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Original Article

Multichannel Intraluminal Impedance and pH Monitoring: A Step Towards Pediatric Reference Values

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Background/Aims
Combined multichannel intraluminal impedance and pH monitoring (MII/pH) is considered the most accurate test to detect gastroesophageal reflux (GER), however lacking reference values. We aim to determine reference values for the pediatric population and to correlate these values with age and postprandial/fasting period.

Methods
We evaluated MII/pH traces from patients (newborns, infants, and children) admitted to 3 Italian hospitals and who underwent MII/pH for suspected GER disease. Patients with MII/pH traces that showed significant symptom-reflux associations and/or a pathological reflux index (> 6% for newborns and infants, > 3% for children) were excluded. Traces were analysed in their entirety, and in the postprandial period (first hour after a meal) and the fasting period (the following hours before the next meal) separately.

Results
A total of 195 patients (46 newborns, 83 infants, and 66 children) were included. Age positively correlated with frequency of acidic GER events ($r = 0.37$, $P < 0.05$) and negatively associated with weakly acidic GER events ($r = 0.46$, $P < 0.05$).

Conclusions
This study describes the distribution of MII/pH values in a pediatric population with normally acidic GER exposure and no significant association between GER events and symptoms. These MII/pH values may be used as reference values in clinical practice for a corrected GER disease diagnosis in the pediatric population.
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Key Words
Child; Esophageal pH monitoring; Gastroesophageal reflux; Infant; Reference values

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Introduction

Gastroesophageal reflux (GER) is a common phenomenon in infants and children.[1] Clinical diagnosis of GER is laborious, as symptoms in infants and young children are not specific and overlap with a wide spectrum of different disorders. However, proper evaluation of GER events is important to identify patients with GER disease (GERD) and to avoid the inappropriate use of acid-suppressors, which are often prescribed on a clinical or own-experience basis.[2-6]

Combined multichannel intraluminal impedance and pH monitoring (MII/pH) provides a detailed description of esophageal events and is considered the most accurate test to detect GER in infants and children, although its use in clinical practice is not widespread.[7,8] Indeed, there are limitations to MII/pH, including the cost of the equipment, the need to carry out 24-hour monitoring sessions with a nasogastric probe, the experience needed to accurately analyse MII/pH traces, and the inter- and intra-observer variability of visual compared to automated analysis.[9]

Nevertheless, in the latest decade, MII/pH data has led to improvements in our knowledge of GER pathophysiology and has helped assess the relationship between GER events and symptoms.[10-13] MII/pH can detect all types of GER events, and these events can be classified according to concomitant changes in the intra-esophageal pH.[7,14] Furthermore, MII/pH allows us to analyze the relationship between GER events and symptoms through methods such as the symptom index (SI), symptom sensitivity index, and symptom association probability (SAP) index.

Among MII/pH parameters, the most known is the reflux index (RI). Other variables that can be derived from MII-channel recordings are GER frequency, duration, and proximal extent, and the bolus exposure index (BEI).[15] While the cut-off for RI has been reported,[1] reference values for other MII parameters in the pediatric population are still uncertain[7] and could be inappropriate as they are derived from adult populations.[16-18] As MII/pH is an invasive test, for ethical reasons it cannot be performed on healthy infants and children, making it challenging to obtain traditional reference values, ie, from a normal, healthy population, for MII/pH parameters. One way to overcome this problem is to consider a large, symptomatic population with negative MII/pH results (ie, results that do not indicate GERD). This population can be defined as a "reference" population because of its negative MII/pH results, even though it is not exactly "normal," because it is symptomatic.

Indexes of the association between GER events and symptoms currently have the same definition and values for all ages and can be helpful to classify a MII/pH trace as pathological or normal.[7-19] In the last 10 years, some authors[19-21] have described reference ranges for infants and children, but unfortunately, the uniqueness of the population recruited, the small sample size, or the lack of newborns younger than 3 weeks limited their use. The aim of the present study is to determine MII/pH reference values for the neonatal and pediatric population and to correlate these values with age and post-prandial/fasting period.

Materials and Methods

In this multicentric retrospective observational study we evaluated MII/pH traces from patients who underwent MII/pH for suspected GERD[22] (symptoms such as weight loss or poor weight gain, irritability, ruminative behavior, heartburn or chest pain, hematemesis, dysphagia, odynophagia, wheezing, stridor, cough, and hoarseness) at 3 Italian hospitals.

