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Airway inflammation and injury in children with prevalent weakly acidic gastroesophageal refluxes

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ABSTRACT

Background: In children with gastroesophageal reflux (GER) both acid refluxes (AR) and weakly acidic refluxes (WAR) can induce respiratory symptoms (RS).

Methods: To characterize the airway inflammation in children with more prevalent WAR or AR (defined according a ROC curve analysis), we performed a 3 year-retrospective review of the medical records of patients who underwent fiberoptic bronchoscopy for difficult-to-treat chronic/recurrent respiratory symptoms and who had a positive multiple intraluminal esophageal impedance (pH/MII) monitoring.

Results: In the 13 WAR and 11 AR children, the number of cells recovered by bronchoalveolar lavage (BAL) was similar [0.78 (0.29–1.28) x 10^6 cells, and 1.05 (0.68–1.64) x 10^6 cells, respectively] ($P = 0.22$). A neutrophilic alveolitis and an elevated lipid-laden-macrophage (LLM) index were detected in both groups: no differences were found in neutrophils and lymphocyte percentages or in LLM index between WAR and AR children. In contrast, higher BAL epithelial cell proportions were seen in WAR [10.4 (4.85–23.45) %], as compared to AR [2.5 (1.25–7.25) %] children ($P = 0.0045$), suggesting greater airway damage in the formers. In the whole patient population a significant correlation was found between the proportions of BAL epithelial cells and the number of WAR events ($r = 0.43$; $P = 0.037$). Finally, elevated BAL concentrations of substance P and of pepsin were observed, not statistically different in the WAR and AR groups.

Conclusions: In this patient population, WAR events can be associated with a significant airway inflammation and injury that, because of the biochemical mechanisms involved, are likely not completely preventable and/or counteracted by anti-acid treatments.

1. Introduction

Respiratory disorders and GER often co-exist in childhood, and a causal relationship between these two conditions is often difficult to prove, also with the aid of specific diagnostic tests [1–3]. The pathogenesis of this condition is multifactorial, being related not only to the frequency and duration of the reflux events and to ability of the patient to avoid or limit aspiration, but also to the physical and biochemical characteristics of the refluxate [2–4]. With the advent of the pH/MII monitoring, it has become possible to show that weakly acidic and alkaline refluxes are frequent in the pediatric population and that they can induce respiratory symptoms, such as persistent and/or nocturnal cough, wheezy bronchitis and asthma, recurrent lower respiratory tract infections, apnea and laryngospasm [2,4–7]. Moreover, a recent prospective study has demonstrated that children with GER-related respiratory symptoms have a significantly higher number of weakly acidid refluxes than children with GERD-related gastro-intestinal symptoms [7]. These findings may at least partially explain the often observed ineffectiveness of acid-suppressive treatments in this patient population, also when proton pump inhibitors (PPI) are prescribed [8,9]. Acid suppression can only change acid into weakly or to weakly acidic or non-acid refluxes, not the frequency of reflux events that, if aspirated, may still produce airway injury [9–11]. With this background, to characterize the inflammatory changes induced by weakly acidic refluxes, we performed a retrospective study in children with GER in whom BAL during fiberoptic bronchoscopy was carried out for the presence of chronic/recurrent respiratory symptoms. Data on the BAL cellularity in those in with more prevalent weakly acidic refluxes (WAR) or acid refluxes (AR) (defined according to a ROC analysis [2]) were compared. In addition, the BAL levels of pepsin, as an index of

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aspiration [12], and of substance P, as a marker of neurogenic inflammation [13], were also evaluated and compared.

2. Materials and methods

2.1. Patients

We retrospectively reviewed the records of children referred in the last three years to the Pulmonary and Allergy Unit of the Giannina Gaslini University Hospital because of “difficult-to-treat” respiratory symptoms (recurrent lower respiratory tract infections, persistent or recurrent cough with or without wheeze, “difficult-to-treat” asthma, recurrent/spasmodic croup) [3], who had a positive 24-hr pH/MII monitoring. We then selected those who underwent BAL during fiberoptic bronchoscopy, as part of the diagnostic procedures. We excluded children with: I) prematurity; II) neurological abnormalities; III) swallowing disorders; IV) underlying ENT problems; V) bronchiectasis; VI) great vessel and gastro-intestinal structural abnormalities; VII) motility disorders of the upper gastro-intestinal tract; VIII), chromosomal abnormalities; IX) cellular/humoral immune-deficiencies; X) current or recent (four weeks) respiratory tract infections; XI) positive microbiological analyses on BAL fluid. We also excluded children treated in the previous eight weeks with acid suppressor (H_2-blockers or PPI) or in the previous week with antacids/alginates. The original protocol was approved by the local ethics committee.

2.2. Clinical assessment

Clinical data were collected in all children. Each patient underwent standard examinations, including lung function testing (when possible because of the age) and radiological, cardio logical and immunological evaluations. In addition, multidetector computed tomography and esophago-gastro-duodeno-scopy were performed, when clinically required [4]. Indication for fiberoptic bronchoscopy and BAL was discussed with the children's parents or tutors. All investigations were carried out with their full/informed consent.

