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PHD PROGRAM IN PEDIATRIC SCIENCES

Pediatric Specialties Curriculum (Code 6675)

Management of children with chronic wet cough: the experience of the Pediatric Pulmonary Unit of the G. Gaslini University Hospital

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I would like to dedicate this thesis to
my wife Maria and my son Marco

Acknowledgements

My sincere thanks go to those who supported me and those who were close to me hitherto.
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I sincerely appreciated the assistance you provide my paper.

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Abstract

Background. Cough is a physiological defense mechanism, but it may also be the first, initial symptom of a variety of respiratory tract disorders and, more rarely, of extrapulmonary diseases. Independently from its nature, when persistent or recurrent, cough can severely impact the quality of life of children and their families and be one of the most common medical complaints for which parents seek medical assistance. Prompt recognition and early management is necessary to prevent inappropriate treatment and, possibly, the onset of persistent damage to the airways.

Materials and methods. We retrospectively studied 110 children, referred to our Unit, diagnosed for chronic/recurrent wet cough in according to international guidance. Fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) and chest CT scan were included in the evaluation, when clinically indicated.

Results. Out of the 71 children in which endoscopy was performed, 38 (53.5%) had evidence of bronchial inflammation with mucopurulent secretions and 23 (32.4%) of airway malacia due to extrinsic pulsatile airway compression in 19 (26.8%). Evaluation BAL fluid showed a neutrophilic alveolitis in 51 of the samples and significant bacterial growth in 48 patients: H. influenzae in 37 samples, Str. pneumoniae and, M. catharralis in 7. Chest CT scan performed in 79 children showed the presence of bronchiectasis in 30 and mediastinal vascular anomalies in 19 of them: aberrant innominate artery (AIA) in 15, right aortic arch (RAA) in 2 and AIA + RAA association in 2. Comparing children with or without bronchiectasis, we found that the median age at admission was significantly higher in the formers, who also tended to have a higher age at symptoms onset and a longer period between symptom onset and diagnosis. Moreover, the “bronchiectasis group” had a higher percentage of children with airway malacia ($p<0.001$), and cultures positive for Haemophilus influenzae ($p<0.02$). Patients were treated with respiratory physiotherapy and high-dose amoxicillin/clavulanic acid (40 mg/kg/ day) up to 6 weeks Evaluation of the long-term outcome showed that 40.6% could be classified as “completely asymptomatic”, 28.1% as “intermittent symptomatic, 18.7% as “recurrent symptoms, with substantial interference upon life-style” and 12.5%, of which 3 with bronchiectasis, as “unsatisfactory result”.

Conclusions. These data may be useful to describe the possible evolutionary conditions in children with chronic wet cough and the best therapeutic approach in these patients even employing prolonged antibiotic therapies plus respiratory physiotherapy. Protection of the airway against respiratory bacteria for a longer period may reduce the risk of recurrence and may be protective against bronchial wall degeneration leading to the development of bronchiectasis. Delayed diagnosis or inappropriate treatments may give enough time for the "vicious cycle": infection, inflammation, and impaired mucociliary clearance to destroy the integrity of the bronchi and proceed toward the development of bronchiectasis.

List of figures legends

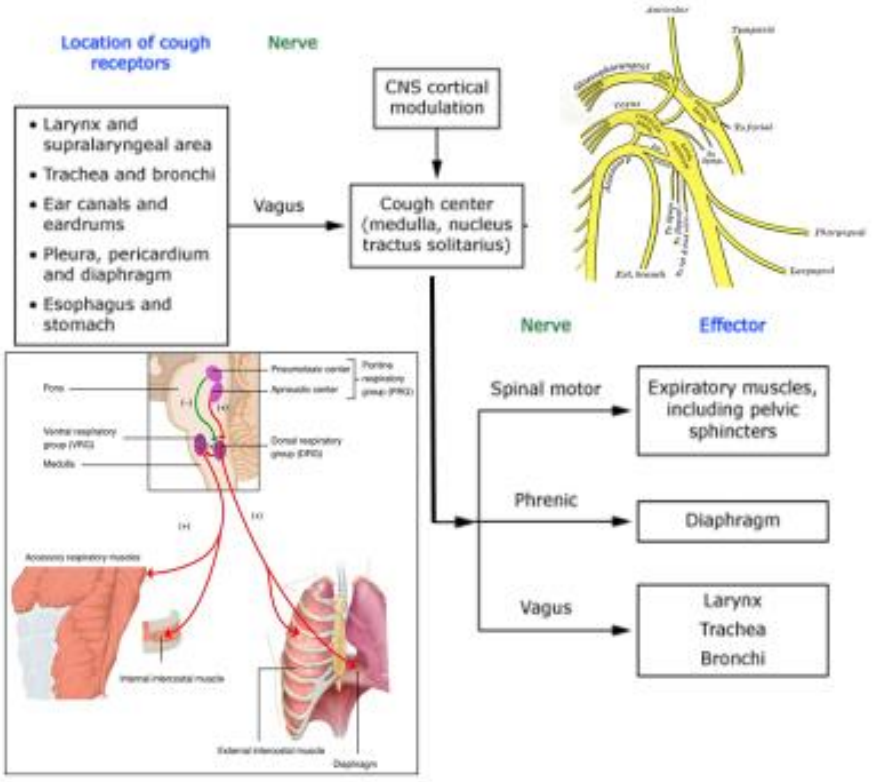
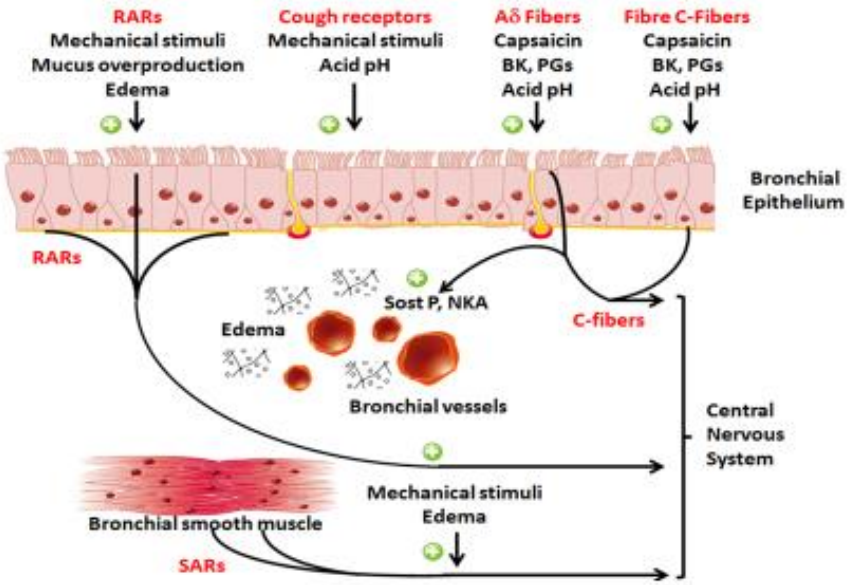
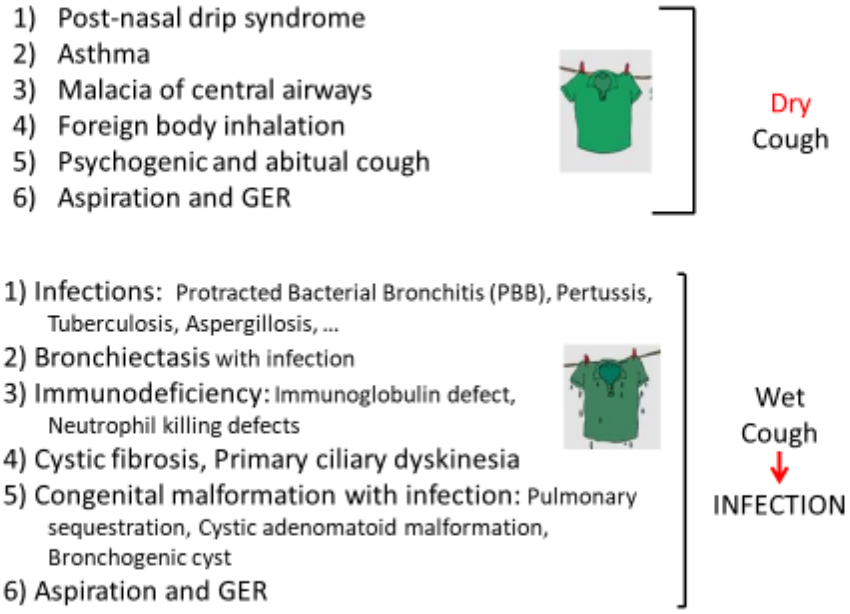


Figure 1. The physiology of cough.



Capristo C. Minerva Pediatr. 2017;69:444-452.

Figure 2. The sensory innervation of the airways. The sensory nerves of the airways can be divided roughly into two groups: those primarily activated by mechanical stimuli, (the quick adaptation receptors RARs, and the receptors of cough) and those primarily activated by chemical stimuli (C-fibers, nociceptors Aδ). The first are sensitive to lung hyperextension, bronchospasm, mucus overproduction and edema but also to the environmental acidity. The latter are sensitive to capsaicin, bradykinin (BK), prostaglandins (PGs) and the acidity of the environment. The activation of C-fibers induces an axon reflex that results in the release of neuropeptides such as substance P and neurokinin A.



