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ORIGINAL ARTICLE

Non-Invasive Assessment of Middle Ear Function: Establishing Age-Specific Reference Ranges Using Innovative Pressureless Acoustic Immittance

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Abstract

Purpose Tympanometry, the current gold standard for assessing middle ear function, has limitations in infants and in cases of tympanic membrane perforation due to its potential invasiveness, as it requires the application of varying pressure in the ear canal. To overcome these issues, the Pressure-Less Acoustic Immittance (PLAI^{TM}) device was recently developed as a non-invasive alternative that does not require pressure application. However, reference values and age-specific norms are not yet established. This study aims to define age-specific reference ranges for key acoustic admittance parameters using PLAI^{TM} in healthy individuals and to offer a preliminary comparison with subjects affected by Otitis Media with Effusion (OME), a common middle ear pathology.

Methods A total of 318 healthy ears and 68 OME-affected ears were analyzed, covering an age range from 4 months to 80 years. Parameters extracted from PLAI^{TM} admittance curves included resonance frequency, admittance peak, minimum and maximum frequencies of the resonance peak, bandwidth, half peak height, and equivalent volume.

Results Results indicate a strong age dependence for all parameters, best modeled with a logarithmic fit. Statistical analysis supports categorizing ages into three groups (0–3, 3–12, 12+ years), reflecting stages of ear development. Most parameters stabilized after age 12. Significant differences were observed between healthy and OME ears across several PLAI^{TM} metrics.

Conclusion These findings establish essential reference values and demonstrate the clinical potential of PLAI^{TM} for diagnosing middle ear dysfunctions, particularly in populations where conventional tympanometry is limited.

Keywords Acoustic impedance test · Ear function · Wideband tympanometry · Otitis Media with Effusion

1 Introduction

Evaluating ear functionality and pathological status is a complex process, typically achieved through a range of examinations, each dedicated to a specific aspect of the auditory system. Among these methodologies, tympanometry is the standard for assessing the functional state of the outer and middle ear by measuring acoustic admittance while varying the pressure (−400 to 400 dPa) applied to the external ear. Acoustic admittance is evaluated by measuring the energy reflected by the eardrum at each pressure level in response to a 226 Hz probe signal [1].

Although this methodology is widely used as a gold standard [2], it does not provide a comprehensive assessment of the underlying causes of hearing impairment. In fact, measurements taken from subjects with different pathological conditions can yield similar results [3]. To improve this diagnostic tool, researchers have also employed tympanometry at multiple frequencies, known as wideband tympanometry (WBT), which produces a 3D tympanogram showing energy reflectance as a function of both pressure and frequency [4]. However, despite the increasing adoption in clinical settings, WBT is not yet well established due to limited clinical data and interpretive challenges [5–7].

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Furthermore, both standard tympanometry and WBT can be considered invasive tools because they apply supplemental pressure during measurement. This makes them unsuitable for infants, due to the high elasticity of their auditory structures [8, 9] as well as for individuals with tympanic membrane perforations, since the applied pressure could further exacerbate their condition [1, 10–12].

Given the limitations in assessment capabilities and the invasive nature of both standard and wideband tympanometry (WBT), early identification of middle ear pathologies, particularly in infants and individuals with tympanic membrane perforations, remains a clinical challenge. To address this need and enable more effective and timely treatment, a novel, non-invasive technology known as Pressure-Less Acoustic Immittance (PLAI™, Neuranix Srl, Napoli, Italy) has been developed.

The system operates by generating a multi-frequency acoustic signal (100–2000 Hz) and measuring the speed of the air returning from the ear at atmospheric pressure, from which it derives the acoustic admittance curve (Fig. 1) as a function of frequency. From this curve, key parameters including the characteristic resonance frequency, which depends on the air volume contained in the ear canal and the mechanical properties (mass, viscosity and elasticity) of the outer and middle ear [13], as well as the corresponding peak values are calculated. Due to its measurement method, conducted exclusively at atmospheric pressure, the approach is non-invasive and suitable for evaluating admittance even in infants and individuals with tympanic membrane perforations.

Since PLAI™ is based on different physical principles than tympanometry, the parameters it obtains are not directly comparable to those derived from the gold standard procedure. This necessitates the evaluation of reference data from healthy subjects as well as from at least a pathologic group

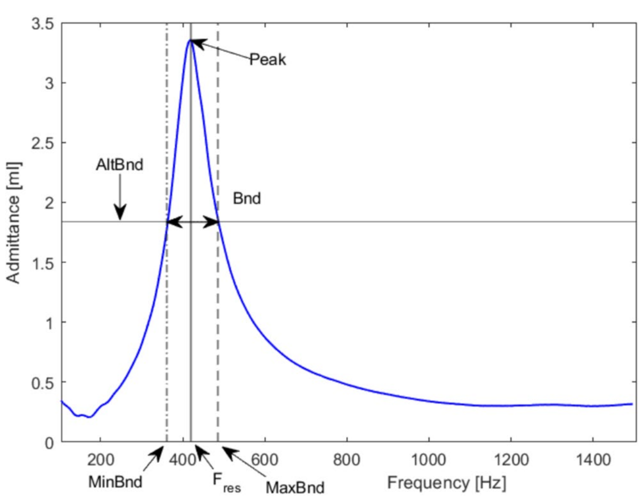

Fig. 1 Example of an Admittance modulus curve from PLAI™ with main parameters extracted from the curve

to establish appropriate cut-off values, enabling potential clinical use of the approach.

