

# Study of acoustic immittance measures with probe tone of 226 and 1000 Hz in neonates

## Estudo das medidas de imitância acústica com tom sonda de 226 e 1000 Hz em neonatos

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### ABSTRACT

**Purpose:** Study the measures of volume, peak pressure and complacency in tympanometric curves of neonates with gender, ears and tone probe of 226 and 1000 Hz and analyze the responses obtained in the survey of the acoustic reflections with the two tone probe tested. **Methods:** transversal study, observational and contemporary. We evaluated 73 neonatal with integrity of outer hair cells observed by transient evoked otoacoustic emissions. The children performed the evaluation of measures of acoustic immittance clinical outcome, observation of the answers obtained in tympanometries and research of acoustic reflexes with tone probe 226 and 1000 Hz. **Results:** We observed statistically significant difference between the curves of single and double peak, with a higher occurrence of single peak with tone probe of 1000 Hz. There was statistically significant difference between the genders as complacency in 226 Hz and between the ears in compliance measures and peak pressure in 226 and 1000 Hz, respectively. In addition, we found statistically significant difference in the average of the ipsilateral and contralateral acoustic reflexes with tone probe of 1000 Hz against the variable gender and ipsilateral and contralateral reflexes between the frequencies of 1000 and 2000 Hz with tone probe of 226 and 1000 Hz. **Conclusion:** The results obtained in this study showed statistically significant difference between analyzed variables with tone probe of 226 and 1000 Hz tone. The compliance values and reflexes were higher in females. All neonates had acoustic reflexes, and reflexes contralateral higher than the ipsilateral.

**Keywords:** Hearing; Compliance; Child; Reflex, Acoustic; Acoustic impedance tests

### RESUMO

**Objetivo:** Estudar as medidas de volume, pressão do pico e complacência obtidas nas curvas tímpanométricas de neonatos, na comparação entre gêneros e orelhas, utilizando tom sonda de 226 e 1000 Hz, e analisar as respostas obtidas na pesquisa dos reflexos acústicos com os dois tons de sonda testados. **Métodos:** Foram avaliados 73 neonatos, com integridade de células ciliadas externas verificadas pelas emissões otoacústicas evocadas transientes. Foi realizada a avaliação das medidas de imitância acústica nas crianças, tendo como desfecho clínico a observação das respostas obtidas nas tímpanometrias e na pesquisa dos reflexos acústicos com tom sonda de 226 e 1000 Hz. **Resultados:** Observamos diferença entre as curvas de pico único e pico duplo, com maior ocorrência de pico único com tom sonda de 1000 Hz. Verificamos diferença entre os gêneros, na medida complacência em 226 Hz e entre as orelhas, nas medidas complacência e pressão do pico em 226 e 1000 Hz, respectivamente. Encontramos, também, diferença nas médias dos reflexos acústicos ipsilaterais e contralaterais com tom sonda de 1000 Hz, em relação a variável gênero, e entre os reflexos ipsilaterais e contralaterais nas frequências de 1000 e 2000 Hz com tom sonda de 226 e 1000 Hz. **Conclusão:** Houve diferença entre as variáveis analisadas com tom sonda de 226 e 1000 Hz. Os valores de complacência e de reflexos foram mais elevados no gênero feminino. Todos os neonatos apresentaram reflexos acústicos, sendo os contralaterais mais elevados que os ipsilaterais.

**Descritores:** Audição; Complacência; Criança; Reflexo acústico; Testes de impedância acústica

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## INTRODUCTION

The measures of acoustic immittance consist of two objective tests: tympanometry and the research of acoustic reflexes (AR)<sup>(1)</sup>. These are tests that do not need the patient's response and make it possible to verify the integrity conditions and the function of the middle ear in adults and neonates<sup>(2)</sup>. The measures of acoustic immittance must be utilized in the clinical practice of neonates, since they constitute important objective assessment when confirming an audiological diagnosis<sup>(3)</sup>.

The tests of acoustic immittance are important because when an alteration of the middle ear exists, it becomes more difficult to interpret in the other tests that compose the audiological evaluation and, therefore, it is harder to establish the distinction between auditory loss of the conductive and the sensorineural<sup>(4)</sup> type. In order to make sure the audiological diagnosis with neonates is made in a reliable and accurate way, it is necessary to evaluate the conditions of the middle ear<sup>(3)</sup>.

