ISSN 2219-2808 (online)

World Journal of *Clinical Pediatrics*

World J Clin Pediatr 2019 April 9; 8(2): 15-42

Published by Baishideng Publishing Group Inc

WJCP

World Journal of Clinical Pediatrics

Contents

Irregular Volume 8 Number 2 April 9, 2019

MINIREVIEWS

ORIGINAL ARTICLE

Prospective Study

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Pediatrics*, Anand Pandey, MAMS, MBBS, Associate Professor, Department of Pediatric Surgery, King George's Medical University, Lucknow 226003, Uttar Pradesh, India

AIMS AND SCOPE

World Journal of Clinical Pediatrics (*World J Clin Pediatr*, *WJCP*, online ISSN 2219-2808, DOI: 10.5409) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

The *WJCP* covers a variety of clinical medical topics, including fetal diseases, inborn, newborn diseases, infant diseases, genetic diseases, diagnostic imaging, endoscopy, and evidence-based medicine and epidemiology. Priority publication will be given to articles concerning diagnosis and treatment of pediatric diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, *etc*.

We encourage authors to submit their manuscripts to *WJCP*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

The *WJCP* is now abstracted and indexed in PubMed, PubMed Central, China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Han Song*

Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Clinical Pediatrics

ISSN
ISSN 2219-2808 (online)

LAUNCH DATE
June 8, 2012

FREQUENCY
Irregular

EDITORS-IN-CHIEF
Consolato Maria Sergi, Toru Watanabe

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2219-2808/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director

PUBLICATION DATE
April 9, 2019

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2019 April 9; 8(2): 15-22

DOI: 10.5409/wjcp.v8.i2.15

ISSN 2219-2808 (online)

MINIREVIEWS

Applications of lung clearance index in monitoring children with cystic fibrosis

Andrew Fretzayas, Konstantinos Douros, Maria Moustaki, Ioanna Loukou

ORCID number: Andrew Fretzayas (0000-0003-0964-8594); Konstantinos Douros (0000-0001-7632-1159); Maria Moustaki (0000-0001-6818-4350); Ioanna Loukou (0000-0002-3617-4526).

Author contributions: Each author did equally to this work.

Conflict-of-interest statement: The authors state that they have no conflicts of interest.

Manuscript source: Invited manuscript

Received: December 5, 2018
Peer-review started: December 5, 2018
First decision: January 21, 2019
Revised: February 3, 2019
Accepted: February 19, 2019
Article in press: February 19, 2019
Published online: April 9, 2019

P-Reviewer: Lin JA, El-Radhi ASM, Agrawal A
S-Editor: Dou Y

Andrew Fretzayas, School of Medicine, University of Athens, Athens 11527, Greece

Andrew Fretzayas, Department of Pediatrics, Athens Medical Center, Athens University Medical School, Maroussi 15125, Greece

Konstantinos Douros, Respiratory Unit, Third Department of Pediatrics, Athens University Medical School, "Attikon" University Hospital, Haidari 12464, Greece

Maria Moustaki, Ioanna Loukou, Department of Cystic Fibrosis, "Agia Sofia", Children's Hospital, Athens 11527, Greece

Corresponding author: Andrew Fretzayas, MD, Doctor, Department of Pediatrics, Athens Medical Center, Athens University Medical School, 5-7 Distomou str, Maroussi 15125, Greece. afretz@med.uoa.gr
Telephone: +30-210-6157269
Fax: +30-210-6862376

Abstract

A sensitive, reproducible and feasible measure of lung function for monitoring the respiratory health is a prerequisite for the optimization of management of the patients with cystic fibrosis (CF). Spirometry has been considered the method of choice, although it is applicable only in children older than 6 years of age, as good cooperation is necessary for its proper performance. However, over the last 15 years, scientific interest in gas dilution techniques and particularly in multiple breath wash out (MBW) method has been revived. The most commonly reported index of MBW is lung clearance index (LCI). The aim of this review is to present the most recent developments in the application of LCI as a monitoring index of respiratory status of CF patients. LCI is a sensitive and reproducible marker of ventilation inhomogeneity. It is more sensitive than spirometry and, unlike spirometry; it can be performed across the whole pediatric age range. Since it is dependent on body size, until at least the age of 6 years, the relative and not the absolute changes are more appropriate for providing clinically meaningful conclusion on ventilation inhomogeneity. Until now, MBW has been mainly used as a research tool. Based on the currently available data LCI cannot safely predict high-resolution computed tomography findings in children with CF, especially in infants. It can be used as an end-point measure for the assessment of beneficial effect of interventions. However, its utility as an outcome measure for the efficacy of therapeutic interventions seems to be dependent on the pathophysiologic mechanisms that underlie each intervention. It seems that more studies, especially longitudinal ones, are required in order to fully clarify the clinical usefulness of LCI, not only in the research setting, but also in every day practice

L-Editor: A
E-Editor: Song H

of CF clinic.

Key words: Cystic fibrosis; Respiratory health; Lung clearance index; Ventilation inhomogeneity

Core tip: We herein present an overview of the applications of lung clearance index (LCI) in monitoring the respiratory health status of children with cystic fibrosis (CF). LCI is a more sensitive marker than spirometry and unlike spirometry it can be performed across the entire pediatric age range. At present, it is mostly used in research settings. However, as more data become available from longitudinal studies, it may be proved to be a very useful marker of respiratory status monitoring in children with CF, able to identify early those who are at risk for deterioration and allowing the early application of more aggressive interventions.

Citation: Fretzayas A, Douros K, Moustaki M, Loukou I. Applications of lung clearance index in monitoring children with cystic fibrosis. *World J Clin Pediatr* 2019; 8(2): 15-22
URL: https://www.wjgnet.com/2219-2808/full/v8/i2/15.htm
DOI: https://dx.doi.org/10.5409/wjcp.v8.i2.15

INTRODUCTION

Cystic fibrosis (CF) is a chronic inherited disorder that mainly impairs the lung health and the nutritional status of the affected individuals. Advances in the early diagnosis of the disease, optimal monitoring of the lung health and timely implementation of the appropriate available therapies have improved the quality of life of CF patients as well as their longevity[1]. The use of a sensitive, reproducible and feasible measure of lung function for monitoring the respiratory health and documenting stability or progression of the pulmonary disease is a prerequisite for the optimization of the patients' management. For decades, conventional spirometry, like in many other respiratory disorders, was the technique of choice for evaluating respiratory status in CF patients[2,3]. However, this technique is not applicable in infants and toddlers as adequate cooperation and coordination are required for performing the test. Additionally, even in cooperative subjects of the appropriate age, peripheral extension of bronchiectasis, as it is seen on the CT scans, may deteriorate faster compared to the spirometric indices[4,5]. This implies that spirometry is not sufficiently sensitive for evaluating lung health in CF patients. For these reasons, scientific interest in gas dilution techniques, in particular multiple breath wash out (MBW) measurements, has been revived over the last decade.

The aim of this review is to present an overview of the recent developments in the application of lung clearance index (LCI), the main MBW outcome, in monitoring the respiratory status of children with CF.

HISTORICAL-TECHNICAL BACKGROUND OF MBW

MBW test was first described by Fowler *et al*[6], in 1952. Fowler developed a method, which could measure the extent of uneven ventilation by obtaining pulmonary nitrogen clearance curves of single breath washouts from healthy subjects and from patients with cardiorespiratory diseases[6]. This method was laborious and initially received little attention. However, technological evolution and modifications in the nitrogen washout curves led to a replenishment of interest in the application of the method in the clinical setting[7]. The method has been used successfully since 1985 in young children who could not cooperate to perform conventional spirometry, as only spontaneous tidal breathing is required for MBW test[8].

The MBW procedure consists of two phases, a wash in and a wash out phase[9]. During the wash in phase the subject breaths an inert exogenous gas of known concentration, more commonly sulfur hexafluoride, until the concentration of the expired gas reaches the concentration of the delivered gas. At this point the wash out phase of the procedure starts. When nitrogen, which is an inert intrinsic gas, is used

there is no wash in period. During the wash out phase, the subject inhales room air (if an exogenous gas was used in the wash in phase), or 100% oxygen (if the gas used in the wash in phase was nitrogen). The wash out period is considered to be complete when the inert gas concentration reaches the 1/40th of the initial level[10].

A number of parameters have been suggested for the description of the wash out curve (flow and gas concentration plotted against time). The most commonly used is LCI, whereas mixing ratio and moment ratio are less often reported[11].

LCI is defined as the number of lung turnovers that are required to reduce the inert gas concentration to the 1/40th of the initial concentration. It is calculated by dividing cumulative expired volume/functional residual capacity. LCI is, therefore, an easy to compute and simple to understand index and it is considered the preferred outcome parameter of ventilation inhomogeneity in CF studies[12].

CHARACTERISTICS OF MBW TECHNIQUE AND LCI MEASUREMENT

Feasibility

As only relaxed tidal breathing is required for MBW test and the derived LCI index[10], the test is expected to be feasible even in preschoolers. Quiet breathing is performed through a facemask or a mouthpiece according to the age of the child. The success rate of the test in the clinical setting, in unsedated preschoolers older than 3.5 years of age, ranged from 75%-100%[13]. Longitudinal monitoring seems to be possible in this age group as preschoolers who completed the first test were also able to complete the test at the follow up visit[13]. Similar success rate (78%-87%) was observed in the research setting[14]. However, the test is feasible only under sedation in infants and preschoolers younger than 3.5 years of age. In this age group the success rate ranged from 78.9%-100% in sedated subjects, who were either healthy or suffered from CF and other lung diseases[15].

Short and long term repeatability of LCI

The mean coefficient of variation of LCI measurements in subjects with CF, within one test occasion, was between 4 to 8% in most studies[16], with wide, however, range, when reported. These values indicate that LCI is a rather reproducible test.

The knowledge of LCI fluctuation over time is essential for understanding when differences in sequential measurements represent clinically meaningful changes of ventilation inhomogeneity, and not simply measurements variability. The short and long term variability of LCI measurements was acceptable in healthy children and adolescents, ranging from 4.2%-5.1% for one and six months intervals respectively[17]. These observations indicate that the absolute change of LCI > 1 unit represents a clinical relevant change[17]. Likewise, Singer *et al*[18] found that the coefficient of repeatability between tests occasions performed 24 h apart, was 0.96 in children with CF compared to 0.62 in controls. Their results also indicate that a change of LCI of at least 1 unit is clinically meaningful in children with CF. However, later it was shown by Svedberg *et al*[19] that higher LCI values were associated with higher variability for both intra and inter test measurements. Therefore expressing in absolute numbers the clinical relevant change of LCI may lead to over-representation of clinical meaningful change. According to Svedberg *et al*[19]'s data a relative increase of > 17% (compared to a previous measurement in a clinically stable CF patient) may be an indicator of lung disease deterioration. Green *et al*[20] suggested that a change of LCI > 25%, between sessions, indicate a clinically relevant change. As the upper limit of normal Svedberg *et al*[19] used 1.64 SD whereas Green *et al*[20] 1.96 SD. In agreement with these findings, Oude Engeberink *et al*[21] corroborated that in preschool children a relative change of LCI > ± 15% is considered clinically relevant and confirmed the observation that LCI variability was proportional to its mean. Therefore, they also supported the view that the expression of LCI change in absolute numbers is biased.

Reference values

Although it was initially considered that LCI was an age independent index in healthy subjects, Lum *et al*[22] showed that LCI was dependent on body size. Absolute reduction of LCI, which was large enough to be considered of clinical significance, was observed till 6 years of age, whereas thereafter LCI was almost stabilized till early adulthood. Therefore, appropriate reference equations are needed throughout childhood in order to reliably interpret the LCI results. However, it should be noted, that reference data should not be generalized to all different MBW systems or inert gases[23].

CORRELATION OF LCI WITH FINDINGS FROM LUNG IMAGING

The main structural abnormalities that characterize the CF lung disease are bronchiectasis and small airways disease[24]. These structural components are clearly depicted by high-resolution computed tomography (HRCT), an accurate modality at delineating not only the extent and severity of advanced lung disease but also early lung disease[25]. However, its routine use for monitoring CF lung disease has been questioned because of the risk posed by the radiation exposure[26]. Nevertheless, spirometric indices cannot predict the HRCT findings as structural abnormalities were not uncommon in children with CF and normal spirometric parameters[4]. By contrast, LCI is a more sensitive indicator than spirometric indices for predicting HRCT abnormalities in CF lung disease[27]. In a retrospective study of school age children and adolescents with CF[27] the sensitivity of LCI for detecting HRCT defined abnormalities ranged from 85% to 94% depending on the type of lung damage (bronchiectasis, air trapping, or HRCT score). Therefore, LCI values within the normal range almost precluded the possibility of CF lung disease detectable by HRCT scan. These findings were corroborated by another prospective cross-sectional study in young patients with CF (aged 6-26 years) who had normal FEV1 (> 80%predicted)[28]. It should be noted that due to technical reasons, in this sample population there was an interval up to 243 d between CT performance and LCI measurement. In this population LCI had a sensitivity of 88% to detect structural lung abnormalities of HRCT, a positive predictive value of 88% but a negative predictive value as low as 63%. Therefore, these findings also supported the notion that an abnormal chest CT is unlikely, but not impossible, in the presence of a normal LCI. Later, another study[29] was conducted in a population of children with CF, aged 6-10 years, who underwent lung function tests, including LCI measurement, and volumetric HRCT on the same day. This study showed that LCI and HRCT scans have similar sensitivity to detect CF lung diseases with an overall concordance of 81% for total CT score. However, they found a positive predictive value of 88% and a low negative predictive value of 44% for LCI in relation to the detection of HRCT structural abnormalities indicating that a normal LCI did not preclude abnormal HRCT findings. The results of this study are not comparable to the respective results of the two preceding ones as there were methodological differences that are beyond the scope of this review to be presented in detail.

It seems that the age of patients is an important factor for the relation of LCI values with structural lung damage detected by HRCT. In a study (AREST CF program)[30] that assessed infants with CF diagnosed with newborn screening, it was shown - after controlling for age and infection status - that LCI values were not correlated with the presence of bronchiectasis but only with air trapping.

In accordance to the AREST study[30], a more recent study[31] that included pediatric patients with CF across the entire pediatric age spectrum (0-16 years) showed that LCI was not sensitive for the detection of structural abnormalities in infancy. By contrast, in preschool and school age children an elevated LCI had an 85% positive predictive value for the detection of bronchiectasis, but a low negative predictive value of 55% indicating that a normal LCI could not rule out bronchiectasis in almost half of the cases.