Indications, methodology and interpretation used for the MII/pH studies were the same in all centers, according Italian guidelines of the Italian Society of Pediatric Gastroenterology, Hepatology, and Nutrition (SIGENP) working group.[22]

Patients were enrolled in the study if they met the following inclusion criteria: (1) a valid MII/pH trace for a minimum 21 hours, of which a minimum of 16 hours excluding artifacts and meals; (2) absence of therapies with effects on GER for at least 1 week before MII/pH; (3) absence of congenital heart defects, infectious, genetic, metabolic, and neurological diseases; and (4) being fed orally without any tube feeding.

All newborns, infants, and children with a pathological RI ($> 3\%$ for children and $> 6\%$ for newborns and infants) and/or a significant association between GER events and symptoms (expressed as SI $\geq 50\%$ and SAP $\geq 95\%$), according to European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) guidelines and Mousa's study,[1,20] were excluded from the study.

During MII/pH recording newborns were kept supine and received breast milk or formula from a feeding bottle every 4 hours (thickened formulas were excluded).

Parents of included patients provided written informed consent to undergo MII/pH. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution's human research committee and it was approved by the Local Committee for Health Research Ethics in Turin, Italy (No. 0046662).

Patients were classified into 3 groups by age: newborns (aged

0-30 days), infants (aged 1-12 months), and children (1-16 years).

A single-use, age-appropriate (infant/pediatric) MII/pH catheter (Sandhill Scientific, Highland Ranch, CO, USA) was used. Each catheter has a diameter of 2.13 mm (6.4 F) and contains 7 impedance sensors, corresponding to 6 impedance channels and an antimony electrode sensitive to pH variation. The antimony electrode was calibrated with 2 different pH solutions (pH 4.0 and pH 7.0) before each MII/pH session. The appropriate catheter size was placed transnasally after a 3-hour fasting period. In newborns and infants, impedance channels were positioned every 1.5 cm, whereas in children they were placed every 2 cm, with the pH-electrode in the middle of the most distal channel, placed at the level of the second vertebra above the diaphragm. The approximate position of the probe was calculated according to Strobel's formula,[22] checked radiologically, and corrected if necessary. The catheter was then connected to an exterior impedance device (Sleuth System; Sandhill Scientific) for signal processing and recording. Parents and older children were instructed to press the "event" buttons on the device in case of specific symptoms. They were also asked to complete a written diary reporting any symptoms, meal and sleeping times, and position changes (supine/upright) that occurred during MII/pH.

The MII/pH traces were visually evaluated by a single expert operator from each center. To reduce inter-observer variability,[9] all operators had similar experience and analyzed the traces with the same software (BioView Analysis, Sleuth System; Sandhill Scientific). A MII-GER event was defined as a decrease in impedance starting in the most distal channel, extending proximally over 2 or more channels, and followed by an increase in impedance back to baseline values. The duration of a GER event was defined as the time (in seconds) between its onset (50% drop in impedance from baseline relative to nadir) and bolus exit (50% recovery point from nadir to baseline) recorded at the distal channel. The pH value of each MII-GER event was defined as the nadir esophageal pH recorded during that event and was classified as acidic (MII-A-GER, pH $<$ 4), weakly acidic (MII-WA-GER, 4 $\leq$ pH $>$ 7), or weakly alkaline (MII-WALK-GER, pH $\geq$ 7). A pH-GER event was defined as a temporary drop of the pH value below 4 lasting at least 5 seconds and not associated with a MII-GER event.[14-24]

Different MII/pH parameters were evaluated: GER frequency, expressed as GER/hour; proximal GER frequency, ie, GER reaching the 2 most proximal MII channels, expressed as GER/hour; bolus clearance time (BCT), expressed in seconds; BEI, defined as percentage of time in which the bolus was detected in the distal impedance channel; and RI, expressed as the percentage of time during which the distal esophagus is exposed to a pH $<$ 4. We chose to calculate GER frequency as GER/hour to provide comparable data in all patients regardless of the duration of MII/pH. In this way, we were able to analyse MII/pH traces in their entirety, and in the postprandial period (first hour after a meal) and the fasting period (the following hours before the next meal) separately.

