 \pm 0.35 | 0.61 \pm 0.19 | 0.78 \pm 0.33 | 548 \pm 94 | 357 \pm 118 | 711 \pm 151 | 354 \pm 177 |
| | 3-12 | 1.29 \pm 0.37 | 0.76 \pm 0.20 | 1.06 \pm 0.40 | 477 \pm 65 | 336 \pm 77 | 595 \pm 82 | 259 \pm 74 |
| | 12+ | 1.57 \pm 0.39 | 0.90 \pm 0.21 | 1.76 \pm 0.55 | 384 \pm 58 | 284 \pm 56 | 477 \pm 73 | 192 \pm 56 |
| OME | 0-3 | 0.99 \pm 0.38 | 0.57 \pm 0.18 | 0.49 \pm 0.18 | 656 \pm 101 | 481 \pm 74 | 852 \pm 94 | 371 \pm 101 |
| | 3-12 | 1.27 \pm 0.44 | 0.69 \pm 0.21 | 0.59 \pm 0.17 | 596 \pm 68 | 453 \pm 71 | 754 \pm 96 | 301 \pm 90 |
| | 12+ | 1.53 \pm 0.69 | 0.85 \pm 0.35 | 1.04 \pm 0.44 | 484 \pm 74 | 366 \pm 81 | 612 \pm 71 | 245 \pm 92 |
| p-val | 0-3 | <i>n.s.</i> | <i>n.s.</i> | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | <i>n.s.</i> |
| | 3-12 | <i>n.s.</i> | <i>n.s.</i> | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.022 |
| | 12+ | <i>n.s.</i> | <i>n.s.</i> | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | 0.013 |

n.s. : not significant ($p > 0.05$)

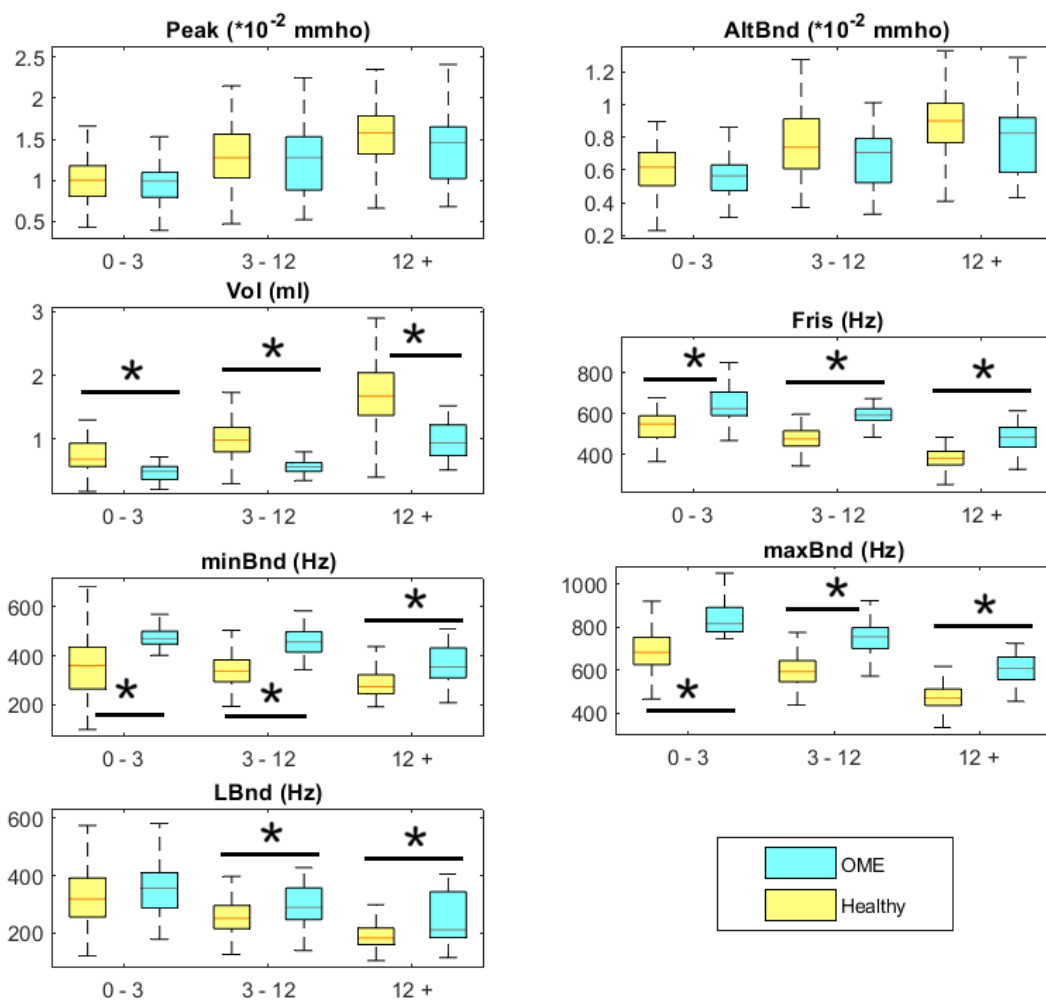


Figure 2.9: Box plots for the examined parameters in healthy and OME subjects using a three-class age subdivision. Statistically significant differences are indicated by asterisks.

2.3.3 Discussion

Both standard tympanometry and wideband tympanometry (WBT) can be considered invasive, as they require the application of supplemental pressure during measurement [93, 142]. This limitation makes them unsuitable for certain populations, including infants with highly elastic auditory structures and individuals with tympanic membrane perforations, for whom applied pressure may worsen the condition [93, 103, 143, 97, 101, 100]. The development of a novel, non-invasive methodology addresses this limitation, enabling assessment in neonatal patients and those with tympanic perforations [127]. However, effective use of this new technology requires comprehensive characterization, including evaluation of age-related influences on measurements in healthy subjects.

Accordingly, this study aimed to establish reference value ranges for each parameter derived from PLAITM curves across all age groups in healthy ears. These reference ranges provide a foundation for distinguishing healthy individuals from those with middle ear pathologies in future clinical applications. By facilitating non-invasive assessment of middle ear function, this approach can benefit populations traditionally underserved by standard tympanometry due to anatomical or age-related constraints.

Measurements were analyzed across a broad age range, from infants (4 months) to older adults (80 years). The behavior of the seven parameters (Figures 2.6, 2.7 and 2.8) revealed general growth or decline trends, alongside notable inter-subject variability. Some of this variability may reflect the multicenter sample, encompassing a wide geographical range and potential regional differences in ear morphology. The observed decline in frequency-related parameters (F_{res} , MaxBnd, MinBnd) and the increase in volume with age likely correspond to ear canal growth, as suggested in previous studies [138]. Similarly, increases in peak admittance (Peak) and AltBnd may reflect reduced tympanic stiffness, which lowers impedance and enhances energy absorption [134]. A slight decrease in Peak and AltBnd after age 50 may indicate increased tympanic stiffness in older adults [43].

The age-related stabilization observed after 12 years allows clinicians to distinguish between physiological changes and pathological findings, improving diagnostics in both pediatric and older adult populations. Age-specific reference values further support accurate interpretation by accounting for natural variability in middle ear parameters, enabling effective clinical use of PLAITM where pressure-based assessments are undesirable.

Comparison with literature values indicates that the equivalent canal volume is slightly

higher in both adults (1.4 ± 0.3 ml [144], compared with 1.3 ml [134]) and school-aged children [138, 134, 145]. Dividing subjects into three age groups (0–3, 3–12, and over 12 years) effectively accounts for age-related influences while maintaining statistical independence among classes, facilitating targeted diagnostic thresholds and early detection of middle ear dysfunction in infants and children at risk of developmental delays [8, 146].

Comparison between healthy and OME-affected ears highlighted several parameters with clear discriminatory power. Ear canal volume (Vol), resonance frequency (F_{res}), minimum frequency (MinBnd), and maximum frequency (MaxBnd) differed significantly across all age groups. In the 3–12 and over 12 year groups, bandwidth (LBnd) also proved informative. In contrast, Peak and AltBnd did not show significant differences, suggesting limited diagnostic utility for these parameters within this non-invasive methodology.

Applying the same age-based stratification to the OME group confirmed that the grouping remains meaningful in a pathological context, supporting direct comparisons and reinforcing the clinical relevance of age-specific baselines.

Clinically, these results highlight PLAI™'s potential as a diagnostic tool for identifying and monitoring middle ear pathologies, particularly in populations where traditional pressure-based techniques are limited or contraindicated. Significant differences in key parameters between healthy and pathological ears demonstrate the method's capacity to detect deviations from typical middle ear behavior. Combined with age-specific reference values, PLAI™ may enable earlier, safer, and more accessible detection of OME, facilitating timely interventions to mitigate developmental delays and auditory complications [8, 146].

In summary, PLAI™, together with age-group classification and parameter analysis, offers a robust, non-invasive approach for assessing middle ear function. Its application could significantly enhance routine screening, particularly for infants, young children, and patients with tympanic membrane perforations, who are challenging to assess using conventional tympanometry.