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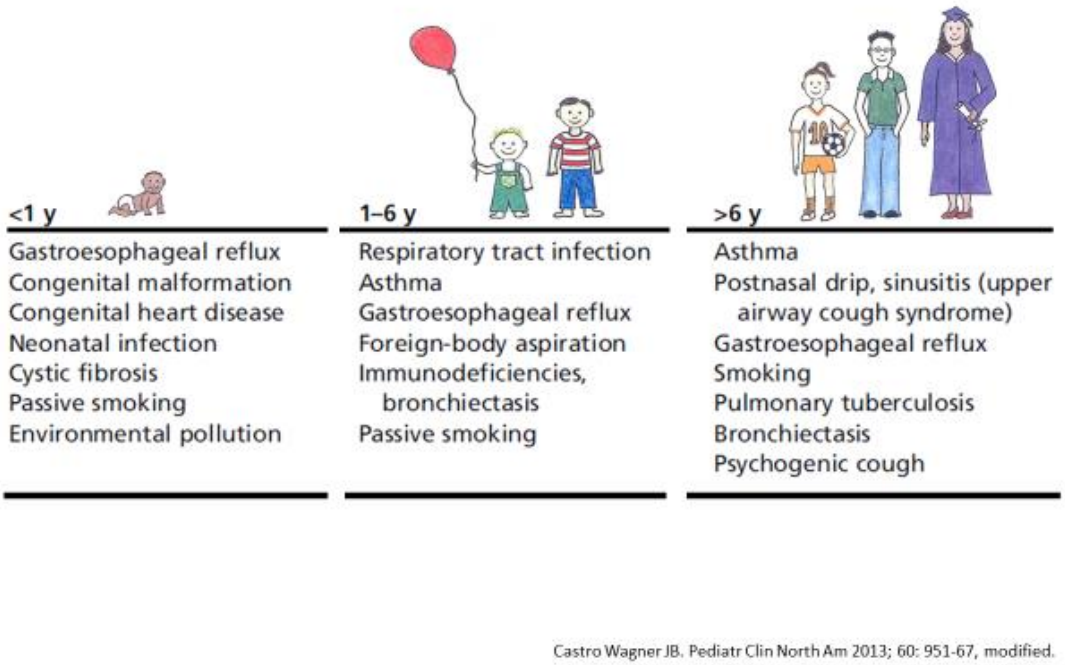

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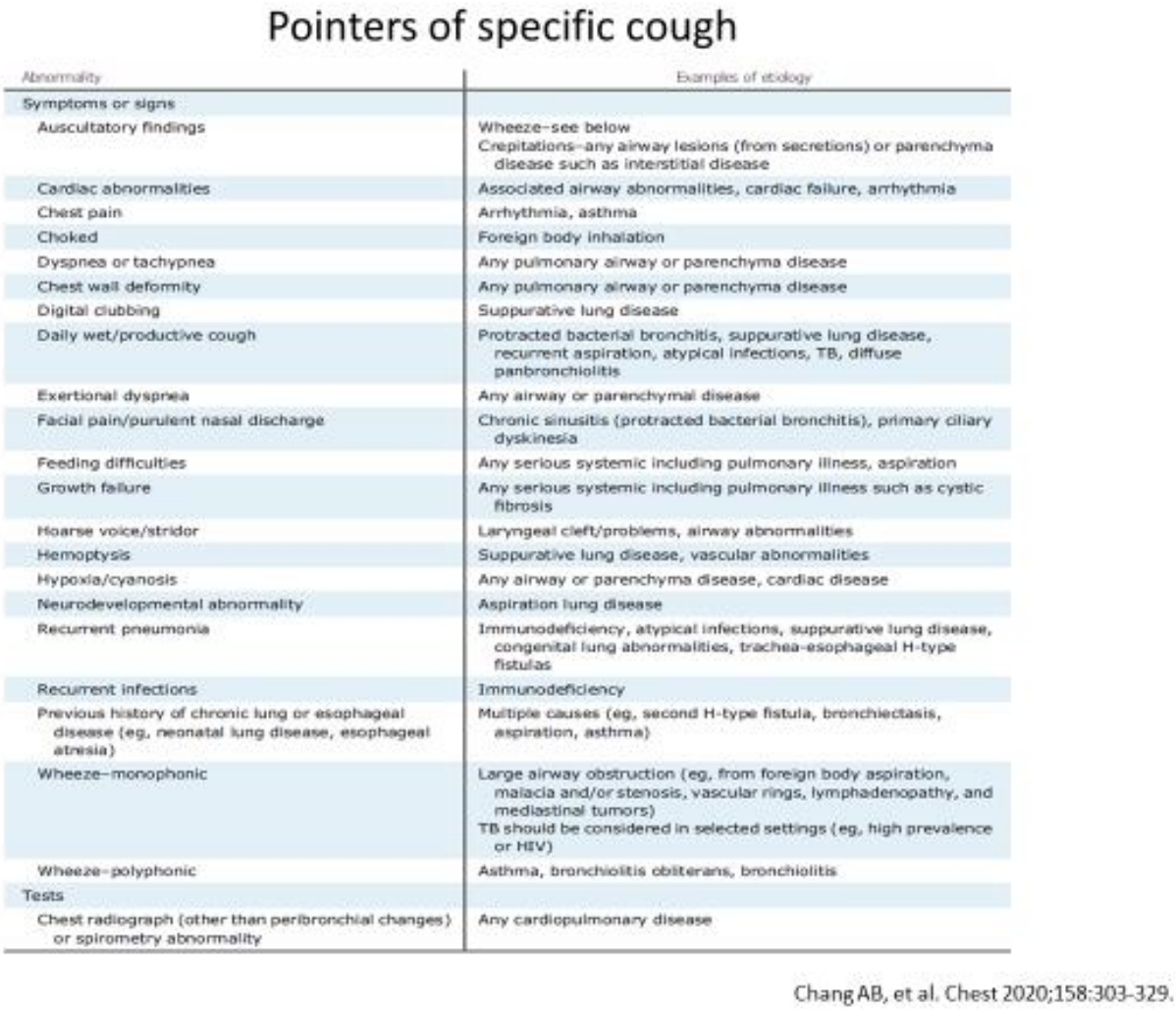
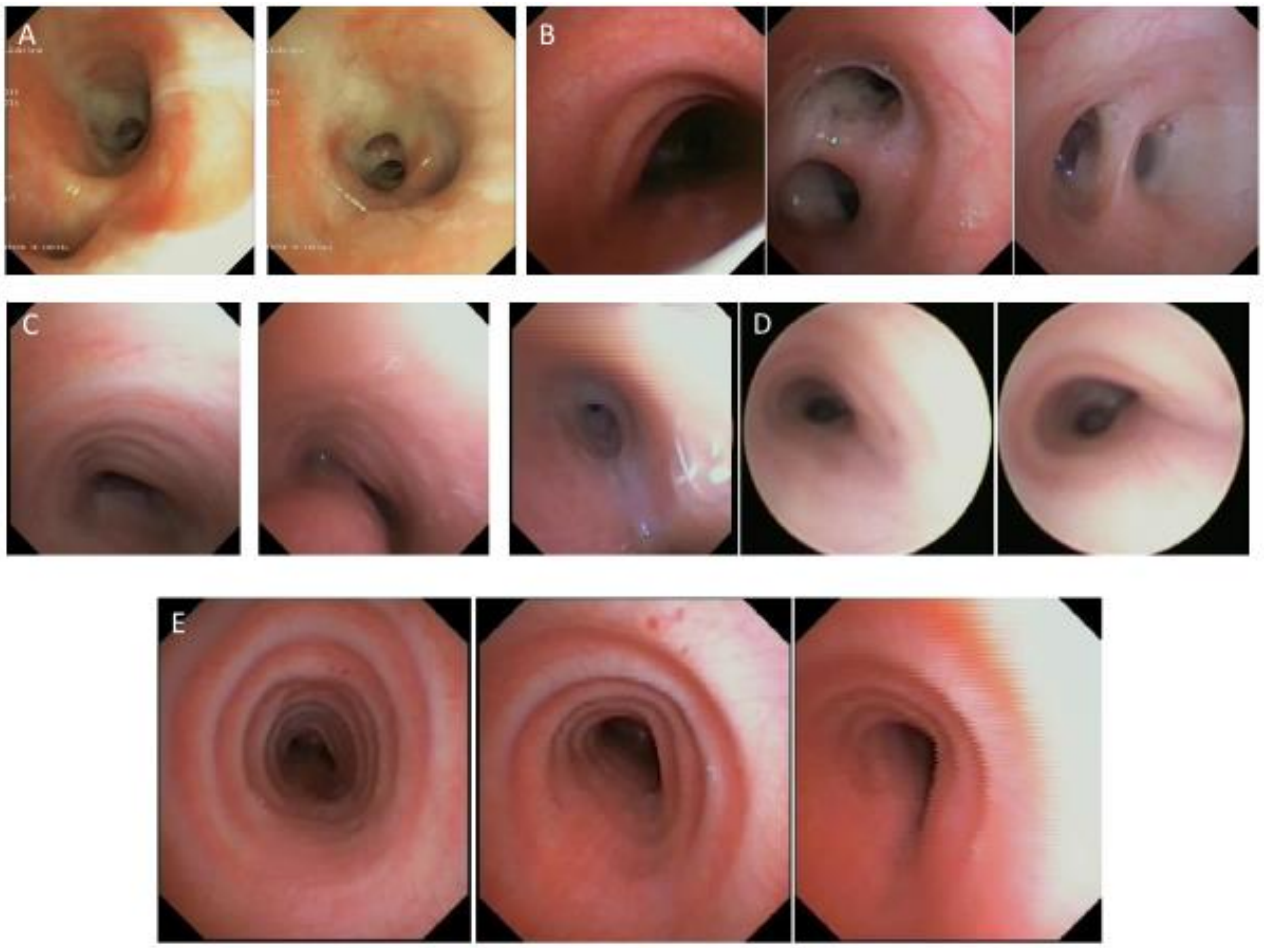