Furthermore, as with classical tympanometry, anatomical differences in the external and middle ear related to patient age mean that measurements are performed differently in newborns and adults [14]. This suggests that age-related dependency may also be present in measurements obtained with PLAI™.

Therefore, the aim of this study is to establish reference value ranges for each parameter derived from PLAI™ curves in healthy ears as well as in one the most frequent middle ear pathology (Otitis Media with Effusion, OME) across all age groups. These reference ranges will serve as a basis for distinguishing between healthy individuals and those with other middle ear pathologies in future clinical applications.

2 Methods

2.1 Population

In this multicentric study, subjects from 6 italian clinical institutions (Gemelli University Hospital IRCCS, Rome; "Guglielmo da Saliceto" Hospital, Piacenza; "Federico II" University Hospital, Napoli; "Giovanni XXIII" Children Hospital, Bari; Fondazione IRCCS Policlinico “S. Matteo” Hospital, Pavia; IRCCS Maternal and Child Health Institute "Burlo Garofolo", Trieste) presenting healthy ears or being affected by OME were enrolled.

The subjects were recruited between May 2023 and April 2024 among outpatients, with no age restrictions, excluding those with a history of otologic surgery, significant comorbidities, and/or cognitive impairments that could affect measurements. All subjects underwent otoscopy and tympanometry to confirm the status of their auditory organs. Only confirmed cases of Otitis Media with Effusion (OME) and healthy subjects were considered for the following analysis. Ears with a confirmed diagnosis of OME and without other otologic comorbidities were included in the pathologic group, while ears without any specific diagnosis for otologic pathologies were included in the healthy group. A total of 360 healthy and 76 pathologic ears were collected from 218 subjects aged between 4 months and 80 years.

The obtained measurements were manually reviewed for errors, such as motion artifacts and probe misplacement, by two experts independently, resulting in 318 healthy and 68 pathologic measurements.

2.1.1 Ethical Approval and Informed Consent

The Clinical Trial was approved by the Ethics Committees of all six participating institutions, beginning with the lead institution, IRCCS "A. Gemelli" Hospital in Rome (Prot. 5389, January 19, 2023), and, as required by Italian regulations, by the Italian Ministry of Health (MEDWAVE-2, Clinical Trial number: IT-23-03−042692, May 9, 2023). Informed consent was obtained from all adult participants, and for minors, consent was provided by a parent or legal guardian.

2.2 Pressure-Less Acoustic Immittance™

The Pressure-Less Acoustic Immittance (PLAI™, Neuranix Srl, Napoli, Italy) device is a non-invasive device specifically developed for clinical audiometric assessment, allowing the measurement of middle ear function without applying external pressure. Unlike conventional tympanometry, which requires pressure manipulation, PLAI™ uses a design that measures both particle velocity and acoustic pressure at the same point [13]. The device's measurement process begins with the emission of a wideband acoustic signal covering frequencies from 100 to 2000 Hz.

The signal, generated by the audio source, excites the auditory canal and the tympanic membrane. As the sound wave travels through the ear canal and reflects off the tympanic membrane, the resulting return signal provides essential information on the external and middle ear's acoustic properties. PLAI™ captures this signal through a dual-sensor setup (Fig. 2), which, by means of two microphones, allows the measurement of both pressure and the speed of movement of air particles along the canal's axis. The simultaneous measurement of velocity and pressure enables the calculation of the acoustic admittance curve (Fig. 1) [13].

From the modulus component of this complex valued curve, the device extracts a series of parameters: (1) resonance frequency (Fres), (2) peak admittance value (Peak)

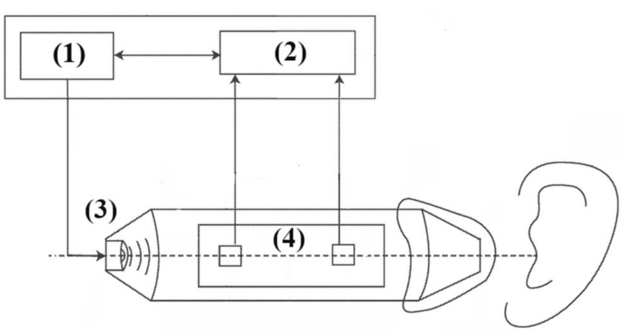

Fig. 2 Simplified schematic of the Pressure-Less Acoustic Immittance (PLAI™) device, showing key components: (1) Signal generator, (2) Processing unit for data analysis, (3) Speaker and (4) Sensors to capture reflected sound. [13]

measured at Fres, (3) minimum and (4) maximum frequency values (minBnd and maxBnd) (5) of a band (LBnd) defined as the frequencies at which admittance is half of its amplitude (6) (AltBnd), calculated as the difference between the maximum and the minimum admittance values (Fig. 1).

