MEDWAVE-2 Clinical Trial Report

Using the device on subjects aged 0-24 months

UNIVERSITÀ DEGLI STUDI DI TRIESTE

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1. DESCRIPTION OF THE CLINICAL TRIAL

The clinical trial on **med·wave®** was conducted in the following six Italian hospitals:

1. **The Agostino Gemelli Foundation University IRCCS Polyclinic - Rome** Principal investigator: **Prof. Guido Conti**;
2. **The Guglielmo da Saliceto Hospital, Piacenza**; Principal investigator: **Prof. Domenico Cuda**;
3. **Federico II University Hospital, Naples**; Principal investigator: **Prof. Gennaro Auletta**;
4. **The Giovanni XXIII Hospital, Bari**; Principal investigator: **Prof. Nicola Antonio Adolfo Quaranta**;
5. **The San Matteo Foundation IRCCS Polyclinic, Pavia**; Principal investigators: **Prof. Marco Benazzo and Prof. Pietro Canzi**;
6. **The Burlo-Garofolo IRCCS Maternal and Child Health Center, Trieste**; Principal investigator: **Dr. Domenico Leonardo Grasso**;

The aims of investigation activities were:

- **to validate and demonstrate that the use of the med·wave® device on subjects younger than 2 years of age is completely safe and free of contraindications and adverse events**;

- **to confirm its effectiveness, which has already been established in previous clinical investigation MEDWAVE-1**.

Specifically, the following objectives have been posed:

1. to analyze the device's output parameters during use on healthy subjects from 0 months upwards after dividing the population under study into the following age groups:

- 0 – 6 months;
- 7 – 12 months;
- 13 – 24 months;
- 25 months - 6 years;
- 7 - 18 years;
- Over 18 years.

2. To analyze the device's output parameters in the following pathologies of the middle ear:

- Endo-tympanic effusion;
- Anatomic outcomes of otitis media;
- Tympanic retraction/Atelectasis;
- Tympanic membrane perforation;
- Otosclerosis;
- Other unperforated eardrum pathologies.

- Inclusion criteria: pediatric subjects (0 months old and upward) who came to the otolaryngology clinics at the hospitals involved for ENT examinations, including those requiring tympanograms and otoscopy.

- Exclusion criteria:

- subjects with previous ear surgery capable of altering parameters under study;
- subjects with severe co-pathologies (severe disability requiring life-support systems, etc.) and cognitive deficits that prevent the autonomous granting and signature of patient consent;
- subjects with behavioral disorders that prevented or impeded the procedure planned.

- Study protocol: investigators were asked to examine each patient by traditional methods including tympanometry to ascertain the condition of the ear and then to take a measurement with the med·wave® device.
The clinical investigation lasted six months at each participating center.

2. RESULTS

2.1 Statistical analysis

Data collected at the six participating hospitals processed using appropriate software developed using Matlab are presented below.
The following graphs provide the number of ears for which measurements were taken divided by hospital and the number of ears for which data was processed and included in the statistical analyses after the requisite "cleaning" operation that consisted of excluding the admittance curves obtained for ears with resonance frequencies of higher than 1000 Hz resulting in shapes entirely different from those recorded in most cases for such reason considered measurement errors caused by incorrect probe positioning in order to avoid compromising the trend otherwise expected. Specifically, 87 ears were discarded from the total 669 acquired; in other words, 13% of the acquired data were not considered in the final processing, a figure within the range of the drop-out value previously envisioned in the study protocol and therefore deemed acceptable (Tab. 1).

The following tables also provide the ear data processed as divided by subject age group and pathology considered (Tab. 2-3).

In particular, this clinical investigation report focuses only on the results for subjects aged 0-2 years.