Impedance is the term normally used to indicate the impedance or admittance which is in opposition to the acoustic energy flow by means of a system, in this case the tympano-ossicular<sup>(1)</sup> system. The impedance measures are capable of supplying quantitative information about the fluid presence in the middle ear and the volume of the external acoustic meatus<sup>(5)</sup>. The values of admittance or impedance of the tympano-ossicular system characterize the tympanometric curves<sup>(2,6)</sup>.

The research of AR is performed by means of a tone stimulus of strong intensity, which will make possible the contraction of the middle ear muscles, especially the stapedius. It can be performed ipsilaterally (on the same side in which the stimulus was presented) or contralaterally (on the opposite side of the stimulus presented) in relation to the tested ear<sup>(6-9)</sup>. The research allows to analyze and measure the thresholds of the acoustic reflexes in the frequencies of 500, 1000, 2000 e 4000 Hz<sup>(7)</sup>. In order that the capture of acoustic reflexes may be carried on, the integrity of the tympano-ossicular system and the afferent and efferent auditory pathways of the reflex arc are necessary<sup>(6)</sup>.

The acoustic reflexes involve auditory nuclei of the encephalic trunk, which relate to auditory processing skills. Acoustic reflex alterations may, therefore, indicate an alteration in one of these nuclei and, consequently, in auditory processing skills such as locating, selective attention and speech with background noise<sup>(10)</sup>.

The alterations of the middle ear may cause conductive auditory loss and influence the research of the cochlear function. Therefore, it is necessary to evaluate these conditions in order to obtain a reliable diagnosis of the neonate<sup>(3)</sup>. The research of the cochlear function can be analyzed by means of Evoked Otoacoustic Emissions and the transient click stimulus (TEOA) is the most recommended by the literature in neonatal auditory triages with children without risk indicators<sup>(3,6)</sup>. However, obtaining a trustworthy diagnosis of the dysfunction of the middle ear in newborns is a very challenging task. Otoscopy is difficult

for this age group, since the osseous walls of the external acoustic meatus are not formed at birth, and the cartilaginous walls are flaccid and narrow. Besides, the presence of vernix in the acoustic meatus may hinder the examiner's view<sup>(11)</sup>.

Conventional tympanometry is performed with probe tones of 226 Hz, and the results obtained have considerable diagnostic value for adults, elderly and children older than six months of age. However, there are controversies in the literature regarding the use of the same tone test with breast-fed new-born babies and neonates<sup>(12,13)</sup>. Some studies show that tympanometry with normal standards breast-fed new-born babies with absence of TEOA and in the occurrence of some conductive alteration may be applied. For this reason, several authors have suggested the investigation with test tone of 1000 Hz, because discreet alterations of the middle ear may not be identified with a 226 Hz probe<sup>(3,6,13,14)</sup>. On the other hand, several studies carried on in Brazil<sup>(15,16)</sup> defend the use of 226 Hz test tone in neonates aged eight months or less, as reliable results have been found. Some researchers<sup>(15,17,18)</sup> have investigated the use of high-frequency probes (678 and 1000 Hz) in neonates in the search for more sensitive results for middle ear alterations. Studies<sup>(17,19)</sup> demonstrate that tympanometry tests with high-frequency tones was more sensitive for middle ear alterations when performed with breast-fed babies of up to six months of age, considering the resonance of the middle ear of this age group.

Starting from the considerations made above, we emphasize the need to carry on more tympanometry researches in neonates making use of both test conditions (226 e 1000 Hz). It is possible to research the thresholds of the acoustic reflexes in breast-fed babies, or even in the neonatal period, and, therefore, obtain information about the auditory pathways<sup>(10,15)</sup>. In literature there are studies which demonstrate the presence of contralateral acoustic reflex in neonates<sup>(20)</sup>, while there are others which demonstrate the presence of ipsilateral acoustic reflex in breast-fed babies<sup>(6,21,22)</sup>.

We believe that the studies obtained in the measures of acoustic immittance in neonates may help outlining procedures and the choice of identification protocols for the identification of middle ear alterations in this group, making a diagnostic and a treatment viable.