This study represents a first step in characterizing the PLAI™ system (Pressure-Less Acoustic Immittance), which measures acoustic admittance without applying external pressure. The instrument provides an admittance curve across 100–2000 Hz. Each of the seven derived parameters exhibits age-related trends that can be modeled with simple logarithmic functions, effectively removing age-related effects. Findings support the use of three independent age groups (0–3, 3–12, and over 12 years), with mean values \pm SD and best-fit

curves provided as reference standards for healthy ears.

Comparison with OME-affected ears demonstrated significant differences in several PLAITM parameters, supporting the method's potential for clinical use. These results establish essential reference standards for non-invasive diagnosis of middle ear pathologies, enabling broader application in infants and patients with tympanic membrane complications.

Chapter 3

Decision Support Systems

Building on the results presented in Chapter 2, it is now possible to develop decision support systems capable of analyzing the large volumes of data generated during acquisition and providing reliable guidance to the operator.

The research [FB4] described in this chapter utilizes the entire population investigated in the previously presented clinical study, including, where available, additional pathology groups, to train a machine learning-based classifier. This phase is preceded by a statistical analysis of the full dataset, allowing the establishment of potential reference values for each pathology across different age groups.

3.1 Materials and methods

Outpatients aged 0 to 80 years, both healthy and affected by various ear pathologies, were enrolled from six Italian hospitals: Gemelli University Hospital IRCCS (Rome), “Federico II” University Hospital (Naples), Fondazione IRCCS Policlinico “San Matteo” Hospital (Pavia), “Giovanni XXIII” Children’s Hospital (Bari), “Guglielmo da Saliceto” Hospital (Piacenza), and the IRCCS Maternal and Child Health Institute “Burlo Garofolo” (Trieste). Each participant underwent a comprehensive otologic examination, including otoscopy and, when feasible, tympanometry to confirm the diagnosis. Following clinical assessment, PLAI™ measurements were collected to evaluate the middle ear’s acoustic properties under pressure-free conditions.

Only patients with ears classified as healthy or affected by a single pathology were included. Exclusion criteria comprised a history of otologic surgery, significant comorbidi-

ties, and cognitive impairments that could interfere with testing procedures. Otoscopy and tympanometry were performed on all participants to confirm ear status. Table 3.1 summarizes the distribution of ears by age group and diagnosis.

Table 3.1: Distribution of individual ears by age and pathology

| Age Group | Healthy | OME | Perforation | Otosclerosis | Retraction | Total |
|------------|---------|-----|-------------|--------------|------------|-------|
| 0-3 years | 71 | 22 | - | - | - | 93 |
| 3-12 years | 90 | 28 | 7 | - | 18 | 143 |
| 12+ years | 157 | 18 | 18 | 63 | 17 | 273 |
| Total | 318 | 68 | 25 | 63 | 35 | 509 |

To evaluate whether PLAITM-derived parameters differed significantly between healthy and pathological ears, each acoustic feature was compared across diagnostic categories within each age group using the Wilcoxon rank-sum test. Bonferroni correction was applied for multiple comparisons, and statistical significance was set at $p < 0.05$.

Subsequent to statistical testing, a classification pipeline was developed to assess the ability of PLAITM parameters to discriminate between healthy and pathological ears. The dataset was stratified by age group and divided into training (80%) and testing (20%) subsets. Feature selection was based on the intrinsic feature importance scores derived from a Random Forest classifier, retaining only the most informative parameters for each age group. To address class imbalance, the Synthetic minority oversampling technique (SMOTE) was applied to the training data to generate synthetic samples for underrepresented diagnostic classes.

Random Forest was selected as the primary classifier due to its robustness against overfitting, capacity to model nonlinear relationships, and inherent interpretability through feature importance metrics. Hyperparameters were optimized using validation split performance. Model performance was evaluated through accuracy, precision, recall, F1-score, and specificity, computed for each class as well as aggregated through micro- and macro-averages. In fact, given the initial imbalance between classes, it is important to evaluate all the performance parameters on the single possible outputs, in order to avoid the adoptions of models which underrepresent a class [147]. Receiver Operating Characteristic (ROC) curves were plotted, and confusion matrices were generated in grayscale for each age cat-

egory.

To enhance model interpretability, SHAP values were calculated to quantify the contribution of individual features to classification outcomes [148]. Feature importance visualizations are presented in the Results section for each age group.

All analyses and visualizations were conducted using Python 3.8 with the Scikit-learn, Imbalanced-learn, and SHAP libraries.

3.2 Results

3.2.1 Descriptive statistics

Age 0-3

Table 3.2 presents the descriptive statistics and comparative analysis of key PLAITM parameters for healthy children and those diagnosed with OME within the 0–3 year age group. Several parameters exhibited statistically significant differences between the two groups. These findings are visually represented in Figure 3.1 through comparative boxplots, highlighting the dispersion and central tendency of each parameter.

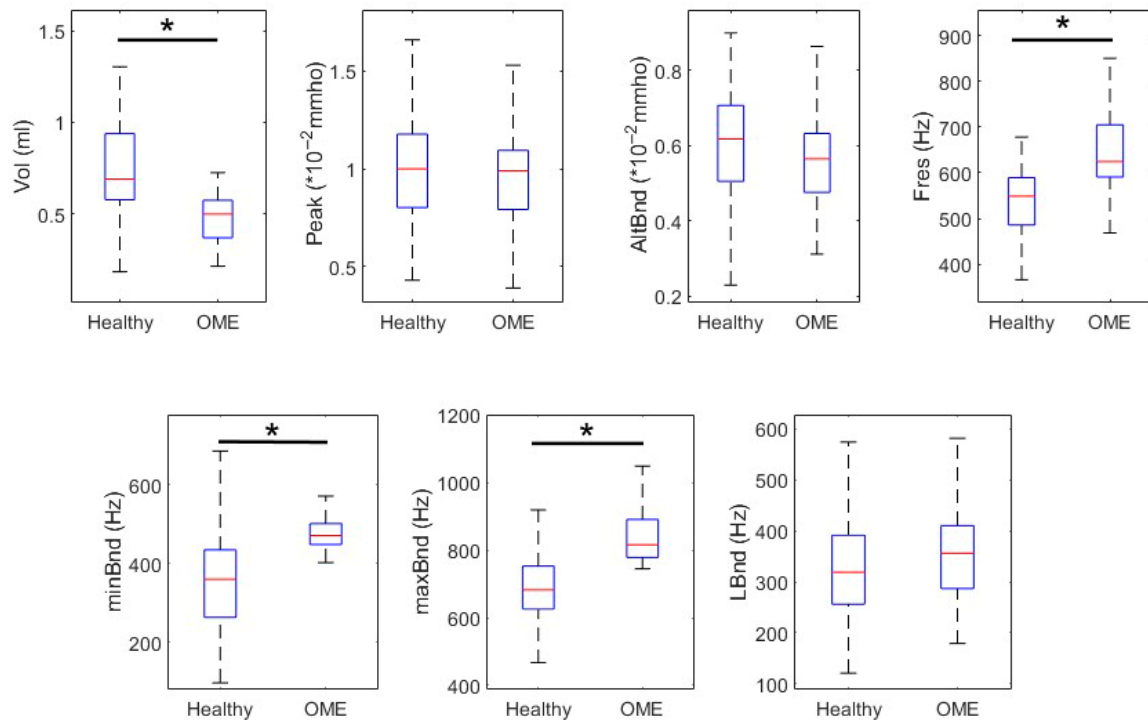


Figure 3.1: Box plots comparing PLAITM parameters between Healthy and OME subjects in the 0–3 age group. Statistically significant differences are indicated by asterisks.

Table 3.2: Descriptive statistics and p-values for PLAITM parameters in the 0–3 year age group.

| Parameter | Group | Mean | SD | p-Value |
|--------------------------------|---------|------|-------|---------|
| Volume [ml] | Healthy | 0.78 | 0.333 | <0.001 |
| | OME | 0.62 | 0.666 | |
| Peak [$\cdot 10^{-2}$ mmho] | Healthy | 1.02 | 0.353 | n.s. |
| | OME | 1.01 | 0.398 | |
| AltBnd [$\cdot 10^{-2}$ mmho] | Healthy | 0.61 | 0.19 | n.s. |
| | OME | 0.58 | 0.19 | |
| F _{res} [Hz] | Healthy | 548 | 94 | <0.001 |
| | OME | 638 | 132 | |
| minBnd [Hz] | Healthy | 357 | 118 | <0.001 |
| | OME | 470 | 90 | |
| maxBnd [Hz] | Healthy | 711 | 151 | <0.001 |
| | OME | 827 | 154 | |
| LBnd [Hz] | Healthy | 354 | 177 | n.s. |
| | OME | 356 | 123 | |

n.s. : not significant ($p > 0.05$)

Age 3-12

Table 3.3 presents the descriptive statistics and comparative analysis of the main PLAITM-derived parameters in children aged 3 to 12 years, categorized into three groups: healthy subjects, those diagnosed with OME, and those with tympanic membrane retraction. Comparisons between groups were conducted using appropriate statistical tests, applying a Bonferroni-corrected significance threshold of $p < 0.0167$. The corresponding boxplots illustrating these results are shown in Figure 3.2.