2.3. Esophageal Impedance–pH monitoring procedure

Combined esophageal impedance–pH was recorded with a 2.1-mm diameter catheter, as previously described [6]. The study was performed after an overnight fast and the catheters were passed transnasally and the esophageal pH sensor was positioned at the second vertebral body above the diaphragm [6]. Recordings was considered “positive” when the reflux index (the total percentage of time of esophageal exposure to pH < 4) was $> 2\%$ [14] and when a temporal association between reflux event and respiratory symptoms were detected during the procedure [6]. The impedance/pH recordings were evaluated only if the duration was at least 20 h.

2.4. Fiberoptic bronchoscopy and BAL cell analysis with quantification of lipid-laden-macrophage (LLM) index

Fiberoptic bronchoscopy and BAL were performed as previously described [15], within five days after the pH/MII procedure. Briefly, after premedication with atropine and sedation with meperidine (0.5–2.0 mg/kg body weight i.v.) or propofol (2.0 mg/kg body weight i.v.) the fiberoptic bronchoscopes were passed through a ventilation mask, and introduced in a nostril. After local anesthesia of the airways, BAL was performed by injecting 3×1 ml/kg body weight aliquots of sterile saline solution, which were aspirated at a negative pressure of 40 mm Hg in siliconized plastic tubes. A sample of BAL fluid was sent to the microbiology department to rule out the presence of bacteria, mycobacteria and viruses [14], and the volume of fluid and the total and differential cell counts were recorded as described. To identify lipid inclusions, BAL cells were stained with Nile-Red and evaluated by fluorescence microscopy [15]. Data were compared with normal values in our laboratory [3,15].

2.5. BAL fluid pepsin and substance P evaluation

The concentration of pepsin and substance P in each BAL fluid supernatant sample was assayed using respectively the Human Pepsin ELISA (detection range: 0.078–5 ng/ml; Uscn Life Science Inc., Wuhan, P.R. China), and Parameter Substance P Assay (detection range: 0.02–25 ng/ml; Peninsula Laboratories, LLC, San Carlos, CA) according to the manufacturer's instructions. Pepsin and substance P concentrations were corrected by the correspondent albumin levels.

2.6. Data and statistical analysis

The impedance/pH records were analyzed using the Sandhill Technologies software and displayed on a single screen for computer-assisted manual analysis [6]. To discriminate WAR *vs.* AR patients we used the cut-off of previously receiver operator characteristic (ROC) curve analysis that was determined in a similar population [4]. The optimal cut off value obtained for the percentage of acid reflux events was 70.37%: children with $\leq$70.37% or with $>$ 70.37% of acid reflux events were respectively defined as “weakly acidic reflux” (WAR) group or as “acid reflux (AR)” group [sensitivity: 73.3%, specificity: 63.3%, AUC: 0.728 (0.650–0.796)]. Arithmetic mean with standard error in parentheses was used to describe parametric variables, whereas median values with lower and upper quartiles in parentheses were used to express non-parametric variables. Student t-test was used to compare differences in parametric variables and Mann-Whitney *U* test for non-parametric variables. Chi-square test was used to evaluate differences in prevalence of respiratory and gastro-esophageal symptoms in WAR *vs.* AR patients. Correlations were determined by Spearman's rank correlation coefficients. *P*-values < 0.05 have been considered as statistically significant.

3. Results

3.1. Patient population and GER disease-related symptoms

We included the records of 24 children, 13 belonging to the WAR group and 11 to the AR group. There were 17 males and 7 females. The mean age was 6.54 (3.83) years, and none were below 1 yr of age. The two groups were homogeneous by males to females ratio, but not by chronological age, being 4.90 (3.48) years in the WAR group and 8.48 (3.40) years in the AR group ($P = 0.019$). There were no significant differences in the most prevalent respiratory and gastro-esophageal symptoms: persistent/nocturnal cough, wheezy bronchitis/asthma, recurrent respiratory infections and vomiting/regurgitations (P =NS, all comparisons) (Fig. 1).

3.2. pH/MII monitoring data

The median numbers of the acid refluxes were 23.00 (13.50–41.00), in the WAR group, and 52.00 (37.50–66.00), in the AR group ($P = 0.008$), whilst the median numbers of the weakly acidic refluxes were 27.00 (16.00–54.00), in the WAR group, and 6.00 (3.50–9.00), in the AR group ($P = 0.003$). No differences in the median numbers of the reflux events > 5 min was detected ($P = 0.10$). In contrast, differences between the WAR and AR groups were observed in: a) the acid percent time, 1.43 (0.25–1.95) and 2.80 (1.70–5.24), respectively ($P = 0.002$); b) the weakly acidic percent time, 1 (0.55–3.05) and 0,25 (0–0.45), respectively ($P = 0.008$); c) the reflux index, 3.20 (2.35–5.15) and 6.00 (5.30–29.70), respectively ($P = 0.014$). Finally, the WAR/AR ratio was 1.36 (0.48–2.21) in the WAR group and 0.13 (0.08–0.23) in the AR group ($P < 0.0001$). The majority of these differences were intentional, according to the design of the study.