Hence, the objective of the present study is to analyze and compare volume measures, complacency and peak pressure in tympanometric curves of neonates, in the comparison between genders and ears, using 226 and 1000 Hz probes, besides identifying the presence or absence of ipsilateral and contralateral acoustic reflex, with the two kinds of probes tested in neonates in their first month of life.

## METHODS

An observational, contemporaneous and transversal study was performed. The sample consisted of 73 neonates aged 4 to 29 days, of which 38 were female and 35, male. They

were cared for between november and december 2011, in the Auditory Health Outpatient Clinic of the Speech-Language Pathology and Audiology Service of the Nossa Senhora da Conceição Hospital in Porto Alegre (HNSC).

The present study was submitted and approved by the Ethics and Research Committee of the Nossa Senhora da Conceição Hospital in Porto Alegre, protocol number 11137. The Term of Free Willing Approval for the inclusion of neonates in the research was properly signed by the parents or guardians.

The following criteria for the inclusion in the sample group was adopted: full-term neonates whose gestational ages varied from 37 weeks or older and with evoked otoacoustic emissions by transient stimulus (TEOA) present in both ears. Neonates with indicators of hearing loss risk were excluded, according to the criteria proposed by the Joint Committee on Infant Hearing (JCIH)<sup>(23)</sup> and who failed in the auditory triage.

The TEOA, which evaluated the integrity of the external ciliated cells, were performed in the Auditory Health Outpatient Clinic of the Speech-Language Pathology and Audiology Service of the Nossa Senhora da Conceição Hospital using Scout model equipment of Biologic®.

Each ear was analyzed at a time with a click stimulus of 70-75 decibels (dB) equivalent peak, on sound pressure level (SPL), considered "pass" when they obtained a signal/noise relation of (S/N) equal or above 6 dB in consecutive 1000 to 4000 Hz frequencies, with reproducibility of 75% in each frequency and general reproducibility equal or above 70%. When these responses were absent they were considered "fail", i.e., only the neonates who presented "pass" parameters in the TEOA were submitted to the evaluation of measures of acoustic immittance.

After this evaluation, identification data of the children were collected with the guardians and the information was registered in their health cards and hospitalization charts in order to select the sample according to the criteria for inclusion in the study. The TEOA test and the immittance measures were performed in silent and appropriate environment. In the evaluation of the measures of acoustic immittance, a probe was inserted in the neonates' external auditory meatus, applying variable pressure of +200 daPa to -300 daPa, with speed of 50 daPa/s. This examination was performed with probes of 226 and 1000 Hz and 425h model equipment of Interacoustics®. The neonates were subjected to the examination without an order of probe presentation; sometimes the 226 Hz probe was presented first, other times it was the 1000 Hz probe. The choice of ear to begin the testing was made randomly, depending on the position of the neonate as he or she was held by the guardian. The neonates were in a quiet state or naturally asleep and there was a pause during the examination, whenever it was necessary.

The following quantitative measures were obtained and evaluated in the tympanograms with 226 Hz and 1000 Hz probes: complacency or static admittance, external acoustic meatus volume and peak pressure in the external acoustic

meatus. When a double peak occurred, the higher peak measures were obtained.

Finally, a research of the ipsilateral (dBNPS) and contralateral (dBNA) acoustic reflexes was carried out with 226 Hz and 1000 Hz probes. The conversion between contralateral and ipsilateral acoustic reflex intensity was not carried out. In the ipsilateral reflex, the thresholds were researched in the 1000 and 2000 Hz frequencies, and in the contralateral reflex, with 500, 1000, 2000 e 4000 Hz frequencies with intensity between 70 and 110 dB.

In the statistical analysis the variance test (ANOVA) for the significance analysis of complacency of static admittance quantitative measures, external acoustic meatus and peak pressure in the external acoustic meatus was used, and the gender factor (male/female) was applied. For the analysis of the ipsilateral and contralateral acoustic reflexes thresholds for each ear, in the different frequencies tested with 226 and 1000 Hz probes, the t-Student test was used for paired samples, as well as the quantitative measures obtained with the tympanograms in relation to the ear variable (right/left). In the comparison of peak quantities (occurrence of single or double peak), McNemar's chi-squared test was used. In the comparisons of the results of the acoustic reflexes thresholds between genders, we used the t-Student test for independent samples. All of these tests were carried out with a significance level of 95% ( $p < 0.05$ ).