Age greater than 12

Table 3.4 reports the descriptive statistics and comparative analyses for all parameters in participants over 12 years of age. The table provides the mean, median, standard devia-

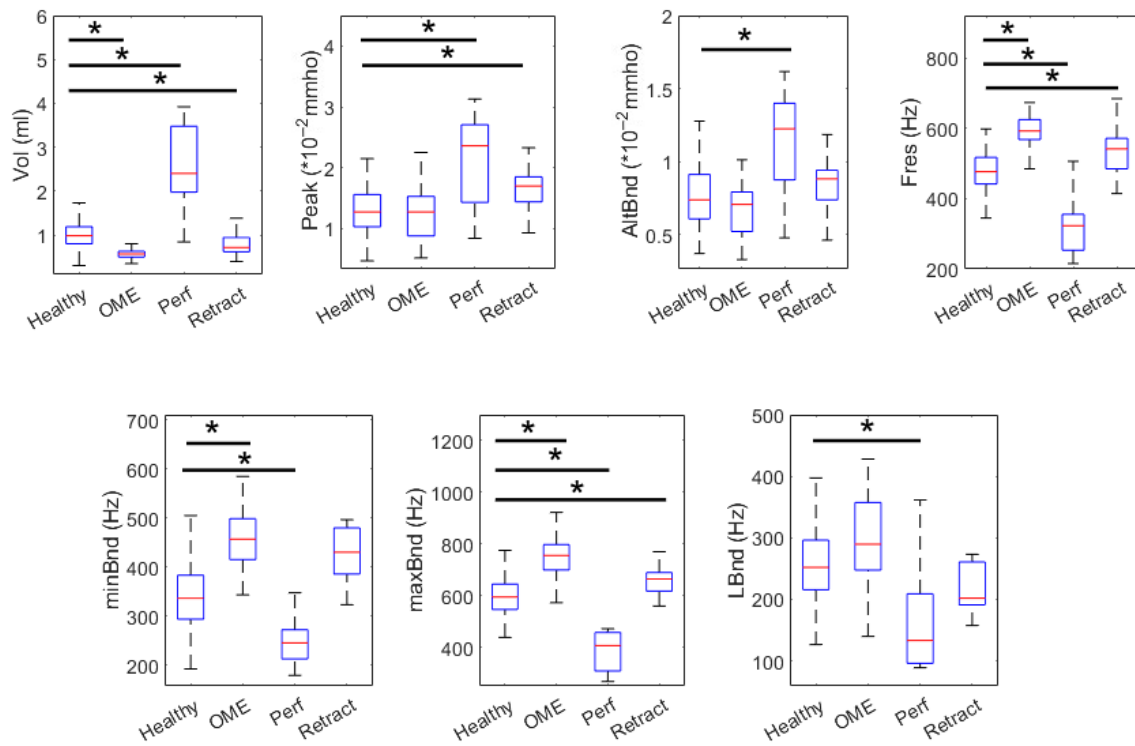


Figure 3.2: Box plots comparing PLAI™ parameters between Healthy, OME, Perforated, and Retracted ears in the 3–12 age group. Statistically significant differences are indicated by asterisks.

Table 3.3: Descriptive statistics and p-values for PLAITM parameters in the 3-12 year age group.

| Parameter | Group | Mean | SD | p-Value | | |
|---------------------------|-------------|------|------|---------|-------------|------------|
| | | | | OME | Perforation | Retraction |
| Volume [ml] | Healthy | 1.06 | 0.40 | | | |
| | OME | 0.59 | 0.17 | | | |
| | Perforation | 2.58 | 1.07 | <0.001 | <0.001 | 0.002 |
| | Retraction | 0.81 | 0.29 | | | |
| Peak [$*10^{-2}$ mmho] | Healthy | 1.29 | 0.37 | | | |
| | OME | 1.27 | 0.44 | | | |
| | Perforation | 2.12 | 0.83 | n.s. | 0.01 | 0.005 |
| | Retraction | 1.59 | 0.45 | | | |
| AltBnd [$*10^{-2}$ mmho] | Healthy | 0.76 | 0.20 | | | |
| | OME | 0.69 | 0.21 | | | |
| | Perforation | 1.13 | 0.39 | n.s. | 0.01 | n.s. |
| | Retraction | 0.83 | 0.21 | | | |
| F _{res} [Hz] | Healthy | 477 | 65 | | | |
| | OME | 596 | 68 | | | |
| | Perforation | 323 | 96 | <0.001 | <0.001 | 0.002 |
| | Retraction | 531 | 69 | | | |
| minBnd [Hz] | Healthy | 336 | 77 | | | |
| | OME | 453 | 71 | | | |
| | Perforation | 248 | 54 | <0.001 | 0.005 | n.s. |
| | Retraction | 427 | 58 | | | |
| maxBnd [Hz] | Healthy | 595 | 82 | | | |
| | OME | 754 | 96 | | | |
| | Perforation | 414 | 148 | <0.001 | 0.002 | 0.003 |
| | Retraction | 656 | 83 | | | |
| LBnd [Hz] | Healthy | 259 | 74 | | | |
| | OME | 301 | 90 | | | |
| | Perforation | 167 | 98 | n.s. | 0.006 | n.s. |
| | Retraction | 229 | 69 | | | |

n.s. : not significant ($p > 0.05$)

Table 3.4: Descriptive statistics and p-values for PLAITM parameters in the 12+ year age group.

| Parameter | Group | Mean | SD | p-Value | | | |
|---------------------------|--------------|------|------|---------|-------------|--------------|------------|
| | | | | OME | Perforation | Otosclerosis | Retraction |
| Volume [ml] | Healthy | 1.76 | 0.55 | | | | |
| | OME | 1.04 | 0.44 | | | | |
| | Perforation | 2.06 | 0.97 | <0.001 | n.s. | n.s. | <0.001 |
| | Otosclerosis | 1.78 | 0.66 | | | | |
| | Retraction | 1.15 | 0.54 | | | | |
| Peak [$*10^{-2}$ mmho] | Healthy | 1.57 | 0.39 | | | | |
| | OME | 1.53 | 0.69 | | | | |
| | Perforation | 1.96 | 1.06 | n.s. | n.s. | n.s. | n.s. |
| | Otosclerosis | 1.66 | 0.38 | | | | |
| | Retraction | 1.83 | 0.81 | | | | |
| AltBnd [$*10^{-2}$ mmho] | Healthy | 0.90 | 0.21 | | | | |
| | OME | 0.85 | 0.35 | | | | |
| | Perforation | 1.12 | 0.55 | n.s. | n.s. | n.s. | n.s. |
| | Otosclerosis | 0.96 | 0.18 | | | | |
| | Retraction | 1.04 | 0.44 | | | | |
| F _{res} [Hz] | Healthy | 384 | 58 | | | | |
| | OME | 484 | 75 | | | | |
| | Perforation | 388 | 161 | <0.001 | n.s. | n.s. | <0.001 |
| | Otosclerosis | 386 | 67 | | | | |
| | Retraction | 489 | 152 | | | | |
| minBnd [Hz] | Healthy | 284 | 56 | | | | |
| | OME | 366 | 81 | | | | |
| | Perforation | 302 | 146 | <0.001 | n.s. | n.s. | <0.001 |
| | Otosclerosis | 286 | 69 | | | | |
| | Retraction | 380 | 143 | | | | |
| maxBnd [Hz] | Healthy | 477 | 73 | | | | |
| | OME | 612 | 70 | | | | |
| | Perforation | 463 | 177 | <0.001 | n.s. | n.s. | 0.003 |
| | Otosclerosis | 473 | 70 | | | | |
| | Retraction | 573 | 172 | | | | |
| LBnd [Hz] | Healthy | 193 | 56 | | | | |
| | OME | 246 | 92 | | | | |
| | Perforation | 161 | 87 | n.s. | 0.002 | n.s. | n.s. |
| | Otosclerosis | 187 | 48 | | | | |
| | Retraction | 193 | 81 | | | | |

n.s. : not significant ($p > 0.05$)

tion (SD), first (25th percentile) and third (75th percentile) quartiles, along with p-values comparing healthy ears to each pathological group. A Bonferroni-corrected significance threshold of $p < 0.0125$ was applied to adjust for multiple comparisons. The corresponding boxplots are illustrated in Figure 3.3. It is noteworthy that no statistically significant differences were found in any parameter for otosclerosis within this age group, indicating that the analyzed features may have limited discriminative power for this condition in the present dataset.