Furthermore, a longitudinal three year study[32], which consisted of patients 6-53 years old, showed that 86% of LCI values in the first year of the study were indicative of the presence or absence of structural lung changes depicted by HRCT scan three years later.

Magnetic resonance imaging has also been used, in research setting, for the detection of early lung disease in children across the entire age range[33]. It was shown that there was a strong age independent correlation of LCI with airway thickening/bronchiectasis detected by MRI. A moderate correlation with mucus plugging/perfusion abnormalities was also observed. The majority of false negative LCI results were detected in the younger children of the study.

Therefore, at present, LCI could not replace HRCT scans in CF children, particularly the younger ones. More longitudinal studies are needed to explore whether LCI could be used as a potential predictor of the presence and the extent of structural lung damage detected by HRCT, in older children and adolescents.

LCI AS A MEASURE OF EFFICACY OF THERAPEUTIC INTERVENTIONS

As LCI is a sensitive marker of ventilation inhomogeneity and it is easily performed

even in infants, clinical trials were performed to evaluate whether LCI is a reliable outcome measure for the detection of treatment effects of variable interventions.

LCI was improved after the administration of dornase alpha for 28 d, in children with CF, older than 6 years of age, who had mild lung disease (FEV1 > 80%)[34]. However, the results of this study should not be generalized in younger children as well as in children with more severe lung disease. Similar findings were observed after the administration of hypertonic saline in pediatric CF patients, aged older than 6 years, with a baseline FEV1 > 70%[35,36]. However, these results were found only in studies with a duration of at least four weeks[35], and children with mild lung disease[36], whereas they were not corroborated in a trial[37] that assessed the short term LCI change 24 h after hypertonic saline inhalation in children with a baseline FEV1 > 40%. This discordance may simply indicate that there is an additive effect of multiple doses of hypertonic saline inhalation and/or a different response in treatment in pediatric CF patients' severe lung disease. It also seems that with the use of LCI z-score (z-LCI) change as an outcome measure, a significant treatment effect was observed in infants and preschool children after the twice-daily administration of hypertonic saline for 48 wk[38]. It needs to be emphasized here that, especially for infancy and early childhood, z-LCI changes should be evaluated in order to adjust for the body size dependence of LCI[22].

Most recently in a randomized controlled trial[39] which evaluated the clinical response to ivacaftor in CF children older than 6 years, with mild lung disease (FEV1 > 90%) and at least one G551D-CFTR allele, it was shown that LCI was more sensitive than spirometry in detecting response in this therapeutic intervention.

In contrast to the above-mentioned findings, the LCI response to antibiotic administration for pulmonary exacerbation was variable. In a recent systematic review[40], it was shown that a significant but not necessarily clinically relevant treatment effect was observed in patients who received antibiotics for pulmonary exacerbations. Although there was a weak correlation with FEV1 changes, discordant results between LCI and FEV1 changes were rather common. There are several hypotheses that could explain the overall small LCI change, the considerable heterogeneity of the observed results, and the discordance with FEV1 changes which, however, are beyond the scope of this review.

It should be noted, however, that even paradoxical increase of LCI was observed after antibiotic administration for pulmonary exacerbation[41]. It is speculated that there are non-ventilating lung units during exacerbation that do not contribute to the LCI values. Following antibiotic treatment, and due to the removal of mucus plugging, these areas become ventilated - though not fully but only partially; their partial aeration increases the LCI index[42].

Overall, it should be recognized that the various therapeutic interventions induce LCI changes to the time interval between the intervention and the assessment of LCI; the severity of the lung disease as it is reflected by baseline spirometric indices; the different mechanisms that underlie the treatment effect of each intervention.

LCI AS A PREDICTOR FOR THE EVOLUTION OF LUNG DISEASE

A question that was raised in the literature was whether LCI values in infancy or early childhood could predict subsequent lung disease status. The London Cystic Fibrosis collaboration (LCFC) followed up a newborn-screened CF cohort up to 2 years of age[43]. They found that z-LCI at 2 years of age were not associated with the respective results at 3 and 12 mo of age. Therefore, it was recognized in this study that up to 2 years of age LCI could not predict the evolution of the disease. It was, however, also acknowledged that a long-term follow up in preschool and school years is essential for determining whether early measured LCI could be served as a predictor of the lung disease status in childhood.

LCFC also measured LCI and spirometric indices in preschool children (3-5 years) with CF and in healthy controls[44] and repeated the measurement during early school age (6-10 years). It was found that LCI at preschool age had a positive predictive value of 94% for predicting spirometry or LCI abnormal results, whereas the negative predictive value was as low as 62%. The future repeat of LCI and spirometry in this cohort during late childhood and adolescence will reveal whether preschool age LCI values are predictors for late childhood and adolescence lung health status in CF subjects.

There is also limited data[45], in children aged 6-19 years, that LCI was a predictor of the occurrence of pulmonary exacerbation during the subsequent 12 mo following the measurement, even in the subgroup of patients with a normal FEV1.

It seems therefore that tracking longitudinal changes of LCI may confer to the prediction of lung health status in patients with CF. However the existing data are rather limited and more longitudinal studies are needed for the clarification of this issue.

CONCLUSION

LCI is a sensitive marker for the assessment of respiratory status of children with CF. However, more data from longitudinal studies is needed, in order to clarify which relative change is of clinical importance for identifying the patients who are at risk for respiratory deterioration. Should this information be available, the LCI would be a potential useful marker for monitoring pediatric patients with CF, in every day clinical practice. Until then several obstacles should also be overcome, taking into consideration that the results from different equipment and different inert gasses are not comparable.

REFERENCES

1 **Paranjape SM**, Mogayzel PJ. Cystic fibrosis in the era of precision medicine. *Paediatr Respir Rev* 2018; **25**: 64-72 [PMID: 28372929 DOI: 10.1016/j.prrv.2017.03.001]
2 **Gibson RL**, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 2003; **168**: 918-951 [PMID: 14555458 DOI: 10.1164/rccm.200304-505SO]
3 **Douros K**, Loukou I, Doudounakis S, Tzetis M, Priftis KN, Kanavakis E. Asthma and pulmonary function abnormalities in heterozygotes for cystic fibrosis transmembrane regulator gene mutations. *Int J Clin Exp Med* 2008; **1**: 345-349 [PMID: 19079680]
4 **de Jong PA**, Lindblad A, Rubin L, Hop WC, de Jongste JC, Brink M, Tiddens HA. Progression of lung disease on computed tomography and pulmonary function tests in children and adults with cystic fibrosis. *Thorax* 2006; **61**: 80-85 [PMID: 16244089 DOI: 10.1136/thx.2005.045146]
5 **de Jong PA**, Nakano Y, Lequin MH, Mayo JR, Woods R, Paré PD, Tiddens HA. Progressive damage on high resolution computed tomography despite stable lung function in cystic fibrosis. *Eur Respir J* 2004; **23**: 93-97 [PMID: 14738238 DOI: 10.1183/09031936.03.00006603]
6 **Fowler WS**, Cornish ER, Kety SS. Lung function studies. VIII. Analysis of alveolar ventilation by pulmonary N2 clearance curves. *J Clin Invest* 1952; **31**: 40-50 [PMID: 14907879 DOI: 10.1172/JCI102575]
7 **Robinson PD**, Goldman MD, Gustafsson PM. Inert gas washout: theoretical background and clinical utility in respiratory disease. *Respiration* 2009; **78**: 339-355 [PMID: 19521061 DOI: 10.1159/000225373]
8 **Wall M**, Misley M, Dickerson D. Moment analysis of multibreath nitrogen washout (mbnw) as a test of lung function in young children. *Pediatr Res* 1984; **18**: 409A-409A [DOI: 10.1203/00006450-198404001-01894]
9 **Subbarao P**, Milla C, Aurora P, Davies JC, Davis SD, Hall GL, Heltshe S, Latzin P, Lindblad A, Pittman JE, Robinson PD, Rosenfeld M, Singer F, Starner TD, Ratjen F, Morgan W. Multiple-Breath Washout as a Lung Function Test in Cystic Fibrosis. A Cystic Fibrosis Foundation Workshop Report. *Ann Am Thorac Soc* 2015; **12**: 932-939 [PMID: 26075554 DOI: 10.1513/AnnalsATS.201501-021FR]
10 **Robinson PD**, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs SI, King GG, Lum S, Macleod K, Paiva M, Pillow JJ, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson BR, Gustafsson PM. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. *Eur Respir J* 2013; **41**: 507-522 [PMID: 23397305 DOI: 10.1183/09031936.00069712]
11 **Aurora P**. Multiple-breath inert gas washout test and early cystic fibrosis lung disease. *Thorax* 2010; **65**: 373-374 [PMID: 20435855 DOI: 10.1136/thx.2009.132100]
12 **Robinson PD**, Lindblad A, Gustafsson PM. Comparison of the utility of multiple breath inert gas washout parameters in cystic fibrosis. *Thorax* 2010; **65**: 659 [PMID: 20627929 DOI: 10.1136/thx.2009.121590]
13 **Downing B**, Irving S, Bingham Y, Fleming L, Bush A, Saglani S. Feasibility of lung clearance index in a clinical setting in pre-school children. *Eur Respir J* 2016; **48**: 1074-1080 [PMID: 27390277 DOI: 10.1183/13993003.00374-2016]
14 **Aurora P**, Bush A, Gustafsson P, Oliver C, Wallis C, Price J, Stroobant J, Carr S, Stocks J; London Cystic Fibrosis Collaboration. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. *Am J Respir Crit Care Med* 2005; **171**: 249-256 [PMID: 15516530 DOI: 10.1164/rccm.200407-895OC]
15 **Stahl M**, Graeber SY, Joachim C, Barth S, Ricklefs I, Diekmann G, Kopp MV, Naehrlich L, Mall MA. Three-center feasibility of lung clearance index in infants and preschool children with cystic fibrosis and other lung diseases. *J Cyst Fibros* 2018; **17**: 249-255 [PMID: 28811149 DOI: 10.1016/j.jcf.2017.08.001]
16 **Kent L**, Reix P, Innes JA, Zielen S, Le Bourgeois M, Braggion C, Lever S, Arets HG, Brownlee K, Bradley JM, Bayfield K, O'Neill K, Savi D, Bilton D, Lindblad A, Davies JC, Sermet I, De Boeck K; European Cystic Fibrosis Society Clinical Trial Network (ECFS-CTN) Standardisation Committee. Lung clearance index: evidence for use in clinical trials in cystic fibrosis. *J Cyst Fibros* 2014; **13**: 123-138 [PMID: 24315208 DOI: 10.1016/j.jcf.2013.09.005]
17 **Fuchs SI**, Eder J, Ellemunter H, Gappa M. Lung clearance index: normal values, repeatability, and reproducibility in healthy children and adolescents. *Pediatr Pulmonol* 2009; **44**: 1180-1185 [PMID: 19911370 DOI: 10.1002/ppul.21093]
18 **Singer F**, Kieninger E, Abbas C, Yammine S, Fuchs O, Proietti E, Regamey N, Casaulta C, Frey U, Latzin P. Practicability of nitrogen multiple-breath washout measurements in a pediatric cystic fibrosis outpatient setting. *Pediatr Pulmonol* 2013; **48**: 739-746 [PMID: 22888105 DOI: 10.1002/ppul.22651]

19 **Svedberg M**, Gustafsson PM, Robinson PD, Rosberg M, Lindblad A. Variability of lung clearance index in clinically stable cystic fibrosis lung disease in school age children. *J Cyst Fibros* 2018; **17**: 236-241 [PMID: 28822728 DOI: 10.1016/j.jcf.2017.08.004]

20 **Green K**, Kongstad T, Skov M, Buchvald F, Rosthøj S, Marott JL, Gustafsson P, Pressler T, Nielsen KG. Variability of monthly nitrogen multiple-breath washout during one year in children with cystic fibrosis. *J Cyst Fibros* 2018; **17**: 242-248 [PMID: 29273421 DOI: 10.1016/j.jcf.2017.11.007]

21 **Oude Engberink E**, Ratjen F, Davis SD, Retsch-Bogart G, Amin R, Stanojevic S. Inter-test reproducibility of the lung clearance index measured by multiple breath washout. *Eur Respir J* 2017; 50 [PMID: 28982773 DOI: 10.1183/13993003.00433-2017]

22 **Lum S**, Stocks J, Stanojevic S, Wade A, Robinson P, Gustafsson P, Brown M, Aurora P, Subbarao P, Hoo AF, Sonnappa S. Age and height dependence of lung clearance index and functional residual capacity. *Eur Respir J* 2013; **41**: 1371-1377 [PMID: 23143552 DOI: 10.1183/09031936.00005512]

23 **Robinson PD**, Latzin P, Ramsey KA, Stanojevic S, Aurora P, Davis SD, Gappa M, Hall GL, Horsley A, Jensen R, Lum S, Milla C, Nielsen KG, Pittman JE, Rosenfeld M, Singer F, Subbarao P, Gustafsson PM, Ratjen F; ATS Assembly on Pediatrics. Preschool Multiple-Breath Washout Testing. An Official American Thoracic Society Technical Statement. *Am J Respir Crit Care Med* 2018; **197**: e1-e19 [PMID: 29493315 DOI: 10.1164/rccm.201801-0074ST]

24 **Tiddens HA**, Stick SM, Davis S. Multi-modality monitoring of cystic fibrosis lung disease: the role of chest computed tomography. *Paediatr Respir Rev* 2014; **15**: 92-97 [PMID: 23830321 DOI: 10.1016/j.prrv.2013.05.003]

25 **Linnane B**, Robinson P, Ranganathan S, Stick S, Murray C. Role of high-resolution computed tomography in the detection of early cystic fibrosis lung disease. *Paediatr Respir Rev* 2008; **9**: 168-174; quiz 174-175 [PMID: 18694708 DOI: 10.1016/j.prrv.2008.05.009]

26 **de Jong PA**, Mayo JR, Golmohammadi K, Nakano Y, Lequin MH, Tiddens HA, Aldrich J, Coxson HO, Sin DD. Estimation of cancer mortality associated with repetitive computed tomography scanning. *Am J Respir Crit Care Med* 2006; **173**: 199-203 [PMID: 16254271 DOI: 10.1164/rccm.200505-810OC]