## RESULTS

We registered 146 tympanograms for each probe tested (226 and 1000 Hz). The research of the acoustic reflexes could only be carried out in 71 neonates, making it a total of 142 researches, because two neonates cried during the examination, so no threshold was obtained.

The tympanometric findings revealed tympanometric curves characterized by a single peak (SP) and a double peak (DP) with 226 and 1000 Hz probes. In the findings with 226 Hz probes, we observed that there were no differences in the comparison between SP and DP in the analyzed ears. With the 226 Hz test, the SP percentage was 47.9% (RE) and 46.6% (LE), while the DP percentage was 52.1% (RE) e de 53.4% (LE). However, with the 1000 Hz probe, we observed a difference between SP and DP ( $p < 0.001$ ), with percentages reaching 87.7% (RE) and 79.5% (LE) for SP and 12.3% (RE) and 20.5% (LE) for DP (Table 1).

Besides the analysis of single peak and double peak occurrences, the quantitative variables of the data obtained in the tympanograms with 226 and 1000 Hz probes were also evaluated. When using the 226 Hz probe, we observed the significance effect of the gender variable for the complacency measure ( $p = 0.011$ ) in the left ear (LE). When using the 1000 Hz probe, there was no difference whatsoever in any of the measures analyzed in relation to this variable (Table 2).

**Table 1.** Comparison between single peak and double peak tympanometric curves with probe tone of 226 and 1000 Hz per ear

Peaks	226 Hz n (%)	1000 Hz n (%)	p-value
Right ear			<0.001*
SP	35 (47.9)	64 (87.7)	
DP	38 (52.1)	9 (12.3)	
Left ear			<0.001*
SP	34 (46.6)	58 (79.5)	
DP	39 (53.4)	15 (20.5)	

\*Significant values ( $p < 0.05$ ) – McNemar's Chi-squared test

**Note:** SP = single peak; DP = double peak

With the 226 Hz probe, the significance effect of the RE and the LE for complacency measure ( $p = 0.002$ ) and pressure ( $p < 0.001$ ) was observed. With the 1000 Hz probe, there was also a difference for the complacency measure ( $p = 0.004$ ) and for the peak pressure ( $p = 0.045$ ), in both ears (Table 3).

The results of the mean complacency with the 226 and 1000 Hz probes, for each ear, obtained with the t-Student test for paired samples can be observed in Figure 1. The circle

represents the average and the lower and upper error bars represent the interval limits of 95% of reliability. We found 100% of A-type curves, according to Jerger's<sup>(24)</sup> classification.

In the analysis of the ipsilateral and contralateral acoustic reflexes in the tested frequencies in relation to the test tone variable in the RE, we verified a difference between the ipsilateral and contralateral reflexes in the 1000 and 2000 Hz frequencies with 226 and 1000 Hz probes, all of them with  $p < 0.001$  and the contralateral reflexes thresholds were superior (Table 4).

In the analysis of the ipsilateral and contralateral acoustic reflexes with the respective tested frequencies in relation to the test tone variable in the LE, we observed a significance effect between the ipsilateral and contralateral reflexes in the 1000 and 2000 Hz frequencies with 226 and 1000 Hz probes, all of them with values of  $p < 0.001$ , except the ipsilateral and contralateral reflexes in the 2000 Hz frequencies with 226 Hz probe, with values of  $p = 0.022$ . In the analysis between the ipsilateral and contralateral acoustic reflexes in the tested frequencies, we verified a difference between the RE and the LE in the 1000 and 2000 Hz frequencies with both test probes. We observed that the threshold averages of the ipsilateral and contralateral acoustic reflexes, in relation to the gender variable, presented

