Gastroesophageal reflux in children: the use of pH-impedance measurements and new insights in treatment
Loots, C.M.

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Esophageal impedance baselines in infants before and after treatment with placebo, antacid and proton pump inhibitor therapy

Clara Loots
Roos Wijnakker
Michiel van Wijk
Geoffrey Davidson
Marc Benninga
Taher Omari

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ABSTRACT

Esophageal impedance monitoring records changes in conductivity. During esophageal rest impedance baseline values represent mucosal integrity.

Objective
Assess the influence of acid suppression therapy on impedance baselines in a placebo controlled setting.

Study design
Impedance recordings from 53 infants (0-6 months) enrolled in randomized placebo controlled trials of antacid and proton pump inhibitor (PPI) were retrospectively analyzed. Infants underwent 24hr pH-impedance monitoring prior to and after two weeks of double blind therapy with placebo, antacid omeprazole or esomeprazole. Typical gastroesophageal reflux (GER) symptoms were recorded and the I-GERQ-R questionnaire was completed.

Results
Median (IQR) baselines increased on omeprazole \[1167 (856-1579) \text{ vs. } 1976 (1649-2067) \ p=0.005]\] and esomeprazole \[1291 (666-1493) \text{ vs. } 1903 (1254-2239) \ p=0.006]\]. Baselines in placebo and antacid group did not change. Lower baselines correlated with higher numbers of GER, acid GER, weakly acid GER, acid exposure and symptoms. Increase in baselines is most strongly driven by a reduction in acid GER. Patients with initial low baselines have no improved symptomatic response to treatment.

Conclusion
Impedance baselines are influenced by GER and increase on PPI therapy but not on placebo or antacids. Clinical impact of this observation remains undefined as targeting therapy at infants with low baselines does not seem to improve symptomatic response to treatment.
INTRODUCTION

Esophageal multichannel intraluminal impedance is used for the detection of gastroesophageal reflux (GER) episodes in infants, children and adults.\(^1\)\(^-\)\(^4\) This is a technique to measure esophageal flow represented by changes in conductivity of adjacent contents between multiple electrode pairs on a catheter.\(^5\) Impedance values, representing changes in conductivity, drop in the presence of highly conductive contents, such as saliva or gastric fluids indicating a liquid swallow or GER. Less conductive bolus, such as air, cause an increase in the impedance signal. When the esophagus is at rest and no swallows or GER occurs, the impedance measured, referred to as the impedance baseline, is likely to reflect the conductivity of the esophageal mucosa.\(^6\)\(^-\)\(^8\) Low baselines have been observed in patients with esophagitis\(^7\) and esophageal motility abnormalities.\(^9\) Recently Farré et al demonstrated in vitro and in vivo that impedance is a useful tool for the evaluation of mucosal integrity and that patients with GER disease and non-erosive reflux disease (NERD) have lower baselines compared to healthy volunteers.\(^6\) The authors report that the changes in baseline are not only related to macroscopic changes and secretion of inflammatory fluids, as seen in esophagitis, but to more subtle changes in the esophageal mucosa such as dilated intracellular spaces (DIS). DIS have been postulated to be the mechanism underlying NERD, providing a pathophysiological explanation for increased acid perception.\(^10\)\(^,\)\(^11\) A significant correlation has been observed between heartburn/pain and baseline values, suggesting a relationship linking baselines, DIS and perception of symptoms.