27 **Gustafsson PM**, De Jong PA, Tiddens HA, Lindblad A. Multiple-breath inert gas washout and spirometry versus structural lung disease in cystic fibrosis. *Thorax* 2008; **63**: 129-134 [PMID: 17675316 DOI: 10.1136/thx.2007.077784]

28 **Ellemunter H**, Fuchs SI, Unsinn KM, Freund MC, Waltner-Romen M, Steinkamp G, Gappa M. Sensitivity of Lung Clearance Index and chest computed tomography in early CF lung disease. *Respir Med* 2010; **104**: 1834-1842 [PMID: 20637585 DOI: 10.1016/j.rmed.2010.06.010]

29 **Owens CM**, Aurora P, Stanojevic S, Bush A, Wade A, Oliver C, Calder A, Price J, Carr SB, Shankar A, Stocks J; London Cystic Fibrosis Collaboration. Lung Clearance Index and HRCT are complementary markers of lung abnormalities in young children with CF. *Thorax* 2011; **66**: 481-488 [PMID: 21422040 DOI: 10.1136/thx.2010.150375]

30 **Hall GL**, Logie KM, Parsons F, Schulzke SM, Nolan G, Murray C, Ranganathan S, Robinson P, Sly PD, Stick SM, AREST CF, Berry L, Garratt L, Massie J, Mott L, Poreddy S, Simpson S. Air trapping on chest CT is associated with worse ventilation distribution in infants with cystic fibrosis diagnosed following newborn screening. *PLoS One* 2011; **6**: e23932 [PMID: 21886842 DOI: 10.1371/journal.pone.0023932]

31 **Ramsey KA**, Rosenow T, Turkovic L, Skoric B, Banton G, Adams AM, Simpson SJ, Murray C, Ranganathan SC, Stick SM, Hall GL, AREST CF. Lung Clearance Index and Structural Lung Disease on Computed Tomography in Early Cystic Fibrosis. *Am J Respir Crit Care Med* 2016; **193**: 60-67 [PMID: 26359952 DOI: 10.1164/rccm.201507-1409OC]

32 **Fuchs SI**, Gappa M, Eder J, Unsinn KM, Steinkamp G, Ellemunter H. Tracking Lung Clearance Index and chest CT in mild cystic fibrosis lung disease over a period of three years. *Respir Med* 2014; **108**: 865-874 [PMID: 24726097 DOI: 10.1016/j.rmed.2014.03.011]

33 **Stahl M**, Wielpütz MO, Graeber SY, Joachim C, Sommerburg O, Kauczor HU, Puderbach M, Eichinger M, Mall MA. Comparison of Lung Clearance Index and Magnetic Resonance Imaging for Assessment of Lung Disease in Children with Cystic Fibrosis. *Am J Respir Crit Care Med* 2017; **195**: 349-359 [PMID: 27575911 DOI: 10.1164/rccm.201604-0893OC]

34 **Amin R**, Subbarao P, Lou W, Jabar A, Balkovec S, Jensen R, Kerrigan S, Gustafsson P, Ratjen F. The effect of dornase alfa on ventilation inhomogeneity in patients with cystic fibrosis. *Eur Respir J* 2011; **37**: 806-812 [PMID: 20693248 DOI: 10.1183/09031936.00072510]

35 **Ellemunter H**, Eder J, Fuchs S, Gappa M, Steinkamp G. Long-term improvement of lung clearance index in patients with mild cystic fibrosis lung disease: Does hypertonic saline play a role? *J Cyst Fibros* 2016; **15**: 123-126 [PMID: 26190829 DOI: 10.1016/j.jcf.2015.06.009]

36 **Amin R**, Subbarao P, Jabar A, Balkovec S, Jensen R, Kerrigan S, Gustafsson P, Ratjen F. Hypertonic saline improves the LCI in paediatric patients with CF with normal lung function. *Thorax* 2010; **65**: 379-383 [PMID: 20435858 DOI: 10.1136/thx.2009.125831]

37 **Amin R**, Stanojevic S, Kane M, Webster H, Ratjen F. A randomized controlled trial to evaluate the lung clearance index as an outcome measure for early phase studies in patients with cystic fibrosis. *Respir Med* 2016; **112**: 59-64 [PMID: 26856191 DOI: 10.1016/j.rmed.2016.01.020]

38 **Subbarao P**, Stanojevic S, Brown M, Jensen R, Rosenfeld M, Davis S, Brumback L, Gustafsson P, Ratjen F. Lung clearance index as an outcome measure for clinical trials in young children with cystic fibrosis. A pilot study using inhaled hypertonic saline. *Am J Respir Crit Care Med* 2013; **188**: 456-460 [PMID: 23742699 DOI: 10.1164/rccm.201302-0219OC]

39 **Davies J**, Sheridan H, Bell N, Cunningham S, Davis SD, Elborn JS, Milla CE, Starner TD, Weiner DJ, Lee PS, Ratjen F. Assessment of clinical response to ivacaftor with lung clearance index in cystic fibrosis patients with a G551D-CFTR mutation and preserved spirometry: a randomised controlled trial. *Lancet Respir Med* 2013; **1**: 630-638 [PMID: 24461666 DOI: 10.1016/S2213-2600(13)70182-6]

40 **Sonneveld N**, Stanojevic S, Amin R, Aurora P, Davies J, Elborn JS, Horsley A, Latzin P, O'Neill K, Robinson P, Scrase E, Selvadurai H, Subbarao P, Welsh L, Yammine S, Ratjen F. Lung clearance index in cystic fibrosis subjects treated for pulmonary exacerbations. *Eur Respir J* 2015; **46**: 1055-1064 [PMID: 26160868 DOI: 10.1183/09031936.00211914]

41 **Saunders C**, Bayfield K, Irving S, Short C, Bush A, Davies JC. Developments in multiple breath washout testing in children with cystic fibrosis. *Curr Med Res Opin* 2017; **33**: 613-620 [PMID: 27931123 DOI: 10.1080/03007995.2016.1268999]

42 **Horsley AR**, Davies JC, Gray RD, Macleod KA, Donovan J, Aziz ZA, Bell NJ, Rainer M, Mt-Isa S, Voase N, Dewar MH, Saunders C, Gibson JS, Parra-Leiton J, Larsen MD, Jeswiet S, Soussi S, Bakar Y, Meister MG, Tyler P, Doherty A, Hansell DM, Ashby D, Hyde SC, Gill DR, Greening AP, Porteous DJ,

Innes JA, Boyd AC, Griesenbach U, Cunningham S, Alton EW. Changes in physiological, functional and structural markers of cystic fibrosis lung disease with treatment of a pulmonary exacerbation. *Thorax* 2013; **68**: 532-539 [PMID: 23396354 DOI: 10.1136/thoraxjnl-2012-202538]

43 **Davies G**, Stocks J, Thia LP, Hoo AF, Bush A, Aurora P, Brennan L, Lee S, Lum S, Cottam P, Miles J, Chudleigh J, Kirkby J, Balfour-Lynn IM, Carr SB, Wallis C, Wyatt H, Wade A; London Cystic Fibrosis Collaboration (LCFC). Pulmonary function deficits in newborn screened infants with cystic fibrosis managed with standard UK care are mild and transient. *Eur Respir J* 2017; 50 [PMID: 29122914 DOI: 10.1183/13993003.00326-2017]

44 **Aurora P**, Stanojevic S, Wade A, Oliver C, Kozlowska W, Lum S, Bush A, Price J, Carr SB, Shankar A, Stocks J; London Cystic Fibrosis Collaboration. Lung clearance index at 4 years predicts subsequent lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 2011; **183**: 752-758 [PMID: 20935113 DOI: 10.1164/rccm.200911-1646OC]

45 **Vermeulen F**, Proesmans M, Boon M, Havermans T, De Boeck K. Lung clearance index predicts pulmonary exacerbations in young patients with cystic fibrosis. *Thorax* 2014; **69**: 39-45 [PMID: 24021874 DOI: 10.1136/thoraxjnl-2013-203807]

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2019 April 9; 8(2): 23-32

DOI: 10.5409/wjcp.v8.i2.23

ISSN 2219-2808 (online)

MINIREVIEWS

Prevention of necrotizing enterocolitis in premature infants – an updated review

Yu-Ting Jin, Yue Duan, Xiao-Kai Deng, Jing Lin

ORCID number: Yu-Ting Jin (0000-0003-2056-9344); Yue Duan (0000-0003-0930-7458); Xiao-Kai Deng (0000-0001-8421-4169); Jing Lin (0000-0002-4405-134X).

Author contributions: Jin YT wrote the first draft; Duan Y and Deng XK contributed some sections of the draft; Lin J initiated the project and finalized the manuscript.

Conflict-of-interest statement: Dr. Lin has nothing to disclose. The other authors did not provide a conflict-of-interest statement.

Manuscript source: Invited manuscript

Received: August 25, 2018
Peer-review started: August 25, 2018
First decision: October 5, 2018
Revised: December 31, 2018
Accepted: January 28, 2019
Article in press: January 28, 2019
Published online: April 9, 2019

Yu-Ting Jin, Yue Duan, Xiao-Kai Deng, Jing Lin, Department of Neonatology, The Second Affiliated Hospital and Yuying Children's Hospital, Wenzhou Medical University, Wenzhou 325027, Zhejiang Province, China

Jing Lin, Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States

Corresponding author: Jing Lin, MD, Associate Professor, Department of Pediatrics, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, NY 10029, United States. jing.lin@mssm.edu
Telephone: +1-212-241-6186
Fax: +1-212-534-5207

Abstract

Necrotizing enterocolitis (NEC) is among the most common and devastating diseases encountered in premature infants, yet the true etiology continues to be poorly understood despite decades of research. Recently, gut bacterial dysbiosis has been proposed as a risk factor for the development of NEC. Based on this theory, several best clinical practices designed to reduce the risk of NEC have been proposed and/or implemented. This review summarizes the results of recent clinical trials and meta-analyses that support some of the existing clinical practices for reducing the risk of NEC in premature infants. It is evident that human milk feeding can reduce the incidence of NEC. While most of the studies demonstrated that probiotic supplementation can significantly reduce the incidence of NEC in premature infants, there are still some concerns regarding the quality, safety, optimal dosage, and treatment duration of probiotic preparations. Antibiotic prophylaxis does not reduce the incidence of NEC, and prolonged initial empirical use of antibiotics might in fact increase the risk of NEC for high-risk premature infants. Lastly, standardized feeding protocols are strongly recommended, both for prevention of postnatal growth restriction and NEC.

Key words: Necrotizing enterocolitis; Prevention; Human milk feeding; Probiotics; Empiric antibiotics; Standardized feeding protocols

Core tip: In this review, we summarize some of the clinical practices recommended to reduce the risk of necrotizing enterocolitis (NEC) in premature infants. Firstly, it is

P-Reviewer: DeLorenzo T, Gazzolo D, Klinger G, Nobile S
S-Editor: Ji FF
L-Editor: Filipodia
E-Editor: Song H

evident that human milk feeding can reduce the incidence of NEC. Secondly, while most of the studies demonstrated that probiotic supplementation can significantly reduce the incidence of NEC in premature infants, there are still some concerns regarding the quality of probiotic preparations, safety, optimal dosage, and treatment duration. Thirdly, initial empiric antibiotic use should be restricted in daily practice to reduce the incidence of NEC. Lastly, standardized feeding protocols are recommended both for prevention of postnatal growth restriction and NEC.

Citation: Jin YT, Duan Y, Deng XK, Lin J. Prevention of necrotizing enterocolitis in premature infants – an updated review. *World J Clin Pediatr* 2019; 8(2): 23-32
URL: https://www.wjgnet.com/2219-2808/full/v8/i2/23.htm
DOI: https://dx.doi.org/10.5409/wjcp.v8.i2.23

INTRODUCTION

Necrotizing enterocolitis (NEC) is among the most common and devastating diseases encountered in premature infants, yet the true etiology continues to be poorly understood, despite decades of research. Prematurity remains the most consistent risk factor, although term babies can develop NEC with a much lower incidence. Based on a recent large study from the Canadian Neonatal Network, approximately 5.1% (1.3%-12.9%) of infants with a gestational age < 33 wk develop NEC, and the incidence increases with decreasing gestational age[1]. Despite advances in care in the neonatal intensive care unit (NICU), the estimated mortality rate associated with NEC ranges between 20% and 30%, with the highest rate among infants requiring surgery. Following recovery from the acute phase of NEC, long term complications include intestinal stricture and short bowel syndrome[2-4].

The classical presentation of NEC includes feeding intolerance, abdominal distension, and bloody stools after 8-10 d of age when feeding enterally. The signs and symptoms are quite variable, ranging from feeding intolerance to evidence of a fulminant intra-abdominal catastrophe with peritonitis, sepsis, shock, and death. Many theories have attempted to elucidate the true pathogenesis since Santulli *et al*[5] first described a series of NEC cases in premature infants with respiratory distress syndrome. Most theories about the pathogenesis of NEC have focused on the most important risk factors, such as immaturity, formula feeding, and the presence of bacteria[6]. More recently, gut bacterial dysbiosis has been proposed as the main risk factor for the development of NEC[7]. Based on this theory, several best clinical strategies are being recommended to reduce the risk of NEC. These include breast milk feeding, restrictive use of antibiotics, supplementation with probiotics, and standardized feeding protocols (SFPs). The purpose of this review is to summarize the results of the recent clinical trials that provide evidence supporting these practices in premature infants as methods to reduce the risk of NEC.

LITERATURE REVIEW

A search was conducted in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) for studies published before 15 June 2018. The search included all terms related to NEC and preventive interventions, including human milk feeding, probiotics, prophylactic antibiotics, and SFPs, utilizing PubMed MeSH terms and free-text words and their combinations through the appropriate Boolean operators. Similar criteria were used for searching MEDLINE. The review was limited to clinical studies involving human subjects. All relevant articles were accessed in full text following PRISMA guidelines. The manual search included references of retrieved articles. We reported the results in tables and text.

HUMAN MILK FEEDING

The unique properties of human milk promote an improved host defense and gastrointestinal function. Several well controlled clinical trials have demonstrated that human milk feeding can reduce the incidence of NEC. The results of the recent

randomized trials are summarized in Table 1[8-12]. Cristofalo *et al*[9] and Schanler *et al*[12] demonstrated that human milk feeding could reduce the incidence of NEC in premature infants compared to those fed with preterm formula in their randomized trials. Sullivan *et al*[10] studied a total of 207 infants and found that feeding with an exclusively human milk-based diet is associated with a significantly lower rate of NEC than a diet of human milk fortified with bovine milk-based products. Human milk feeding also reduces the incidence of late onset sepsis in premature infants[12].