Data are presented as numbers and percentages for categorical variables. Continuous variables were expressed as mean and standard deviation if normally distributed, or as median and interquartile range if normality could not be accepted. The paired sample *t* test was used to compare values in the postprandial and fasting periods. ANOVA was used to evaluate MII/pH variables among newborns, infants, and children. Pearson's test was used to evaluate the correlation between values of MII/pH parameters and age. A *P*-value $<$ 0.05 was considered statistically significant. Statistical analyses were performed using the STAT software package for Windows, release 5.5 (StatSoft, Tulsa, OH, USA).

Results

A total of 195 patients (46 newborns, 83 infants, and 66 children) had MII-pH traces that satisfied the inclusion criteria. The 3 centers analysed 75 (37 newborns, 37 infants, and 1 child), 65 (8 newborns, 20 infants, and 37 children), and 55 traces (1 newborn,

Table 1. Characteristics of the Study Population

Characteristics	Newborns	Infants	Children	All children
Cases	46	83	66	195
Age	18 (3-30) day	64 (32-328) mo	5 (1-16) yr	0 (0-16) yr
Gender	29 M/17 F	42 M/41 F	33 M/33 F	104 M/91 F
Time of analysis (min)	1356 (1228-1441)	1232 (1139-1363)	1267 (1191-1324)	1271 (1165-1370)
Meal period (min)	67 (48-100)	90.5 (61-134)	83 (59-109)	81 (56-121)
Postprandial (min)	354 (315-413)	354 (295-436)	239 (189-295)	354 (246-413)
Fasting (min)	883.5 (830-976)	797 (670-924)	942 (806-987)	852 (720-963)

Data are presented as number, median (range), or median (interquartile range).

Table 2. Median and Interquartile Range (95th Percentile) of Values of Combined Multichannel Intraluminal Impedance and pH Monitoring Parameters in Newborns, Infants, and Children

Reflux type	Parameters	Newborns (0-30 day)	Infants (1-12 mo)	Children (1-16 yr)	Overall population (0-16 yr)	ANOVA
	Cases	46	83	66	195	*P*-value
MII-A-GER	GER frequency (GER/hr)	0.83 (0.48-1.13; 2.69)[a]	0.85 (0.49-1.43; 2.35)[b]	1.17 (0.74-1.82; 2.45)[a,b]	0.97 (0.55-1.53; 2.49)	< 0.001
	Proximal GER frequency (GER/hr)	0.38 (0.06-0.74; 1.06)	0.19 (0.00-0.66; 1.31)	0.00 (0.00-0.79; 1.70)	0.22 (0.00-0.74; 1.50)	0.804
	BCT (sec)	20.80 (17.69-30.27; 44.73)	20.61 (14.64-26.71; 45.07)[b]	22.87 (17.32-27.17; 41.83)	21.37 (16.67-27.90; 45.48)	0.390
MII-WA -GER	GER frequency (GER/hr)	2.17 (1.49-2.53; 4.06)[a]	1.65 (1.10-2.38; 3.72)[b]	0.74 (0.39-1.34; 2.73)[a,b]	1.46 (0.79-2.25; 3.62)	< 0.001
	Proximal GER frequency (GER/hr)	0.83 (0.22-1.43; 2.22)[a]	0.38 (0.00-1.11; 1.99)	0.00 (0.00-0.74; 1.53)[a]	0.39 (0.00-1.07; 2.00)	< 0.001
	BCT (sec)	21.59 (16.77-27.98; 40.26)[a]	16.70 (11.60-21.83; 32.63)	12.58 (7.82-17.75; 29.26)[a]	16.50 (9.50-22.80; 33.74)	0.004
MII-WALK -GER	GER frequency (GER/hr)	0.00 (0.00-0.04; 0.41)[c]	0.00 (0.00-0.00; 0.06)[c]	0.00 (0.00-0.00; 0.15)	0.00 (0.00-0.00; 0.18)	0.017
	Proximal GER frequency (GER/hr)	0.00 (0.00-0.00; 0.12)[a,c]	0.00 (0.00-0.00; 0.04)[c]	0.00 (0.00-0.00; 0.02)[a]	0.00 (0.00-0.00; 0.05)	< 0.001
	BCT (sec)	12.72 (9.97-14.90; 33.95)	14.35 (10.35-31.13; 80.34)	7.89 (0.00-13.80; 20.07)	12.29 (7.50-17.73; 47.06)	0.094
MII-ALL -GER	GER frequency (GER/hr)	3.01 (2.23-3.78; 5.67)[a]	2.74 (1.95-3.76; 4.88)[b]	2.09 (1.31-3.12; 4.40)[a,b]	2.57 (1.86-3.63; 5.08)	< 0.001
	Proximal GER frequency (GER/hr)	1.31 (0.29-2.15; 3.16)	0.56 (0.00-1.77; 3.11)	0.00 (0.00-1.68; 3.16)	0.73 (0.00-1.95; 3.24)	0.051
	BCT (sec)	21.72 (17.39-28.15; 33.43)[a]	17.87 (14.67-22.75; 39.47)	16.00 (13.00-19.85; 26.87)[a]	17.98 (14.09-23.37; 34.34)	0.001
	BEI (%)	2.03 (1.44-2.83; 4.42)[a,c]	1.50 (1.03-2.52; 3.69)[b,c]	1.25 (0.87-1.69; 2.73)[a,b]	1.48 (1.03-2.30; 3.71)	< 0.001
pH-GER	GER frequency (GER/hr)	0.64 (0.36-1.38; 3.83)	1.13 (0.61-1.79; 3.47)	1.10 (0.64-1.70; 2.49)	1.08 (0.51-1.62; 3.15)	0.531
	RI (%)	2.80 (1.08-5.25; 5.89)[a]	2.00 (1.04-3.97; 4.69)[b]	1.52 (0.73-2.24; 2.48)[a,b]	1.96 (0.92-3.39; 4.68)	0.004