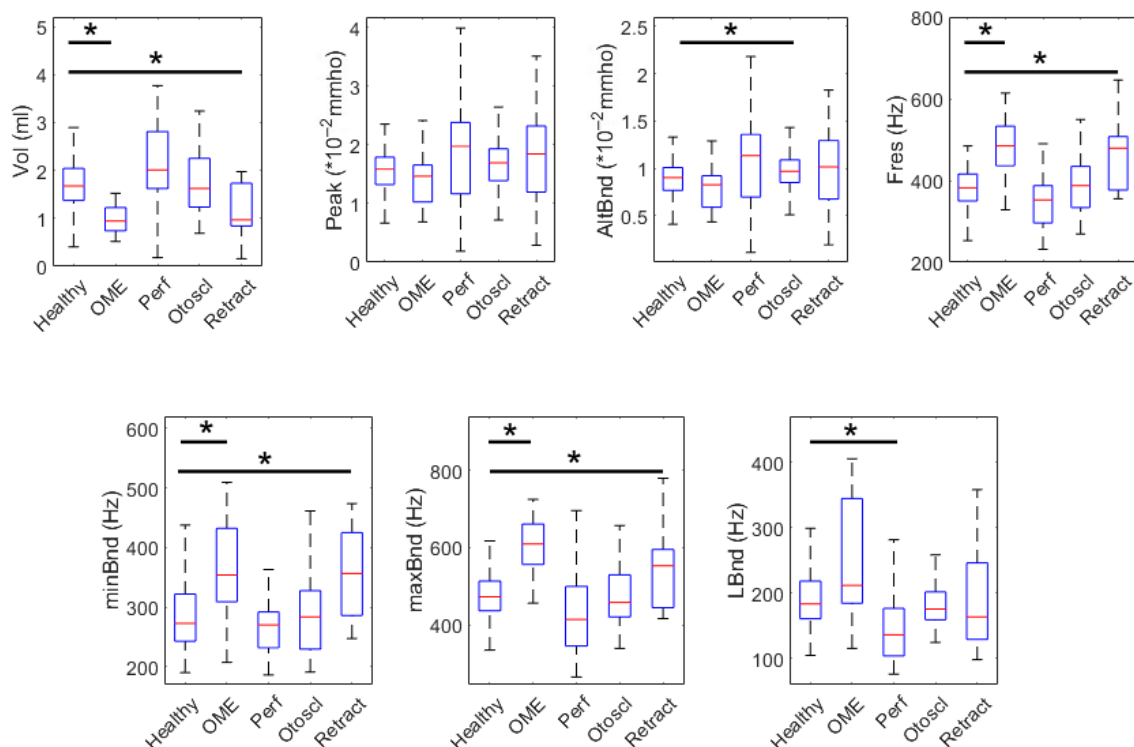


Figure 3.3: Box plots comparing PLAITM parameters between Healthy, OME, Perforated, and Retracted ears in the 12+ age group. Statistically significant differences are indicated by asterisks.

3.2.2 Feature Relevance via SHAP analysis

To assess the contribution of individual features to the classification outcomes, SHAP analysis was conducted for each age group. Table 3.5 summarizes the mean SHAP values of the top-ranked features identified by the Random Forest classifier across the three age categories. These values represent the average influence of each feature on the model's predictions.

Table 3.5: Mean SHAP values for PLAITSTM derived features

| Feature | 0-3 years | 3-12 years | 12+ years |
|------------------|-----------|------------|-----------|
| Vol | 0.1461 | 0.0908 | 0.1573 |
| Peak | 0.0306 | 0.0374 | 0.0343 |
| AltBnd | 0.0572 | 0.0484 | 0.0739 |
| F _{res} | 0.1603 | 0.0858 | 0.1242 |
| minBnd | 0.1110 | 0.0739 | 0.0516 |
| maxBnd | 0.1461 | 0.0380 | 0.0529 |
| LBnd | 0.0967 | 0.0696 | 0.0384 |

In the 0–3 year group, the most influential parameter was F_{res}, followed by maxBnd, minBnd, and Vol, indicating that frequency-related measures were the main drivers in distinguishing middle ear conditions. For the 3–12 year group, Vol and F_{res} again showed strong importance, together with minBnd and LBnd, suggesting that these features retain diagnostic relevance throughout early development. In the 12+ group, Vol, F_{res}, and AltBnd were the primary contributors, confirming that volumetric and resonance-related parameters remain significant in older individuals. Overall, these results align with physiological expectations, as tympanic volume and resonance characteristics are frequently altered in middle ear pathologies such as effusions, retractions, and perforations.

3.2.3 Random Forest Classification Performance

Random Forest classifiers were developed and evaluated separately for each age group to perform a five-class diagnostic classification. To address class imbalance, synthetic oversampling was applied using the SMOTE technique [149]. Model performance was evaluated using both macro- and micro-averaged precision, recall, F1-score, and specificity metrics, while class-level accuracy was derived from the classification reports. For the 0–3 year age group, the classifier achieved a macro F1-score of 0.79 and a micro F1-score of 0.80, demonstrating strong overall performance despite anatomical variability typical of early development. The model also achieved a macro specificity of 0.95, confirming its robustness in differentiating pathological from healthy cases. The corresponding confusion matrix and ROC curves are shown in Figures 3.4a and 3.4b, respectively.

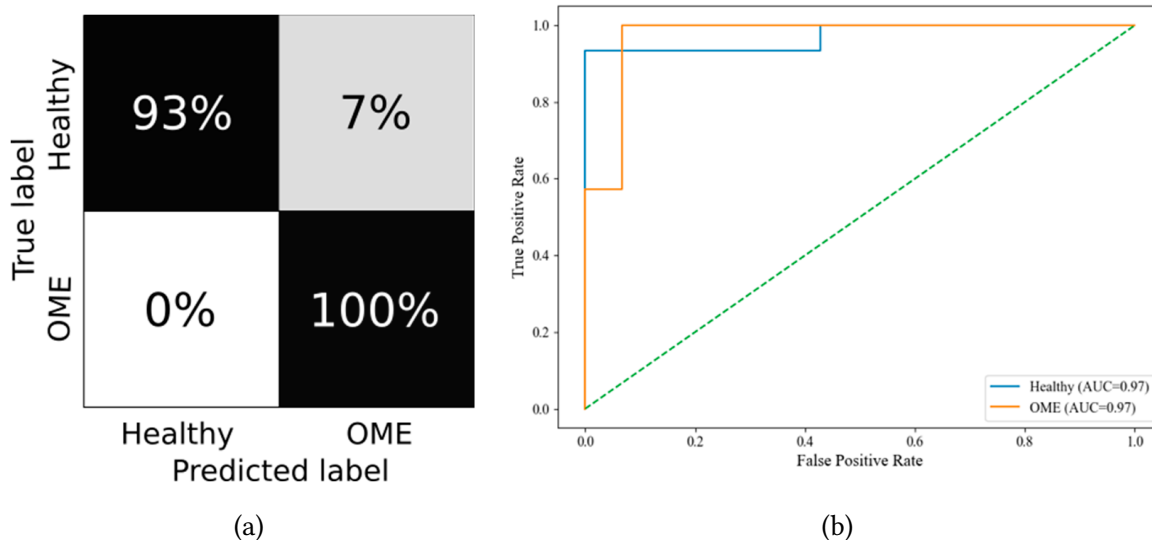


Figure 3.4: 0-3 age group RF metrics. (a) Confusion Matrix; (b) ROC Curve

In the 3–12-year age group, the classifier achieved its best overall performance, with a macro F1-score of 0.84 and a micro F1-score of 0.86. The high macro precision (0.85) and specificity (0.96) further highlight its strong ability to discriminate between diagnostic categories in this cohort, which commonly exhibits well-defined middle ear pathologies. The corresponding confusion matrix and ROC curves are shown in Figures 3.5a and 3.5b, respectively.

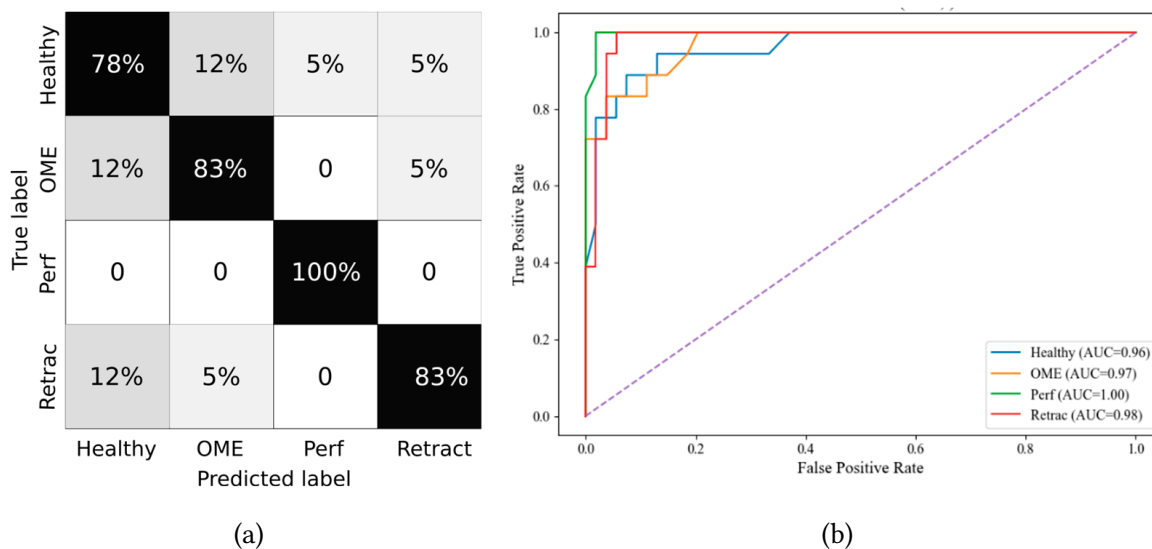


Figure 3.5: 3-12 age group RF metrics. (a) Confusion Matrix; (b) ROC Curve

In the 12+ age group, the model maintained solid performance, though slightly lower than in younger cohorts, achieving a macro F1-score of 0.78 and a micro F1-score of 0.80. The corresponding confusion matrix and ROC curves are shown in Figures 3.6a and 3.6b, re-

spectively. This modest reduction in accuracy likely reflects greater anatomical variability and the wider range of underlying pathophysiological conditions observed in adolescents and adults.