In infants and children, antacids and proton pump inhibitors (PPIs) are the most commonly used therapeutic agents for GER disease. Data on the influence of antacids on GER, acid exposure and symptomatic relieve in infants are sparse.\(^12\)\(^,\)\(^13\) PPI therapy is proven effective for healing esophagitis in adult GER disease,\(^14\)\(^-\)\(^16\) for reducing acid exposure in infants,\(^13\)\(^,\)\(^17\)\(^,\)\(^18\) and is suggested to heal erosive esophagitis in 89% of children one to 11 years of age.\(^19\) However the effectiveness of PPI’s to relieve symptoms of GER disease in infants has not been proven.\(^20\)\(^,\)\(^21\) The best way to diagnose and treat infantile GER disease remains controversial with, on balance, no evidence supporting empirical PPI therapy for treating typical GER symptoms, such as irritability, vomiting and feed refusal.\(^21\)\(^,\)\(^22\) With endoscopy being difficult to perform in infants, impedance baselines may potentially be a marker of changes to mucosal integrity likely in increase symptom perception and therefore supportive of a diagnosis of GER disease and a justification for PPI therapy. We have recently reported an increase in impedance baseline values in infants on PPI therapy in an open label, non placebo controlled trial.\(^8\) These data suggest that PPIs produce changes in mucosal integrity, especially in those with initial low baselines. These findings are however uncontrolled, and therefore we retrospectively analyzed impedance recordings from infants with symptoms of GER disease who were enrolled in randomized placebo controlled trials of antacid and PPI to examine the effects of treatment on impedance baselines and the relationships between impedance baselines and typical GER symptoms.
METHODS

Patient data from a research database compiled of data from previously conducted randomized controlled trials (RCT) of anti-reflux therapies were reanalyzed. RCT protocols were approved by the Human Research Ethics and Drug Therapeutics Committees of the Women’s and Children’s Hospital, Adelaide, Australia.

Preterm and term infants from zero to six months of age were enrolled. Infants were included if they presented with symptoms suggestive of GER such as irritability, crying, excessive vomiting, regurgitation, coughing, feed refusal, unsettled behavior, back arching, failure to thrive or apneas and had failed to respond to non-pharmacological therapy. Patients underwent eight hour pH-impedance monitoring and symptoms were continuously recorded by trained staff. Episodes of vomiting, regurgitation, irritability, crying, fussing, cough, sneeze, backarchning, choking, gagging were scored during the study. Primary caretakers completed a validated infant questionnaire, the I-GERQ-R. After the eight hour hospital based study, the pH-impedance probe was left in place for 24 hr GER assessment either in hospital or at home.

GER and impedance baseline values were recorded using a single use infant pH-impedance catheter with seven sensors (six impedance channels) spaced 1.5 cm apart (ComforTec MII/pH probe, Sandhill Scientific, Highlands Ranch, CO, USA). The pH sensor was placed at the third vertebrae above the diaphragm as confirmed by a thoracic X-ray.

After the initial study patients were randomized (double blind), and dependent on the specific nature of each trial, received two weeks of any of the following treatments: 1. Placebo 2. Antacid (1.5, 3 or 5ml Mylanta™ once daily in infants 0-2, 2-4 and 4-6months of age) 3. Omeprazole (1 mg/kg/day once daily) 4. Esomeprazole (0.5 mg/kg/day once daily).

Eight hour pH-impedance, manual symptoms scoring, I-GERQ-R and 24hr pH-impedance monitoring was repeated on therapy after two weeks.

Data analysis

Impedance analysis

The eight hour and 24hr pH-impedance tracings (Bioview; Sandhill Scientific) were analyzed by two observers for the presence of liquid and mixed bolus GER. Distal esophageal acid exposure time, reflux index (RI) was calculated as the % time pH<4. I-GERQ-R scores were calculated as previously described.

Baseline calculation by automated analysis

Raw impedance values for all catheter channels were exported from each recording in text format at one sample per second. The baseline value per channel was estimated for both the initial 8h symptom assessment period and the full 24h study period using automated analysis procedures performed on the raw impedance data using a Matlab™ based algorithm. The algorithm was designed to filter the data, by removing the influence of rapid impedance dips and rises typically associated with reflux episodes and swallowing.
The algorithm operated as follows:
Firstly all data samples >5000 Ohm (representing air) were excluded. The algorithm calculates the nadir impedance point per 10 seconds (Figure 1 A). The mean and standard deviation of 60 nadir impedance data points (equivalent to 10 minutes in real time) was then calculated and samples above and below one standard deviation of the mean were removed (Figure 1B). The mean of the residual samples was then calculated and this was taken as the estimate of baseline impedance for each 10 minute interval (Figure 1B). The analysis was repeated for consecutive 10 minute intervals of the complete dataset and then the median of all 10 minute intervals was used to estimate impedance baseline of the eight hour symptom assessment period and the 24 hour study (Figure 1C).