[a]$P = 0.013$ newborns vs children.
[b]$P < 0.034$ infants vs children.
[c]$P = 0.042$ newborns vs infants.
GER, gastroesophageal reflux; MII, multichannel intraluminal impedance; MII-A-GER, MII acidic GER; MII-WA-GER, MII weakly acidic GER; MII-WALK-GER, MII weakly alkaline GER; MII-ALL-GER, all MII-GER events combined; BCT, bolus clearance time; BEI, bolus exposure index; pH-GER, reflux detected by pH-metry and not associated with MII-GER events; RI, reflux index.
Data are presented as number or median (interquartile range; 95th percentile).

26 infants, and 28 children), respectively. Characteristics of the population and the median duration of analyzed traces are listed in Table 1.

Multichannel Intraluminal Impedance Reference Values in Newborns, Infants, and Children

The mean GER frequency, BCT, and BEI for all MII-GER events combined (MII-ALL-GER) significantly decreased from newborns to children. We found a significantly ($P < 0.001$) higher MII-A-GER frequency in children than in newborns and infants. No difference was observed in MII-A-GER BCT between the 3 groups. On the other hand, the greatest MII-WA-GER frequency was observed in newborns, with a progressive decline towards children ($P < 0.001$). Longer MII-WA-BCT was observed in newborns as compared to infants ($P = 0.051$) and children ($P < 0.001$) (Table 2). MII-WALK-GER was very rarely represented in all age groups. Pearson correlation models demonstrated a positive correlation between age and MII-A-GER ($r = 0.37$, $P = 0.039$) and a negative correlation between age and MII-WA-GER ($r = -0.46$, $P = 0.028$).

Postprandial and Fasting Periods

We found that MII-A-GER was significantly ($P < 0.002$) more frequent in newborns during the fasting period, while MII-WA-GER was predominant in newborns and infants during the postprandial period ($P < 0.001$) (Fig. 1). In contrast, a significantly higher MII-A-GER frequency occurred in children during the postprandial period ($P < 0.001$) (Fig. 1), and a reduction of MII-WA-GER occurred during the fasting period ($P < 0.001$) (Table 3).

Pearson analysis revealed a positive correlation between age and MII-A-GER in the postprandial period ($r = 0.30$, $P = 0.021$) and a negative correlation between age and MII-WA-GER in postprandial periods ($r = -0.28$, $P = 0.031$), and between age and MII-A-GER during fasting periods ($r = -0.33$, $P = 0.042$). A positive correlation was also demonstrated between MII-WA-GER frequency during the fasting period and MII-A-GER frequency during both the postprandial ($r = 0.40$, $P = 0.043$) and the fasting period ($r = 0.20$, $P = 0.029$). MII-WA-GER frequency during the postprandial period strongly correlated with MII-A-GER frequency during the fasting period ($r = 0.60$, $P = 0.014$) (Fig. 2).