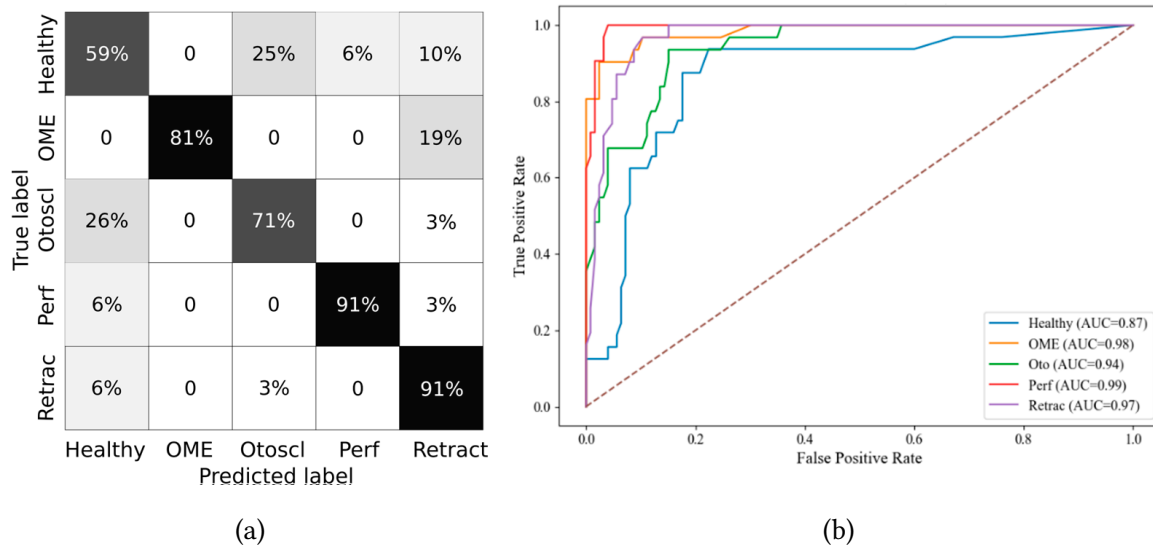


Figure 3.6: 12+ age group RF metrics. (a) Confusion Matrix; (b) ROC Curve

The overall classification performance is summarized in Table 3.6, while the per-class classification accuracy is reported in Table 3.7.

Table 3.6: Classification performance metrics of Random Forest classifiers for each age group.

| Metric | 0-3 years | 3-12 years | 12+ years |
|-------------------|-----------|------------|-----------|
| Macro Precision | 0.80 | 0.85 | 0.77 |
| Micro Precision | 0.81 | 0.86 | 0.80 |
| Macro Recall | 0.78 | 0.84 | 0.79 |
| Micro Recall | 0.81 | 0.86 | 0.80 |
| Macro F1-score | 0.79 | 0.84 | 0.78 |
| Micro F1-score | 0.81 | 0.86 | 0.80 |
| Macro Specificity | 0.95 | 0.96 | 0.93 |

Table 3.7: Per class accuracy of obtained classifiers.

| Per class accuracy | 0-3 years | 3-12 years | 12+ years |
|--------------------|-----------|------------|-----------|
| Healthy | 0.93 | 0.78 | 0.59 |
| OME | 1.0 | 0.83 | 0.81 |
| Otosclerosis | - | - | 0.71 |
| Perforation | - | 1.0 | 0.90 |
| Retraction | - | 0.83 | 0.90 |

3.3 Discussion

This study explored whether dividing pediatric participants into distinct age groups enhances the diagnostic modeling of middle ear conditions using quantitative PLAITM parameters. Given the well-documented developmental changes in auditory anatomy and physiology during infancy and childhood [150, 36], we hypothesized that age-related differences significantly affect tympanometric outcomes and, consequently, influence the accuracy of models predicting conditions such as OME, tympanic membrane perforation (Perf), otosclerosis (Otoscl), and tympanic membrane retraction (Retract).

The primary objective was to determine whether age stratification reveals statistically significant differences between diagnostic groups and to evaluate the performance of machine learning classifiers trained within these age categories for non-invasive detection of ear pathologies. Statistical analysis of PLAITM-derived parameters confirmed that age-based grouping enhances the differentiation between healthy and pathological ears—particularly in the youngest (0–3 years) and oldest (12+ years) cohorts. This finding justified the development of age-specific models, which subsequently demonstrated strong classification performance. Random Forest classifiers trained on SMOTE-balanced datasets achieved macro F1-scores exceeding 80% in both the 0–3 and 12+ groups. SHAP analysis consistently identified Peak, Fres, and Vol as the most influential parameters across all age strata.

Crucially, incorporating age stratification led to a notable reduction in false negatives, especially for OME and tympanic retractions. This improvement strengthens diagnostic confidence and highlights the method’s potential as a non-invasive screening tool suitable for clinical practice. In particular, the PLAITM system could be integrated into primary care settings, enabling general practitioners and pediatricians to conduct rapid, low-complexity ear

health assessments. By automatically flagging atypical patterns suggestive of pathology, PLAI™ could facilitate early detection and referral to otolaryngology specialists, which is especially valuable in contexts with limited access to advanced diagnostic equipment.

From a physiological perspective, these results align with established knowledge of auditory development. In infants and toddlers (0–3 years), the shorter and more compliant ear canal, coupled with the ongoing maturation of the ossicular chain and tympanic membrane, significantly influences acoustic transfer characteristics [143]. This explains the distinct parameter distributions observed in early childhood and underscores the need for age-specific diagnostic thresholds. In contrast, adolescents (12+ years) exhibit more mature auditory anatomy, likely accounting for the clearer parameter separation and improved model performance seen in this group.

Among the PLAI™ features, Peak admittance, representing energy transmission efficiency, emerged as a strong predictor, while F_{res} (resonant frequency) also showed high discriminative value, likely reflecting stiffness-related changes in the tympanic and ossicular systems. Volume (Vol), although particularly effective for identifying perforations, exhibited reduced distinctiveness in overlapping pathologies such as OME and retraction, especially within the heterogeneous 3–12 age group.

Despite these promising findings, certain limitations must be acknowledged. The dataset exhibited class imbalance, particularly for less common conditions like otosclerosis and retraction, which may limit generalizability. Although SMOTE was used to alleviate imbalance, it generates synthetic examples by interpolating existing data, potentially reducing variability and realism in rare conditions. Moreover, age stratification was limited to three broad categories; finer age segmentation could provide further insight but would require larger, more balanced datasets. Small sample sizes for rare diagnoses also introduce potential sampling bias, as synthetic data may amplify existing distributional patterns rather than correct them. Future studies should therefore aim to collect additional real-world data to improve model robustness and reduce dependence on synthetic augmentation.

While the current models demonstrated strong diagnostic potential, especially in early childhood and adolescence, external validation on independent datasets remains necessary to confirm reproducibility. Expanding the sample base, ensuring balanced class representation, and incorporating longitudinal data would enhance model reliability and clinical applicability.

Overall, this work emphasizes the importance of age-aware modeling in pediatric audiology and supports the potential of PLAITM as a non-invasive diagnostic tool for early and reliable identification of middle ear disorders.

In conclusion, this study demonstrated that age-stratified analysis improves the classification of common otologic pathologies using non-invasive acoustic parameters. By grouping participants into physiologically meaningful categories (0–3, 3–12, and 12+ years), we achieved clearer parameter discrimination and higher classification accuracy, especially in the youngest and oldest age brackets. Random Forest classifiers combined with SHAP-based feature interpretation consistently identified Peak admittance, F_{res} , and Volume as key contributors to diagnostic accuracy.

These findings reinforce the influence of developmental anatomy and physiology on acoustic immittance responses and highlight the need for age-specific diagnostic frameworks. Although limitations related to sample size and class imbalance remain, the results demonstrate the strong potential of integrating machine learning with tympanometric data for early, pressure-free detection of middle ear pathologies. Future research will aim to validate these models across larger and more diverse cohorts, refine age-dependent diagnostic thresholds, and establish condition-specific PLAITM criteria benchmarked against clinical gold standards such as otoscopy and audiometry. Ultimately, this approach could improve diagnostic precision, facilitate early intervention, and enhance care in pediatric and general audiology.

Chapter 4

Ear models based on PLAITM

This chapter begins by outlining theoretical ear models, with particular attention to lumped-parameter representations of the external and middle ear, and subsequently introduces the preliminary work carried out toward developing an electrical lumped-element equivalent model informed by PLAITM measurements.

Establishing an equivalent model within the context of a novel diagnostic system plays a dual role. First, it offers a structured means for identifying pathological patterns and therefore has the potential to enhance diagnostic accuracy. Second, it serves as a tool for investigating the measurement process itself, enabling the identification of potential sources of error and informing strategies to improve measurement reliability and robustness.