**Table 2.** Comparison between genders relating to the measures of acoustic immittance with probe tones of 226 Hz

Variables	Total sample (n=73)	Male gender (n=35)	Female gender (n=38)	p-value
	Average $\pm$ SD [min – max]	Average $\pm$ SD [min – max]	Average $\pm$ SD [min – max]	
226 Hz – OD				
Volume	0.44 $\pm$ 0.18 [0.12 to 1.04]	0.48 $\pm$ 0.21 [0.14 a 1.04]	0.41 $\pm$ 0.15 [0.12 a 0.77]	0.133
Complacência	0.64 $\pm$ 0.20 [0.0 to 1.2]	0.61 $\pm$ 0.23 [0.0 to 1.2]	0.66 $\pm$ 0.17 [0.3 to 1.1]	0.262
Pressão	31.7 $\pm$ 24.7 [-17 to 78]	33.8 $\pm$ 24.5 [-17 to 75]	29.8 $\pm$ 25.1 [-16 to 78]	0.499
226 Hz – OE				
Volume	0.50 $\pm$ 0.26 [0.19 to 1.44]	0.54 $\pm$ 0.33 [0.19 to 1.44]	0.46 $\pm$ 0.17 [0.19 to 0.79]	0.206
Complacency	0.55 $\pm$ 0.13 [0.3 to 0.8]	0.59 $\pm$ 0.12 [0.4 to 0.8]	0.51 $\pm$ 0.13 [0.3 to 0.8]	0.011*
Pressure	15.8 $\pm$ 34.4 [-85 to 95]	10.7 $\pm$ 33.6 [-85 to 95]	20.5 $\pm$ 34.9 [-85 to 75]	0.224

\*Significant values ( $p < 0.05$ ) – One-Way Variance Analysis (ANOVA)

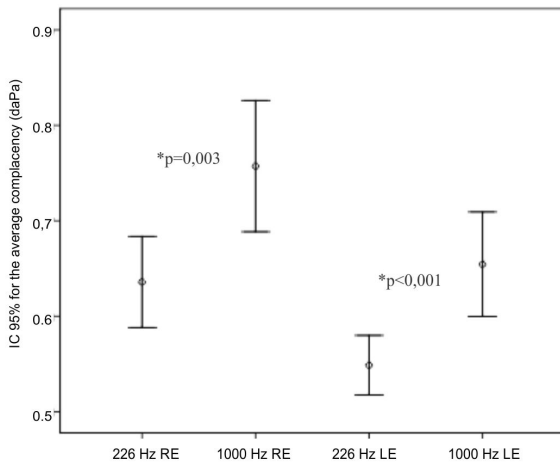
**Note:** RE = right ear; LE = left ear; SD = standard deviation

**Table 3.** Comparison between ears relating to the measures of acoustic immittance with probe tones of 226 and 1000 Hz

Variables	RE	LE	p-value
	Average $\pm$ SD [min – max]	Average $\pm$ SD [min – max]	
226 Hz			
Volume	0.44 $\pm$ 0.18 [0.12 to 1.04]	0.50 $\pm$ 0.26 [0.19 to 1.44]	0.087
Complacency	0.64 $\pm$ 0.20 [0.0 to 1.2]	0.55 $\pm$ 0.13 [0.3 to 0.8]	0.002*
Pressure	31.7 $\pm$ 24.7 [-17 to 78]	15.8 $\pm$ 34.4 [-85 to 95]	<0.001*
1000 Hz			
Volume	0,50 $\pm$ 0,24 [0.13 to 1.00]	0.47 $\pm$ 0.26 [0.15 a 1.44]	0.482
Complacency	0,76 $\pm$ 0,29 [0,0 to 1,6]	0.66 $\pm$ 0.23 [0.2 a 1.3]	0.004*
Pressure	28.0 $\pm$ 27.5 [-50 to 82]	18.2 $\pm$ 35.5 [-85 to 95]	0.045*

\*Significant values ( $p < 0.05$ ) – t-Student test for paired samples

**Note:** RE = right ear; LE = left ear; SD = standard deviation



\*Significant values ( $p < 0.05$ ) – t-Student test for paired samples

Note: RE = right ear; LE = left ear

**Figure 1.** Analysis of mean complacency with probe tone of 226 and 1000 Hz

a difference with the 1000 Hz probe in the ipsilateral reflexes tested and in two of the four contralateral tested reflexes, all of them with stimuli in the 1000 Hz frequency. The averages were greater in the female gender (Table 5).

We verified a difference in the threshold averages of the ipsilateral and contralateral acoustic reflexes with 1000 Hz probes, in relation to both genders, as it can be observed in Figure 2.

## DISCUSSION

All of the neonates evaluated in this research presented a cochlear function which was considered normal, as they passed the TEOA test, which evaluates the function of the external ciliated cells, and they presented acoustic reflexes in both ears. In the classification of the tympanograms, we found 100% of type-A curves according to Jerger's<sup>(24)</sup> classification.