Finally, (7) equivalent volume (Veq) is automatically computed by PLAI™ from Fres using a polynomial relationship derived empirically from a proprietary PLAI™ calibration curve.

2.3 Statistical Analysis

To assess the potential age dependency of the parameters in healthy ears, various analytical functions were applied to fit the data and approximate age-related trends. To determine the most appropriate fitting function, we tested the following options: (1) a logarithmic curve, (2) a ramp superimposed on a logarithmic curve, (3) a polynomial curve up to the third order, (4) an exponential curve, and (5) an inverse exponential curve. The root mean square error (RMSE) between measured values and the fitting curves was used as the goodness of fit metric, along with the number of model coefficients.

$$parameter = a * log_{10}(age) + b \tag{1}$$

$$parameter = a * log_{10}(age) + b * age + c \tag{2}$$

$$parameter = a + b * age + c * age^2 + d * age^3 \tag{3}$$

$$parameter = a * e^{b*age} \tag{4}$$

$$parameter = a + b * e^{-c*age} \tag{5}$$

Among these fits, the analytical model with the overall lowest RMSE and fewest coefficients was selected. To account for age influence, the data were divided into discrete classes, with class size determined by the rate at which parameters varied with age.

For each age class and for each parameter the mean and standard deviation were then calculated using an ad hoc MATLAB® script. Pairs of age classes were then compared using the Wilcoxon rank-sum test with Bonferroni correction.

Subsequently, a group of subjects with ears affected by OME was analyzed to evaluate differences between healthy and pathological cases. The analysis aimed to verify the potential age dependency observed in the healthy cohort by applying the same stratification to pathological cases. This approach serves both to validate the relevance of age-based grouping in a clinical context and to illustrate the potential

Table 1 Mean values of RMSE of each group of the fit functions for each parameter

	First group	Second roup
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 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

diagnostic utility of the method in distinguishing between healthy and OME-affected ears.

3 Results

3.1 Selection of Fit Functions Based on RMSE Performance

The analysis of the fit functions revealed distinct grouping patterns based on RMSE values and that the fitting functions could be categorized into two main groups (clusters) based on their RMSE performance. The group consisting of second- and third-order polynomial curves, as well as logarithmic and logarithmic-plus-linear approximations, showed lower RMSE values with respect to the second group of fitting curves (linear, exponential, and inverse exponential) that exhibited slightly higher RMSE values for the Peak, AltBnd, and Volume parameters and substantially higher values for Fres, MinBnd, MaxBnd, and Bnd (Table 1). Within the best-fitting group, the logarithmic function was selected due to its lowest RMSE values and complexity in terms of the number of coefficients. Table 1 presents the mean RMSE values for each parameter within the two fit groups, while Table 2 provides the median values and 95% confidence intervals for the coefficients of the logarithmic approximation curves, along with their respective RMSE.

3.2 Age-Related Trends and Variability in Acoustic Parameters

In Fig. 3 the seven parameters are reported as a function of age together with the corresponding logarithmic fitting curves.

Figure 3 shows scatter plots of the parameters, revealing substantial variability across the entire age range. A clear trend of rapid increase in parameters such as Peak, AltBnd and Volume (or decrease for Fres, MinBnd, MaxBnd and Bnd) is observed with increasing age up to approximately 10–14 years. Specifically, it was observed that for all the parameters the logarithmic fit reaches 85% of the final value before the age of 12. Beyond this age, the parameters stabilize, showing a constant course.

3.3 Statistical Comparison of Parameter Variability Across Age Groups

To determine the number of age groups with significantly different values while maintaining sufficient cases in each group, subjects were divided into four three-year classes from newborn to 12 years, with an additional class for subjects older than 12 years. The number of ears in each age class was 76 for 0–3 years, 46 for 3–6 years, 29 for 6–9 years, 19 for 9–12 years, and 162 for those over 12 years.

In Fig. 4 the boxplot for each parameter is shown while Table 3 presents the mean values (±1SD) for each parameter and class together with the p-values of the comparison between each pair of age groups.

The patterns shown in Fig. 4 and the p-values in Table 3 indicate no significant differences among the 3–6, 6–9, and 9–12 age classes, allowing them to be combined. Consequently, we consider only three classes: 0–3 years (76 ears), 3–12 years (94 ears), and over 12 years (162 ears), as represented in the box plots in Fig. 5. The mean values ± 1 SD and p-values for differences between class pairs are reported in Table 4.