The introductory model presented in the second section focuses on the combined effects of ear canal volume and tympanic membrane stiffness, characteristics that hold relevance for detecting conditions such as OME, where membrane tension is altered, and for refining the estimation of ear canal volume. Drawing from established physical principles and models described in the literature, this framework is evaluated through an experimental protocol in which the ears of healthy volunteers were measured before and during the Valsalva maneuver. This approach induces changes in tympanic membrane tension, allowing assessment of the model's capacity to capture tension-dependent variations. The resulting admittance curves were fitted and analyzed, providing empirical support for the model and confirming that membrane biomechanics significantly influence measurement outcomes. These results further suggest that adjustments to the current acquisition protocol may enhance measurement precision.

Finally, the chapter considers the feasibility of constructing physical reference models for

testing and calibration. Preliminary results obtained from 3D-printed acoustic cavities are presented, demonstrating the potential of this approach for replicating specific anatomical conditions, assessing measurement limits, and evaluating the influence of individual parameters on the instrument's output. Such physical models represent a promising tool for controlled experimentation and for validating future model developments.

4.1 Analytical ear models

As discussed in Chapter 1, the ear is a complex system in which acoustic, mechanical, and even electrical phenomena interact as the result of underlying biological processes. Nevertheless, it is often essential to construct models of individual ear components or of the entire auditory system. Such models allow us to better understand how each structural element contributes to sound transmission and how these contributions change when a pathological condition arises. They are also useful in the design of improved prosthetic devices in cases of structural damage due to trauma or disease [151].

Different modeling strategies have been proposed over time, depending on the objectives of the analysis and the expertise of the researchers involved. Three main categories can be identified:

- **Lumped Element Model (LEM).** Electrical circuit analogies are employed to simplify the analysis and exploit standard circuit theory tools to predict the behavior of the ear across frequencies and to evaluate how modifications to individual structures affect the overall response. Thanks to a limited number of parameters, these models are compact and computationally efficient, making them suitable for integration in systems with limited processing resources [152].
- **Finite Element and numerical models.** These approaches provide a high level of detail and can accurately represent complex anatomical structures and material properties. They are typically used to characterize the response of specific patient ears or to construct general models for numerical simulation and research on biomaterials [153].
- **Machine learning models.** Based on large data sets obtained from techniques such as wideband acoustic immittance, these models can detect characteristic changes

associated with middle ear pathologies and support clinical decision making through pattern recognition [154].

Since the PLAI™ system is designed as an embedded solution, this work concentrates on lumped element models, which are generally more portable and well suited for implementation in devices with constrained computational capabilities.

Lumped element models are typically developed for individual anatomical regions, specifically the outer, middle, and inner ear. Therefore, multiple models often need to be integrated to represent the complete auditory system.

One widely cited representation of the external ear using a lumped approach is the model by Zwislocki and Feldman [155], shown in Figure 4.1, where the ear canal is described as a simple transmission line.

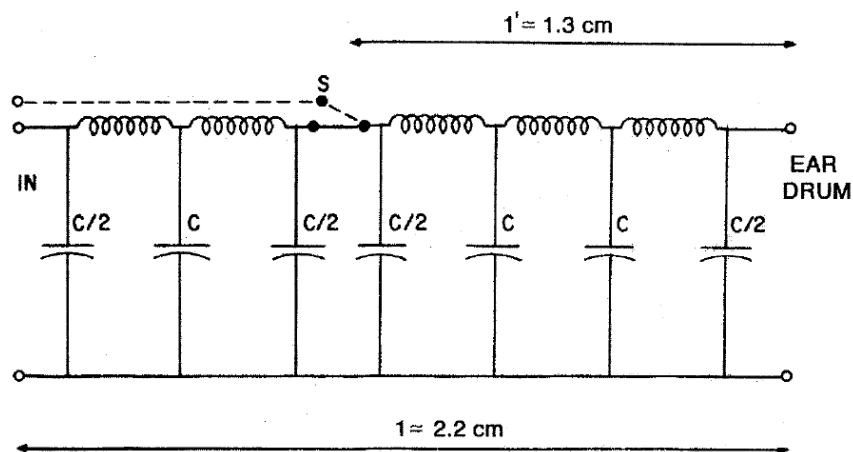


Figure 4.1: Network analog of the ear canal. The switch S marks the location of the tips of most ear inserts [155]

In this formulation, inductance and capacitance are determined directly from the average cross section and volume of the external auditory canal.

Moving to the middle ear, one of the first comprehensive lumped models was also proposed by Zwislocki [156], illustrated in Figure 4.2. This model describes the ear as an equivalent electrical network and allows computation of the equivalent impedance and corresponding frequency response.

While highly influential, the original model has several limitations. Some parameters were estimated from cadaveric tissue, not all components were analytically derived, and the tympanic membrane was not fully characterized. Despite these drawbacks, the model and

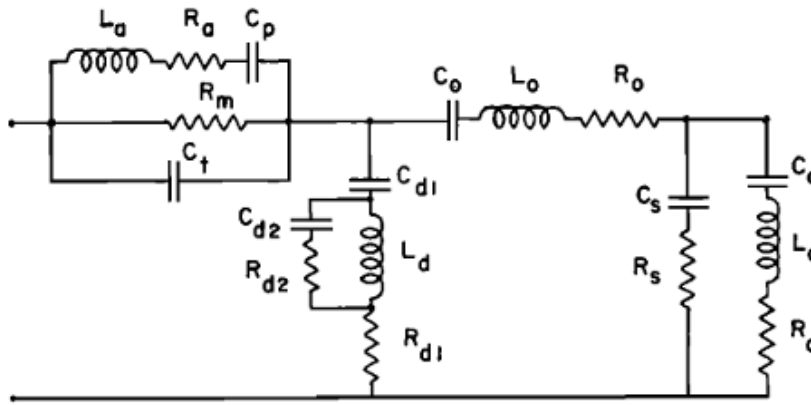


Figure 4.2: Zwislocki's model of the middle ear [156]

its refinements continue to represent a fundamental reference [157]. It is important to note that purely lumped models typically hold validity up to frequencies of approximately 4kHz, beyond which the dimensions of the ear structures become comparable to the wavelength of sound and distributed effects emerge. More advanced models, such as those by Puria and Allen, introduce distributed components to extend frequency accuracy [158, 159].

Since the goal in this work is to study acoustic immittance at the tympanic membrane, the cochlear interface included in the Zwislocki formulation is sufficient, and an additional inner ear model is not required.

Overall, the complete auditory representation results from combining multiple elements and impedance contributions. Estimating these parameters from a single measurement remains challenging, and at present it is not feasible to recover a complete parametric description of the ear in a routine clinical setting [160].

4.2 Simplified lumped element ear model

With the already existing models in mind, a simplified model was developed. Given that PLAITM returns a measurement of acoustic immittance up to 1.5 kHz, and thus the minimum wavelength is larger than the anatomical structures being analyzed, a fully lumped-element model was adopted.

As a starting point for parameterization, a literature review on electrical equivalent models of resonant cavities was performed. In fact, the PLAITM probe inserted into the external auditory canal can be viewed as a Helmholtz resonator, where the probe represents the neck and the EAC acts as the resonant cavity coupled with a compliant membrane [141].

The model was then refined by incorporating a membrane at the termination of the cavity to represent the tympanic membrane [161].

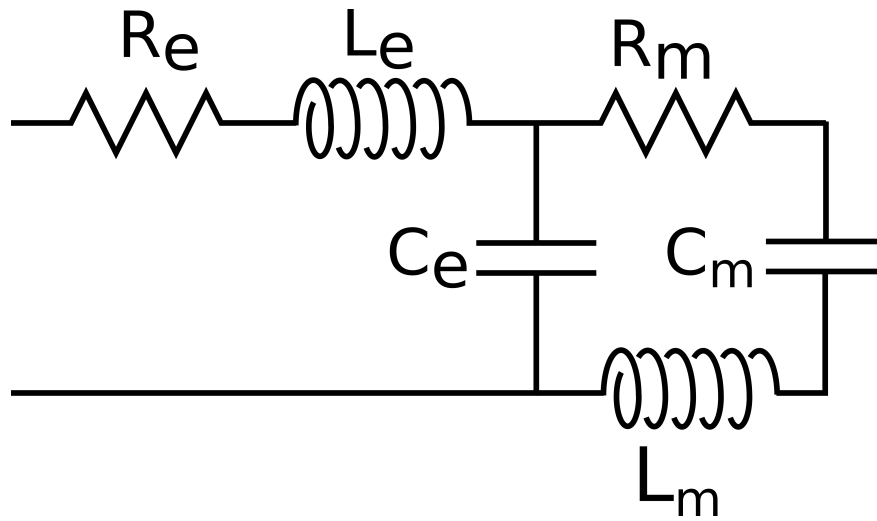


Figure 4.3: Simplified equivalent ear model

The resulting model is shown in Figure 4.3, where R_e , L_e , and C_e represent the EAC components, and R_m , L_m , and C_m represent the middle and inner ear. In particular, the equations for the external ear components are:

$$R_e = \frac{l\sqrt{2\eta\omega\rho_0}}{\pi r_0^3} + \frac{\omega^2\rho_0}{2\pi c_0} \quad (4.1)$$

$$L_e = \frac{\rho_0 l'}{S_0} \quad (4.2)$$

$$C_e = \frac{V_{EAC}}{\rho_0 c_0^2} \quad (4.3)$$

Where:

| | |
|--------------------------------------|--------------------------------|
| $l = 0.105$ m | probe length |
| $\eta = 1.813 \times 10^{-5}$ Pa * s | air viscosity |
| ω | angular frequency |
| $\rho_0 = 1.21$ kg/m ³ | air density |
| $r_0 = 0.0015$ mm | probe radius |
| $c_0 = 343$ m/s | speed of sound in air |
| V_{EAC} | EAC volume |
| $l' = l + 1.4$, $r_0 = 0.1071$ m | equivalent length of the probe |

A brief examination of the proposed model reveals that, in the case of a rigid tympanic membrane, the middle ear can be substituted with an open circuit, leaving only an RLC series, and thus returning to the standard Helmholtz resonator. In this case, the resonance frequency is equal to $f = \frac{c_0}{2\pi} \sqrt{\frac{S_0}{V_{EAC}'}}$, where $S_0 = \pi r_0^2 = 7.06 * 10^{-6} \text{ m}^2$ is the cross-sectional area of the probe.