NUMBER OF EARS AVAILABLE

	ROMA	PIACENZA	BARI	NAPOLI	PAVIA	TRIESTE	TOTAL
ACQUIRED DATA	166	57	71	76	163	136	**669**
VALID DATA	154	54	65	61	145	103	**582**

13% of the acquired data were discarded due to: curves altered by noise, wobbly curves or those with F_{ris} > 1000 Hz

EARS DIVIDED BY PATHOLOGY CONSIDERED

	ROMA	PIACENZA	BARI	NAPOLI	PAVIA	TRIESTE	TOTAL
HEALTHY	82	32	43	46	67	44	**314**
TYMPANIC PERFORATION	10	0	1	2	13	5	**31**
OTOSCLEROSIS	24	13	1	0	13	0	**51**
UNPERFORATED EARDRUM PATHOLOGIES	19	6	6	1	35	2	**69**
TYMPANIC RETRACTION	7	1	5	0	4	21	**38**
ENDO-TYMPANIC EFFUSION	10	0	4	7	13	30	**64**
ANATOMIC OUTCOMES OF OTITIS MEDIA	2	2	5	5	0	1	**15**
TOTAL	154	54	65	61	145	103	**582**

EARS DIVIDED BY AGE GROUP

	0-2 YEARS	2-6 YEARS	6-18 YEARS	> 18 YEARS	TOTAL
HEALTHY	67	55	63	129	**314**
TYMPANIC PERFORATION	1	3	7	20	**31**
OTOSCLEROSIS	0	0	0	51	**51**
UNPERFORATED EARDRUM PATHOLOGIES	1	0	9	59	**69**
TYMPANIC RETRACTION	6	9	12	11	**38**
ENDO-TYMPANIC EFFUSION	16	22	7	19	**64**
ANATOMIC OUTCOMES OF OTITIS MEDIA	0	2	2	11	**15**
TOTAL	91	91	100	300	**582**

Tab. 1-2-3 / Number of measurements acquired at each hospital by pathology and age group.

The average admittance curves derived from the measurements acquired on subjects aged 0-2 years are presented below: the curves show the modulus of admittance on the ordinates and the frequency of stimulation on the abscissae; the different types of subjects are distinguished by color (Fig.1):

Black = Healthy,
Light blue = Tympanic retraction
Violet = Endo-tympanic effusion (or Otitis media - OM)

The following (Tab.4) shows the mean values and standard deviations (SD) of the seven parameters calculated in subjects aged 0 to 2 years and their calculated p-values:

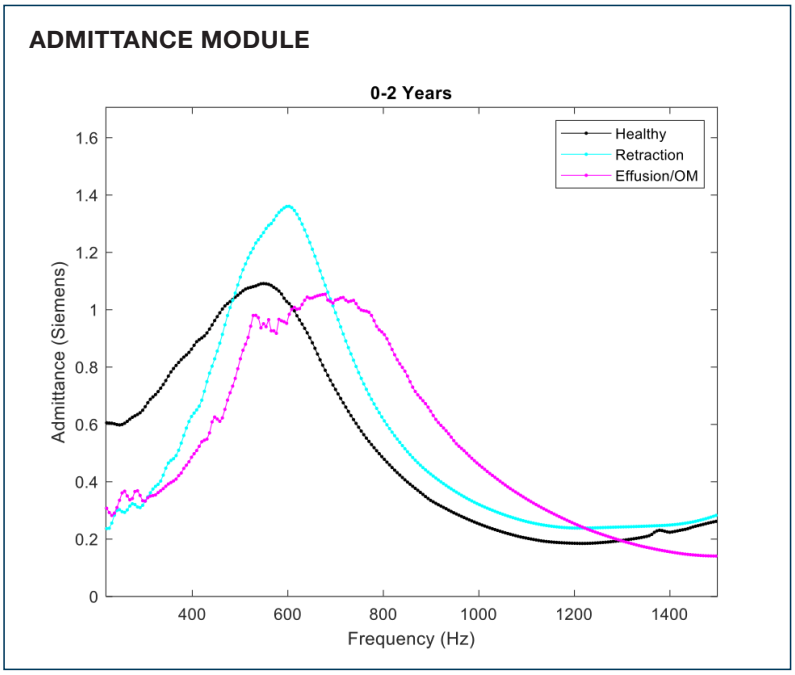


Figure 1. / Admittance module on the y-axis; Stimulation frequency on the x-axis.