Pointers of specific cough

Abnormality	Examples of etiology
Symptoms or signs	
Auscultatory findings	Wheeze–see below Crepitations–any airway lesions (from secretions) or parenchyma disease such as interstitial disease
Cardiac abnormalities	Associated airway abnormalities, cardiac failure, arrhythmia
Chest pain	Arrhythmia, asthma
Choked	Foreign body inhalation
Dyspnea or tachypnea	Any pulmonary airway or parenchyma disease
Chest wall deformity	Any pulmonary airway or parenchyma disease
Digital clubbing	Suppurative lung disease
Daily wet/productive cough	Protracted bacterial bronchitis, suppurative lung disease, recurrent aspiration, atypical infections, TB, diffuse panbronchiolitis
Exertional dyspnea	Any airway or parenchymal disease
Facial pain/purulent nasal discharge	Chronic sinusitis (protracted bacterial bronchitis), primary ciliary dyskinesia
Feeding difficulties	Any serious systemic including pulmonary illness, aspiration
Growth failure	Any serious systemic including pulmonary illness such as cystic fibrosis
Hoarse voice/stridor	Laryngeal cleft/problems, airway abnormalities
Hemoptysis	Suppurative lung disease, vascular abnormalities
Hypoxia/cyanosis	Any airway or parenchyma disease, cardiac disease
Neurodevelopmental abnormality	Aspiration lung disease
Recurrent pneumonia	Immunodeficiency, atypical infections, suppurative lung disease, congenital lung abnormalities, trachea-esophageal H-type fistulas
Recurrent infections	Immunodeficiency
Previous history of chronic lung or esophageal disease (eg, neonatal lung disease, esophageal atresia)	Multiple causes (eg, second H-type fistula, bronchiectasis, aspiration, asthma)
Wheeze–monophonic	Large airway obstruction (eg, from foreign body aspiration, malacia and/or stenosis, vascular rings, lymphadenopathy, and mediastinal tumors) TB should be considered in selected settings (eg, high prevalence or HIV)
Wheeze–polyphonic	Asthma, bronchiolitis obliterans, bronchiolitis
Tests	
Chest radiograph (other than peribronchial changes) or spirometry abnormality	Any cardiopulmonary disease

Chang AB, et al. Chest 2020;158:303-329.

Figure 5. Pointers of specific cough etiology.



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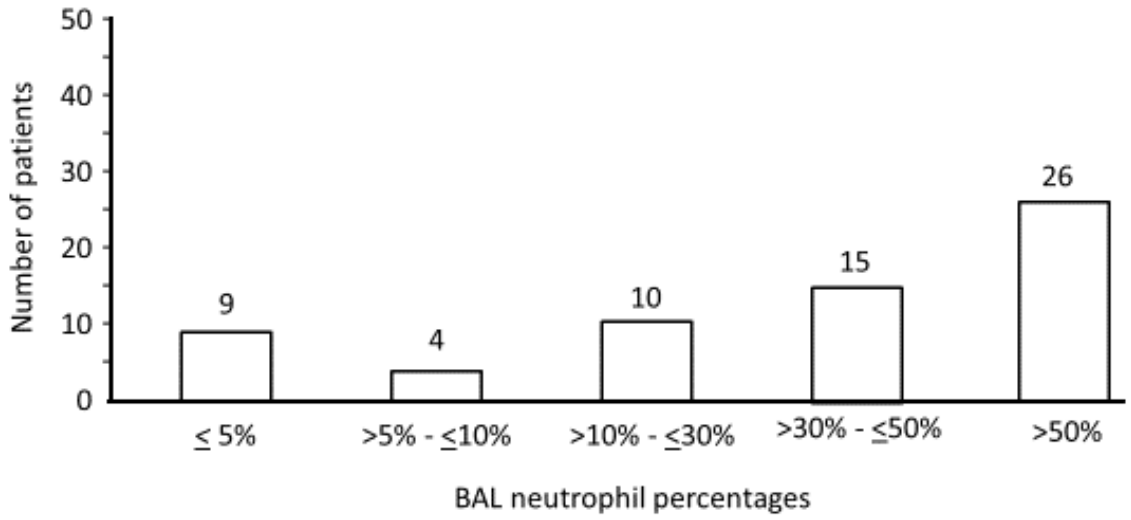

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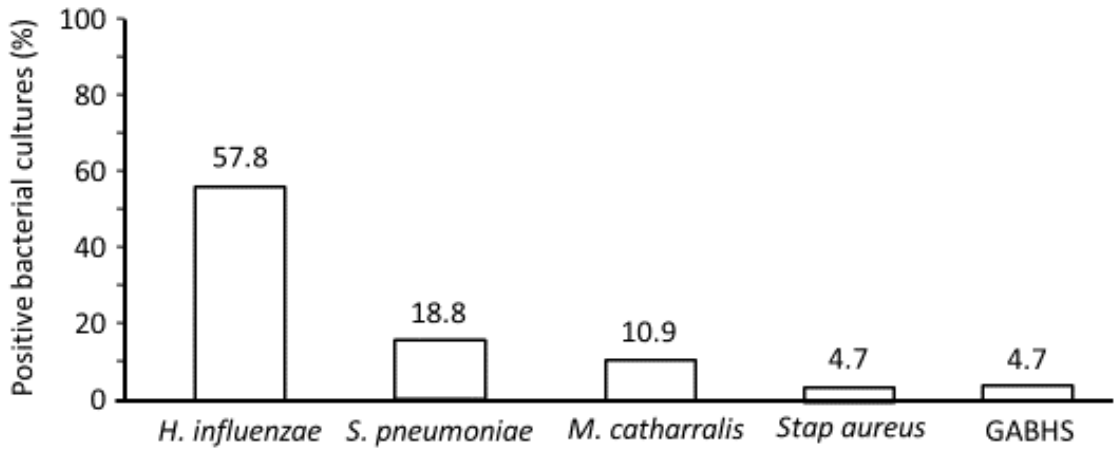
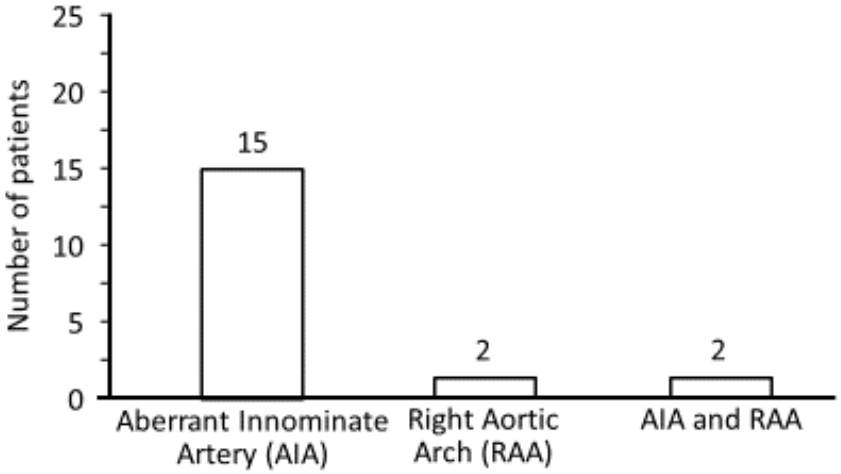


* GABHS: group A β-hemolytic streptococci

Figure 8. Percentage of bronchoalveolar lavage sample cultures with bacterial load > 10^4 CFU/mL for *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and Group A β-hemolytic streptococci (GABHS).



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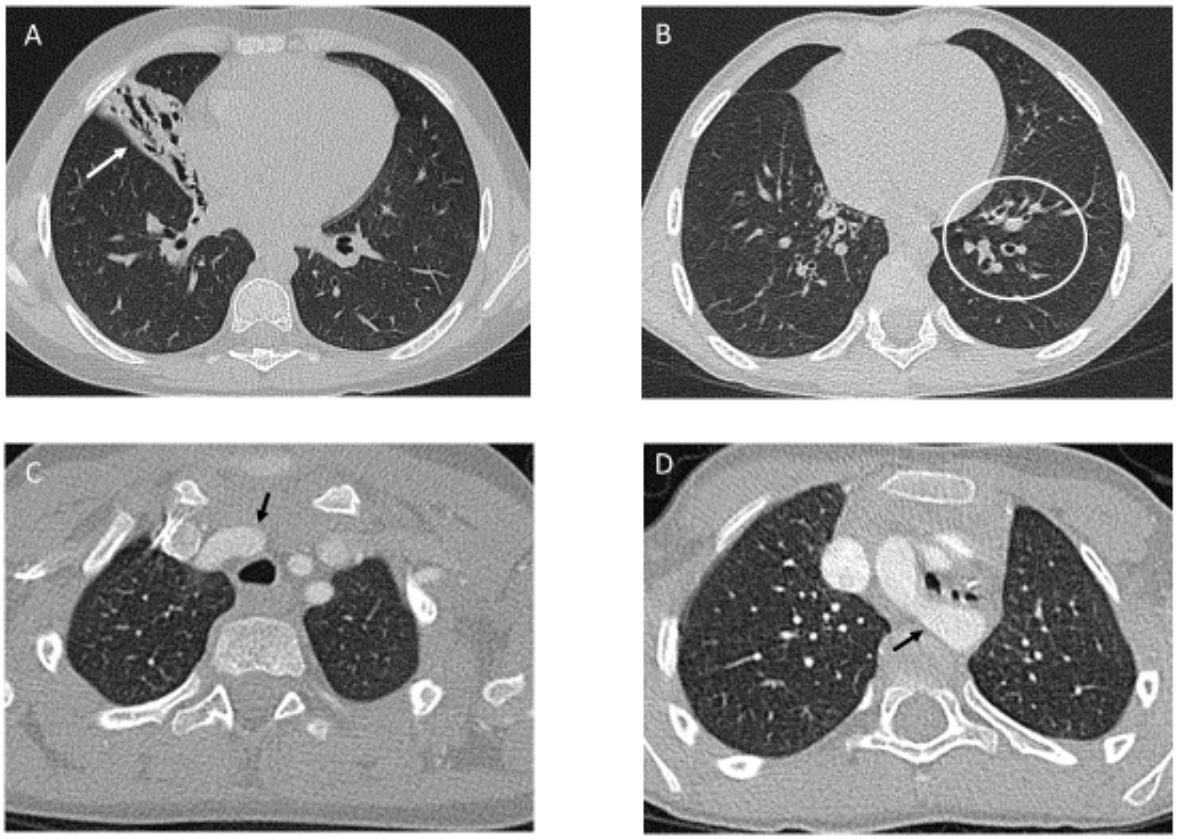

Figure 10. Examples of the CT scans of children with chronic wet cough: A. Medium lobe bronchiectasis and atelectasis (white arrow) in a 9 years patient with recurrent wet cough and fever, BAL bacterial load $\geq 10^4$ CFU/mL and cultures positive for *Haemophilus influenzae*. B. Left lower lobe bronchiectasis (white circle) in an 8 years old patient with recurrent wet cough, BAL bacterial load $\geq 10^4$ CFU/mL and cultures positive for *Haemophilus influenzae*. C. Tracheal compression by aberrant innominate artery (black arrow) without bronchiectasis in a 5-years-old, with BAL bacterial load $\geq 10^4$ CFU/mL and cultures positive for *Staphylococcus aureus*. D. Right aortic arch in a 4 years old patient. The TC scan shows the right circumflex aortic arch with the left thoracic descending aorta (black arrow). BAL bacterial load was $\geq 10^4$ CFU/mL and cultures positive for *Streptococcus pneumoniae*.