Human donor milk is considered a safe alternative when the mother's own milk is not available. When the mother's breast milk supply was deficient, the short-term outcomes related to safety and efficacy were similar in very low birth weight (VLBW) infants who were fed with pasteurized donor milk or with preterm formula in the first 10 d of life[8]. For feeding extremely preterm infants, donor milk offered little short-term preponderance over preterm formula[8,11]. However, by using the Cochrane Neonatal search strategy, Quigley *et al*[13] performed a systematic review and meta-analysis of formula versus donor breast milk for feeding preterm or low birth weight (LBW) infants. They identified 11 randomized or quasi-randomized trials in which 1809 infants participated, and they concluded that donor milk feeding decreased the risk of NEC based on the meta-analysis. However, formula-fed infants had higher in-hospital rates of weight gain, linear growth and head growth[13]. Furthermore, infants fed with donor human milk-based fortifier had approximately 64% lower odds of developing NEC compared to those fed with bovine-based fortifiers[14]. It is clear that human milk feeding can reduce the risk of NEC. Recently, the policy statement from the American Academy of Pediatrics on the use of human breast milk states that preterm infants should only receive their own mother's milk or pasteurized human donor milk when their own mother's milk is not available[15].

Human milk colostrum is high in protein, fat-soluble vitamins, minerals, and immunoglobulins. The benefit of colostrum for newborn infants has been well established. However, most extremely premature infants are usually not ready to be fed in the first few days of life for a variety of reasons. Several studies support the use of colostrum for oral care to provide immunotherapy in preterm infants. The efficacy of oropharyngeal colostrum therapy (OCT) in the prevention of NEC in VLBW infants has been reviewed, and a meta-analysis on this topic was recently published[16]. Only randomized controlled trials and quasi-randomized trials performed in VLBW infants or preterm infants with gestational age < 32 wk were included for the meta-analysis. As a result, a total of 148 subjects (77 in OCT arm and 71 in control arm) in four trials were analyzed, and no statistically significant difference in the incidence of NEC was demonstrated. The authors concluded that the current evidence was not sufficient to enable the recommendation of OCT as a routine clinical practice in the prevention of NEC[16].

ADMINISTRATION OF PROBIOTICS

Establishment of a normal intestinal microbial colonization after birth is vital for proper maturity of the innate immune system and maintenance of intestinal barrier function. It has been proposed that disruption of the normal gut microbiota formation may play a major role in the pathogenesis of NEC in premature infants[17]. Probiotics are live micro-organisms that, upon ingestion at certain amounts, confer health to the host. It is known that probiotics can produce bacteriostatic and bactericidal substances, thus having immunomodulatory effects; furthermore, they prevent colonization of pathogens by competing for adhesion to the intestinal mucosa[18]. One strategy to prevent NEC is oral administration of probiotics to alter the balance of the gut microbiome in favor of non-pathogenic bacteria. In the past two decades, multiple randomized clinical trials in preterm infants have been performed to evaluate the effect of probiotic administration on NEC prevention. The results of these studies are summarized in Table 2. A total of 10520 infants have now been enrolled in probiotic-NEC studies, and a cumulative pooled meta-analysis of the effects of probiotics on NEC was recently published[19]. In these trials, a wide variety of probiotic strains, dosages, and durations were used. Despite the clinical heterogeneity, the conclusion of the cumulative meta-analysis was that probiotic treatment decreased the incidence of NEC (average estimate of treatment effect, relative risk: 0.53; 95%CI: 0.42-0.66)[19]. Therefore, it is clear that some oral probiotics can prevent NEC and decrease mortality in preterm infants. However, it is unclear whether a single probiotic or a mixture of probiotics is most effective for the prevention of NEC. Furthermore, some questions remain unanswered regarding the quality of probiotic products, safety, optimal dosage, and treatment duration.

Probiotics are not all equally effective in preventing NEC in preterm infants. A

Table 1 Summary of five randomized controlled trials of human milk feeding on the risk of necrotizing enterocolitis

Ref.	Yr	GA	BW	*N*	Study	Control	NEC ≥ stage II, %			Mortality, %			LOS, %		
							Study	Control	*P* < 0.05	Study	Control	*P* < 0.05	Study	Control	*P* < 0.05
Corpeleijn *et al*[8]	2016	-	< 1500	373	DM	PF	9.3	8.9	No	13.7	12.7	No	36.6	34.7	No
Cristofalo *et al*[9]	2013	-	500-1250	53	HM	PF	3	21	Yes	0	8	No	55	79	No
Sullivan *et al*[10]	2010	-	500-1250	207	HM	Bovine milk	5.8	15.9	Yes	-	-	-	19	13	No
Schanler *et al*[11]	2005	< 30	-	243	DM	PF	6	9	No	3	3.6	No	1	4.8	No
Schanler *et al*[12]	1999	26-30	-	108	HM	PF	1.6	13	Yes	-	-	-	31	48	Yes

BW: Birth weight; LOS: Late onset sepsis; NEC: Necrotizing enterocolitis.

variety of probiotic strains have been tested in different trials. A detailed analysis of the published data on the effects of probiotics for preterm infant regarding specific probiotic strains was recently performed by the ESPGHAN Working Group on Probiotics, Prebiotics and Committee on Nutrition[20]. They concluded that both *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb-12/B94 appeared to be effective in reducing NEC. Both the combination of *Lactobacillus rhamnosus* GG with *Bifidobacterium longum* BB536 and the combination of *Bifidobacterium lactis* Bb-12 with *Bifidobacterium longum* BB536, however, showed no measurable effect. They suggest that we need to more precisely define the optimal treatment strategies before the routine clinical use of probiotics in preterm infants for NEC prevention can be recommended. Another recent meta-analysis concluded that multiple strains of probiotics were associated with a significantly lower incidence of NEC, with a pooled OR of 0.36 (95%CI: 0.24-0.53; $P < 0.00001$)[21]. As probiotics are neither drugs nor devices, they fall into a peculiar category of medical intervention and are therefore not strictly regulated. Because the cost of probiotics is low and the consequences of NEC can be devastating, given the available evidence and safety profile of probiotics from the large number of infants studied, a strong argument can be made for the routine use of probiotics in all preterm infants during their NICU stay[22]. By offering donor milk to infants at high risk when no maternal milk was available, along with the routine use of probiotics, no confirmed NEC cases were reported in a NICU in Canada for over a year[22]. A much lower incidence of NEC was also observed in Japan where the use of probiotics in preterm infants was routine[23]. Based upon this strong evidence, Dr. Taylor[22] argues that NEC can be easily prevented by the routine use of human milk and prophylactic probiotics.

In fact, a large number of commercially available probiotic preparations have been used in different clinical settings. However, all of these products have not been approved as prescription medications through routine vigorous rules and regulations. Concerns regarding the quality of probiotics and the risk of probiotic-associated sepsis have been raised. For example, an increased incidence of NEC associated with routine administration of a particular probiotic preparation, Infloran™, in extremely preterm infants was recently reported[24]. In this observational study, the routine use of probiotics was implemented in 2008 in one NICU. Infants born at < 28 wk gestational age were prospectively followed and compared with historical controls. Routine use of Infloran™ in infants was associated with an increase in stage II or higher NEC (13.3% *vs* 5.9%, $P = 0.010$). Surgical NEC was 12.1% *vs* 5.9% ($P = 0.029$). Adjusting for confounders (sex, gestational age, antenatal steroids and human milk) did not change those trends ($P = 0.019$). Therefore, some experts propose that if an NICU plans to pursue the use of probiotics as a routine supplementation for preterm infants, a quality improvement approach should be utilized to measure the desired effect of probiotics on the risk of NEC and to assess their safety[19].

RESTRICT EMPIRIC ANTIBIOTIC USE

Empiric antibiotics are commonly used in preterm infants immediately after birth due to the possibility that infection caused preterm labor and the relatively high risk for sepsis in VLBW infants. Because the presence of bacteria is one of the main risk factors

Table 2 Summary of 23 randomized controlled trials of probiotics on the risk of necrotizing enterocolitis

Ref.	Yr	GA	BW	*N*	Study	NEC ≥ stage II, %			Mortality, %			LOS, %		
						Study	Control	*P* < 0.05	Study	Control	*P* < 0.05	Study	Control	*P* < 0.05
Shashidhar *et al*[25]	2017	-	750-1499	104	*L. acidophilus, L. rhamnosus, B. longum and S. boulardii*	4.1	12.5	No	1.9	5.7	No	-	-	-
Güney-Varal *et al*[26]	2017	≤ 32	≤ 1500	110	*L. rhamnosus, L. casei, L. plantorum and B. animalis*	0	10	Yes	1.4	22.5	Yes	17.1	35	No
Xu *et al*[27]	2016	30-37	1500-2500	125	*S. boulardii*	0	0	No	-	-	-	7.8	12.2	No
Hays *et al*[28]	2016	25-31	700-1600	199	*B. lactis, B. longum, B. lactis and B. longum.*	5.5	5.8	No	-	-	-	-	-	-
Costeloe *et al*[29]	2016	23-30	-	1310	*B. breve*	9.4	10.0	No	8.3	8.5	No	11.2	11.7	No
Patole *et al*[30]	2014	< 33	-	159	*B. breve*	0	1.3	No	0	0	No	22	16	No
Totsu *et al*[31]	2014	-	< 1500	283	*B. bifidum*	0	0	No	1.3	0	No	8.5	13.1	No
Benor *et al*[32]	2014	-	≤ 1500	58	*L. acidophilus and B. lactis*	4	18.2	No	1	2	NO	24	18	No
Oncel *et al*[33]	2014	≤ 32	≤ 1500	454	*L. reuteri*	4.0	5.0	No	7.5	10	No	6.5	12.5	Yes
Jacobs *et al*[34]	2013	< 32	< 1500	1099	*B. infantis, S. thermophilus, and B. lactis*	2.0	4.4	Yes	4.9	5.1	No	14.2	16.5	No
Serce *et al*[35]	2013	≤ 32	≤ 1500	208	*S. boulardii*	6.7	6.7	No	3.8	4.8	No	24.3	18.3	No
Fernández-Carrocera *et al*[36]	2013	-	< 1500	150	*L. acidophilus, L. rhamnosus, L. casei, L. plantarum, B. infantis and S. thermophillus*	8.0	16.0	No	1.3	9.3	No	-	-	-
Demirel *et al*[37]	2013	≤ 32	≤ 1500	271	*S. boulardii*	4.4	5.1	No	3.7	3.6	No	14.9	15.4	No
Rojas *et al*[38]	2012		≤ 2000	750	*L. reuteri*	2.4	4.0	No	5.9	7.4	No	9.1	10.6	No
Al-Hosni *et al*[39]	2012	-	501-1000	101	*L. rhamnosus and B. infantis*	6.0	7.8	No	6	7.8	No	26.0	31.4	No
Braga *et al*[40]	2011	-	750-1499	231	*B. breve and L. casei*	0	3.6	Yes	21.8	24.1	No	33.6	37.5	No

Sari *et al*[41]	2011	< 33	< 1500	221	*L. sporogenes*	6.5	9	No	2.7	3.6	No	26.4	23.4	No
Mihatsch *et al*[18]	2010	< 30	< 1500	183	*B. lactis*	2.2	4.5	No	2.2	1.1	No	-	-	-
Samanta *et al*[42]	2009	< 32	< 1500	274	*B. infantis, B. bifidum, B. longum and L. acidophilus*	5.5	15.8	Yes	4.4	14.7	Yes	14.3	19.5	Yes
Lin *et al*[43]	2008	< 34	< 1500	434	*B. bifidum and L. acidophilus*	1.8	6.5	Yes	0.9	4.1	No	19.8	11.5	No
Lin *et al*[44]	2005	-	< 1500	367	*L. acidophilus and B. infantis*	1.1	5.3	Yes	3.9	10.7	Yes	12.3	19.3	Yes
Bin-Nun *et al*[45]	2005	-	≤ 1500	145	*B. infantis, S. thermophilus, and B. bifidus*	4	16.4	Yes	0	20.5	Yes	-	-	-
Dani *et al*[46]	2002	< 33	< 1500	585	*L. rhamnosus*	1.4	2.8	No	-	-	-	4.7	4.1	No

BW: Birth weight; LOS: Late onset sepsis; NEC: Necrotizing enterocolitis.

for NEC, some believe that the use of prophylactic antibiotics may decrease the risk of NEC. Others feel that the opposite is true and that the altered normal postnatal gut colonization due to antibiotic use may contribute to the pathogenesis of NEC[17]. Several randomized controlled clinical trials have been performed to evaluate the effect of prophylactic antibiotic administration on the risk of NEC. The results of randomized controlled trials are summarized in Table 3[47-51]. Although Siu *et al*[50] found that prophylactic oral vancomycin conferred some protection against NEC in VLBW infants, Tagare *et al*[47], Kenyon *et al*[48] and Owen *et al*[51] found no protective effect of routine antibiotic use in low risk preterm neonates. Rather, their data suggest that antibiotic may increase the risk of NEC. The efficacy of prophylactic antibiotic usage in the prevention of NEC in premature infants was reviewed, and a meta-analysis on this topic was recently published[52]. Only randomized controlled trials or retrospective cohort studies in LBW infants or preterm infants were included in the meta-analysis. As a result, a total of 5207 infants were included in nine studies. Based on their meta-analysis, the authors conclude that the current evidence does not support the use of prophylactic antibiotics to reduce the incidence of NEC for high-risk premature infants[52].

On the other hand, restricting the use of initial empiric antibiotics course may be important. There is increasing recognition that prolonged empirical antibiotic use might increase the risk of NEC for high-risk premature infants. Cotton *et al*[53] investigated initial empirical antibiotic practices for 4039 extremely low birth weight (ELBW) infants, and 2147 infants in the study cohort received initial empirical antibiotic treatment for more than 5 d. The data suggest that the administration of empiric antibiotics for more than 4 d when the blood culture is negative increases odds of NEC or death in ELBW infants. They suggest that prolonged initial empirical antibiotic therapy for infants with sterile cultures may be associated with increased risk of subsequent death or NEC and should be used with caution. In another retrospective 2:1 control-case analysis from Yale, 124 cases of NEC were matched with 248 controls. Infants with NEC were less likely to have had respiratory distress syndrome (P = 0.018) and more likely to have achieved full enteral feeding (P = 0.028) than were the controls. The risk of NEC significantly increased with duration of antibiotic exposure when infants with culture-confirmed sepsis were removed from the cohort, and exposure to antibiotics for more than 10 d resulted in an approximately three-fold increase in NEC risk[54].