Discussion

In the present study, we provide reference values for MII/pH parameters for pediatric patients, including newborns. These results reinforce the current scientific knowledge on this topic,[20,21] adding data on newborns, with advantages for clinicians reporting MII/pH pediatric traces.

Our study sample of newborns, infants, and children referred to MII/pH for suspected GERD, is one in which participants showed neither a significant association between GER events and symptoms, nor a pathological RI on MII/pH tracings. As such, they might be considered the most representative of a healthy population. In order to align our results with ESPGHAN and the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) guidelines[1] and 2 previously studies on pediatric reference data,[19,20] we only included patients with negative SI and SAP and a normal RI,[1] using exactly the same

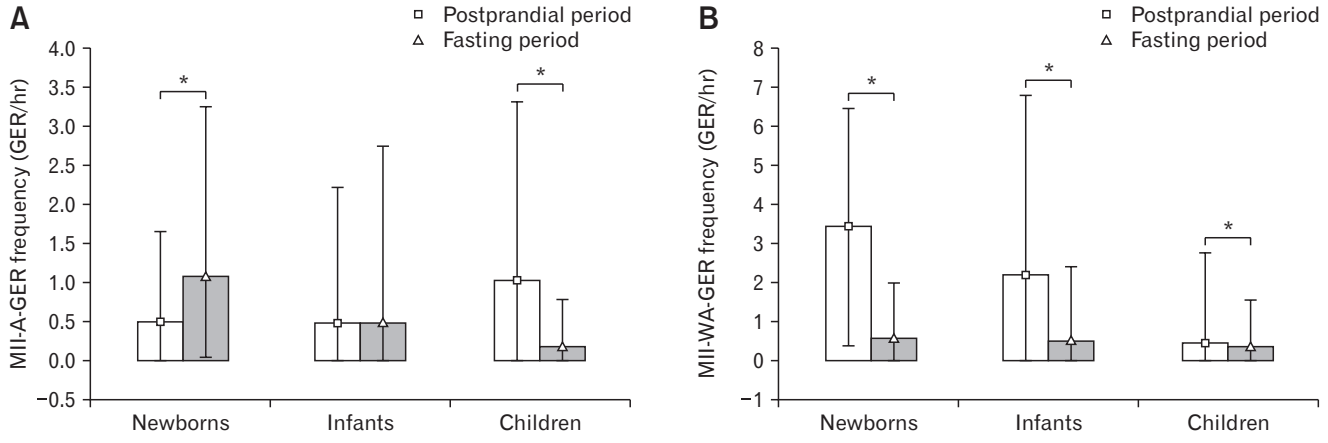

Figure 1. Differences in multichannel intraluminal impedance acidic gastroesophageal reflux (MII-A-GER) frequency (A) and multichannel intraluminal impedance weakly acidic gastroesophageal reflux (MII-WA-GER) frequency (B) between postprandial and fasting periods in newborns, infants, and children. *$P < 0.05$.

Table 3. Median and Interquartile Range (95th Percentile) of Values of Combined Multichannel Intraluminal Impedance and pH Monitoring Parameters in Postprandial and Fasting Periods