0-2 YEAR OLD AGE GROUP COMPARISON								
	HEALTHY		OM		OM+RETRACT		P-VALUE	
	mean	SD	mean	SD	mean	SD	H VS OM	H VS OM+RETR
Vol (cm3)	0.80	0.33	0.49	0.22	0.62	0.14	0.00017	0.00015
Fq	2.36	0.69	2.32	1.60	2.30	0.61	n.s.	n.s.
Peak (Siemens)	1.33	0.47	1.40	0.82	1.54	0.56	n.s.	n.s.
Altbnd (Siemens)	0.85	0.24	0.83	0.39	0.91	0.29	n.s.	n.s.
Fris (Hz)	538	81	662	120	580	50	0.00017	0.00015
minBnd (Hz)	413	72	490	77	457	45	0.0011	0.0009
maxBnd (Hz)	668	116	839	128	718	86	0.00003	0.00004

Tab. 4 / Mean and Standard Deviation values of the 7 parameters

Examining the significance ($p<0.05$) of the pairwise difference from a statistical point of view using Wilcoxon's nonparametric test with Bonferroni's correction (to account for the number of comparisons to be made), we see that the parameters Fq (curve quality factor), peak (Peak), and band height (AltBnd) do not differ significantly between any pair of case types, whereas the resonance frequency (Fris) and its correlates (Vol, MinBnd and maxBnd) significantly differentiate the healthy from the other groups, both at individual and group level.

The box plot (Fig.2) below illustrate in graphic form the numerical description provided in the table above.

Red lines in the box plots indicate the median values; the edges of the box indicate the 25th and 75th percentiles. The black bars outside the blue boxes indicate the range in which the values that cannot be considered outliers fall, which are instead marked with red crosses above or below the black bars and are the most extreme data points.