Symptom analysis
For symptoms analysis we calculated the sum of all symptoms recorded by trained staff continuously monitoring infants during the eight hour study. Symptoms of vomiting, crying and coughing episodes were assessed separately as well, as these were consistently observed in all infants. GER symptom association probability (SAP) was calculated for all symptoms together and for vomiting, crying and coughing separately. The SAP is based on the Fisher’s exact test calculating the probability that GER and the symptoms are unrelated. The SAP is calculated as \(1 - p\) x 100% and a SAP of >95% is referred to as a positive SAP.\(^1\)

Statistical analysis
We report on the influence of therapy on baselines based on the 24 hour pH-impedance recordings. The data on correlation between baselines, GER, acid exposure and symptoms are based on the eight hour pH-impedance study as symptoms were only continuously and reliably monitored during this period.
The baseline data were not normally distributed and are shown as medians (interquartile range). Comparisons were made using Wilcoxon’s signed rank test and Spearman’s correlation statistics. Furthermore, Spearman partial correlations were performed to assess the influence of individual variables, while correcting for other interacting variables. A Spearman’s \(r\) of 0 - 0.3 was considered a weak correlation, 0.3 - 0.6 a moderate correlation, >0.6 a strong correlation. Statistical significance is defined as \(p<0.05\).

RESULTS
Data were derived from 53 preterm and term infants, 25 (47%) male between the age of zero and six months, mean age was eight weeks (IQR 4-15 weeks).
Bolus GER parameters such as total number of GER episodes, acid GER, weakly acid GER, reflux index (RI) and number of symptoms scored during eight hour monitoring for the different treatment groups are presented in Table 1.
Figure 1. Automated calculation of impedance baseline values.
A. One minute time interval. The circles in the figure represent the 6 minimum impedance data points per minute used for the calculations.
B. Ten minute time interval to calculated mean and standard deviation 60 samples (obtained from panel A). Samples above and below 1 standard deviation of the mean were removed (open circles). The mean of the remaining samples (closed circles) was calculated and this number was taken as the estimate of baseline impedance for each 10min interval.
C. Eight hour time interval. The analysis (panel B) was repeated for consecutive 10min intervals of the complete dataset and the median of all 10min intervals was used to estimate the overall impedance baseline value.
Impedance baseline values in the esophagus

Median (IQR) impedance baseline values measured in the initial study at the six impedance segments on the catheter ranged from 1428 (1109-1726) Ohm in the most distal channel to 1498 (981-2092) Ohm in the most proximal channel, the third most distal segment showed the highest impedance baseline 2108 (1549-2566), probably caused by imprint of the aortic arch on the esophagus. Baseline values measured over 24hrs (1482 (1247-1659) Ohm) are slightly lower than the values measured in the eight hour study (1619 (1266-1899) Ohm). This is presumably due to the prolonged sleep period during the night with fewer body movement artifacts, swallowing and reflux. The most significant changes in impedance baselines occurred in the single most distal segment. Therefore throughout the manuscript we report the baseline values in the most distal impedance segment.

| Placebo N=13 | | Antacid N=13 | |
|-------------| | | | |
| GER total 76 (60-102) | 70 (56-101) | 0.81 | GER total 49 (29-68) | 31 (22-41) | 0.023 |
| GER acid 30 (12-48) | 22 (11-28) | 0.39 | GER acid 15 (4-26) | 6 (2-22) | 0.05 |
| GER WA 45 (33-67) | 55 (33-72) | 0.44 | GER WA 27 (21-44) | 20 (15-29) | 0.093 |
| Reflux index 31 (15-59) | 26 (20-40) | 0.6 | Reflux index 1.2 (0.7-12.3) | 4.6 (0.1-10.7) | 0.05 |
| Symptoms 146 (135-201) | 166 (131-209) | 0.25 | Symptoms 131 (83-208) | 138 (105-220) | 0.2 |

Table 1. GER parameters pre and post treatment.
GER parameters per treatment group. Reflux index in % acid exposure during the study. Symptoms are the total number of symptoms recorded during the eight hour study.