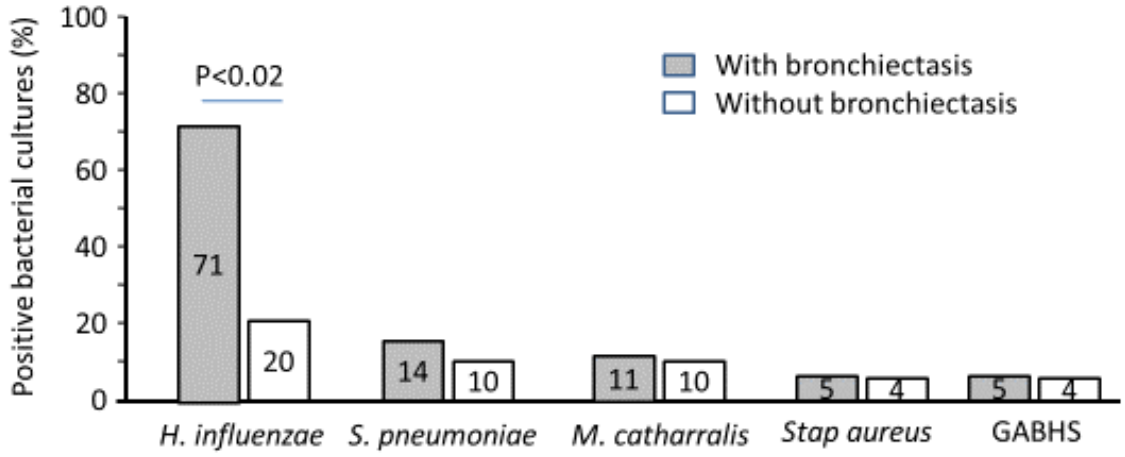

Figure 11. Percentage of bronchoalveolar lavage sample cultures with bacterial load > 10^4 CFU/mL for *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and Group A β-hemolytic streptococci (GABHS) in children with and without bronchiectasis.

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Table 1. Fiberoptic bronchoscopy	[N (%)]
• Airways inflammation	64 (90.1)
• Diffuse airways inflammation with mucopurulent secretions	38 (53.5)
• Airways malacia	23 (32.4)
- Primary malacia	4 (5.6)
- Secondary malacia (due to extrinsic pulsatile airway compression)	19 (26.8)
• Bronchial hypoplasia	10 (14.1)

• Table 2. Neutrophilia in BAL samples with bacterial load (BL) < or $\geq 10^4$ CFU/mL

	BL $<10^4$ CFU/mL (N=25)	BL $\geq 10^4$ CFU/mL (N=39)	p values
• Neut ≤5%	8 (32.0)	1 (2.6)	p<0.001
• Neut >5-<10%	1 (4.0)	3 (7.7)	p=0.55
• Neut 10-<30%	5 (20.0)	5 (12.8)	p=0.44
• Neut 30-<50%	7 (28.0)	8 (20.5)	p=0.49
• Neut ≥50%	4 (16.0)	22 (56.4)	p<0.001

Part I

Cough in children

Part I - Cough in children

1. Introduction

Cough is a physiological defense mechanism to accelerate mucus clearance, to clear excessive secretions and to remove inhaled foreign material from the airways. The mean number of coughing episodes in healthy children has been measured as 11 (range 1-34) per 24 hours[1]. However, cough may also be the first, initial symptom of a variety of respiratory tract disorders and rarely of extrapulmonary diseases but also be initiated and suppressed voluntarily and represent habit a tic or psychogenic cough that has no underlying organic cause and does not respond to conventional medical treatment[2]. Independently from the nature, when persistent or recurrent, cough can severely impact the quality of life and be one of the most common medical complaints for which parents seek medical assistance. 75% to 80% of children with cough have sought more than 5 consultations prior to presentation to a respiratory specialist[3-5]. Despite the recent advances in understanding the mechanisms that regulate cough, in physiological and pathological conditions, when a specific etiology is not detectable, current therapeutic options are little or only moderately effective. When specific cough pointers cannot be identified through medical history and physical examination and routine tests, more “aggressive” approaches, such as fiberoptic bronchoscopy with cytological and microbiological evaluation of bronchoalveolar lavage fluid, and eventually chest computed tomography (CT) scan, should be performed to avoid unnecessary diagnostic examinations and ineffective chronic treatments.

2. The physiology of cough

Cough is a forced expulsive maneuver usually against a closed glottis and constitutes a vital protective reflex to clear the respiratory tract from inhaled foreign materials or secretions[6]. Cough reflex has both sensory (afferent) mainly via vagus nerve and motor (efferent) components (figure 1). Cough receptors are rapidly adapting irritant receptors, located mainly in pharynx, larynx on the posterior wall of the trachea and at the tracheal carina level[2]. The receptors are less abundant in the distal airways, i.e. in respiratory bronchioles and alveolar septa. Pharynx, larynx and the major conducting airways are sensitive to both mechanical and chemical stimuli, whilst terminal bronchioles and even the alveoli are sensitive to chemical stimuli such as sulfur dioxide gas or chlorine gas[7]. More airway receptors are found in the external auditory canals, eardrums, paranasal sinuses, diaphragm, pleura, pericardium, and stomach[2]. These are probably mechanical receptors only, which can be stimulated by triggers such as touch or displacement. When triggered, receptors send impulses traveling via the afferent neural pathway, i.e. through the internal laryngeal nerve, a branch of the superior laryngeal nerve which stems from the vagus nerve, to the medulla oblongata of the brain, were the cough center is located[2]. In the efferent pathway, impulses then travel from via the vagus, phrenic, and spinal motor nerves to

diaphragm, abdominal wall and muscles[8]. The *nucleus retroambigualis*, by phrenic and other spinal motor nerves, sends impulses to the inspiratory and expiratory muscles, whilst the *nucleus ambiguus*, by the laryngeal branches of the vagus sends impulses to the larynx[9]. These impulses promote the four phases that characterize the cough: 1) the inspiratory phase, a rapid deep inspiration through a widely open glottis; 2) the compression phase, characterized by contraction of the respiratory muscles producing an expiratory effort against a closed glottis, with rapid generation of high intrapulmonary pressure; 3) the expiratory phase, with sudden opening of the glottis causing explosive expiration and a high linear velocity of gas flow; 4) the cessation stage[10]. At least three broad classes of receptor classes can be identified based on their myelination, conduction velocity, physicochemical sensitivity, adaptation to sustained lung inflation, neurochemistry, origin, sites of termination in the airways. These include the rapidly adapting receptors (RARs), the slowly adapting stretch receptors (SARs) and the C-fibers, which are sensitive to different mechanical and chemical stimuli[11] (figure 2). Most of the afferent nerves innervating the airways are the unmyelinated C-fibers, relatively insensitive to mechanical stimulation and lung inflation but are directly activated by chemical stimuli such as capsaicin, citric acid, bradykinin, hypertonic saline solution and sulfur dioxide (SO_2), which interact with two non-selective ion channels: the transient receptor potential ankyrin 1 (TRPA1) and the transient receptor potential vanilloid 1 (TRPV1)[12]. At least in physiological conditions, it is very likely that cough reflex can be regulated by complex interactions between subtypes of sensory nerve pathways, diverse according to the characteristics of the stimulus. The disruption of the balance among complex interactions may increase the sensitivity of the peripheral nerve endings, leading to the generation of excessive, difficult to control, coughing not only in patients with chronic bronchial pulmonary disease, but also in normal subjects during acute infections of the upper or lower airways[13].

3. Classification of cough based on symptom duration and its epidemiology

In the pediatric population, cough can be classified as acute, subacute, or chronic depending on how long it lasts. Acute coughs last less than two weeks and usually are caused by the common cold or other infections such as sinusitis or pneumonia. Subacute coughs last two to four weeks and remain after the initial cold or respiratory infection is over. Chronic coughs last more than four weeks and can be caused by gastroesophageal reflux disease (GERD), postnasal drip from sinus infections, allergies, long-lasting infections, chronic lung conditions, airway malformations and psychogenic disorders. There are no studies that have clearly defined when cough should be labelled as chronic: it is described variously as a persistent daily cough of more than 4,6 and 8 weeks. The British Thoracic Society (BTS) defined as chronic a cough lasting longer than 8 weeks, rather than the 4 weeks recommended

by the American College of Chest Physicians (ACCP) and the Thoracic Society of Australia and New Zealand (TSANZ)[5,14-17]. An intermediate time zone, defined as "prolonged acute cough", is considered

in the BTS guideline to include a 2-3 weeks period for cough resolution in children with post-viral cough meeting the criteria for chronic cough who may undergo unnecessary investigations. A proportion of children with uncomplicated pertussis may also find their symptoms resolve during this time. Recurrent cough refers to >3 episodes/year, each lasting more than 7 to 14 days. If the cough-free periods between the exacerbations are short, it can be difficult to distinguish recurrent from chronic cough[5,18].