Among all the parameters, only minBnd does not show significant differences between the 0–3 and 3–12 year classes, primarily due to the high variability of this parameter within the 0–3 year class and the relatively small difference in values between these two classes. For all other parameters, the differences were statistically significant, despite the large standard deviations observed for the MinBnd, MaxBnd, and Bnd parameters.

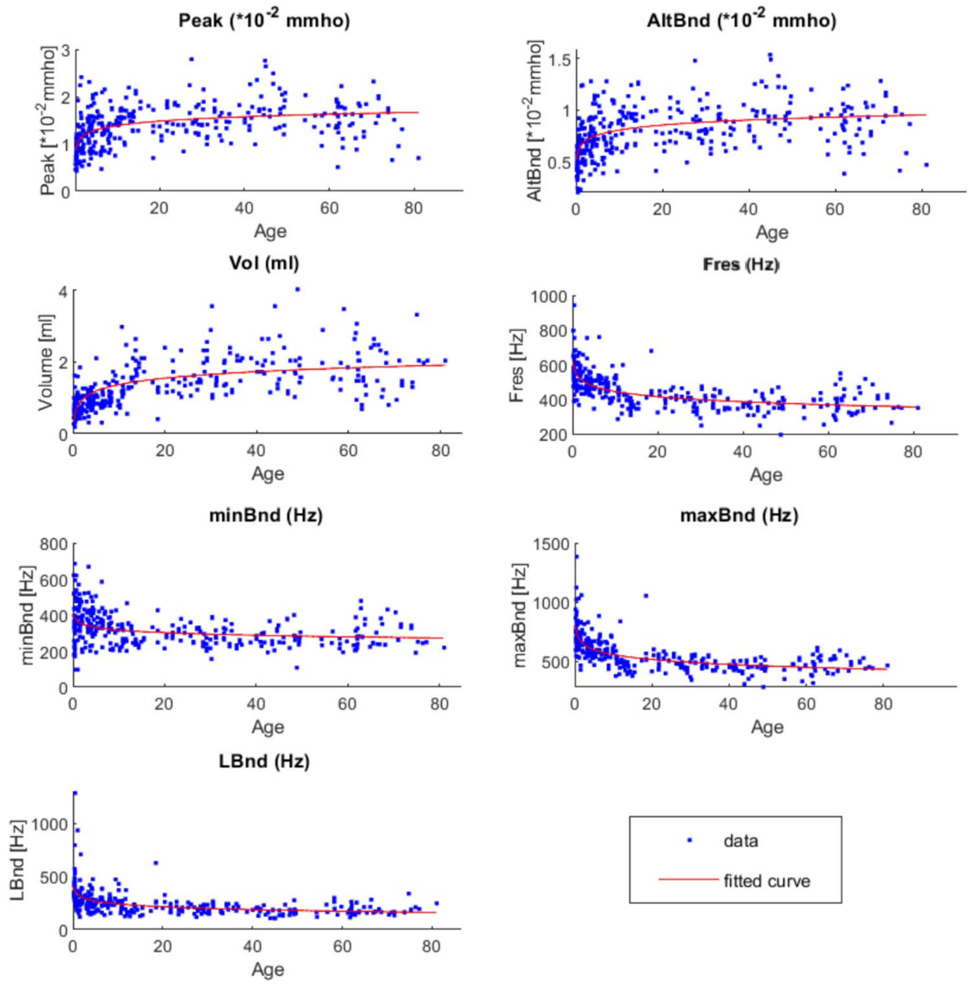

Fig. 3 Age distribution of the seven considered PLAI™ parameters, with the logarithm-fitted curves

3.4 Comparison Between Healthy and OME Subjects

The pathologic group was composed of 68 ears, divided in the three classes as follows: 0–3 years (22 ears), 3–12 years (28 ears), and over 12 years (18 ears).

Table 5 and Fig. 6 present the parameters that exhibited statistically significant differences between healthy and OME-affected ears across the three defined age groups.

Statistically significant differences between healthy and OME ears were observed in ear canal volume (Vol), Resonance Frequency (Fres), Minimum Frequency (MinBnd), and Maximum Frequency (MaxBnd) across all age groups. In the 3–12 year and 12+ groups, band width (LBnd) also showed a significant difference. Peak at resonance frequency (Peak) and height of the band (AltBnd) did not show significant differences in any age group.

4 Discussion

Both standard tympanometry and WBT can be considered invasive, as they require the application of supplemental pressure during measurement [1, 4]. This renders them unsuitable for certain populations, such as infants, whose auditory structures are highly elastic, and individuals with tympanic membrane perforations, for whom applied pressure may exacerbate their condition [1, 8–12]. The development of a new, non-invasive methodology addresses one of the primary limitations of tympanometry by allowing for evaluation in neonatal patients and those with tympanic perforations [13]. However, this advancement necessitates a comprehensive characterization of the innovative device, including an assessment of age-related influences on measurement outcomes in healthy subjects.