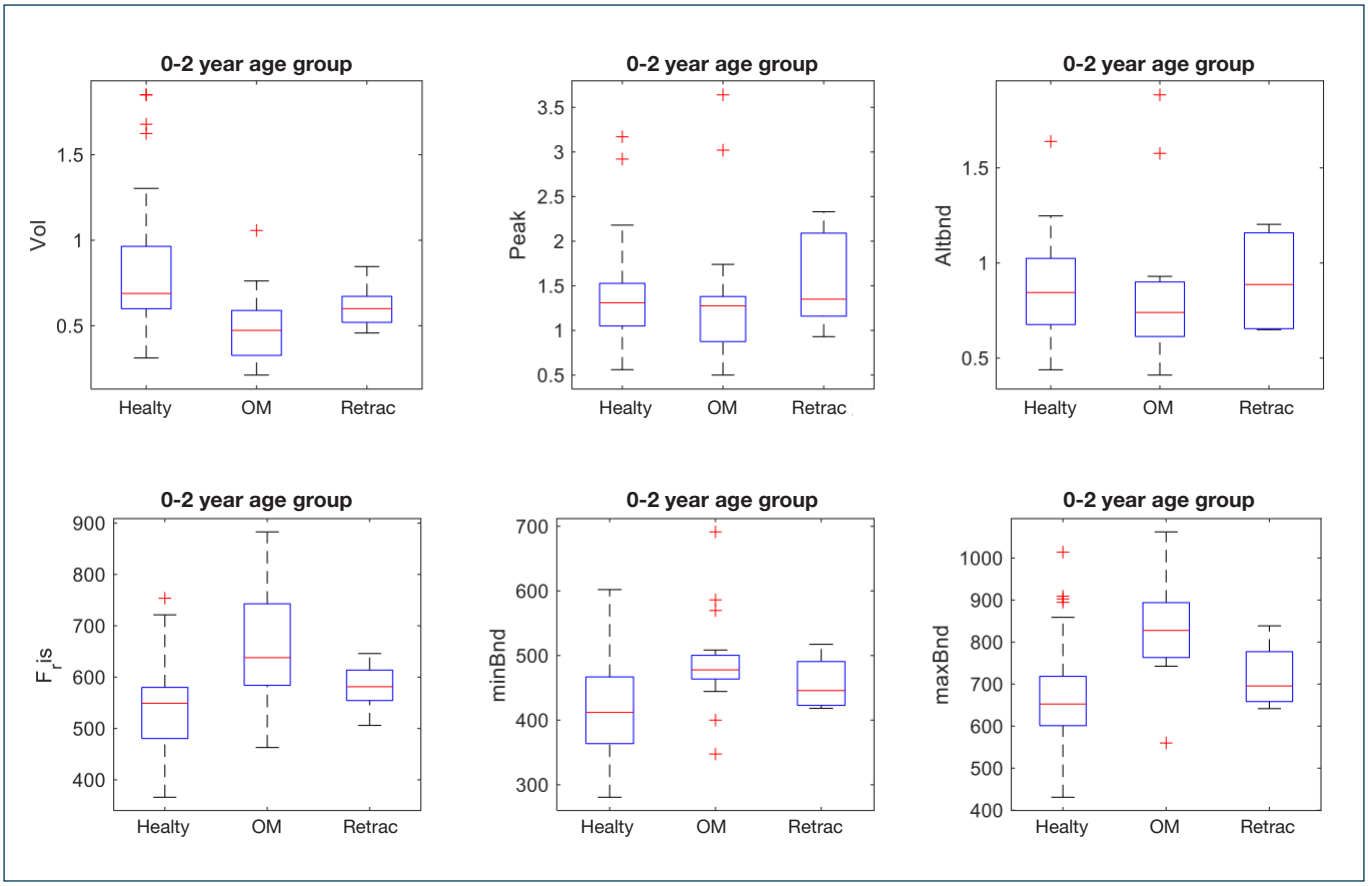


Figure 2. / Box plot for Resonance Frequency.

2.2 Classifiers

In order to test whether the use of multiple parameters at the same time allows identification in single cases, a series of classifiers capable of quantifying the performance of the device in distinguishing between PASS and REFER cases was considered, in other words, differentiation between healthy and pathological.

For this purpose, 3 Machine Learning classification models (Neural Network, Weighted Logistic Regression, and Tree) were applied, and then the one that provided the best performance was selected, starting from the Confusion Matrix.

The Confusion matrix generally assumes the dimension N × N where N is the number of classes C0, C1, . . . CN; here, N = 2. The specific TP(Ci), TN(Ci), FP(Ci) and FN(Ci) values for each class can be defined by using the approach illustrated in the figure. The metrics calculated for each class are: Sensitivity (or Recall), Specificity, and Accuracy, whereas the metrics used to evaluate the overall performance of the network are the Accuracy and Area under ROC curve (AUC). These metrics are described below.

Sensitivity or Recall of Class Ci (when Class Ci is considered positive)

Sensitivity estimates the probability of obtaining a positive test result with subjects grouped in Class Ci considered 'positive'. In other words, it indicates the ratio of classified positive cases to the sum of all true positive cases, or rather, the ability of a test to detect what fraction of all true positive samples was correctly predicted as positive by the classifier.

$$\text{SENSITIVITY (CI)} = \frac{TP(CI)}{TP(CI)+FN(CI)}$$

Specificity of Class Ci (when Class Ci is considered positive)

Specificity indicates the percentage of correctly recognized negatives, i.e., the probability of obtaining a negative test result in a negative case. In other words, it indicates the ability of a diagnostic procedure to recognize negative cases.

$$\text{SENSITIVITY (CI)} = \frac{TN(CI)}{TN(CI)+FP(CI)}$$

Precision of Class Ci (when Class Ci is considered positive)

Precision indicates the ratio of correctly classified positive cases to all cases classified as positive.

$$\text{SENSITIVITY (CI)} = \frac{TP(CI)}{TP(CI)+FP(CI)}$$

Classification accuracy (CA)

Classification accuracy is defined as the ratio of correctly classified cases out of all cases considered. The closer CA comes to 1, the more correctly the algorithm has correctly classified all cases.

$$CA = \frac{TP+TN}{TP+TN+FP+FN}$$

ROC and AUC Curve

AUC (Area Under the Curve) is a parameter indicating the validity of the test, and is calculated from the ROC curve of the classifier. As shown in the figure, this curve is derived from a graph constructed by plotting the pairs of specificity on the x-axis and of sensitivity on the y-axis in accordance with the variations in probability.

Figure 3. / ROC and AUC Curve

The shape of a ROC curve and the AUC (the area subtended by the ROC curve) let us estimate how high a test's discriminating power may be. The closer the curve comes to the upper left corner, the higher the AUC will be, and the better the test is at discriminating between the different situations considered.