4. Chronic cough epidemiology in pediatrics

No studies have systematically compared the prevalence of chronic cough in children worldwide. Reports of chronic cough in populations vary between 1% in India, 9% in Eastern Europe and 5-12% in China with increases in areas with higher air pollution[19-21]. Many studies highlighted that there is a substantial and frequently unrecognized burden of morbidity attributable to chronic cough in childhood with prevalence of around 10%. There is no precise data on the burden of chronic cough, probably because chronic cough was previously perceived not as a clinical entity but as the subsequent symptom from other respiratory conditions. Moreover, subjective perception and parental reporting of symptoms further biases prevalence reports. Therefore, the charge of chronic cough in children is influenced by age range, gender, air pollution and the quality of the health care system [5,22-29]. Chronic wet cough is very common among pre-school (0-6 years aged) children whose parents sought medical consultation, particularly in the first two-three years of life [30,31]. Some authors found that 63.9% of children newly referred to pediatric pulmonologists for chronic cough had wet or productive cough[32]. Unlike the symptom of dry cough, chronic wet cough is more reliably reported by parents when compared to clinician's assessment and bronchoscopic findings[33]. The mean or median age of children with chronic wet cough is 10 months to 8.4 years and ranged from 0.3 to 17 years. Children with chronic wet cough are significantly younger than those with chronic dry cough. A median duration cough 28 weeks (minimum quartile value 11.5 weeks and maximum quartile 56.3 weeks) is observed in affected children[34,35].

5. Classification of chronic cough based on characteristics

In the anamnestic evaluation of the child the characteristics of the cough should be carefully defined. There are certain cough types which can be readily led to a specific diagnosis. Pertussis or whooping cough is characterized by severe paroxysms of coughing, followed by a gasping inspiration producing

cough may be characteristic of tracheomalacia, and particularly when associated with tracheoesophageal fistula but, again, may not be heard or reported in infants and young children[37].

Psychogenic coughs may appear as dry repetitive habit 'tic-like' coughs or honking with the child not being very disturbed by the cough, which is typically non present during sleep or when the child is

absorbed in an activity. Chronic cough with short, staccato sounds is a characteristic sign of the lung infection by *Chlamydia pneumoniae*, especially in infants. Cough can also be differentiated in wet and dry (figure 3). A chronic productive or wet cough suggests some underlying cause inducing mucous hypersecretion that needs to be investigated. Purulent secretions are most likely associated with diseases that promote chronic or recurrent bacterial infection, such as bronchiectasis, primary ciliary dyskinesia and cystic fibrosis. A dry cough suggests airway irritation, inflammation and can be associated with bronchial hyperreactivity with or without wheezing, and allergic sensitization. It is important to note that some children with dry coughing may have periods with wet cough e.g. during respiratory infections[14,15,38-41].

6. Chronic wet cough

Since infants and pre-school children are not usually able to expectorate, in pediatrics cough associated with substantial respiratory secretions should be defined as "wet" not as "productive"[14,15]. The etiology of chronic cough are, at least partially, age dependent (figure 4). Several conditions should be considered in the differential diagnosis of chronic wet cough, bronchiectasis, primary ciliary dysfunctions, cystic fibrosis, pulmonary aspiration, primary and secondary airway malacia, immunodeficiency, missed foreign body aspirations and chronic infections[14,15,18,32,34,35,42-47]. Prompt recognition and early management of child with chronic wet cough is important to prevent inappropriate treatment and the onset of persistent functional and structural damage to the airways[48]. In recent years, increasing attention has been focused on the most common cause of chronic wet cough, chiefly in young children: protracted bacterial bronchitis [22,23,30,31,34].

7. Protracted bacterial bronchitis: the initial definition

The term protracted bacterial bronchitis (PBB) was first described as a diagnostic entity by the Brisbane group in 2006[49] and then recognized in National and International Guidelines as a cause of chronic wet cough in children[17]. The initial definition included: a) a history of chronic wet cough, b) positive bronchoalveolar (BAL) cultures for a respiratory pathogen, and c) response to a 2-week course of oral amoxicillin–clavulanic acid treatment[49]. In that study by Chang AB and co-workers, evidence of airway neutrophilic inflammation was detected by bronchoalveolar lavage (BAL) and the most

common pathogens isolated were *Haemophilus influenzae* in 47%, *Streptococcus pneumoniae* in 35% and in 26% *Moraxella catarrhalis*[49]. Trachea-bronchomalacia was found in 33% of PBB children[49]. In a follow-up study, the same researchers group evaluated the 2-year outcomes PBB children and found that high *Haemophilus influenzae* titers in BAL fluid were major risk factors for bronchiectasis suggesting a role of this specific infection in bronchiectasis pathogenesis[50]. The response to antibiotics (positive in only 48% of children) was confirmed in that relatively small randomized controlled trial[50], used a cough score as an endpoint, rather than any more scientific biomarker or objective cough counting. Children included in that report did not have any signs and symptoms associated with underlying illnesses that could explain the presence of prolonged wet cough[50].

8. Persistent bacterial bronchitis: the revised definition

Because of the inappropriateness to perform bronchoscopy in many not severely affected young children[22], the original protracted bacterial bronchitis (PBB) definition was modified by the European Respiratory Society (ERS) statement and termed "PBB-micro", because it includes microbiological analysis of BAL. To this term, the new term "PBB-clinical" has been associated, which eliminates the need for BAL, but overtly acknowledges the need to exclude other causes of chronic wet cough, which is implied but not stated in PBB-micro[14,23]. The "PBB-clinical" clinical entity is characterized by an isolated chronic wet or productive cough, lasting for four weeks or more, without any evidence of the signs and symptoms known as specific cough pointers (figure 5) without signs of another cause. Many cases of PBB responding to a relatively short course of oral antibiotics have a resolution of cough after a course of appropriate antibiotic (usually oral amoxicillin-clavulanic acid) for at least two weeks[15]. If the affected children do not respond to appropriate treatment with antibiotics it is probable that PBB is misdiagnosed and further investigation is required to reach an accurate diagnosis, avoiding the abuse of antibiotic therapy and the occurrence of respiratory complications such as the onset of bronchiectasis[23]. Unfortunately, in the ERS statement there is no requirement to try to define infection non-bronchoscopically[51,52]. This is a sad example of the current "don't measure" culture of pediatric pulmonology, which is a significant omission, as is the absence of any requirement to the appropriate use of antibiotics and to test if infection has resolved after treatment. The ERS statement, defining "PBB-clinical" allows up to 4 weeks of antibiotics to resolve symptoms gut, again, there is no requirement for positive bacteriology[23]. Moreover, "PBB-micro" or "PBB-clinical" with resolution necessitating 4 weeks of antibiotics is termed "PBB-extended" and if there is a recurrence of more than 3 episodes of PBB/year is termed "recurrent PBB" [23]. As to what investigations should be performed to exclude other causes of chronic wet cough, this is left to judgment of the treating physician.

9. Conclusions

Therefore, PBB is a description, not a diagnosis, which should be included in a broader umbrella together with all the other chronic suppurative lung disease endotypes. It is perfectly clear that in children with chronic wet cough negative response to antibiotic treatment may be explained, for example, by anatomical airway obstruction, a local or systemic immunodeficiency, or any one of many aspiration syndromes. These children should be referred for further investigations when specific cough pointers are present or when the wet cough does not respond to 4 weeks of antibiotics[53].Presence of bronchiectasis and other structural airway abnormalities detectable with chest computed tomography scans can be clearly associated with longer duration of chronic wet cough and with failure of the cough to respond to 4 weeks of antibiotics[23]. In children with the “extended” and “recurrent” PBB phenotypes, understanding of disease pathophysiology defining those at high risk of progressing to bronchiectasis will likely lead to novel and highly specific treatments.

Part II

Management of children with chronic wet cough: our experience

Part II - Management of children with chronic wet cough: our experience

1. Materials and Methods

1.1 Study population

A retrospective study was performed on records of 110 children admitted to the Pulmonary Disease Unit of the Giannina Gaslini Children Hospital, from January 2016 to February 2020. These children had been consecutively enrolled from a population referred to our Unit for a clinical history of chronic/recurrent wet cough not responding to medical therapies. Most of the patients had history of recurrent lower respiratory tract infections, but were evaluated while in stable condition, at least two months after the last episode requiring antibiotic treatment. We excluded children presenting congenital anomalies or malformations of the esophagus (such as esophageal atresia or tracheoesophageal fistula), history suggestive of inhaled foreign body aspiration, swallowing disorders, primary ciliary dyskinesia, cystic fibrosis and known cellular/humoral immune deficiencies. The original protocol was approved by the local ethics committee but, because of the retrospective nature, ethical approval and patient consent were judged unnecessary for the present study.

1.2 Patient evaluation

Demographic data were collected uniformly in all children, focusing specifically on age at onset, type, severity and persistency of respiratory symptoms, and presence of comorbidities. Physical examination and routine blood tests, serum immunoglobulin levels and blood lymphocyte subpopulations, microbiological analysis of nasopharyngeal swabs, which included specific Polymerase Chain Reaction (PCR) for respiratory viruses, *Bordetella pertussis* and *Mycoplasma pneumoniae* and skin prick tests for the most common classes of allergens, were performed in all patients. Flows and volumes were measured by spirometry in those able to perform reproducible forced expiratory maneuvers[54]. Esophageal impedance/pH monitoring, barium contrast fluoroscopy and fiberoptic esophagogastroscopy were executed when gastroesophageal reflux, aspiration or structural abnormalities of the gastroesophageal tract (pyloric stenosis, malrotation, hiatal hernia, or esophageal stricture) were suspected[55]. Furthermore, in patients with recurrent respiratory tract infections ciliary beat frequency on nasal epithelial cells and cystic fibrosis transmembrane regulator gene mutations were evaluated. Finally, when clinically indicated, rhino laryngoscopy was performed to diagnose malformation or obstruction of the upper airways. Video bronchoscopy and bronchoalveolar

lavage (BAL) was performed when required, followed by chest radiography and/or computed tomography (CT) with or without radiocontrast agents.