Impedance baseline pre vs post treatment per treatment group

The median (IQR) 24hr impedance baseline increased significantly following both omeprazole and esomeprazole treatment but not with placebo or antacid therapy (Table 2, Figure 2). By chance the initial baseline in the esomeprazole group is (not significantly) lower than the antacid group (p=0.056) and the omeprazole group (p=0.065). The change in baseline was significantly larger in the omeprazole and esomeprazole group compared to placebo (Figure 2). Including only those patients with a lower baseline (of <750, <1000, <1250, <1500, <1750, <2000 Ohm) before treatment did not change these findings.
Impedance baselines in relation to other parameters based on the eight hour study

Before therapeutic intervention, lower impedance baselines are significantly correlated to a higher number of GER episodes, higher number of acid GER episodes, higher number of weakly acid GER episodes, higher reflux index, higher number of symptoms, higher number of GER related symptoms and a higher number of vomiting episodes (Table 3). I-GERO-R outcomes, number of cough episodes and number of crying episodes did not correlate to baseline values at any time.

The difference in impedance baseline during therapy correlated inversely with the difference in reflux index and the number of acid GER episodes across all groups (Spearman r = -0.38 (moderate), p=0.005 and Spearman r = -0.427 (moderate), p=0.001 respectively).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline Pre</th>
<th>Baseline Post</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>1445 (1033-1791)</td>
<td>1650 (1292-1983)</td>
<td>0.13</td>
</tr>
<tr>
<td>Antacid</td>
<td>1619 (860-2215)</td>
<td>1546 (869-2408)</td>
<td>0.237</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>1167 (856-1579)</td>
<td>1976 (1649-2067)</td>
<td>0.005</td>
</tr>
<tr>
<td>Esomeprazole</td>
<td>1291 (666-1493)</td>
<td>1903 (1254-2239)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Table 2. Baseline values per 24 hr study pre and post treatment for the most distal channel. Wilcoxon signed rank test.

Figure 2. Difference in baseline post – pre treatment per treatment group.
Difference in baseline value on treatment – pre treatment per treatment group. Baselines in the omeprazole and esomeprazole group are significantly increased compared to placebo. The difference between antacid and omeprazole and esomeprazole is p=0.055 and p=0.051 respectively.
However, these parameters influence each other and it is likely that differences between the treatment groups exist. Partial correlation calculations revealed that impedance baselines changes most strongly correlate to changes in number of acid GER (Spearman r -0.35 (moderate), p=0.01). Subdividing groups based on treatment did not reveal other correlations.

**Outcome for different initial baselines**

Although we observed a correlation between low baselines and higher numbers of symptoms, we did not observe a different response to treatment in patients with low baselines in terms of the total number of symptoms recorded, crying, vomiting or coughing episodes. We used cut off values for low baselines of <1000, <1250, <1500, <1750 and <2000 Ohms. Including only patients with a positive GER – symptom association based on a positive SAP before treatment did not change these results.

**DISCUSSION**

In this study we assessed the influence of commonly used acid suppression therapies on impedance baseline values and the relation between impedance baseline and symptoms in patients enrolled in RCTs performed in our centre. We demonstrated that PPI treatment significantly increased impedance baseline, whereas antacid and placebo did not. Esomeprazole showed a larger increase in baseline on therapy compared to omeprazole, however this finding may be attributed to the lower initial baselines in the esomeprazole group. Lower impedance baselines pre treatment correlate with a higher number of GER episodes, number of acid GER episodes, number of weakly acid GER episodes, reflux index and total number of symptoms. The increase in impedance baselines with therapy is most strongly driven by the reduction in acid GER episodes. These findings suggest that impedance baseline values may reflect integrity of the esophageal mucosa. Mucosal integrity appears to be driven by the balance between damage caused by bolus GER,
acid exposure and possibly other factors\textsuperscript{11,24,25} and protection by the tight squamous epithelium of the esophageal mucosa.