Accordingly, this study aims to establish reference value ranges for each parameter derived from PLAI™ curves across all age groups in healthy ears. These reference ranges are intended to support future clinical applications by serving as potential information for differentiating healthy

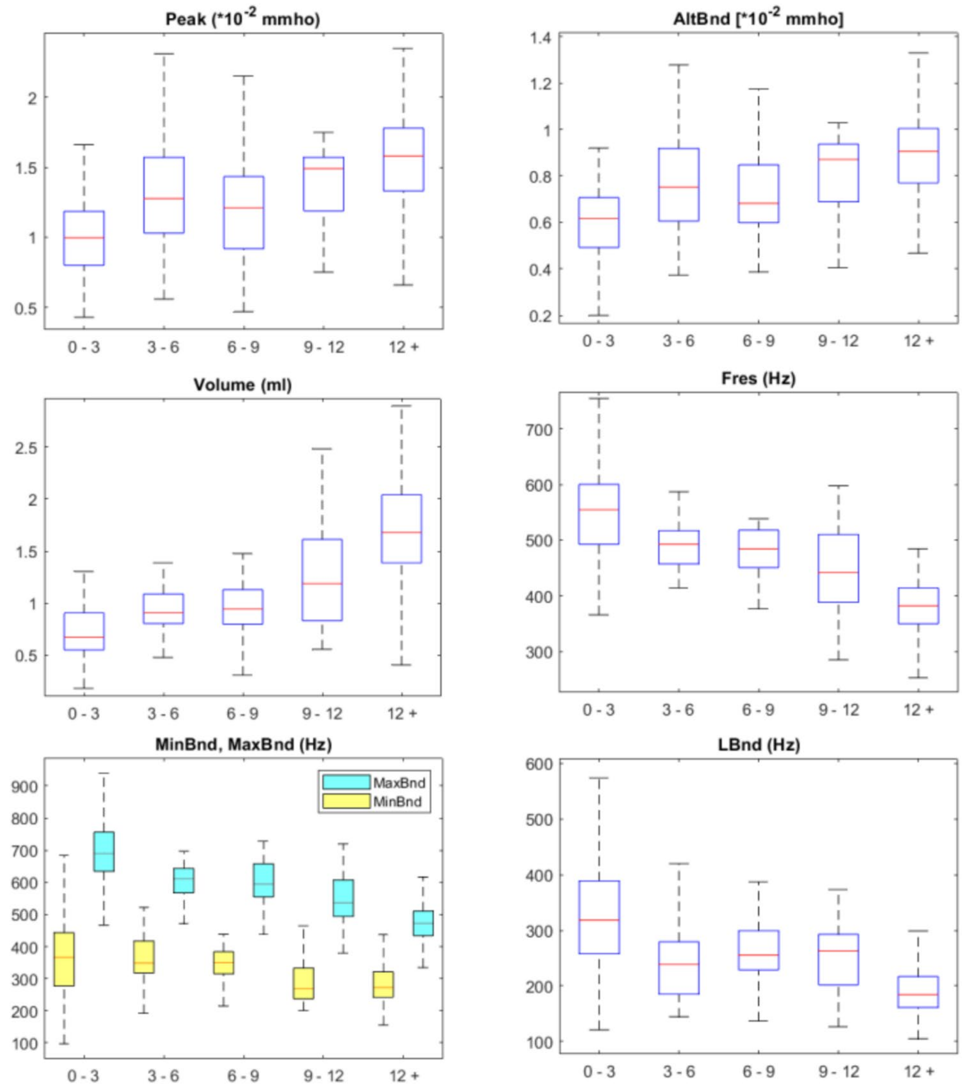

Fig. 4 Box plots for the seven examined parameters using a five-class age subdivision

Table 3 Mean values (± 1SD) of each parameter in the five classes together with the p-values

	Peak	AltBnd	Vol	Fres	MinBnd	MaxBnd	LBnd
	$*10^{-2}mmho$	$*10^{-2}mmho$	ml	Hz	Hz	Hz	Hz
0–3 years	1.02 ± 0.35	0.61 ± 0.19	0.78 ± 0.33	548 ± 94	357 ± 118	711 ± 151	354 ± 177
3–6 years	1.27 ± 0.38	0.75 ± 0.21	0.98 ± 0.27	485 ± 47	350 ± 72	604 ± 57	254 ± 76
6–9 years	1.25 ± 0.40	0.74 ± 0.22	0.99 ± 0.28	487 ± 68	345 ± 80	607 ± 87	262 ± 58
9–12 years	1.41 ± 0.28	0.82 ± 0.17	1.33 ± 0.64	444 ± 85	292 ± 72	559 ± 112	267 ± 91
12+ years	1.57 ± 0.39	0.90 ± 0.21	1.76 ± 0.55	384 ± 58	284 ± 56	477 ± 73	192 ± 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 ($p > 0.05$)