1.3 Video bronchoscopy and bronchoalveolar lavage (BAL) cell analysis

Video bronchoscopy and BAL were performed as previously described[56]. 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 video bronchoscope (BF-3C160 or BF-P190, Olympus, Tokyo, Japan) was passed through a ventilation mask and introduced in a nostril, with the patient spontaneously breathing. Ventilation was assisted manually, when needed. After local anesthesia of the airways with 1% lidocaine solution, BAL was performed by injecting in a bronchial subsegment 3×1 ml/kg body weight aliquots of sterile saline solution, which were aspirated at a negative pressure of 40 mmHg in siliconized plastic tubes. When no segmental abnormalities or localized inflammatory lesions were detected, BAL was performed in right middle lobar bronchus or in the lingula. During the procedure, suctioning was begun only in lower airway tract to decrease the operating channel contamination by germs from upper respiratory tract. All bronchoscopic procedures were videotaped. BAL fluid samples were sent to the microbiology department to detect the presence of bacteria, mycobacteria and viruses. The volume of fluid recovered and the total and differential cell counts were recorded, as previously described[55].

1.4 Chest computed tomography (CT) scan

Multidetector CT was performed with a 64-slice CT scanner (Siemens Somatom Sensation 64, Siemens, Erlangen, Germany), using an intravenous radiocontrast agent (Iomeron 300, Bracco, Milan, Italy), which was administered, in all patients, with a power injector via a peripheral vein. The examination was performed, when possible, holding the breath in the inspiratory phase, but quiet breathing was tolerated in younger and non-cooperative patients[57].

1.5 Lung functions

Lung function test by spirometry was performed in cooperative children able to perform acceptable and reproducible forced expiratory manoeuvres[54,58]. Forced vital capacity (FVC), forced expiatory volume in 1 second (FEV1), and their ratio (FEV1/FVC) were measured (Med

Graphics, Pulmonary Function System 1070 series 2, Med Graphics Corporation, Saint Paul, Minnesota, USA), following the ATS/ERS guideline[59].

1.6 Follow-up

The follow up data were collected, 12-18 months after diagnosis, from clinical records obtained from following hospital admissions to the hospital or to out-patients clinic visits, or by phone interviews. A questionnaire was used designed to assess the (i) presence, frequency, and severity of symptoms and the (ii) quality of life (QoL) of children and their families, as previously described[60]. A satisfaction test using a Visick symptom scale was used to assess the "general" outcome rated by means of the Visick symptom scale[60], which includes 4 steps: VS1—perfect result=asymptomatic patient; VS2—mild=intermittent symptoms controlled by therapy without a substantial interference on life-style; VS3—moderate=recurrent symptoms, with substantial interference upon life-style; VS4—unsatisfactory result[60].

1.7 Data analysis

Qualitative variables (such as age, neutrophil percentage in BAL, FCV, FEV_1, FEF_{25-75}) were presented as median, with inter-quartile range, along with maximum and the minimum value in case of large distribution data from the mean, or in the form of median, with standard deviation, if number of outliers were irrelevant. Quantitative variables (such as high versus low bacterial load in BAL, patients with bronchiectasis versus without bronchiectasis) were presented in the form of absolute and relative frequency. Explorative analysis was the first step in collected data evaluating, to describe general characteristics of our study population and its qualitative/quantitative data. In order of type of variables, we used different statistical analysis. For quantitative variables, parametric and nonparametric methods were used. Parametric method, such as t-test, were performed with the data following a normal distribution and when variances of the distributions were equal. Nonparametric method, such as Mann-Whitney test, were employed in case of data following an abnormal distribution. Chi-square test (or Fisher's exact test if the numbers are very small) were used to compare categorical or qualitative variables. A p-value ≤ 0.05 was considered statistically significant. All statistical analysis procedures were performed using GraphPad Prism3 program (GraphPad Software Inc.).

2. Results

2.1 Patients population

110 children, 50 males (45.5%) and 60 females (54.5%), mean age at time of referral of 5.79 (4.10-7.78) years, were included in the study. Median age at the symptom onset was 24 (12.0-48.0) months and the median period between symptoms onset and diagnosis was 3.0 (2.0-5.0) years.

2.2 Pulmonary functions

Spirometry was performed in 47 cooperative children. 27 patients (57.4 %) showed normal (>90% than predicted) values for pulmonary volume (FVC and FEV1). 6 patients (12.8 %) showed a restrictive spirometry pattern and 15 children (32.0%), an obstructive pattern.

2.3 Video bronchoscopy

Video bronchoscopy was performed when clinically indicated because of the high frequency and/or the severity of respiratory symptoms. Out of 71 children (64.5% of the total patient population) in which endoscopy was performed, 64 (90.1%) had evidence of airways inflammation, associated with mucopurulent secretions in 38 (53.5%). Moreover, airways malacia was detected in 23 (32.4%) children, primary in 4 (5.6%) and secondary, due to extrinsic pulsatile airway compression, in 19 (26.8%) (table 1) (figures 6 A, B, C, D and E).

2.4 Cytological and microbiological analysis of BAL fluid

Cytologic evaluation was performed in 64 (90.1%) BAL fluid and showed a neutrophil count $\geq$10% in 51 (79.7%) of the samples. The median neutrophil count in BAL fluid was 48.8% (14.8% - 83.0%): $\leq$5% in 9 children (14.1%), >5-<10% in 4 (6.3%), 10-<30% in 10 (15.6%), 30-<50% in 15 (23.4%), ≥50% in 26 (40.6%) (figure 7). BAL fluid cultures were performed in 64 children (90.1%) and demonstrated bacterial growth in 48 patients (75.0%): *Haemophilus influenzae* in 37 samples (57.8%), *Streptococcus pneumoniae* in 12 (18.8%), *Moraxella catharralis* in 7 (10.9%), *Staphylococcus aureus* in 3 (4.7%), group A beta-hemolytic Streptococcus (GAS) in 3 (4.7%) (figure 8). Bacterial load was $\geq 10^4$ CFU/mL in BAL fluid from 39 children (60.9%) of whom 4 with neutrophil count <10% and 35 with neutrophil counts $\geq$10% ($p<0.001$). The degree of neutrophilia in BAL samples with bacterial load < or $\geq 10^4$ CFU/mL is shown in table 2.

2.5 Roentgenographic evaluation

Chest x-rays, performed in 90 children (81.8%), demonstrated peri bronchial interstitial thickening in 49 (54.4%), parenchymal lung consolidation in 32 (35.6%) and atelectasis in 9, (10.0%). Chest CT scan with contrast agent, performed in 79 children (71.8%), allowed a more detailed examination of the lungs and showed bronchial obstruction and air trapping (n=45, 63.4%), bronchiectasis in 30 (42.3%), parenchymal lung consolidation in 24 (33.8%), peribranchial interstitial thickening in 23, (32.4%). Extrinsic tracheal compression was shown in 19 children (26.8%), induced by aberrant innominate artery (AIA) in 15 (79.0%), right aortic arch (RAA) in 2 (10.5%) and AIA + RAA association in 2 (10.5%) (figure 9) (figures 10 A, B, C and D).

2.6 Airway inflammation and infection in children with and without bronchiectasis

As compare with the "without bronchiectasis" group, the median age at hospitalization was significantly higher in the "with bronchiectasis" group [7.4 (5.5-10.7) years vs. 5.6 (4.1-7.0) years; p <0.001], which also showed a tendency to have a higher median age at symptoms onset [33.5 (24.0-48.0) months vs. 24.0 (12.0-36.0) months; p 0.07], and a longer period between symptoms onset and diagnosis [4.0 (2.0-7.0) years vs. 3.0 (2.0-4.0) years: p 0.08]. BAL cytological examination showed a significant higher median neutrophil count in the "with bronchiectasis" group [80.3 (30.1-87.6)], as compared with the "without bronchiectasis" group [21.0 (2.7-60.3); (p< 0.0001). Moreover, as compared with the "without bronchiectasis" group, the "bronchiectasis group" had a higher percentage of children with airway malacia [20/30 (66.7%) vs. 3/34 (8.8%), p<0.00001), and cultures positive for *Haemophilus influenzae* (p<0.02) (Figure 11).

2.7 Treatment and follow-up

The approach to treatment was based on respiratory physiotherapy with PEPmask twice a day for several months, associated with high-dose amoxicillin/clavulanic acid (40 mg/kg/ day) for 2 weeks and reviewing response to treatment. If the cough did not clear, the antibiotic was continued for a further 4–6 weeks in the first instance[61]. Improvement in symptoms, shortly after the 2-weeks course was detected in 34 of 39 (87.18%) patients: only 5 (all belonging to bronchiectasis and with a bacterial load ≥10^4 cfu/mL group) required a longer treatment for

symptom resolution. Evaluation of the long-term outcome was obtained in 32 of 39 the antibiotic-treated children: 13 (40.6%) could be classified as "completely asymptomatic" (Visick symptom scale 1), 9 (28.1%) as "intermittent symptomatic, without a substantial interference on life-style" (Visick symptom scale 2), 6 (18.7%), of which 4 with bronchiectasis, as "recurrent symptoms, with substantial interference upon life-style" (Visick symptom scale 3) and 4 (12.5%), of which 3 with bronchiectasis, as "unsatisfactory result" (Visick symptom scale 4). Airways malacia, BAL bacterial load $\geq 10^4$ CFU/mL and cultures positive for *Haemophilus influenzae* occurred in all children with Visick symptom scale 3 and 4.