Table 3 Summary of five randomized controlled trials of prophylactic antibiotics on the risk of necrotizing enterocolitis

Ref.	Yr	GA	BW	*N*	Study	NEC ≥ stage II, %			Mortality, %			LOS, (%)		
						Study	Control	*P* < 0.05	Study	Control	*P* < 0.05	Study	Control	*P* < 0.05
Tagare *et al*[47]	2010	< 37	-	140	Amoxicillin clavulanic acid and amikacin	13.0	4.2	Yes	2.9	2.8	No	1.4	14.1	Yes
Kenyon *et al*[48]	2002	< 37	-	4809	Co-amoxiclav	1.6	0.3	Yes	5.7	6.2	No	6.2	7.9	No
Oei *et al*[49]	2001	≤ 32	-	43	Erythromycin	4.5	4.8	No	4.5	4.8	No	-	-	-
Siu *et al*[50]	1998	-	< 1500	140	Vancomycin	12.7	27.5	Yes	15.5	18.8	No	-	-	-
Owen *et al*[51]	1993	24-33	-	117	Ampicillin	14	3.5	Yes	6.8	12	No	3.4	10	No

BW: Birth weight; LOS: Late onset sepsis; NEC: Necrotizing enterocolitis.

STANDARDIZED FEEDING PROTOCOL

A current challenge in clinical NEC research is the high variation in feeding practices. It is clear that consistency in approach to feeding intolerance, feeding advancement and breast milk promotion all impact NEC. SFPs address a consistent approach to the: (1) preferred feeding substance; (2) advancement and fortification of feeding; (3) criteria to stop and specifying how to re-start feedings once held; (4) identification and handling of feeding intolerance; and (5) initiation and duration of trophic feeding. SFPs are simple, inexpensive, effective, and transmissible methods for prevention of postnatal growth restriction in premature infants.

In 2015, a total of 482 infants were enrolled in a feeding bundle study, which was a prospective quality improvement project to standardize a protocol for initiating and advancing enteral feeds, and to improve the nutritional care of neonates admitted to the NICU[55]. In this study, the feeding bundle included breast milk feeding, initiating feedings within 24 h of birth, fortification of breast milk with additional calcium, phosphorus and vitamin D, and the use of trophic feeding for 5 d for ELBW infants followed by daily increases of 10 to 20 mL/kg per day if criteria for tolerance are met. The rate of NEC after bundle implementation was decreased compared to the baseline rate of NEC prior to bundle implementation. Therefore, the authors suggest that early initiation and advancement of enteral feedings does not increase NEC risk, but may actually improve the outcomes[55]. In 2016, Gephart *et al*[14] reviewed papers published and found that studies consistently showed lower or unchanged NEC rates when SFPs were used. They combined data from nine observational studies of infants with birth weight < 1500 g and showed overall reduced odds of NEC by 67% (OR = 0.33, 95%CI: 0.17, 0.65, $P = 0.001$) when SFPs were used. Therefore, it is possible that SFPs reduce the risk of NEC.

CONCLUSION

In this review, we summarize the results of the recent clinical trials and meta-analyses that support some of the common clinical practices to reduce the risk of NEC in premature infants. Firstly, it is evident that human milk feeding can reduce the incidence of NEC. We suggest enhanced lactation support in all NICUs, as well as the establishment of more human milk banks in NICUs. Secondly, while most of the studies demonstrated that probiotic supplementation can significantly reduce the incidence of NEC in premature infants, there are still some concerns in regards to the quality of probiotic preparations, safety, optimal dosage, and treatment duration. Thirdly, antibiotic prophylaxis does not reduce the incidence of NEC, and prolonged empirical use of antibiotics may in fact increase the risk of NEC for high-risk premature infants. Therefore, restricting initial empiric antibiotic use should be implemented in daily practice. Lastly, SFPs are recommended both for prevention of postnatal growth restriction and NEC.

ACKNOWLEDGEMENTS

We would like to thank Dr. Robert Green for critical review of the manuscript.

REFERENCES

1 **Yee WH**, Soraisham AS, Shah VS, Aziz K, Yoon W, Lee SK; Canadian Neonatal Network. Incidence and timing of presentation of necrotizing enterocolitis in preterm infants. *Pediatrics* 2012; **129**: e298-e304 [PMID: 22271701 DOI: 10.1542/peds.2011-2022]

2 **Fitzgibbons SC**, Ching Y, Yu D, Carpenter J, Kenny M, Weldon C, Lillehei C, Valim C, Horbar JD, Jaksic T. Mortality of necrotizing enterocolitis expressed by birth weight categories. *J Pediatr Surg* 2009; **44**: 1072-1075; discussion 1075-6 [PMID: 19524719 DOI: 10.1016/j.jpedsurg.2009.02.013]

3 **Hull MA**, Fisher JG, Gutierrez IM, Jones BA, Kang KH, Kenny M, Zurakowski D, Modi BP, Horbar JD, Jaksic T. Mortality and management of surgical necrotizing enterocolitis in very low birth weight neonates: a prospective cohort study. *J Am Coll Surg* 2014; **218**: 1148-1155 [PMID: 24468227 DOI: 10.1016/j.jamcollsurg.2013.11.015]

4 **Thyoka M**, de Coppi P, Eaton S, Khoo K, Hall NJ, Curry J, Kiely E, Drake D, Cross K, Pierro A. Advanced necrotizing enterocolitis part 1: mortality. *Eur J Pediatr Surg* 2012; **22**: 8-12 [PMID: 22434227 DOI: 10.1055/s-0032-1306263]

5 **Sántulli TV**, Schullinger JN, Heird WC, Gongaware RD, Wigger J, Barlow B, Blanc WA, Berdon WE. Acute necrotizing enterocolitis in infancy: a review of 64 cases. *Pediatrics* 1975; **55**: 376-387 [PMID: 1143976]

6 **Gibbs K**, Lin J, Holzman IR. Necrotising enterocolitis: the state of the science. *Indian J Pediatr* 2007; **74**: 67-72 [PMID: 17264459 DOI: 10.1007/s12098-007-0031-0]

7 **Warner BB**, Deych E, Zhou Y, Hall-Moore C, Weinstock GM, Sodergren E, Shaikh N, Hoffmann JA, Linneman LA, Hamvas A, Khanna G, Rouggly-Nickless LC, Ndao IM, Shands BA, Escobedo M, Sullivan JE, Radmacher PG, Shannon WD, Tarr PI. Gut bacteria dysbiosis and necrotising enterocolitis in very low birthweight infants: a prospective case-control study. *Lancet* 2016; **387**: 1928-1936 [PMID: 26969089 DOI: 10.1016/S0140-6736(16)00081-7]

8 **Corpeleijn WE**, de Waard M, Christmann V, van Goudoever JB, Jansen-van der Weide MC, Kooi EM, Koper JF, Kouwenhoven SM, Lafeber HN, Mank E, van Toledo L, Vermeulen MJ, van Vliet I, van Zoeren-Grobben D. Effect of Donor Milk on Severe Infections and Mortality in Very Low-Birth-Weight Infants: The Early Nutrition Study Randomized Clinical Trial. *JAMA Pediatr* 2016; **170**: 654-661 [PMID: 27135598 DOI: 10.1001/jamapediatrics.2016.0183]

9 **Cristofalo EA**, Schanler RJ, Blanco CL, Sullivan S, Trawoeger R, Kiechl-Kohlendorfer U, Dudell G, Rechtman DJ, Lee ML, Lucas A, Abrams S. Randomized trial of exclusive human milk versus preterm formula diets in extremely premature infants. *J Pediatr* 2013; **163**: 1592-1595.e1 [PMID: 23968744 DOI: 10.1016/j.jpeds.2013.07.011]

10 **Sullivan S**, Schanler RJ, Kim JH, Patel AL, Trawöger R, Kiechl-Kohlendorfer U, Chan GM, Blanco CL, Abrams S, Cotten CM, Laroia N, Ehrenkranz RA, Dudell G, Cristofalo EA, Meier P, Lee ML, Rechtman DJ, Lucas A. An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. *J Pediatr* 2010; **156**: 562-567.e1 [PMID: 20036378 DOI: 10.1016/j.jpeds.2009.10.040]

11 **Schanler RJ**, Lau C, Hurst NM, Smith EO. Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. *Pediatrics* 2005; **116**: 400-406 [PMID: 16061595 DOI: 10.1542/peds.2004-1974]

12 **Schanler RJ**, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. *Pediatrics* 1999; **103**: 1150-1157 [PMID: 10353922 DOI: 10.1542/peds.103.6.1150]

13 **Quigley M**, Embleton ND, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. *Cochrane Database Syst Rev* 2018; **6**: CD002971 [PMID: 29926476 DOI: 10.1002/14651858.CD002971.pub4]

14 **Gephart SM**, Hanson C, Wetzel CM, Fleiner M, Umberger E, Martin L, Rao S, Agrawal A, Marin T, Kirmani K, Quinn M, Quinn J, Dudding KM, Clay T, Sauberan J, Eskenazi Y, Porter C, Msowoya AL, Wyles C, Avenado-Ruiz M, Vo S, Reber KM, Duchon J. NEC-zero recommendations from scoping review of evidence to prevent and foster timely recognition of necrotizing enterocolitis. *Matern Health Neonatol Perinatol* 2017; **3**: 23 [PMID: 29270303 DOI: 10.1186/s40748-017-0062-0]

15 **Section on Breastfeeding**. Breastfeeding and the use of human milk. *Pediatrics* 2012; **129**: e827-e841 [PMID: 22371471 DOI: 10.1542/peds.2011-3552]

16 **Garg BD**, Balasubramanian H, Kabra NS, Bansal A. Effect of oropharyngeal colostrum therapy in the prevention of necrotising enterocolitis among very low birthweight neonates: A meta-analysis of randomised controlled trials. *J Hum Nutr Diet* 2018; **31**: 612-624 [PMID: 30073712 DOI: 10.1111/jhn.12585]

17 **Huang XZ**, Zhu LB, Li ZR, Lin J. Bacterial colonization and intestinal mucosal barrier development. *World J Clin Pediatr* 2013; **2**: 46-53 [PMID: 25254174 DOI: 10.5409/wjcp.v2.i4.46]

18 **Mihatsch WA**, Vossbeck S, Eikmanns B, Hoegel J, Pohlandt F. Effect of Bifidobacterium lactis on the incidence of nosocomial infections in very-low-birth-weight infants: a randomized controlled trial. *Neonatology* 2010; **98**: 156-163 [PMID: 20234140 DOI: 10.1159/000280291]

19 **Patel RM**, Underwood MA. Probiotics and necrotizing enterocolitis. *Semin Pediatr Surg* 2018; **27**: 39-46 [PMID: 29275816 DOI: 10.1053/j.sempedsurg.2017.11.008]

20 **van den Akker CHP**, van Goudoever JB, Szajewska H, Embleton ND, Hojsak I, Reid D, Shamir R; ESPGHAN Working Group for Probiotics, Prebiotics & Committee on Nutrition. Probiotics for Preterm Infants: A Strain-Specific Systematic Review and Network Meta-analysis. *J Pediatr Gastroenterol Nutr* 2018; **67**: 103-122 [PMID: 29384838 DOI: 10.1097/MPG.0000000000001897]

21 **Chang HY**, Chen JH, Chang JH, Lin HC, Lin CY, Peng CC. Multiple strains probiotics appear to be the most effective probiotics in the prevention of necrotizing enterocolitis and mortality: An updated meta-analysis. *PLoS One* 2017; **12**: e0171579 [PMID: 28182644 DOI: 10.1371/journal.pone.0171579]

22 **Taylor RS**. Probiotics to prevent necrotizing enterocolitis: Too cheap and easy? *Paediatr Child Health* 2014; **19**: 351-352 [PMID: 25332671 DOI: 10.1093/pch/19.7.351]

23 **Isayama T**, Lee SK, Mori R, Kusuda S, Fujimura M, Ye XY, Shah PS; Canadian Neonatal Network; Neonatal Research Network of Japan. Comparison of mortality and morbidity of very low birth weight