The tympanometries of the evaluated neonates presented curves with single peak (SP) and double peak (DP) with 226

**Table 4.** Analysis of ipsilateral and contralateral acoustic reflexes in the right ear in the different frequencies tested with probe tone of 226 and 1000 Hz

Frequencies	226 Hz	1000 Hz	p-value
	Average $\pm$ SD [min – max]	Average $\pm$ SD [min – max]	
500 Hz			
Contralateral	101.1 $\pm$ 7.0 [80 – 110]	100.8 $\pm$ 7.4 [85 – 110]	0.719
1000 Hz			
Ipsilateral	95.9 $\pm$ 7.0 [80 – 110]	95.4 $\pm$ 7.0 [80 – 110]	0.623
Contralateral	100.4 $\pm$ 7.4 [80 – 110]	100.2 $\pm$ 7.1 [80 – 110]	0.837
p-value*	<0.001*	<0.001*	
2000 Hz			
Ipsilateral	96.7 $\pm$ 6.5 [80 – 110]	95.9 $\pm$ 7.2 [80 – 110]	0.448
Contralateral	98.7 $\pm$ 7.2 [85 – 110]	99.0 $\pm$ 7.3 [80 – 110]	0.802
p-value *	0.001*	<0.001*	
4000 Hz			
Contralateral	98.9 $\pm$ 7.4 [80 – 110]	98.5 $\pm$ 7.1 [80 – 110]	0.648

\* Significant values ( $p < 0.05$ ) – t-Student test for paired samples

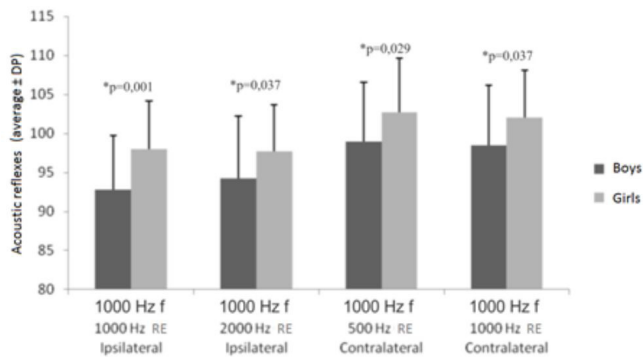
Note: SD = standard deviation

**Table 5.** Analysis of ipsilateral and contralateral acoustic reflexes in the left ear in the different frequencies tested with probe tone of 226 and 1000 Hz

Frequencies	226 Hz	1000 Hz	p-value
	Average $\pm$ SD [min – max]	Average $\pm$ SD [min – max]	
500 Hz			
Contralateral	101.8 $\pm$ 6.5 [85 – 110]	101.8 $\pm$ 6.7 [85 – 110]	0.937
1000 Hz			
Ipsilateral	95.3 $\pm$ 6.4 [80 – 110]	96.1 $\pm$ 7.1 [80 – 110]	0.398
Contralateral	101.4 $\pm$ 6.5 [80 – 110]	101.1 $\pm$ 6.3 [85 – 110]	0.747
p-value*	<0.001*	<0.001*	
2000 Hz			
Ipsilateral	96.8 $\pm$ 5.8 [85 – 110]	95.6 $\pm$ 6.2 [80 – 110]	0.204
Contralateral	100.6 $\pm$ 7.0 [80 – 110]	100.6 $\pm$ 6.6 [85 – 110]	1.000
p-value *	0.022*	<0.001*	
4000 Hz			
Contralateral	99.9 $\pm$ 6.9 [85 – 110]	99.9 $\pm$ 5.6 [90 – 110]	0.937

\* Significant values ( $p < 0.05$ ) – t-Student test for paired samples

Note: SD = standard deviation



\*Significant values ( $p < 0.05$ ) – t-Student test for paired samples  
**Note:** f = primary frequency; RE = right ear; LE = left ear

**Figure 2.** Analysis of averages of ipsilateral and contralateral acoustic reflexes thresholds in relation to the gender variable with probe tone of 1000 Hz

and 1000 Hz probe tones. In Jerger's<sup>(24)</sup> classification, only the SP type curve was classified as similar to type A, as previously defined by this author. In the literature, there is no reason that makes the occurrence of the other tympanometric curve types evident in neonates. However, there are several studies which consider the PD-type curve as an indicator of normalcy in this population, as well as the SP-type curve<sup>(18,25,26)</sup>. Jerger<sup>(24)</sup> classified the double peak curve as a D-type curve.