4. Discussion

We studied patients referred to our Unit for chronic/recurrent wet cough. Most of the patients were previously treated with repeated and short (6-8 days) courses of antibiotic therapy with limited and transient clinical benefit. Our study population underwent routine blood tests and various diagnostic procedures when indicated, primarily to rule out noninfectious causes of chronic wet cough, but the two main qualifying procedures we performed to evaluate the most symptomatic patients were bronchoscopy with BAL and chest CT scan. In pediatric age, sputum collection from the lower airways is difficult to perform, even with an induced sputum procedure, and video bronchoscopy is an essential diagnostic tool to investigate the presence of possible lower airway abnormalities and to assess the cytology and the infectious status of the more distant airways through bronchoalveolar lavage[63]. CT scan of the chest with contrast medium was performed whenever indicated by the clinical history and/or video bronchoscopy finding. Out of the 71 children in which endoscopy was performed, 38 (53.5%) had evidence of bronchial inflammation with mucopurulent secretions and 23 (32.4%) of airway malacia due to extrinsic pulsatile airway compression in 19 (26.8%). The presence of an aberrant innominate artery (AIA), as the cause of the extrinsic compression, was shown by CT scan in 15 of these children. Airway malacia is not a rare condition in the general paediatric population, as reported by Boogaard et al.[64] In that study, out of total of 512 bronchoscopies performed in children with a median age of 4.0 years, 160 cases of airway malacia were detected and classified as primary in 136 cases and secondary in 24 cases[64]. Children with airway malacia often have protracted courses of airway infections, because dynamic collapse of the airway lumen during coughing results in impaired mucociliary clearance, favouring airway plugging by bronchial secretions and,

a possible sequence of bronchiectasis formation[65]. The impaired bronchial clearance with persistence of mucus in the bronchial lumen due to airway malacia, can act as a potent trigger of chronic airway inflammation, probably through several mechanisms including hypoxic epithelial necrosis, retention of inhaled irritants or allergens, and potential immunomodulatory effects[66]. Chronic wet cough, evidence of bronchitis at bronchoscopy and airway neutrophilic inflammation are the hallmark of protracted bacterial bronchitis (PBB)[49]. In the first study defining PBB, the Chang AB group reported that trachea-bronchomalacia was present in 33% of children with chronic cough and in a further report showed that BAL cultures grew *Haemophilus influenzae* in 47% of samples, *Streptococcus pneumoniae* in 35% and *Moraxella catarrhalis* in 26%[46]. In our patient population, out of the 71 children in which endoscopy was performed, 38 (53.5%) had evidence of bronchial inflammation with mucopurulent secretions. *Haemophilus influenza* were isolated in 57,8% of the BAL fluids, *Streptococcus pneumoniae* in 18.8% and *Moraxella catarrhalis* in 10,9%. Moreover, chest CT scan showed the presence of bronchiectasis in 30 out of the 79 children. Interestingly, the median age at admission was significantly higher in patients with than in those without bronchiectasis. Also, the age at symptoms onset and the period between symptom onset and diagnosis tended to be longer in the bronchiectasis group, although the differences were not statistically significant. That in patients with bronchiectasis lower airway infection tends to become chronic and is the real culprit in the development of bronchiectasis is demonstrated by BAL cultural examinations, showing that, as compared with the "without bronchiectasis" group, the "bronchiectasis group" had a higher percentage of children with airway malacia, associated with a bacterial load $\geq 10^4$ CFU/mL and cultures positive for *Haemophilus influenzae*. Impaired mucociliary clearance in bronchiectasis patients was associated with a higher neutrophil count, a higher bacterial load and higher percentage of BAL cultures positive for *Haemophilus influenzae***,** higher evidence of airways malacia. These results can only be interpreted as evidence that a delayed therapeutic intervention (prolonged antibiotic therapy plus respiratory physiotherapy) gives enough time for the "vicious cycle": infection, inflammation, and impaired mucociliary clearance, to destroy the integrity of the bronchi and proceed toward the development of bronchiectasis[67]. The aim of antibiotic treatment is to eradicate bacteria and to allow regeneration of the epithelium in the absence of infection. However, in children with chronic wet cough the airway epithelium is likely to be damaged and impaired mucociliary clearance will persist beyond organism clearance. Differing from community acquired pneumonia for which 5-7 days' treatment is usually enough, in chronic wet

cough short courses of antibiotics tend to result in only a partial resolution of the cough or a cough that will relapse after a few days or weeks off treatment[62]. In a follow-up study performed two

years after the diagnosis, the Chang AB group found that a great proportion (43.5%) of 161 PBB children had more than three episodes of bacterial bronchitis per year, 8.1% were diagnosed with bronchiectasis and *Haemophilus influenzae* infection was demonstrated in BAL fluid of 85% of children with bronchiectasis and of 49% of children without bronchiectasis[50]. Our follow up data show also that the presence of bronchiectasis at CT scan is an unfavorable prognostic factors since this patient group had a higher frequency of airway malacia, positive cultures positive for *Haemophilus influenzae* ($p<0.02$), and, despite prolonged antibiotic therapy cycles, had a less favorable long-term outcome.

The data collected in our patients may be useful to describe the possible evolutionary transitions from one condition to another and the best therapeutic approach in these pediatric patients even employing prolonged antibiotic therapies plus respiratory physiotherapy. Protection of the airway against respiratory bacteria for a longer period may reduce the risk of recurrence and may be protective against bronchial wall degeneration leading to the development of bronchiectasis.

References

1. Munyard P, Bush A. How much coughing is normal? Arch Dis Child 1996;74(6):531-534.

2. Canning BJ. Functional implications of the multiple afferent pathways regulating cough. Pulm Pharmacol Ther 2011;24(3):295-299.

3. Chamberlain SA, Garrod R, Douiri A, Masefield S, Powell P, Bücher C, Pandyan A, Morice AH, Birring SS. The impact of chronic cough: a cross-sectional European survey. Lung 2015;193(3):401-408.

4. Chang AB. Cough. Pediatr Clin North Am 2009;56(1):19-31.

5. Shields MD, Bush A, Everard ML, McKenzie S, Primhak R; British Thoracic Society Cough Guideline Group. BTS guidelines: Recommendations for the assessment and management of cough in children. Thorax 2008;63(3):1-15.

6. Morice AH, Fontana GA, Belvisi MG, Birring SS, Chung KF, Dicpinigaitis PV, Kastelik JA, McGarvey LP, Smith JA, Tatar M, Widdicombe J, European Respiratory Society (ERS). ERS guidelines on the assessment of cough. Eur Respir J 2007 Jun; 29(6):1256-1276.

7. Hall, John (2011). Guyton and Hall Textbook of Medical Physiology with Student Consult Online Access (12th ed.). Philadelphia: Elsevier Saunders. p. 473. ISBN 978-1-4160-4574-8.

8. Ashok Mahashur. Chronic dry cough: diagnostic and management approaches. Lung India 2015;32(1):44-49.

9. Polverino M, Polverino F, Fasolino M, Andò F, Alfieri A, De Blasio F. Anatomy and neuro-pathophysiology of the cough reflex arc. Multidiscip Respir Med 2012;7(1):5.

10. Brooks SM. Perspective on the human cough reflex. Cough 2011;7(1):10.

11. Mazzone SB, McGovern AE. Sensory neural targets for the treatment of cough. Clin Exp Pharmacol Physiol 2007; 34(10): 955-962.

12. Taylor-Clark TE, McAlexander MA, Nassenstein C, Sheardown SA, Wilson S, Thornton J, Carr MJ, Undem BJ. Relative contributions of TRPA1 and TRPV1 channels in the activation of vagal bronchopulmonary C-fibres by the endogenous autacoid 4-oxononenal. J Physiol 2008; 586(14):3447-3459.

13. Capristo C, Rossi GA. Post-infectious persistent cough: pathogenesis and therapeutic options. Minerva Pediatr 2017; 69(5):444-452.

14. Chang AB, Oppenheimer JJ, Irwin RS; CHEST Expert Cough Panel. Managing Chronic Cough as a Symptom in Children and Management Algorithms: CHEST Guideline and Expert Panel Report. Chest 2020;158(1):303-329.

15. Chang AB, Oppenheimer JJ, Weinberger MM, Rubin BK, Grant CC, Weir K, Irwin RS; CHEST Expert Cough Panel. Management of children with chronic wet cough and protracted bacterial bronchitis: CHEST Guideline and Expert Panel Report. Chest 2017;151(4):884-890.

16. Chang AB, Glomb WB. Guidelines for evaluating chronic cough in pediatrics: ACCP evidence-based clinical practice guidelines. Chest 2006;129(1):260-283.

17. Chang AB, Landau LI, Van Asperen PP, Glasgow NJ, Robertson CF, Marchant JM, Mellis CM. Thoracic Society of Australia and New Zealand. Cough in children: definitions and clinical evaluation. Medical Journal of Australia 2006;184(8):398-403.

18. de Benedictis FM, Carloni I, Comberiati P, Shields MD, Bush A, Chang AB. Wet Cough and Nasal Symptoms in Children: Can We Do Better? Front Pediatr 2019;7(1):459.

19. Singh D, Arora V, Sobti PC. Chronic/recurrent cough in rural children in Ludhiana, Punjab. Indian Pediatr 2002;39(1):23-29.

20. Leonardi GS, Houthuijs D, Nikiforov B, Volf J, Rudnai P, Zejda J, Gurzau E, Fabianova E, Fletcher T, Brunekreef B. Respiratory symptoms, bronchitis and asthma in children of Central and Eastern Europe. Eur Respir J 2002;20(4):890-898.

21. Pan G, Zhang S, Feng Y, Takahashi K, Kagawa J, Yu L, Wang P, Liu M, Liu Q, Hou S, Pan B, Li J. Air pollution and children's respiratory symptoms in six cities of Northern China. Respir Med 2010;104(12):1903-1911.

22. Chang AB, Upham JW, Masters IB, Redding GR, Gibson PG, Marchant JM, Grimwood K. Protracted bacterial bronchitis: The last decade and the road ahead. Pediatr Pulmonol 2016;51(3):225-242.