Population	Reflux type	Parameters	Postprandial period	Fasting period	*P*-value
Newborns (0-30 day)	MII-A-GER	Frequency (GER/hr)	0.50 (0.17-1.02; 2.88)	1.08 (0.61-1.82; 3.12)	0.002
		BCT (sec)	17.15 (14.45-28.12; 43.88)	24.88 (17.06-29.73; 44.26)	0.143
	MII-WA-GER	Frequency (GER/hr)	3.43 (1.47-4.83; 6.19)	0.59 (0.35-1.06; 1.74)	< 0.001
		BCT (sec)	22.31 (14.73-26.97; 44.72)	25.49 (16.62-33.06; 58.76)	0.142
Infants (1-12 mo)	MII-A-GER	Frequency (GER/hr)	0.49 (0.14-1.05; 2.19)	0.49 (0.13-1.21; 2.64)	0.679
		BCT (sec)	21.14 (13.98-30.93; 68.31)	19.54 (14.30-30.89; 79.49)	0.357
	MII-WA-GER	Frequency (GER/hr)	2.21 (1.30-3.58; 6.02)	0.52 (0.22-1.23; 1.94)	< 0.001
		BCT (sec)	20.06 (14.18-24.72; 35.49)	20.00 (13.69-26.45; 51.98)	0.363
Children (1-16 yr)	MII-A-GER	Frequency (GER/hr)	1.03 (0.55-1.68; 3.85)	0.19 (0.01-0.32; 0.77)	< 0.001
		BCT (sec)	22.17 (16.36-30.51; 63.13)	17.45 (12.00-24.00; 48.57)	0.355
	MII-WA-GER	Frequency (GER/hr)	0.44 (0.22-1.25; 3.86)	0.37 (0.12-0.77; 1.41)	< 0.001
		BCT (sec)	19.34 (11.66-25.33; 61.82)	22.50 (14.99-27.58; 48.33)	0.223
Overall population (0-16 yr)	MII-A-GER	Frequency (GER/hr)	0.65 (0.20-1.35; 3.29)	0.43 (0.12-1.13; 2.63)	0.051
		BCT (sec)	21.10 (14.57-30.00; 58.60)	19.65 (13.75-29.82; 65.48)	0.421
	MII-WA-GER	Frequency (GER/hr)	1.78 (0.59-3.49; 5.93)	0.48 (0.18-1.03; 1.75)	< 0.001
		BCT (sec)	19.88 (14.14-25.45; 43.53)	21.88 (14.64-30.10; 51.79)	0.036

MII, multichannel intraluminal impedance; GER, gastroesophageal reflux; MII-A-GER, MII acidic GER; MII-WA-GER, MII weakly acidic GER; BCT, bolus clearance time.
Paired *t* test was used for statistical analysis.
Data are presented as median (interquartile range; 95th percentile).

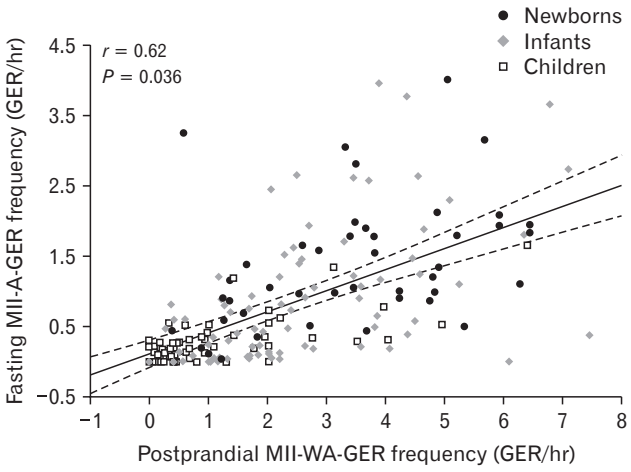

Figure 2. Relationship between fasting multichannel intraluminal impedance acidic gastroesophageal reflux (MII-A-GER) events and postprandial multichannel intraluminal impedance weakly acidic gastroesophageal reflux (MII-WA-GER) events.

cut-off considered by Mousa et al.[20] Furthermore, lower RI cut offs also selected a population who is more likely to be similar to a healthy reference.

The German Pediatric Impedance Group previously published values from 700 MII/pH traces from infants and children presenting with symptoms of GER.[19] However, they only provided data on MII-GER events from a subgroup of 511 children who had confirmed pathological traces. Thus, to the best of our knowledge, ours is the first study to present MII/pH reference values for pediatric patients, including a representative subgroup of newborns.

It has been widely demonstrated that GER characteristics and frequency vary with age.[1,19] In agreement with other authors,[19-21,25] our analysis confirmed that newborns have a higher frequency and longer duration of GER events, mainly secondary to a higher proportion of MII-WA-GER events, compared to infants and children. These features might be ascribed to immature esophageal peristalsis, increased inappropriate transitory lower esophageal sphincter (LES) relaxations,[26] slow gastric emptying, and the significant buffering effect of milk on gastric acidity due to more frequent meals.[25] Our data are consistent with those reported by López-Alonso et al[21] in 21 preterm newborns fed by a modified nasogastric tube. They also observed a greater prevalence of MII-WA-GER events (median: 2.2 events/hour) than MII-A-GER events (median: 0.8 events/hour), especially during meals.