The tympanometric findings with 226 Hz and 1000 Hz probe tests, when comparing SP and DP, in the 146 ears analyzed, confirmed other studies which report a greater occurrence of the SP type with the 1000 Hz probe<sup>(17,18)</sup>.

We verified that the single peak curve classified by Jerger<sup>(24)</sup> suggests normalcy. In fact, it must be an indicator of a middle ear without alterations in the evaluated neonates, since all of them had the TEOA present in both ears and did not show indications of hearing loss risk. Therefore, their cochlear function was considered normal. Other authors consider the single peak tympanometric curve an indicator of adequate function and integrity of the middle ear<sup>(18,27)</sup>. In the researched literature, we have found authors who consider the double peak curve as a sign of a middle ear without alterations<sup>(18,25)</sup>. In this study, we observed a small occurrence of double peak curve in the analysis with the 1000 Hz probe, which proved the findings of another study<sup>(6)</sup>.

In the analysis of volume quantitative measures, complacency and peak pressure obtained in tympanometry, we observed a difference in the complacency values, considering the gender variable with the 226 Hz probe. However, with the 1000 Hz probe, we observed no difference in any of the measures analyzed, unlike the study researched in the literature<sup>(28)</sup>, which found a significance effect in relation to gender for the complacency measure with 1000 Hz and for the volume measure with 226 Hz probe.

In relation to the ear variable, we observed a difference in the complacency and peak pressure measures with the two probe tones tested, unlike the study<sup>(28)</sup>, which found no

significance effect for any measure with the 226 and 1000 Hz test probes. The indications of use of high-frequency probe (1000 Hz) in breast-fed babies occur due to the anatomical and physiological differences in the middle ear of this population<sup>(29)</sup>. Other studies report that the mass components are higher in the high-frequency probe tones, and lower in the low-frequency probe tones<sup>(17,30)</sup>.

In the analysis of mean complacency, in relation to the ear variable, we observed a difference, with a greater average in the right ear with 226 and 1000 Hz probe. However, we have found in the literature no reason that makes the occurrence of this difference between ears evident.

In the research of the ipsilateral and contralateral acoustic reflexes, 100% of the 142 ears analyzed showed the presence of reflex. This finding confirmed what was described in another study<sup>(30)</sup>, which found 100% of ipsilateral acoustic reflex in the breast-fed babies of its research with no hearing loss risk indicators and with a normal tympanometry. Even in neonates with a tympanometric curve with DP, it was possible to obtain the acoustic reflex thresholds, as referred to in another study in the scientific literature<sup>(20)</sup>.

The mean values of ipsilateral acoustic reflex found in this study, using the 1000 and 2000 Hz frequencies were between 95.3 and 101.8 dBNA. Smaller values, between 91 and 96 dBNA were found in the literature with research in neonates<sup>(21)</sup>.

We believe that the acoustic immittance measures must be utilized in the auditory diagnosis in neonates. However, they should not be considered in an isolated way; instead, they should be considered in conjunction with the results of other audiological procedures. Studies with the analysis of acoustic immittance in neonates are scarce. They are also necessary to improve the use of tympanometry in the auditory diagnosis. We believe that more researches approaching this theme are necessary, so that a greater amount of data can be compared and discussed, making, therefore, the reliability of the interpretation of the obtained results possible.

## CONCLUSION

We have observed a difference between the single peak and double peak curves, with greater occurrence of the single peak curve with 1000 Hz probe, as well as complacency values higher for the female gender with different pressure values. We have also observed ipsilateral and contralateral acoustic reflexes in all of the evaluated neonates, with superior values in the contralateral reflexes in the 1000 and 2000 Hz frequencies. All reflexes presented higher values in the neonates of the female gender.

We believe in the need for other comparative researches in neonates, with greater samples, so that the clinical application of 226 and 1000 Hz probe tones in the identification of alterations in the workings of the middle ear in this age group can be investigated.

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