It is also possible to observe that two of the three parameters (R_e and L_e) are fixed, given that the probe dimensions do not vary. Therefore, fitting the curve of a rigid-walled cavity only requires estimating the value of V_{EAC} .

It is then important to infer from both the literature and the circuit analysis that including the middle ear in the model lowers the resonance frequency of the system.

This behavior can be verified with a simple experiment: cavities of known volume were designed to interface either with a silicone-cast membrane or with a rigid wall (Figure 4.4).

The two assemblies were then constructed and measured.

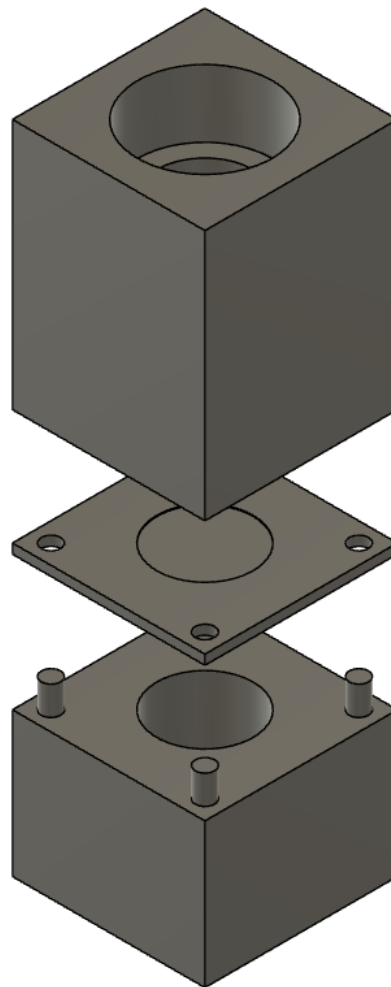


Figure 4.4: Test cavities for membrane influence testing

The results, shown in Figure 4.5, confirm the expected behavior and additionally demonstrate the potential to design custom acoustic cavities for calibration or experimentation tailored to specific patient ear anatomies.

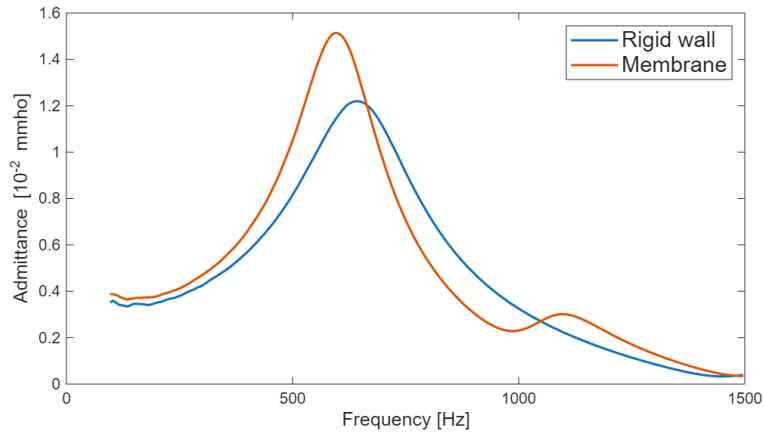


Figure 4.5: Admittance curves of rigid-walled and membrane-walled physical models

To verify the validity of the model, 55 healthy subjects were tested in normal conditions and during the Valsalva maneuver[162], a breathing technique in which air is forced against the closed glottis. This action increases pressure in the oral cavity and subsequently in the middle ear through the Eustachian Tube, stretching the tympanic membrane.

The measured curves from the Valsalva maneuver were fitted using the rigid-walled model, yielding an estimate of the EAC volume. With this value fixed, C_e was computed, and the three parameters R_m , L_m , and C_m were obtained by fitting the normally measured ear admittance.

Only participants with no known or previous otologic disorders and without nasal or ear congestion at the time of measurement were included in the study. Furthermore, because the procedure required performing the Valsalva maneuver, individuals with cardiac or respiratory conditions were excluded. All participants were informed about the potential risks of the procedure and provided written informed consent prior to participation.

This introductory study, conducted on 110 healthy ears, demonstrated the expected shift in resonance frequency (see Figure 4.6) and enabled identification of a mean EAC volume of $1.24 \pm 0.4\text{ml}$, in agreement with normative tympanometric ECV values for the evaluated age group (19 to 40 years).

Moreover, this experiment might also highlight the need to modify the PLATM measurement protocol for patients. Specifically, the protocol could include an initial evaluation of

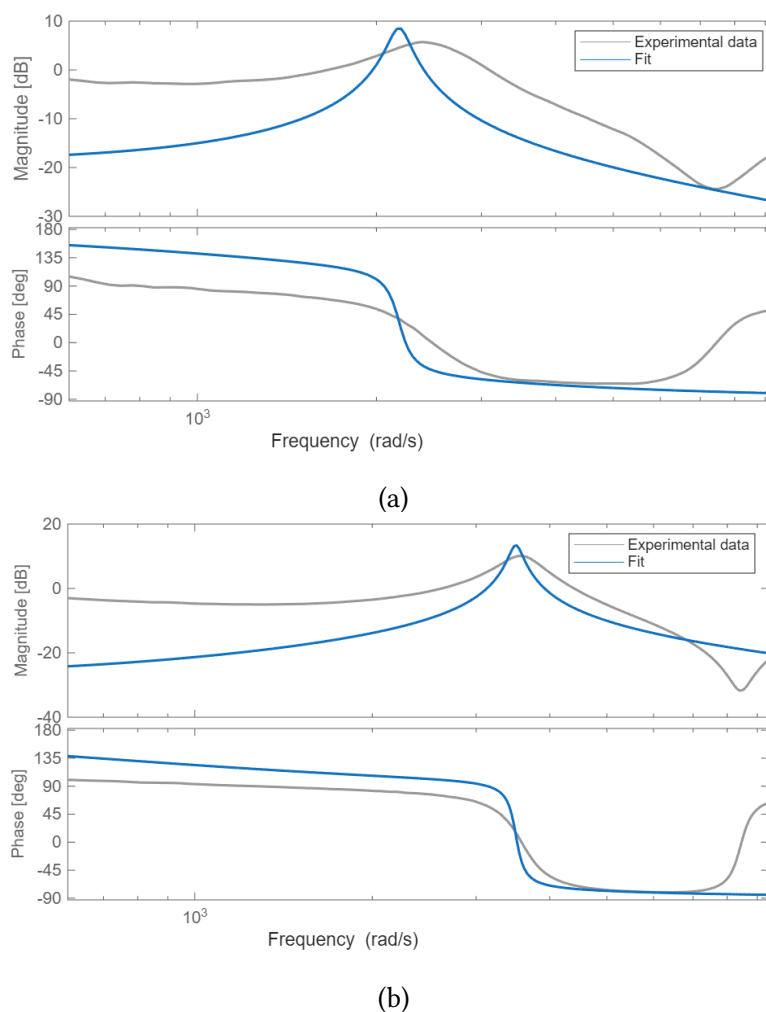


Figure 4.6: Fitted curves obtained from the normal (a) and Valsalva (b) measurements

both ears at ambient pressure followed by a second measurement during the Valsalva maneuver. It is important to note that such a protocol change must first be validated through a dedicated study comparing tympanometric data, PLAI™ measurements, and ear-canal volume estimates obtained from highly reliable methods (e.g., CT or MRI scans). If approved, this procedure should only be applied to patients without heart conditions or tympanic membrane perforation, the latter of which can usually be identified during the initial measurement.

Lastly, this experiment may be regarded as a proxy for the condition observed in OME patients. Therefore, the ability to infer middle ear parameters could provide an early indication supporting diagnostic decision-making.

In conclusion, this study demonstrated the feasibility of developing an equivalent lumped-element ear model and its usefulness in improving the estimation accuracy of parameters derived from the admittance curve, while also offering a preliminary means to identify

pathological alterations.

Furthermore, a physical model for calibration and for testing membrane-induced measurement variations was designed and evaluated, opening the possibility to experiment with specific anatomies or simulated pathological conditions.