Several authors confirmed that infants with GERD experience more MII-WA-GER events and more GER events overall than children.[13,19,25] The reference values for infants reported by Mousa et al[20] were similar to ours in MII-ALL-GER frequency (recalculated as 2.25 events/hour), MII-A-GER frequency (0.83 events/hour), MII-WA-GER frequency (1.33 events/hour), and BEI

(1.4%), while they found a shorter BCT (13 seconds).

In children, we found a significant, lower MII-ALL-GER and MII-WA-GER frequency, with an increase of MII-A-GER events. The change in diet, including the reduction of milk in favor of solid foods, a more competent LES, less frequent meals, and more hours in the upright position may explain this finding. The same pattern was observed by Mousa et al,[20] although the MII-WA-GER, MII-MA-GER, and MII-ALL-GER frequency that we reported was 2 or 3 times higher. As a consequence, the median BEI in children in our study was higher than the 0.6% reported by Mousa et al,[20] although no difference in BCT was observed in the 2 populations (median of 15 seconds). A possible explanation for these differences might be secondary to the different age distribution in the 2 series; indeed, we studied more pre-school children (median age 5.0 vs 7.2 years in Mousa et al[20]), whose habits are still similar to those of infants. The positive correlation between age and MII-A-GER frequency, as well as the negative correlation between age and MII-WA-GER events, supports this hypothesis: a progressive acquisition of LES competence and the progressive modification of dietary habits play a key role in the modification of the physiological pattern of GER in growing children.

MII-WALK-GER events were nearly absent in all age groups, so they were not analyzed further in our study. On this basis, we can speculate that MII-WALK-GER events could be considered a pathological finding in all pediatric ages.

Differently from other authors,[20,21] we analyzed data from postprandial and fasting periods separately, as some GER symptoms occurred mainly in these periods. We found a significant reduction in MII-ALL-GER frequency in the 3 groups during fasting, while MII-A-GER frequency increased in newborns but not the other age groups. Although this finding is consistent with studies in healthy adult populations,[16,17] pediatric studies focusing on postprandial GER analysis generally involved patients with GERD,[27] and therefore cannot be used for comparison. This trend should be considered in the results of a MII/pH trace: observing a higher frequency of postprandial events and rarer events during fasting periods could be representative of a physiological phenomenon, while the inverse, ie, more frequent events during the fasting period, could be considered a warning sign. Moreover, the characterisation of postprandial and fasting GER events may help individual clinical management. Patients with more fasting MII-A-GER events compared to the reference population may benefit from anti-acid therapy, whereas more postprandial MII-WA-GER is likely related to delayed gastric emptying, and might best be treated with prokinetic agents, alginate, and/or anti-regurgitation formulas.

Limitations of this study include the potential bias inherent in retrospective observational studies, as well as the *a priori* hypothesis of absence of pathological findings as an expression of health rather than the use of a healthy, asymptomatic population. Moreover, it might be speculated that the symptoms for which patients were referred could underlie other conditions and affect MII/pH outcomes. However, normal values based on a voluntary, healthy pediatric population will never be available, and we argue that this limitation also represents a strength of our study. Indeed, pediatricians who perform MII/pH would never study asymptomatic, healthy newborns, infants, or children, and they may take greater advantage of reference values based on a population that is similar to that of their patients. The observation of values that are also coherent with those of other authors[19-21] reinforce the accuracy our findings. Furthermore, to limit inter-observer variability in the interpretation of MII/pH traces, only one well-trained investigator per center reviewed the visual analysis. All used the same device and software and reached an agreement with the other investigators in case of unclear GER events.

In conclusion, we describe reference values for pediatric MII/pH parameters in a large population, including newborns and infants, which support and reinforce previous evidence[19-21] and provide a tool for a better interpretation of MII/pH. In addition, this study highlights the changing physiological pattern of GER during growth, characterized by more MII-WA-GER events in younger patients and in postprandial periods. We believe that our findings might help fill existing gaps to allow for more standardized analyses of MII/pH results, and we advocate their routine use.

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Umberto De Rose: wrote the first draft of the manuscript; Francesco Cresi and Elena Andrea Cester: performed the statistical analysis; and Francesco Cresi, Elena Andrea Cester, Silvia Salvatore, Anna Maria Magistà, Claudia Fontana, Elena Maggiora, Alessandra Coscia, and Fernanda Cristofori: revised the manuscript. All the authors approved the final version of the manuscript as submitted.

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