infants between Canada and Japan. *Pediatrics* 2012; **130**: e957-e965 [PMID: 22966031 DOI: 10.1542/peds.2012-0336]
24 **Escribano E**, Zozaya C, Madero R, Sánchez L, van Goudoever J, Rodríguez JM, de Pipaon MS. Increased incidence of necrotizing enterocolitis associated with routine administration of Infloran™ in extremely preterm infants. *Benef Microbes* 2018; **9**: 683-690 [PMID: 29888655 DOI: 10.3920/BM2017.0098]
25 **Shashidhar A**, Suman Rao PN, Nesargi S, Bhat S, Chandrakala BS. Probiotics for Promoting Feed Tolerance in Very Low Birth Weight Neonates - A Randomized Controlled Trial. *Indian Pediatr* 2017; **54**: 363-367 [PMID: 28368269 DOI: 10.1007/s13312-017-1106-2]
26 **Güney-Varal İ**, Köksal N, Özkan H, Bağcı O, Doğan P. The effect of early administration of combined multi-strain and multi-species probiotics on gastrointestinal morbidities and mortality in preterm infants: A randomized controlled trial in a tertiary care unit. *Turk J Pediatr* 2017; **59**: 13-19 [PMID: 29168358 DOI: 10.24953/turkjped.2017.01.003]
27 **Xu L**, Wang Y, Wang Y, Fu J, Sun M, Mao Z, Vandenplas Y. A double-blinded randomized trial on growth and feeding tolerance with Saccharomyces boulardii CNCM I-745 in formula-fed preterm infants. *J Pediatr (Rio J)* 2016; **92**: 296-301 [PMID: 26946967 DOI: 10.1016/j.jped.2015.08.013]
28 **Hays S**, Jacquot A, Gauthier H, Kempf C, Beissel A, Pidoux O, Jumas-Bilak E, Decullier E, Lachambre E, Beck L, Cambonie G, Putet G, Claris O, Picaud JC. Probiotics and growth in preterm infants: A randomized controlled trial, PREMAPRO study. *Clin Nutr* 2016; **35**: 802-811 [PMID: 26220763 DOI: 10.1016/j.clnu.2015.06.006]
29 **Costeloe K**, Bowler U, Brocklehurst P, Hardy P, Heal P, Juszczak E, King A, Panton N, Stacey F, Whiley A, Wilks M, Millar MR. A randomised controlled trial of the probiotic Bifidobacterium breve BBG-001 in preterm babies to prevent sepsis, necrotising enterocolitis and death: the Probiotics in Preterm infantS (PiPS) trial. *Health Technol Assess* 2016; **20**: 1-194 [PMID: 27594381 DOI: 10.3310/hta20660]
30 **Patole S**, Keil AD, Chang A, Nathan E, Doherty D, Simmer K, Esvaran M, Conway P. Effect of Bifidobacterium breve M-16V supplementation on fecal bifidobacteria in preterm neonates--a randomised double blind placebo controlled trial. *PLoS One* 2014; **9**: e89511 [PMID: 24594833 DOI: 10.1371/journal.pone.0089511]
31 **Totsu S**, Yamasaki C, Terahara M, Uchiyama A, Kusuda S; Probiotics Study Group in Japan. Bifidobacterium and enteral feeding in preterm infants: cluster-randomized trial. *Pediatr Int* 2014; **56**: 714-719 [PMID: 24617812 DOI: 10.1111/ped.12330]
32 **Benor S**, Marom R, Ben Tov A, Armoni Domany K, Zaidenberg-Israeli G, Dollberg S. Probiotic supplementation in mothers of very low birth weight infants. *Am J Perinatol* 2014; **31**: 497-504 [PMID: 23934538 DOI: 10.1055/s-0033-1353490]
33 **Oncel MY**, Sari FN, Arayici S, Guzoglu N, Erdeve O, Uras N, Oguz SS, Dilmen U. Lactobacillus Reuteri for the prevention of necrotising enterocolitis in very low birthweight infants: a randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed* 2014; **99**: F110-F115 [PMID: 24309022 DOI: 10.1136/archdischild-2013-304745]
34 **Jacobs SE**, Tobin JM, Opie GF, Donath S, Tabrizi SN, Pirotta M, Morley CJ, Garland SM; ProPrems Study Group. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. *Pediatrics* 2013; **132**: 1055-1062 [PMID: 24249817 DOI: 10.1542/peds.2013-1339]
35 **Serce O**, Benzer D, Gursoy T, Karatekin G, Ovali F. Efficacy of Saccharomyces boulardii on necrotizing enterocolitis or sepsis in very low birth weight infants: a randomised controlled trial. *Early Hum Dev* 2013; **89**: 1033-1036 [PMID: 24041815 DOI: 10.1016/j.earlhumdev.2013.08.013]
36 **Fernández-Carrocera LA**, Solis-Herrera A, Cabanillas-Ayón M, Gallardo-Sarmiento RB, García-Pérez CS, Montaño-Rodríguez R, Echániz-Aviles MO. Double-blind, randomised clinical assay to evaluate the efficacy of probiotics in preterm newborns weighing less than 1500 g in the prevention of necrotising enterocolitis. *Arch Dis Child Fetal Neonatal Ed* 2013; **98**: F5-F9 [PMID: 22556209 DOI: 10.1136/archdischild-2011-300435]
37 **Demirel G**, Erdeve O, Celik IH, Dilmen U. Saccharomyces boulardii for prevention of necrotizing enterocolitis in preterm infants: a randomized, controlled study. *Acta Paediatr* 2013; **102**: e560-e565 [PMID: 24028629 DOI: 10.1111/apa.12416]
38 **Rojas MA**, Lozano JM, Rojas MX, Rodriguez VA, Rondon MA, Bastidas JA, Perez LA, Rojas C, Ovalle O, Garcia-Harker JE, Tamayo ME, Ruiz GC, Ballesteros A, Archila MM, Arevalo M. Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. *Pediatrics* 2012; **130**: e1113-e1120 [PMID: 23071204 DOI: 10.1542/peds.2011-3584]
39 **Al-Hosni M**, Duenas M, Hawk M, Stewart LA, Borghese RA, Cahoon M, Atwood L, Howard D, Ferrelli K, Soll R. Probiotics-supplemented feeding in extremely low-birth-weight infants. *J Perinatol* 2012; **32**: 253-259 [PMID: 21546942 DOI: 10.1038/jp.2011.51]
40 **Braga TD**, da Silva GA, de Lira PI, de Carvalho Lima M. Efficacy of Bifidobacterium breve and Lactobacillus casei oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a double-blind, randomized, controlled trial. *Am J Clin Nutr* 2011; **93**: 81-86 [PMID: 20980486 DOI: 10.3945/ajcn.2010.29799]
41 **Sari FN**, Dizdar EA, Oguz S, Erdeve O, Uras N, Dilmen U. Oral probiotics: Lactobacillus sporogenes for prevention of necrotizing enterocolitis in very low-birth weight infants: a randomized, controlled trial. *Eur J Clin Nutr* 2011; **65**: 434-439 [PMID: 21245887 DOI: 10.1038/ejcn.2010.278]
42 **Samanta M**, Sarkar M, Ghosh P, Ghosh JK, Sinha MK, Chatterjee S. Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birth weight newborns. *J Trop Pediatr* 2009; **55**: 128-131 [PMID: 18842610 DOI: 10.1093/tropej/fmn091]
43 **Lin HC**, Hsu CH, Chen HL, Chung MY, Hsu JF, Lien RI, Tsao LY, Chen CH, Su BH. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. *Pediatrics* 2008; **122**: 693-700 [PMID: 18829790 DOI: 10.1542/peds.2007-3007]
44 **Lin HC**, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, Oh W. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2005; **115**: 1-4 [PMID: 15629973 DOI: 10.1542/peds.2004-1463]
45 **Bin-Nun A**, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, Hammerman C. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. *J Pediatr* 2005; **147**: 192-196 [PMID: 16126048 DOI: 10.1016/j.jpeds.2005.03.054]
46 **Dani C**, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. *Biol Neonate* 2002; **82**: 103-108 [PMID: 12169832 DOI: 10.1159/000063096]
47 **Tagare A**, Kadam S, Vaidya U, Pandit A. Routine antibiotic use in preterm neonates: a randomised

controlled trial. *J Hosp Infect* 2010; **74**: 332-336 [PMID: 19926166 DOI: 10.1016/j.jhin.2009.09.010]
48 **Kenyon S**, Taylor DJ, Tarnow-Mordi WO; ORACLE Collaborative Group. ORACLE--antibiotics for preterm prelabour rupture of the membranes: short-term and long-term outcomes. *Acta Paediatr Suppl* 2002; **91**: 12-15 [PMID: 12200889 DOI: 10.1111/j.1651-2227.2002.tb00153.x]
49 **Oei J**, Lui K. A placebo-controlled trial of low-dose erythromycin to promote feed tolerance in preterm infants. *Acta Paediatr* 2001; **90**: 904-908 [PMID: 11529540 DOI: 10.1111/j.1651-2227.2001.tb02455.x]
50 **Siu YK**, Ng PC, Fung SC, Lee CH, Wong MY, Fok TF, So KW, Cheung KL, Wong W, Cheng AF. Double blind, randomised, placebo controlled study of oral vancomycin in prevention of necrotising enterocolitis in preterm, very low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 1998; **79**: F105-F109 [PMID: 9828735 DOI: 10.1136/fn.79.2.F105]
51 **Owen J**, Groome LJ, Hauth JC. Randomized trial of prophylactic antibiotic therapy after preterm amnion rupture. *Am J Obstet Gynecol* 1993; **169**: 976-981 [PMID: 8238160 DOI: 10.1016/0002-9378(93)90038-K]
52 **Fan X**, Zhang L, Tang J, Chen C, Chen J, Qu Y, Mu D. The initial prophylactic antibiotic usage and subsequent necrotizing enterocolitis in high-risk premature infants: a systematic review and meta-analysis. *Pediatr Surg Int* 2018; **34**: 35-45 [PMID: 29128874 DOI: 10.1007/s00383-017-4207-z]
53 **Cotten CM**, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sánchez PJ, Ambalavanan N, Benjamin DK; NICHD Neonatal Research Network. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. *Pediatrics* 2009; **123**: 58-66 [PMID: 19117861 DOI: 10.1542/peds.2007-3423]
54 **Alexander VN**, Northrup V, Bizzarro MJ. Antibiotic exposure in the newborn intensive care unit and the risk of necrotizing enterocolitis. *J Pediatr* 2011; **159**: 392-397 [PMID: 21489560 DOI: 10.1016/j.jpeds.2011.02.035]
55 **Graziano PD**, Tauber KA, Cummings J, Graffunder E, Horgan MJ. Prevention of postnatal growth restriction by the implementation of an evidence-based premature infant feeding bundle. *J Perinatol* 2015; **35**: 642-649 [PMID: 25880797 DOI: 10.1038/jp.2015.35]

World Journal of Clinical Pediatrics

Submit a Manuscript: https://www.f6publishing.com

World J Clin Pediatr 2019 April 9; 8(2): 33-42

DOI: 10.5409/wjcp.v8.i2.33

ISSN 2219-2808 (online)

ORIGINAL ARTICLE

Prospective Study

Prevalence of respiratory syncytial virus infection among children hospitalized with acute lower respiratory tract infections in Southern India

Sandesh Kini, Bhuvanesh Sukhlal Kalal, Sara Chandy, Ranjani Shamsundar, Anita Shet

ORCID number: Sandesh Kini (0000-0002-6250-2968); Bhuvanesh Sukhlal Kalal (0000-0002-2560-3778); Sara Chandy (0000-0003-3325-9396); Ranjani Shamsundar (0000-0001-5265-352X); Anita Shet (0000-0002-7204-8164).

Author contributions: Kini S and Kalal BS contributed equally to this work. Shet A and Kini S designed the study; Kini S and Kalal BS recruited the children, collected samples and data; Kalal BS and Shamsundar R performed laboratory experiments; Kini S and Kalal BS analysed data and wrote the manuscript; Chandy S, Shamsundar R and Shet A gave technical support and conceptual advice; Shet A critically reviewed the manuscript and supervised the whole study process; all authors read and approved the final manuscript.

Institutional review board statement: Ethical clearance was obtained from the Institutional Ethics Committee (IRB No 134/2008) at St. Johns Medical College Hospital prior to initiating the study.

Informed consent statement: Informed consent was obtained from the caregivers of eligible children.

Conflict-of-interest statement: The authors declare no conflict of interest.

Sandesh Kini, Department of Paediatrics, Kasturba Medical College, Manipal Academy of Higher Education, Manipal 576104, Karnataka, India

Bhuvanesh Sukhlal Kalal, Department of Biochemistry, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangaluru 575018, Karnataka, India

Sara Chandy, Pushpagiri Research Centre, Pushpagiri Institute of Medical Science and Research Centre, Thiruvalla 689101, Kerala, India

Ranjani Shamsundar, Department of Microbiology, St. John's Medical College, Bengaluru 560034, Karnataka, India

Anita Shet, International Vaccine Access Center, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, United States

Corresponding author: Anita Shet, MD, PhD, Full Professor, International Vaccine Access Center, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, United States. ashet1@jhu.edu
Telephone: +1-410-5022629

Abstract

BACKGROUND

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory infections among children.

AIM

To investigate the proportion of RSV and non-RSV respiratory viral infections among hospitalized children ≤ 5 years.

METHODS

Hospitalized children aged < 5 years, with a diagnosis of acute lower respiratory infections (ALRI), admitted between August 2011-August 2013, were included. Cases were defined as laboratory-confirmed RSV and non-RSV respiratory viruses by direct fluorescence assay from the nasopharyngeal wash.

RESULTS

Of 383 1-59 mo old children hospitalized with an acute lower respiratory infection, 33.9% (130/383) had evidence of viral infection, and RSV was detected in 24.5% (94/383). Co-infections with RSV and other respiratory viruses

Manuscript source: Unsolicited manuscript

Received: November 30, 2018
Peer-review started: November 30, 2018
First decision: January 9, 2019
Revised: February 20, 2019
Accepted: February 27, 2019
Article in press: February 27, 2019
Published online: April 9, 2019

P-Reviewer: Kenar T, Pandey A, Pavone P, Yang L
S-Editor: Ji FF
L-Editor: A
E-Editor: Song H

(influenza A or B, adenovirus, para influenza 1, 2 or 3) were seen in children 5.5% (21/383). Over 90% of the RSV-positive children were under 2 years of age. RSV was detected throughout the year with peaks seen after the monsoon season. Children hospitalized with RSV infection were more likely to have been exposed to a shorter duration of breastfeeding of less than 3 mo. RSV positive children had a shorter hospital stay, although there were significant complications requiring intensive care. Use of antibiotics was high among those with RSV and non-RSV viral infections.

CONCLUSION
Our study provides evidence of a high proportion of RSV and other virus-associated ALRI among hospitalized children in India. RSV infection was associated with fewer days of hospital stay compared to other causes of lower respiratory infections. A high level of antibiotic use was seen among all respiratory virus-associated hospitalizations. These results suggest the need for implementing routine diagnostics for respiratory pathogens in order to minimize the use of unnecessary antibiotics and plan prevention strategies among pediatric populations.

Key words: Respiratory syncytial virus; Acute lower respiratory infections; Children; Epidemiology; India; Respiratory viral infection

Core tip: The study shows that a significant proportion of young children hospitalized for acute lower respiratory tract infection were associated with respiratory syncytial virus (RSV) and other viral infections. Early diagnosis of viral infections using a simple test such as the RSV and viral direct fluorescence assay test, in settings where PCR is not feasible, would be useful in the timely institution of appropriate care, minimization of antibiotic overuse, and appropriate follow-up care for complications and sequelae, potentially leading to a reduction of costs of medical care.

Citation: Kini S, Kalal BS, Chandy S, Shamsundar R, Shet A. Prevalence of respiratory syncytial virus infection among children hospitalized with acute lower respiratory tract infections in Southern India. *World J Clin Pediatr* 2019; 8(2): 33-42
URL: https://www.wjgnet.com/2219-2808/full/v8/i2/33.htm
DOI: https://dx.doi.org/10.5409/wjcp.v8.i2.33

INTRODUCTION

Globally, acute lower respiratory infections (ALRI) are an important cause of morbidity and mortality in children < 5 years of age[1]. Molecular diagnostic methods have identified respiratory syncytial virus (RSV), as the most common viral cause of ALRI-related death; other prominent viruses are human metapneumovirus, parainfluenza viruses, influenza viruses A and B and adenoviruses[2,3]. In 2015, Shi *et al*[4] reported an estimated annual burden of RSV of 33.1 million globally (22% of all episodes of ALRI) resulting in 3.2 million hospitalizations and 59600 deaths of children < 5 years, with about 99% of RSV-related childhood mortality occurring in low and middle-income countries (LMICs)[4].