23. Kantar A, Chang AB, Shields MD, Marchant JM, Grimwood J, Grigg J, Priftis KN, Cutrera R, Midulla F, Brand PLP, Everard ML. ERS statement on protracted bacterial bronchitis in children. Eur Resp J 2017; 50(2):1602139.

24. Kantar A, Bernardini R, Paravati F, Minasi D, Sacco O. Chronic cough in preschool children. Early Hum Dev 2013;89(3):19-24.

25. Demoulin-Alexikova S, Plevkova J, Mazurova L, Zatko T, Alexik M, Hanacek J, Tatar M. Impact of Air Pollution on Age and Gender Related Increase in Cough Reflex Sensitivity of Healthy Children in Slovakia. Front Physiol 2016;7(1):54.

26. Morice AH, Millqvist E, Bieksiene K, Birring SS, Dicpinigaitis P, Domingo Ribas C, Hilton Boon M, Kantar A, Lai K, McGarvey L, Rigau D, Satia I, Smith J, Song WJ, Tonia T, van den Berg JWK, van Manen MJG, Zacharasiewicz A. ERS guidelines on the diagnosis and treatment of chronic cough in adults and children. Eur Respir J 2020;55(1):1901136.

27. Song WJ, Chang YS, Faruqi S, Kang MK, Kim JY, Kang MG, Kim S, Jo EJ, Lee SE, Kim MH, Plevkova J, Park HW, Cho SH, Morice AH. Defining chronic cough: a systematic review of the epidemiological literature. Allergy Asthma Immunol Res 2016;8(2):146-155.

28. Marchant JM, Newcombe PA, Juniper EF, Sheffield JK, Stathis SL, Chang AB. What is the burden of chronic cough for families? Chest 2008;134(2):303-309.

29. Petsky HL, Acworth JP, Clark R, Thearle DM, Masters IB, Chang AB. Asthma and protracted bronchitis: who fares better during an acute respiratory infection? J Paediatr Child Health 2009;45(1-2):42-47.

30. Chang AB, Robertson CF, Van Asperen PP, Glasgow NJ, Mellis CM, Masters IB, Teoh L, Tjhung I, Morris PS, Petsky HL, Willis C, Landau LI. A multicenter study on chronic cough in children: burden and etiologies based on a standardized management pathway. Chest 2012;142(4):943-950.

31. Shields MD, Thavagnanam S. The difficult coughing child: prolonged acute cough in children. Cough 2013; 9(1):11.

32. Chang AB, Robertson CF, van Asperen PP, Glasgow NJ, Masters IB, Mellis CM, Landau LI, Teoh L, Morris PS. Can a management pathway for chronic cough in children improve clinical outcomes: protocol for a multicentre evaluation. Trials 2010;11(1):103.

33. Chang AB, Gaffney JT, Eastburn MM, Faoagali J, Cox NC, Masters IB. Cough quality in children: a comparison of subjective vs. bronchoscopic findings. Respir Res 2005;6(1):3.

34. Chang AB, Oppenheimer JJ, Weinberger M, Rubin BK, Irwin RS. Children with chronic wet or productive cough--treatment and investigations: a systematic review. Chest 2016;149(1):120-142.

35. Wurzel DF, Marchant JM, Clark JE, Masters IB, Yerkovich ST, Upham JW, Chang AB. Wet cough in children: infective and inflammatory characteristics in broncho-alveolar lavage fluid. Pediatr Pulmonol 2014;49(6):561-568.

36. Wang K, Harnden A. Pertussis-induced cough. Pulm Pharmacol Ther 2011;24(3):304-307.

37. Shields MD, Doherty GM. Chronic cough in children. Paediatr Respir Rev 2013;14(2):100-105.

38. Galway NC, Shields MD. The child with an incessant dry cough. Paed Resp Rev 2019;30(1):58-64.

39. Swarnkar V, Abeyratne UR, Chang AB, Amrulloh YA, Setyati A, Triasih R. Automatic identification of wet and dry cough in pediatric patients with respiratory diseases. Ann Biomed Eng 2013;41(5):1016-1028.

40. Grarup AP, Pedersen JL. Chronic cough in children. Ugeskr Laeger 2015;177(16):V11140593.

41. Donnelly D, Everard ML. “Dry”and”wet” cough: how reliable is parental reporting? BMJ Open Resp Res 2019;6(1):e000375.

42. Douros K, Everard ML. Time to say goodbye to bronchiolitis, viral wheeze, reactive airways disease, wheeze bronchitis and all that. Front Pediatr 2020;8(1):218.

43. Kantar A. Phenotypic presentation of chronic cough in children. J Thorac Dis 2017;9(4):907-913.

44. Castellani C, Linnane B, Pranke I, Cresta F, Sermet-Gaudelus I, Peckham D. Cystic Fibrosis Diagnosis in Newborns, Children, and Adults. Semin Respir Crit Care Med 2019;40(6):701-714.

45. de Benedictis FM, Bush A. Recurrent lower respiratory tract infections in children. BMJ 2018;362(1):2698.

46. Wurzel DF, Marchant JM, Yerkovich ST, Upham JW, Mackay IM, Masters IB, Chang AB. Prospective characterization of protracted bacterial bronchitis in children. Chest 2014;145(6):1271-1278.

47. Lucas JS, Barbato A, Collins SA, Goutaki M, Behan L, Caudri D, Dell S, Eber E, Escudier E, Hirst RA, Hogg C, Jorissen M, Latzin P, Legendre M, Leigh MW, Midulla F, Nielsen KG, Omran H, Papon JF, Pohunek P, Redfern B, Rigau D, Rindlisbacher B, Santamaria F, Shoemark A, Snijders D, Tonia T, Titieni A, Walker WT, Werner C, Bush A, Kuehni CE. European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia. Eur Respir J 2017;49(1):1601090.

48. Gilchrist FJ. An approach to the child with a wet cough. Paediatr Respir Rev 2019;31(1):75-81.

49. Marchant JM, Masters IB, Taylor SM, Cox NC, Seymour GJ, Chang AB. Evaluation and outcome of young children with chronic cough. Chest 2006;129(5):1132-1141.

50. Wurzel DF, Marchant JM, Yerkovich ST, Upham JW, Petsky HL, Smith-Vaughan H, Masters B, Buntain H, Chang AB. Protracted bacterial bronchitis in children: natural history and risk factors for bronchiectasis. Chest 2016;150(5):1101-1108.

51. Zar HJ, Hanslo D, Apolles P, Swingler G, Hussey G. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. Lancet 2005;365(9454):130-134.

52. Jochmann A, Artusio L, Robson K, Nagakumar P, Collins N, Fleming L, Bush A, Saglani S. Infection and inflammation in induced sputum from preschool children with chronic airways diseases. Pediatr Pulmonol 2016;51(8):778-786.

53. Bush A. Persistent Bacterial Bronchitis: Time to Venture beyond the Umbrella. Front Pediatr 2017;5(1):264.

54. Silvestri M, Spallarossa D, Battistini E, Brusasco V, Rossi GA. Dissociation between exhaled nitric oxide and hyperresponsiveness in children with mild intermittent asthma. Thorax 2000;55(6):484-488.

55. Sacco O, Silvestri M, Ghezzi M, Capizzi A, Rossi GA. Airway inflammation and injury in children with prevalent weakly acidic gastroesophageal refluxes. Respir Med 2018;143(1):42-47.

56. Sacco O, Fregonese B, Silvestri M, Sabatini F, Mattioli G, Rossi GA. Bronchoalveolar lavage and esophageal pH monitoring data in children with "difficult to treat" respiratory symptoms. Pediatr Pulmonol 2000;30(4):313-319.

57. Ghezzi M, Silvestri M, Sacco O, Panigada S, Girosi D, Magnano GM, Rossi GA. Mild tracheal compression by aberrant innominate artery and chronic dry cough in children. Pediatr Pulmonol 2016;51(3):286-294.

58. Silvestri M, Crimi E, Oliva S, Senarega D, Tosca MA, Rossi GA, Brusasco V. Pulmonary function and airway responsiveness in young competitive swimmers. Pediatr Pulmonol 2013;48(1):74-80.

59. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J; ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J 2005;26(2):319-338.

60. Gardella C, Girosi D, Rossi GA, Silvestri M, Tomà P, Bava G, Sacco O. Tracheal compression by aberrant innominate artery: clinical presentations in infants and children, indications for surgical correction by aortopexy, and short- and long-term outcome. J Pediatr Surg 2010;45(3):564-573.

61. Donnelly D, Critchlow A, Everard ML. Outcomes in children treated for persistent bacterial bronchitis. Thorax 2007;62(1):80-84.

62. Craven V, Everard ML. Protracted bacterial bronchitis: reinventing an old disease. Arch Dis Child 2013;98(1):72-76.

63. Barbato A, Magarotto M, Crivellaro M, Novello A Jr, Cracco A, de Blic J, Scheinmann P, Warner JO, Zach M. Use of the paediatric bronchoscope, flexible and rigid, in 51 European centres. Eur Respir J 1997;10(8):1761-1766.

64. Boogaard R, Huijsmans SH, Pijnenburg MW, et al. Tracheomalacia and bronchomalacia in children: incidence and patient characteristics. Chest 2005;128(5):3391-3397.

65. Boogaard R, de Jongste JC, Vaessen-Verberne AA, Hop WC, Merkus PJ. Recombinant human DNase in children with airway malacia and lower respiratory tract infection. Pediatr Pulmonol 2009;44(10):962-969.

66. Zhou-Suckow Z, Duerr J, Hagner M, Agrawal R, Mall MA. Airway mucus, inflammation and remodeling: emerging links in the pathogenesis of chronic lung diseases. Cell Tissue Res 2017;367(3):537-550.

67. Goyal V, Grimwood K, Marchant J, Masters IB, Chang AB. Pediatric bronchiectasis: No longer an orphan disease. Pediatr Pulmonol 2016;51(5):450-469.