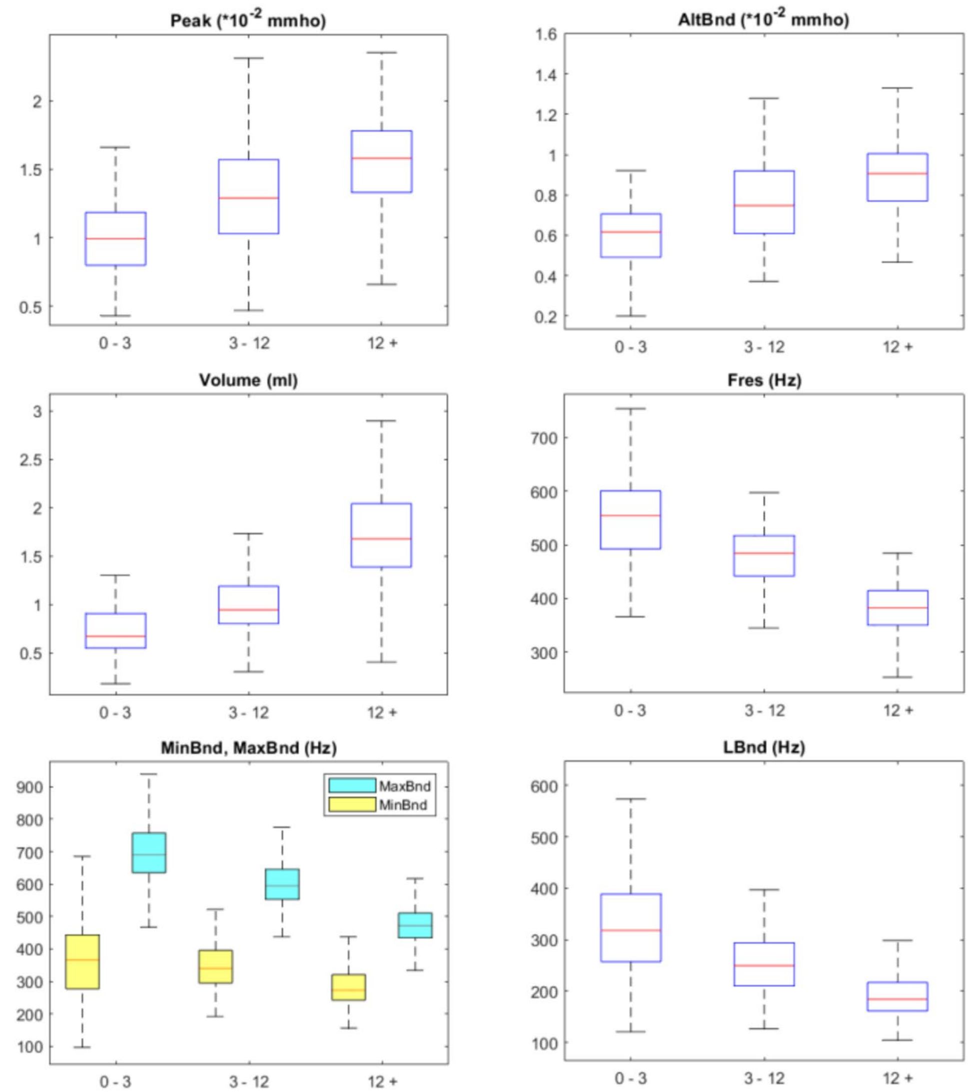

Fig. 5 Box plots for the seven examined parameters using a three-class age subdivision

Table 4 Mean values (± 1SD) 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 ± 0.35	0.61 ± 0.19	0.78 ± 0.33	548 ± 94	357 ± 118	711 ± 151	354 ± 177
	3–12 years	1.29 ± 0.37	0.76 ± 0.20	1.06 ± 0.40	477 ± 65	336 ± 77	595 ± 82	259 ± 74
	12+ years	1.57 ± 0.39	0.90 ± 0.21	1.76 ± 0.55	384 ± 58	284 ± 56	477 ± 73	192 ± 56
p-value	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 ($p > 0.05$)

individuals from those with middle ear pathologies. Our findings have potentially important clinical implications, especially by facilitating the non-invasive assessment of middle ear function in a wider patient population, including those traditionally underserved by standard tympanometry due to anatomical or age-related constraints.

In this study, we analyzed measurements from healthy ears across a broad age range, from infants (4 months) to 80-year-olds. The behavior of the seven parameters examined across different ages (Fig.s 3, 5) reveals general growth or decline trends, along with notable variability among subjects of similar ages. This variability may partly be attributed to the broad geographical range of the multicenter sample, potentially reflecting regional differences in ear morphology. The observed decline in frequency-related parameters (Fres, MaxBnd, and MinBnd) and the increase in Volume