In our population we have established that impedance values throughout the esophagus are rather consistent, with the exception of the channel nearest to the heart and aortic arch. In that channel the narrowing of the esophagus most likely explains the rise in impedance baseline measured. Largest differences with therapy were seen in the most distal segment. This was also observed by Farre et al\textsuperscript{6} and suggests that the distal esophagus is the most suitable site to assess impedance baseline values.

Although correlations do not prove causality, it is interesting that we observed an inverse correlation between impedance baselines and total numbers of GER, both weakly acidic and acidic, acid exposure, and symptoms before treatment. It has been shown that acid and weakly acid solutions can cause DIS in adults\textsuperscript{6,11}. Furthermore, DIS can present in adult patients with NERD\textsuperscript{26-28}. Moreover, increased DIS has been associated with increased perception of heartburn in NERD patients\textsuperscript{11}. It can be argued that infants are similar to adults with NERD in terms of GER like symptoms without erosive esophagitis. Barlow has postulated a unifying hypothesis for the pathogenesis of heartburn in patients with NERD;\textsuperscript{10} the presence of low tissue resistance enables the diffusion of H\textsuperscript{+} ions into the intercellular space, activating chemosensitive nociceptors whose signals are transmitted to the brain and perceived as heartburn. This hypothesis could explain the observed correlation between more acid GER and lower baselines and between lower baselines and increased number of symptoms.

The role of weakly acidic GER has not been addressed in this hypothesis. It has been shown that infusions with weakly acid solutions cause similar DIS to acid solutions\textsuperscript{11}. This is not supported by our findings that patients on PPI treatment, who have more weakly acid GER have higher impedance baselines. The exact relation between weakly acid solutions, weakly acid GER, DIS and baseline levels remains to be established.

Anti reflux treatment in infants has been controversial, largely due to the fact that no treatment has been proven effective for reducing symptoms of GER\textsuperscript{21}. Based on the observation that low baselines correlate to acid induced heartburn in adults\textsuperscript{6} we hypothesized that patients with low baselines before treatment benefit more from treatment. However we did not observe a change in symptoms on treatment in any of the groups, neither did we observe a correlation in change in baseline and change in symptoms. The numbers of patients with low baselines were low so this negative finding may be due to under power of the study. It should be noted however that an increasing number of placebo controlled trial have failed to demonstrate symptomatic improvement with PPI. Hence the most likely conclusion is that it can be attributed to the fact that symptoms suggestive of GER such as crying, irritability and coughing are very non-specific to GER disease in infants.
A limitation of this study is that endoscopic data are unavailable to correlate the change in baseline to esophageal macroscopic mucosal findings and histology. This data is difficult to acquire because endoscopy is infrequently performed in infants and only performed in those who have severe complications and are therapy resistant. However, based on Farrés recent observations that clearly link impaired esophageal mucosal integrity to impedance baseline measurements, this study suggests that PPI’s can improve mucosal integrity while placebo and antacid do not.

In conclusion, we have demonstrated that PPI therapy increases esophageal baseline levels suggesting that PPI’s improve esophageal mucosal integrity whereas placebo and antacid do not have this effect. Patients with low baselines before therapy do not have a better response to treatment in terms of numbers of symptoms compared to patients with high initial baselines, leaving the question of the clinical importance of esophageal impedance baselines in infants unanswered. This study further supports the hypothesis that impedance baselines reflect the integrity of the esophageal mucosa, hence the balance between damage and protection of the mucosa. Whilst symptomatic changes do not appear to correlate with changes in impedance baselines in the patient cohort studied, this approach may still have potential and is worthy of further investigation in older patients, in whom concurrent endoscopy in possible.

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