In this specific case, the best classifier was the one based on the Logistic Regression Balanced algorithm; the confusion matrix of the comparison between healthy subjects and subjects with otitis media is provided below:

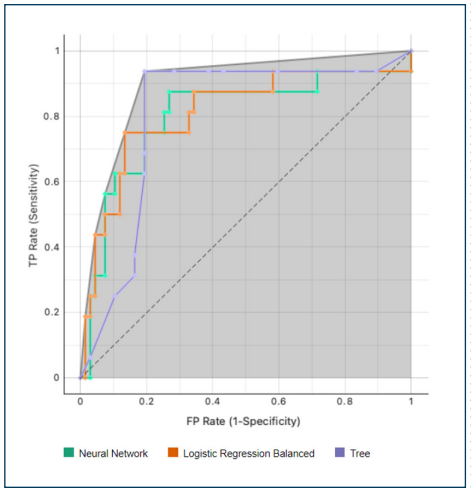
Confusion matrix (HEALTHY vs. OM)
Confusion Matrix:

		Predicted		
		OM	HEALTHY	Σ
ACTUAL	**OM**	12	4	**16**
	HEALTHY	12	55	**67**
	Σ	**24**	**59**	**83**

The overall performance metrics were:

AUC	CA	Precision	Recall	Specificity
0.81	0.81	0.85	0.81	0.76

The ROC curve of this classifier shows an AUC of 0.81. The overall accuracy is 81%, which indicates a good performance of the classifier.



In order to make the distinction between healthy and pathological subjects subsequently more clear, an additional binary classifier based on comparing the healthy group with the pathological group was applied that considered all cases of otitis and retraction together, and the Confusion matrix and the respective metrics were calculated.

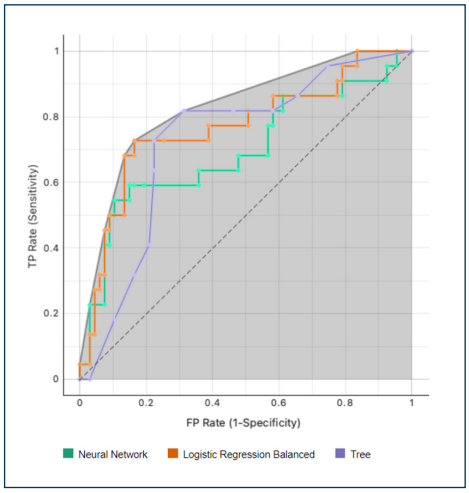
Confusion Matrix (HEALTHY vs. OM+RETRACT.)

		Predicted		
		OM	HEALTHY	Σ
ACTUAL	**OM**	56	11	**67**
	HEALTHY	6	16	**22**
	Σ	**62**	**27**	**89**

The overall performance metrics were:

AUC	CA	Precision	Recall	Specificity
0.77	0.81	0.83	0.81	0.75

The ROC curve of this classifier shows an AUC of 0.77. The overall accuracy here is also 81%, which indicates a good performance of the classifier.



3. CONCLUSIONS

Ultimately, it may be inferred that the objective of validating the use of med·wave® device as a PASS/REFER instrument in the 0-2 age group was fulfilled by the fact that the device proved capable of distinguishing healthy subjects from those with pathologies.
Several of the parameters proposed and calculated—the best of which was Resonance frequency—permitted the significant distinction of the group of healthy subjects from those with the pathologies effectively found.
Lastly, the binary classifiers based on logistic regression enabled 80% accuracy, in such way confirming the possibility of using the med·wave® device as an instrument to make PASS/REFER distinctions in newborns.

All the analyses have been conducted by the Bioengineering Group of the University of Trieste.

About Neuranix

Neuranix is an innovative company that develops and produces diagnostic devices for pathologies of the middle ear. Neuranix turns conventional sealed or closed diagnostics into open ear diagnostics. It has developed med·wave®, the first OPEN middle ear assessment device based on proprietary PLAI® Pressure-Less Acoustic Immittance technology, able to determine conditions in the middle ear painlessly and in a matter of seconds.

The Neuranix mission is to enlarge access to sensitive paediatric patients and post-surgery patients patients for whom current impedance technology cannot thereby also improving access to treatment for the 700 million people (World Health Organization 2021) who suffer from acute middle ear infections every year.

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