Table 5 Mean values (± 1SD) 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	Fres	MinBnd	MaxBnd	LBnd
	Years	$*10^{-2}mmho$	$*10^{-2}mmho$	ml	Hz	Hz	Hz	Hz
Healthy	0–3	1.02 ± 0.35	0.61 ± 0.19	0.78 ± 0.33	548 ± 94	357 ± 118	711 ± 151	354 ± 177
	3–12	1.29 ± 0.37	0.76 ± 0.20	1.06 ± 0.40	477 ± 65	336 ± 77	595 ± 82	259 ± 74
	12+	1.57 ± 0.39	0.90 ± 0.21	1.76 ± 0.55	384 ± 58	284 ± 56	477 ± 73	192 ± 56
OME	0–3	0.99 ± 0.38	0.57 ± 0.18	0.49 ± 0.18	656 ± 101	481 ± 74	852 ± 94	371 ± 101
	3–12	1.27 ± 0.44	0.69 ± 0.21	0.59 ± 0.17	596 ± 68	453 ± 71	754 ± 96	301 ± 90
	12+	1.53 ± 0.69	0.85 ± 0.35	1.04 ± 0.44	484 ± 74	366 ± 81	612 ± 71	245 ± 92
p-value	0–3	*n.s.*	*n.s.*	< 0.0001	< 0.0001	< 0.0001	< 0.0001	*n.s.*
	3–12	*n.s.*	*n.s.*	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.022
	12+	*n.s*	*n.s.*	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.013

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

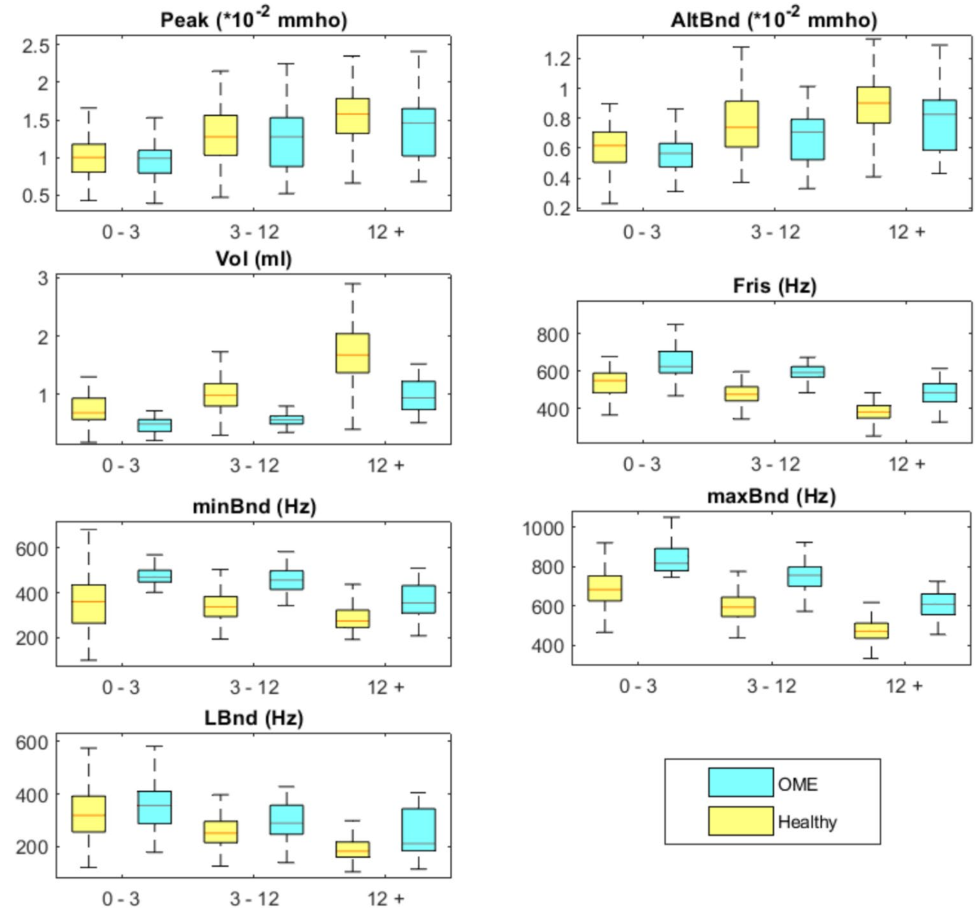

Fig. 6 Box plots for the main examined parameters using only a three-class age subdivision and comparison between healthy and OME subjects

with age may be associated with ear canal growth, as suggested by previous research [15]. Additionally, the increase in peak admittance and AltBnd may be linked to reduced tympanic stiffness, which corresponds to lower impedance and increased energy absorption [16]. Furthermore, Fig. 3 shows a slight mean decrease in admittance peak after age 50 within the 12+ age group, possibly due to a physiological increase in tympanic stiffness in older adults [17]. This age-related stabilization, especially after 12 years, allows clinicians to distinguish between age-appropriate changes and abnormal findings, benefiting both pediatric assessments and older adult diagnostics where subtle pathological changes may otherwise go unnoticed. Furthermore, the age-specific reference values established here enable more precise diagnostics by accounting for natural age-related variability in middle ear parameters, allowing PLAI™ to be used effectively in clinical contexts where pressure-based assessment is undesirable. The only parameter directly comparable with literature values is the equivalent volume of the external ear. Our findings indicate a slightly higher volume both in adults (1.4 ± 0.3 ml, based on data from 244 healthy ears of subjects aged 10 to 89 years [18]; approximately 1.3 ml in [16]) and in school-aged children [15, 16, 19]. Finally, the division into three age groups (0–3, 3–12, and over 12 years) effectively accounts for age-related influences on the parameters while ensuring statistical independence among classes.