Recent literature on pediatric ALRI in LMICs highlights increasing incidence of RSV, a major cause of death of infants < 1 year of age and of ALRI during the first few months of an infant's life[5]. RSV is also gaining prominence in India; recent hospital-based studies indicate that RSV constitutes up to 16% of children hospitalized with acute lower respiratory tract infections (LRTIs), with incidence highest in infants aged below 6 mo[6]. The common respiratory symptoms are recurrent episodes of wheezing mimicking early childhood asthma and may persist as lung function abnormality till adolescence[7]. Known risk factors include low birth weight, smoking during pregnancy, attendance at a child care facility, crowded household, low parental education, exposure to second-hand smoke, history of atopy and lack of breast-feeding[8]. Furthermore, RSV infections tend to be associated with hospitalization and mortality in high-risk cases[7].

Detection of RSV using rapid, sensitive and specific diagnostic tests aids good clinical management. Viral isolation by tissue culture is regarded as the gold standard but has limited availability. Real-time reverse transcriptase PCR (RT-PCR) is more sensitive but not routinely performed in clinical diagnostic laboratories in LMICs due to high expense, the need for technical expertise and high laboratory standards to prevent contamination[9]. Direct fluorescent antibody assay (DFA) has been used as a simple detection tool for RSV antigen detection[10]. DFA has a sensitivity and specificity of 77.8% and 96.8% respectively and can detect RSV antigens even in conditions where the virus cannot be isolated[10,11].

The objective of the study was to investigate the proportion of RSV and non-RSV respiratory viral infections as a cause of ALRI among 1-59 mo old children admitted to a tertiary care hospital in southern India. The study also sought to assess the seasonality, clinical features, risk factors and outcome of RSV and non-RSV respiratory viral infections among these hospitalized children.

MATERIALS AND METHODS

Patients and settings

This prospective study was conducted at a tertiary care center in south India between August 2011 and August 2013, on children aged between 1 mo and 5 years, hospitalized for ALRI. The study site, St. John's Medical College Hospital, Bengaluru, Karnataka, India center to local as well as to referred cases from neighboring states like Andhra Pradesh and Tamil Nadu[12]. ALRI was defined as acute respiratory infection with evidence of respiratory distress and age-specific fast breathing (≤ 2 mo age: ≥ 60 breaths/min; 2-12 mo, ≥ 50 breaths/min; 1-5 years, ≥ 40 breaths/min)[13]. Features of respiratory distress include chest indrawing, stridor, nasal flaring or grunting, inability to breastfeed or drink, with or without danger signs such as central cyanosis, lethargy or unconsciousness. Bronchiolitis was defined as a viral LRTI in children < 2 years of age characterized byclinical features of small airways obstruction causing symptoms of ALRI. Bronchopneumonia was defined as symptoms of ALRI with chest X-ray findings of hyperinflation with bilateral interstitial infiltrates and peribronchial cuffing. Lobar pneumonia was defined as symptoms of ALRI with confluent lobar consolidation. Reactive airway disease was defined as respiratory illness associated with wheeze secondary to allergen exposure. The study excluded children with empyema, hydropneumothorax or tuberculosis and those with non-respiratory causes of respiratory distress. Informed consent was obtained from the caregivers of eligible children. Detailed patient history and clinical observations were recorded. Vital signs and oxygen saturation were monitored and routine laboratory investigations were performed as indicated. Ethical clearance was obtained from the Institutional Ethics Committee (IRB No 134/2008) at St. Johns Medical College Hospital prior to initiating the study.

Sample collection

Nasopharyngeal (NP) wash were collected using a 5 French infant feeding tube cut to 4 cm length, 1.5 mL of sterile saline was quickly introduced into each nostril and immediately aspirated back into a 2 mL disposable sterile syringe. The aspirated sample was transferred into a sterile centrifuge tube, maintained at 4 ^{o}C and transported to the microbiology laboratory immediately on an ice pack (4 °C) within 1 h of collection[14].

Slide preparation and direct immunofluorescence assay

The NP sample was centrifuged and the pallet was smeared on a slide, air dried and placed in cold acetone (-20 ^{o}C) for a minimum of 30 min for fixation. Slides were stained using SimulFluor Respiratory Screen kit (Chemicon International, United States), in accordance with the manufacturer's instructions.

Statistical analysis

The data was compiled in an excel spreadsheet. Descriptive statistics were reported using mean and standard deviation for variables with a normal distribution, and median for variables without a normal distribution. The association between the presence of RSV infection and RSV-related risk factors were assessed by χ^2 test or Fisher's exact test (for categorical variables) and by independent *t*-test (for continuous variables). The analysis was done by using the Statistical software STATA/IC version 12.1 and $P < 0.05$ was considered significant.

RESULTS

Study population

Between August 2011 and August 2013, 9600 children were admitted to the inpatient ward; of these 408 were 1-59 mo old at the time of admission, had ALRI, and were considered eligible for the study. There were 20 caregivers of patients who declined consent citing reasons of non-interest or refusal of permission for the NP wash procedure, and 5 were excluded due to the presence of other diagnoses (2 were diagnosed with pulmonary tuberculosis, and 3 were diagnosed with bacterial pneumonia (one blood culture positive for *Staphylococcus aureus* and two broncho-alveolar lavage fluid positive for *Pseudomonas aeuruginosa* and *Enterobacter* species). The remaining 383 children were included in the study. The median age of subjects was 8 mo (inter quartile range 5-15 mo), and 89.0% were less than 2 years of age. There were 68.7% males, and the subjects resided in Karnataka (64.2%), Andhra Pradesh (19.1%) and Tamil Nadu (15.9%). The majority lived in urban areas (66.8%).

Etiology of respiratory illness

Viral etiology (RSV, influenza A or B, adenovirus, para influenza 1, 2 or 3) was confirmed in 130 (33.9%) of 383 children hospitalized for ALRI. RSV was positive in 94 (24.5%), non-RSV viruses in 57 (14.8%), while co-infection with RSV and non-RSV viruses was seen in 21 (5.5%) children (Table 1). Among the children with RSV infection, 86 (91.5%) children were in the age group 1 mo to 24 mo, and 32 (34%) were children 1-6 mo of age. A peak of RSV positive cases was seen after the rainy season during the months of August through November (Figure 1). A smaller peak was also noted during January and February. The seasonality of other viruses mirrored the pattern of RSV infection.

Clinical features and correlates

The predominant physician-assigned clinical diagnosis was bronchiolitis (173/383, 45.1%). Others were bronchopneumonia (135/383, 35.2%), lobar pneumonia (24/383, 6.2%) and reactive airway disease (51/383, 13.3%). Those with RSV infection had fever (23.8%), rhinorrhea (24.6%), cough, (23.5%), chest retractions (25.6%), auscultatory wheeze (22.9%), and auscultatory crepitations (25.7%). Chest radiographs were obtained in 189 of 383 children who had severe ALRI at admission or those who did not respond to first-line treatment. Radiographic abnormalities were detected in 162 children; 135 had evidence of hyperinflation with bilateral interstitial infiltrates suggestive of bronchopneumonia, 24 had focal lung consolidative changes and 3 had evidence of cardiomegaly without lung field abnormalities.

In univariate analysis, RSV infection was significantly associated with being exclusively breastfed for less than 3 mo, compared to those exclusively breastfed for 3-12 mo (unadjusted OR 1.98, 95%CI: 1.69-3.22). There was no significant association between RSV infection and independent variables such as low birth weight, prematurity, complicated neonatal course, family history of asthma, household smoking or indoor wood fuel usage (Tables 2 and 3). There was no significant association between other viruses (influenza A or B, adenovirus, para influenza 1, 2 or 3) and risk factors mentioned in Table 2. Laboratory parameters including total white blood cell count, and absolute lymphocyte count was similar between RSV-positive and RSV-negative patients.

Outcome

Mean hospital stay was 4.6 d (SD 5.1); and 8.3 d (SD 6.5) in RSV-positive and RSV-negative children, respectively (P = 0.031) (Table 4). The length of PICU stay (P = 0.547), oxygen use (P = 0.176), and qualitative antibiotic use (P = 0.110) were similar in both groups. Antibiotics were used among 56 (59.5%) and 21 (36.8%) of RSV-positive and non-RSV virus-positive children, respectively. Most commonly used first-line antibiotics were amoxicillin/amoxicillin-clavulanic acid/or ceftriaxone. Respiratory complications such as acute respiratory distress syndrome and respiratory failure requiring PICU admission took place among 13.8% (13/94) of RSV-positive, 8.7% (5/57) of non-RSV positive and 16.6% (42/253) of viral negative children. Of the 57 children admitted to PICU, 46 recovered well while 11 had residual respiratory symptoms including persistent tachypnea and persistent oxygen requirement. There was no mortality recorded in this study (Table 5).

DISCUSSION

Viral-associated respiratory illness among hospitalized children 1-59 mo old at our single center tertiary care hospital in southern India was seen among a third of those

Table 1 Respiratory syncytial virus and non-respiratory syncytial viruses associated with lower respiratory tract infection in young children

RSV	94 (24.5%)
All virus (RSV and/or other virus[1])	130 (34.0%)
Co-infection with multiple viruses	21 (5.5%)
Non-RSV viruses alone[1]	57 (14.8%)
Virus-negative	253 (66%)
Total enrolled	383

[1]Other viruses include infection with parainfluenza viruses, influenza viruses A and B and adenoviruses. RSV: Respiratory syncytial virus.

with ALRI. RSV was the predominant viral etiological agent, and over a quarter had evidence of mixed viral pathogens in the respiratory tract that may be etiologically linked to the respiratory illness.RSV and other respiratory viruses are a major cause of pediatric ALRI in India. ALRI constitutes one of the principal causes of death among children under-five of the developing countries[8,15] and RSV has been documented as an important cause of ALRI in pediatric age group[8,14]. In India, studies have shown high ALRI incidence rates of 15.0 per 1000 child years in the under-five age group with the incidence being 3.6 times higher among boys as compared to girls[16]. The highest rates of ALRI generally occur in the first year of life. RSV infection in the very young causes substantial complications such as respiratory failure, prolonged hospitalization, and high mortality similar to seasonal influenza. Previous hospital-based studies from India reported hospital RSV prevalence of 11.4-26.0% (22.1%[14]; 20.2%[17]; 21.3%[18]; 12.0%[19]; 26.0%[20]), although the variability in these studies precludes any direct comparison with the present study. RSV-associated ALRI incidence ranging from 2.4% to 21.2% have been reported in different countries[21]. Hospital-based studies have reported a significant association between being male and having RSV-related ALRI[22]. Nair *et al*[5] concluded in a recent meta-analysis that RSV is the most common cause of childhood LRTI and a major cause of admission to hospital as a result of severe LRTI and that 99.0% of the RSV-related deaths take place among resource-limited countries. The study showed that India, China, Nigeria, Pakistan, and Indonesia together account for a total of 16 million cases of RSV infections, accounting for half of the global cases of under-5 childhood deaths in the world[1].

In India, routine laboratory diagnoses of viral ALRI remains unavailable and unexplored, even in tertiary care centers. Viral etiology of ALRI largely remains unknown and most cases are empirically treated with antibiotics. Gold standard test like viral isolation and RT-PCR are more sensitive but not routinely performed in clinical diagnostic laboratories in LMICs due to high expense LMICs due to high expense. However, in resource-limited settings, DFA still has value for the diagnosis of ALRI[23]. Compared to culture, DFA has sensitivities of 72% to 94% and specificities of 95% to 100%[24]. The DFA kit used in this study, SimulFluor Respiratory Screen Kit (Chemicon) was easy to perform and results were available within 4-5 h. It tested for a panel of seven respiratory viruses; RSV, Influenza A, and B, Parainfluenza 1 to 3 and Adenovirus[10,11]. Limitations of DFA include the degree of subjectivity in the evaluation of the result, as well as the need for a high level of technical skill and sample quality for the assay[25].

Early diagnosis facilitates early management and helps combat ALRIs. In India, antibiotics use in pediatric ALRI is very common even if a viral cause is suspected. A Cochrane review on the effectiveness of antibiotics in children fewer than two years old diagnosed with bronchiolitis did not find enough evidence to support the use of antibiotics[26]. Antibiotics can be used if there is clear documented evidence of secondary bacterial infections[8]. Early diagnosis of a viral respiratory infection has the potential to reduce the rampant use of antibiotics.

As established by the results, this study demonstrates that exclusive breastfeeding for over 3 mo of age, seems to have some protective effect against RSV and other respiratory viral infections. Significance between exclusive breastfeeding and decreased incidence of RSV positive illnesses have been reported[27]. However, there is no association between exclusive breastfeeding and wheezing illnesses associated with RSV as wheezing can be secondary to a maternal history of allergy and asthma or pet exposure[28]. This possibly can be explained by the fact that RSV is caused by infection and breastfeeding is often thought to confer some protection against it[29], whereas wheezing may be immune to any protective effects of breastfeeding since

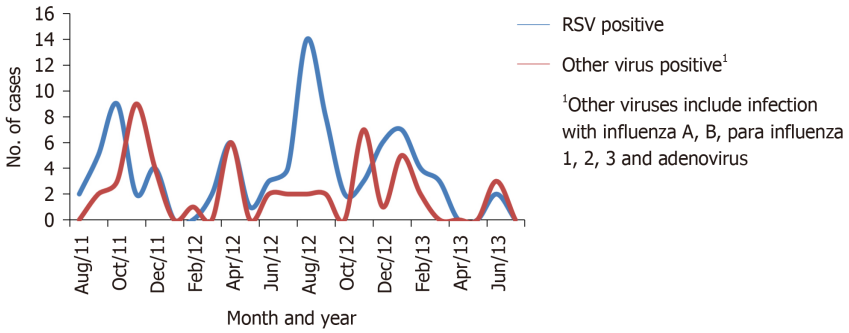

Figure 1 Seasonality patterns of respiratory syncytial virus-positive and non-respiratory syncytial virus viral-associated lower respiratory tract infection in young children.[1]Other viruses include infection with influenza A, B, para influenza 1, 2, 3 and adenovirus.

high IgE levels have been found to share an association with the presence of wheezing.