Chapter 5

Conclusions

The present doctoral project was primarily focused on the characterization of the Pressure-Less Acoustic Immittance (PLAITM) methodology in order to allow for a safe, effective and broad use of the new technology and to develop analytical models and decision support systems systems that can be useful in a clinical setting for the diagnosis of common otological diseases.

Starting from an analysis of the anatomical and physiological properties of the auditory system and of the most common otologic pathologies, the research aimed to characterize this new technology both from a physiological and diagnostic standpoint, establishing quantitative age-dependent reference values and assessing its potential integration into automated decision-support tools.

The first phase of the study, focused on the characterization of the device, was divided in three parts, namely the comparison between PLAITM and tympanometric parameters, the investigation of age dependency of the novel technique parameters and the definition of age group and a first normative value schema for healthy and pathologic subjects. The comparison between tympanometry and F_{res} from PLAITM was conducted on a population of 57 adult subjects, both healthy and pathologic, and allowed to identify a relation with ECV, allowing for an estimation of this tympanometric parameter without the application of an external pressure gradient. A relation with tympanometric Compliance was also identified, although not sufficient to allow for an estimation of this parameter with F_{res} alone. The successive analysis, conducted on 134 healthy individuals, was aimed at the identification of age variability of PLAITM parameters and a comparison of tympanometric and pressureless parameters. This study allowed the identification of logarithmic relations

of parameters with age and confirmed the possibility to estimate tympanometric parameters like ECV, TW and Compliance with pressureless ones, although with limitation due to pathologic conditions, aspect which implies a need for ulterior studies and characterizations. Last step of the first phase was the definition of normative reference ranges for the parameters extracted from the PLAITM admittance curves in healthy individuals and comparing them with pathological cases, primarily OME. Through a multicentric clinical study involving 218 subjects aged from four months to eighty years, the results demonstrated clear age-dependent logarithmic relationships for all PLAITM parameters, allowing the identification of three physiologically distinct age classes: 0–3 years, 3–12 years, and over 12 years. These findings provided the first normative database for the PLAITM device and confirmed its strong correlation with traditional tympanometric measures. Importantly, significant differences between healthy and OME-affected ears were identified for several key parameters, including resonance frequency, band limits, and equivalent ear canal volume, confirming the method's sensitivity to functional changes in the middle ear.

The second phase, explored the diagnostic potential of the PLAITM parameters using machine learning-based classification models. Random Forest classifiers were trained on the same multicenter dataset, stratified by age, to automatically distinguish between healthy ears and several common middle ear pathologies. The models achieved high diagnostic accuracy, with macro F1-scores exceeding 0.8 across most age groups, and demonstrated strong robustness to anatomical variability. The interpretability analysis performed with SHAP values highlighted the physiological coherence of the most influential features, such as resonance frequency, admittance peak, and equivalent volume, confirming their relevance in characterizing middle ear mechanics across ages. These findings established a solid foundation for the future implementation of artificial intelligence-driven diagnostic support systems based on PLAITM data.

This research achieved several significant results. It introduced and clinically validated a new, fully non-invasive diagnostic technique capable of assessing middle ear functionality without the application of pressure-based methods. It also created age-specific reference values for healthy ears across the entire human lifespan, identified diagnostic parameters with significant discriminatory power between healthy and pathological cases, and developed machine learning classifiers capable of automatically detecting pathological deviations based on PLAITM features. The work also demonstrated the clinical feasibility of the

method, which can be operated by non-specialized personnel and therefore has potential applications in primary care and low-resource settings.

Despite these positive outcomes, the study presents some limitations that should be acknowledged. The number of pathological cases included in the analysis was relatively limited compared to healthy controls, particularly for less frequent conditions such as otosclerosis, tympanic retraction, and tympanic membrane perforation. This class imbalance, although partially mitigated through statistical and synthetic data augmentation techniques, may have influenced model generalizability. Moreover, the dataset did not encompass the full spectrum of middle ear pathologies, restricting the evaluation of PLAITM performance to a subset of common disorders. Finally, while the multicentric design improved representativeness, inter-operator variability and minor calibration differences across centers may have contributed to measurement dispersion.

The third phase of the doctoral research focused on the development of an equivalent Lumped Element Model and its preliminary validation on two studies on bench replicas and healthy subjects. The preliminary results demonstrate the possibility to model ears with PLAITM data, opening a new promising phase for model-based decision support systems.

Building upon these encouraging results, several directions for future research are proposed. Expanding the clinical dataset to include a larger and more balanced population of pathological cases will be essential, extending the investigation to additional ear diseases such as otosclerosis, tympanic membrane retraction and chronic suppurative otitis media. Future studies should also focus on developing analytical equivalent models that link PLAITM parameters to specific mechanical and acoustic properties of the middle ear, enabling precise identification of lesion types and severity. Parallel to this, the creation of physical ear models and phantoms will support device calibration, reproducibility testing, and algorithm validation under controlled conditions. Another important direction involves the integration of PLAITM data into machine learning-based clinical platforms for real-time diagnostic support and longitudinal monitoring of ear health. Longitudinal studies could further assess the predictive and prognostic capabilities of PLAITM parameters in patients undergoing treatment or surgical interventions.

In conclusion, this work represents a significant step toward the realization of a non-invasive and accessible diagnostic approach for the evaluation of middle ear function. The

PLAI™ system demonstrated both technical reliability and clinical relevance, showing the potential to complement or, in specific contexts, replace traditional tympanometry. By combining advanced signal analysis, age-aware modeling, and machine learning–based interpretation, this research lays the foundation for a new possibility in otologic diagnostic tools capable of delivering precise, safe, and scalable screening for hearing disorders across all ages.

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- [FB1] F. Bassi and A. Accardo, “Estimation of Middle Ear Characteristics by an Innovative Pressure-Less Acoustic Immittance (PLAI™) Device,” in *9th European Medical and Biological Engineering Conference* (T. Jarm, R. Šmerc, and S. Mahnič-Kalamiza, eds.), vol. 113, pp. 36–42, Cham: Springer Nature Switzerland, 2024. Series Title: IFMBE Proceedings.
- [FB2] F. Bassi, A. Miladinovic, and A. Accardo, “Relations between age, tympanometric parameters and pressureless measurements,” in *9th National Bioengineering Group (GNB) Congress, 2025*.
- [FB3] F. Bassi, A. Miladinović, and A. Accardo, “Non-Invasive Assessment of Middle Ear Function: Establishing Age-Specific Reference Ranges Using Innovative Pressureless Acoustic Immittance,” *Journal of Medical and Biological Engineering*, Dec. 2025.
- [FB4] A. Miladinovic, F. Bassi, M. Ajcevic, and A. Accardo, “Age-Stratified Classification of Common Middle Ear Pathologies Using Pressure-Less Acoustic Immittance (PLAI™) and Machine Learning,” *Healthcare*, 2025.

Publications outside the scope of the doctoral thesis

As part of the doctoral research, the author also contributed to studies on additional topics within the field of biomedical signal processing. The findings of these works are presented in the following publications, which are not included in this thesis:

- **Bassi F.**, Raffini A., Ajčević M., Miladinović A., Di Blas L., Accardo A.; Relationship between personality and kinematic parameters of handwriting. In proceedings of the Mediterranean Conference on Medical and Biological Engineering and Computing (MEDICON) and International Conference on Medical and Biological Engineering (CMBEBIH), september 14–16, 2023, Sarajevo, Bosnia and Herzegovina, IFMBE proceedings, vol 93. Springer. DOI: 10.1007/978-3-031-49062-0_21
- Raffini A., **Bassi F.**, Ajčević M., Miladinović A., Accardo A.; Kinematic characterization of movements during the Tinetti test. In proceedings of the Mediterranean Conference on Medical and Biological Engineering and Computing (MEDICON) and International Conference on Medical and Biological Engineering (CMBEBIH), september 14–16, 2023, Sarajevo, Bosnia and Herzegovina, IFMBE proceedings, vol 93. Springer. DOI: 10.1007/978-3-031-49062-0_18
- **Bassi F.**, Krešević S., Biscontin A., Miladinović A., Ajčević M., Accardo A.; Optimizing electroporation responses in genetically engineered HEK cells: An ensemble learning approach. In 9th European Medical and Biological Engineering Conference. EMBEC 2024. IFMBE Proceedings, vol 113. Springer, Cham. DOI: 10.1007/978-3-031-61628-0_21
- Miladinović A., Ajčević M., Iskra K., **Bassi F.**, Raffini A., Jarmolowska J., Marusić U., Accardo A.; Trade-off between real-time and classification performance in motor im-

agery BCI. In 9th European Medical and Biological Engineering Conference. EMBEC 2024. IFMBE proceedings, vol 113. Springer, cham. DOI: 10.1007/978-3-031-61628-0_37

- Miladinović, A., Biscontin, A., Bonini, A., **Bassi F.**, Kresevic, S., Raffini, A., Iskra, K., Accardo, A., Ajčević, M.; Towards efficient wound management: An automatic deep learning model for accurate wound image segmentation; IFMBE proceedings vol.131, joint 20th NBC & 24th PCBEE, june 16-18, 2025;DOI: 10.1007/978-3-031-96538-8_37