This grouping not only enables more targeted diagnostic thresholds across life stages but also improves the clinical utility of PLAI™ in settings where early detection of middle ear dysfunction is critical, particularly for infants and children at risk of developmental delays due to undiagnosed or late diagnosed hearing issues [20, 21].

The comparison between healthy and pathologic subjects revealed several key findings. Specifically, some PLAI™-derived parameters demonstrated clear discriminatory power in distinguishing healthy ears from those affected by OME. Significant differences were consistently observed across all age groups in ear canal volume (Vol), Resonance Frequency (Fres), Minimum Frequency (MinBnd) and Maximum Frequency (MaxBnd). In older age brackets (3–12 and 12+ years), LBnd also proved to be a distinguishing parameter. Height of the band (AltBnd) and Peak at resonance frequency (Peak) did not show significant differences in any age category, suggesting they may be less effective as diagnostic markers within this non-invasive methodology.

Importantly, the same age group stratification used for the healthy population was applied to the OME group, enabling a direct comparison and validation of age-dependent trends in a pathological context. This approach confirmed that the age-based grouping remains relevant and meaningful when applied to ears affected by OME. The ability to preserve this stratification across both groups strengthens its utility in clinical settings, where age-specific baselines are essential for accurate interpretation of diagnostic data.

From a clinical standpoint, these results underline the potential of the PLAI™ method to serve as a diagnostic aid in the identification and monitoring of middle ear pathologies, particularly in populations where traditional pressure-based techniques are limited or contraindicated. The observed differences in key parameters between healthy and pathological ears, especially among older children and adults, highlight the method's capacity to detect deviations from typical middle ear behavior. When paired with the age-specific reference values established in this study, PLAI™ may enable earlier, safer, and more accessible detection of OME, facilitating interventions that could mitigate developmental delays and auditory complications [20, 21].

In summary, the PLAI™ system, combined with the proposed age grouping and parameter analysis, provides a robust and non-invasive alternative for assessing middle ear function. Its application could significantly enhance routine screening protocols, particularly for infants, young children, and patients with tympanic membrane perforations, who have historically been difficult to evaluate using conventional tympanometric approaches.

5 Conclusion

This paper represents the first step in characterizing an innovative instrument, PLAI™ (Pressure-Less Acoustic Immittance), which measures acoustic admittance without applying external pressure. The instrument provides an admittance curve as a function of frequency from 100 to 2000 Hz. Our results indicate that each of the seven parameters derived from the admittance curve shows an age-related trend that can be approximated with a simple logarithmic curve, allowing for the removal of age influence on each parameter. Additionally, findings support the use of three independent age groups (0–3, 3–12, and over 12 years) for classification. Mean values ± SD are provided for each parameter, along with the best-fit curve, serving as a healthy ear reference.

Furthermore, when comparing healthy and OME ears, significant differences between healthy and OME ears were found in several PLAI™ parameters.

The results will enable future clinical use of this approach to detect deviations from typical measurements in healthy ears. Our findings offer essential reference standards for diagnosing middle ear pathologies without pressure application, thereby supporting broader diagnostic use in infants and patients with tympanic membrane complications.

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Author Contributions Francesco Bassi: Writing—Original Draft, Formal analysis, Visualization, Methodology, Investigation, Software. Aleksandar Miladinović: Writing—Review and Editing, Methodology, Validation, Investigation. Agostino Accardo: Supervision, Writing—Review and Editing, Project administration, Funding acquisition, Conceptualization, Data Curation, Resources.

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Declarations

Competing Interests Agostino Accardo and Aleksandar Miladinović report a relationship with Neuranix Srl, Napoli, Italy that includes: consulting or advisory. Francesco Bassi declares no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Human Ethics and Consent to Participate The Clinical Trial was approved by the Ethics Committees of all six participating institutions, beginning with the lead institution, IRCCS "A. Gemelli" Hospital in Rome (Prot. 5389, January 19, 2023), and, as required by Italian regulations, by the Italian Ministry of Health (MEDWAVE-2, Clinical Trial number: IT-23-03-042692, May 9, 2023). Informed consent was obtained from all adult participants, and for minors, consent was provided by a parent or legal guardian.

Clinical Trial Details The Clinical Trial was approved by the Italian Ministry of Health with the following details: Study code "MEDWAVE-2", Study number IT-23-03-042692, date of approval 9 May 2023, Study title "Utilizzo dello strumento Med-Wave per la diagnosi di patologie dell'orecchio in pazienti pediatrici e adulti".

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