Our finding showed a high rate of respiratory complications in 13.8% (13/94), 8.7% (5/57) and 16.6% (42/253) of children with RSV positive, non-RSV positive and viral negative results. Out of these, 6 children were mechanically ventilated with a median of 4 d (range 3-5 d). Our findings were consistent with previous studies done by Kholy *et al*[30] in which 10% (24/240) of patients were admitted to PICU with a median duration of 6.5 d and 14 patients required mechanical ventilation.

Limitations of the study

In this study, we focused only on the viral causes of ALRI as they are the most common etiological agents and there are limited studies from south India. However, we did not address key questions about bacterial etiology and the possible role of viral and bacterial co-infections. We had a small sample size which was insufficient to make finer observations regarding differences in age, clinical profile, or factors determining severity between specific viral species.

We find that a significant proportion of young children hospitalized for acute LRTI is associated with RSV and other viral infections. Early diagnosis of viral infections using a simple test such as the RSV and viral DFA test, in settings where PCR is not feasible, would be useful in the timely institution of appropriate care, minimization of antibiotic overuse, and appropriate follow-up care for complications and sequelae, potentially leading to a reduction of costs of medical care.

Table 2 Risk factors for respiratory syncytial virus infection

Variable		RSV negative (*n* = 289)	RSV positive (*n* = 94)	*P* value
Birth weight (≥ 2500 g)	No	55 (75.3)	18 (24.7)	0.980
	Yes	234 (75.4)	76 (24.6)	
Breast fed exclusively for ≥ 3 mo	No	66 (68.0)	31 (32.0)	0.049
	Yes	223 (77.9)	63 (22.1)	
Gestational age (≥ 37 wk)	No	33 (75.0)	11 (25.0)	0.940
	Yes	256 (75.5)	83 (24.5)	
Neonatal complications	No	241 (74.8)	81 (25.2)	0.522
	Yes	48 (78.6)	13 (21.4)	
Family history of asthma	No	256 (75.9)	81 (24.1)	0.532
	Yes	33 (71.7)	13 (28.3)	
History of smoking in the household	No	220 (73.8)	78 (26.2)	0.165
	Yes	69 (81.1)	16 (18.9)	
Kitchen type	Indoor with partition	202 (74.2)	70 (25.8)	0.484
	Indoor without partition	85 (78.7)	23 (21.3)	
	Open air	2 (66.6)	1 (33.4)	
Cooking fuel	Electric	2 (100)	0 (0)	0.695
	Kerosene	6 (66.6)	3 (33.4)	
	Liquefied petroleum gas	241 (74.8)	81 (25.2)	
	Wood/dung	40 (80.0)	10 (20.0)	
PICU admission	No	245 (75.2)	81 (24.8)	0.741
	Yes	44 (77.2)	13 (22.8)	

RSV: Respiratory syncytial virus; PICU: Pediatric intensive care unit.

Table 3 Risk factors among respiratory viral infection

Variable		No virus detected (*n* = 253)	Respiratory viral positive (*n* = 130)	*P* value
Birth weight (≥ 2500 g)	No	48	22	0.623
	Yes	205	108	
Breast fed exclusively for ≥ 3 mo	No	198	92	0.106
	Yes	55	38	
Gestational age (≥ 37 wk)	No	30	14	0.752
	Yes	223	116	
Neonatal complications	No	214	111	0.836
	Yes	39	19	
Family history of asthma	No	214	114	0.751
	Yes	27	16	
History of smoking in the household	No	195	106	0.315
	Yes	58	24	
Kitchen type	Indoor with partition	174	99	0.313
	Indoor without partition	77	30	
	Open air	2	1	
PICU admission	No	214	112	0.683
	Yes	39	18	

RSV: Respiratory syncytial virus; PICU: Pediatric intensive care unit.

Table 4 Comparison of outcome between respiratory syncytial virus positive and respiratory syncytial virus negative

Days of	RSV		*P* value
	Positive	Negative	
Hospital stay	4.0 (1.0-17.0)	4.0 (1.0-65.0)	0.031
Oxygen use	1.5 (1.0-9.0)	2.0 (1.0-65.0)	0.176
Antibiotic use	4.0 (1.0-12.0)	4.0 (1.0-29.0)	0.303
Nebulization use	3.0 (1.0-17.0)	4.0 (1.0-65.0)	0.012
PICU stay	2.5 (1.0-10.0)	2.00 (1.0-20.0)	0.547

RSV: Respiratory syncytial virus; PICU: Pediatric intensive care unit.

Table 5 Association of signs and symptoms with respiratory syncytial virus infection

Variable		RSV positive(*n* = 94)	RSV negative(*n* = 289)	*P* value
Fever	Yes	63 (23.8)	201 (76.2)	0.645
	No	31 (26.0)	88 (74.0)	
Rhinorrhea	Yes	81 (24.6)	248 (75.4)	0.931
	No	13 (24.0)	41 (76.0)	
Cough	Yes	85 (23.5)	276 (76.5)	0.066
	No	9 (40.9)	13 (59.1)	
Fast breathing	Yes	62 (24.5)	191 (75.5)	0.981
	No	32 (24.6)	98 (75.4)	
Wheezing	Yes	45 (22.9)	151 (77.1)	0.461
	No	49 (26.2)	138 (73.8)	
Auscultatory crepitations	Yes	73 (25.7)	210 (76.3)	0.338
	No	21 (21.0)	79 (79.0)	
Chest retractions	Yes	52 (25.6)	151 (74.4)	0.604
	No	42 (23.3)	138 (76.6)	

RSV: Respiratory syncytial virus; PICU: Pediatric intensive care unit.

ARTICLE HIGHLIGHTS

Research background

Respiratory syncytial virus (RSV) is the most frequent agent of viral-associated acute lower respiratory diseases (ALRI) and is known to be associated with hospitalization and mortality among high-risk cases.

Research motivation

Early diagnosis of viral infections using a simple test such as the RSV and viral fluorescent antibody assay (DFA) test, in settings where PCR is not feasible, would be useful in the timely institution of appropriate supportive care, minimization of antibiotic overuse, and appropriate follow-up care for complications and sequelae, potentially leading to a reduction of costs of medical care.

Research objectives

The principal objective of the study was to investigate the proportion of RSV and non-RSV respiratory viral infections as a cause of ALRI among 1-59 mo old children admitted to a tertiary care hospital in India. The study also assesses the seasonality, clinical features, risk factors and outcome of RSV and non-RSV respiratory viral infections among these hospitalized children.

Research methods

The prospective study was conducted on hospitalized children aged < 5 years, with a diagnosis of acute lower respiratory infections (ALRI), admitted between August 2011-August 2013, were included. Nasopharyngeal (NP) swabs were obtained from eligible children, and transported to the laboratory in suitable media. Slides were prepared from the media, and DFA staining was performed using SimulFluor Respiratory Screen kit on NP wash samples.

Research results

The median age of subjects was 8 mo (inter quartile range 5-15 mo), and 89.0% were less than 2 years of age. Viral etiology (RSV, influenza A or B, adenovirus, para influenza 1, 2 or 3) was confirmed in 33.9% (130/383) of children hospitalized for ALRI. RSV was positive in 24.5% (94/383) non-RSV viruses in 14.8% (57/383) while co-infection with RSV and non-RSV viruses was seen in 5.5% (21/383) children. A peak of RSV positive cases was seen after the rainy season during the months of August through November. The RSV infection was significantly associated with being exclusively breastfed for less than 3 mo. There was no significant association between RSV infection and independent variables such as low birth weight, prematurity, complicated neonatal course, family history of asthma, household smoking or indoor wood fuel usage. Mean hospital stay was 4.6 ± 5.1 d and 8.3 ± 6.5 d in RSV-positive and RSV-negative children, respectively. The respiratory complications such as acute respiratory distress syndrome and respiratory failure requiring PICU admission, were 13.8% (13/94), 8.7% (5/57) and 16.6% (42/253) of children with RSV positive, non-RSV positive and viral negative results.

Research conclusions

A high proportion of RSV and other virus-associated ALRI were seen among hospitalized children in India. The study demonstrates that exclusive breastfeeding for over 3 mo of age, may have a protective effect against RSV and other respiratory viral infections. The viral DFA test was easy to perform and results were available within 4-5 h.

Research perspectives

Early diagnosis of viral infections using a simple test such as the RSV and would be useful in the timely institution of appropriate supportive care, minimization of antibiotic overuse, and appropriate follow-up care for complications and sequelae, potentially leading to a reduction of costs of medical care.

ACKNOWLEDGEMENTS

We thank all medical staff and faculty members from the Department of Paediatrics and Microbiology at St. John's Medical College Hospital for extending their support for this study.

REFERENCES

1 **Liu L**, Oza S, Hogan D, Chu Y, Perin J, Zhu J, Lawn JE, Cousens S, Mathers C, Black RE. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet* 2016; **388**: 3027-3035 [PMID: 27839855 DOI: 10.1016/S0140-6736(16)31593-8]

2 **Oumei H**, Xuefeng W, Jianping L, Kunling S, Rong M, Zhenze C, Li D, Huimin Y, Lining W, Zhaolan L, Xinmin L, Hua X, Zhiyan J, Yanning L, Yan H, Baoqing Z, Xiaochun F, Chunhui H, Yonghong J, Xue Z, Wei W, Zi W. Etiology of community-acquired pneumonia in 1500 hospitalized children. *J Med Virol* 2018; **90**: 421-428 [PMID: 28975629 DOI: 10.1002/jmv.24963]

3 **Brini I**, Guerrero A, Hannachi N, Bouguila J, Orth-Höller D, Bouhlel A, Boughamoura L, Hetzer B, Borena W, Schiela B, Von Laer D, Boukadida J, Stoiber H. Epidemiology and clinical profile of pathogens responsible for the hospitalization of children in Sousse area, Tunisia. *PLoS One* 2017; **12**: e0188325 [PMID: 29149199 DOI: 10.1371/journal.pone.0188325]

4 **Shi T**, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, Gessner BD, Polack FP, Balsells E, Acacio S, Aguayo C, Alassani I, Ali A, Antonio M, Awasthi S, Awori JO, Azziz-Baumgartner E, Baggett HC, Baillie VL, Balmaseda A, Barahona A, Basnet S, Bassat Q, Basualdo W, Bigogo G, Bont L, Breiman RF, Brooks WA, Broor S, Bruce N, Bruden D, Buchy P, Campbell S, Carosone-Link P, Chadha M, Chipeta J, Chou M, Clara W, Cohen C, de Cuellar E, Dang DA, Dash-Yandag B, Deloria-Knoll M, Dherani M, Eap T, Ebruke BE, Echavarria M, de Freitas Lázaro Emediato CC, Fasce RA, Feikin DR, Feng L, Gentile A, Gordon A, Goswami D, Goyet S, Groome M, Halasa N, Hirve S, Homaira N, Howie SRC, Jara J, Jroundi I, Kartasasmita CB, Khuri-Bulos N, Kotloff KL, Krishnan A, Libster R, Lopez O, Lucero MG, Lucion F, Lupisan SP, Marcone DN, McCracken JP, Mejia M, Moisi JC, Montgomery JM, Moore DP, Moraleda C, Moyes J, Munywoki P, Mutyara K, Nicol MP, Nokes DJ, Nymadawa P, da Costa Oliveira MT, Oshitani H, Pandey N, Paranhos-Baccalà G, Phillips LN, Picot VS, Rahman M, Rakoto-Andrianarivelo M, Rasmussen ZA, Rath BA, Robinson A, Romero C, Russomando G, Salimi V, Sawatwong P, Scheltema N, Schweiger B, Scott JAG, Seidenberg P, Shen K, Singleton R, Sotomayor V, Strand TA, Sutanto A, Sylla M, Tapia MD, Thamthitiwat S, Thomas ED, Tokarz R, Turner C, Venter M, Waicharoen S, Wang J, Watthanaworawit W, Yoshida LM, Yu H, Zar HJ, Campbell H, Nair H; RSV Global Epidemiology Network. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet* 2017; **390**: 946-958 [PMID: 28689664 DOI: 10.1016/S0140-6736(17)30938-8]

5 **Nair H**, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, O'Brien KL, Roca A, Wright PF, Bruce N, Chandran A, Theodoratou E, Sutanto A, Sedyaningsih ER, Ngama M, Munywoki PK, Kartasasmita C, Simões EA, Rudan I, Weber MW, Campbell H. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* 2010; **375**: 1545-1555 [PMID: 20399493 DOI: 10.1016/S0140-6736(10)60206-1]

6 **Saha S**, Pandey BG, Choudekar A, Krishnan A, Gerber SI, Rai SK, Singh P, Chadha M, Lal RB, Broor S. Evaluation of case definitions for estimation of respiratory syncytial virus associated hospitalizations among children in a rural community of northern India. *J Glob Health* 2015; **5**: 010419 [PMID: 26649172 DOI: 10.7189/jogh.05.020419]

7 **Resch B**. Burden of respiratory syncytial virus infection in young children. *World J Clin Pediatr* 2012; **1**:

8-12 [PMID: 25254161 DOI: 10.5409/wjcp.v1.i3.8]
8 **Shi T**, Balsells E, Wastnedge E, Singleton R, Rasmussen ZA, Zar HJ, Rath BA, Madhi SA, Campbell S, Vaccari LC, Bulkow LR, Thomas ED, Barnett W, Hoppe C, Campbell H, Nair H. Risk factors for respiratory syncytial virus associated with acute lower respiratory infection in children under five years: Systematic review and meta-analysis. *J Glob Health* 2015; **5**: 020416 [PMID: 26682048 DOI: 10.7189/jogh.05.020416]
9 **Midgley CM**, Haynes AK, Baumgardner JL, Chommanard C, Demas SW, Prill MM, Abedi GR, Curns AT, Watson JT, Gerber SI. Determining the Seasonality of Respiratory Syncytial Virus in the United States: The Impact of Increased Molecular Testing. *J Infect Dis* 2017; **216**: 345-355 [PMID: 28859428 DOI: 10.1093/infdis/jix275]
10 **Shafik CF**, Mohareb EW, Youssef FG. Comparison of direct fluorescence assay and real-time rt-PCR as diagnostics for respiratory syncytial virus in young children. *J Trop Med* 2011; **2011**: 781919 [PMID: 22220181 DOI: 10.1155/2011/781919]
11 **Tang YW**, Crowe Jr JC. Respiratory syncytial virus and human metapneumovirus. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (ed.). Manual of clinical microbiology, vol. 2. 9th ed. Washington, DC: American Society for Microbiology Press 2007; 1361-1377
12 **Kalal BS**, Puranik P, Nagaraj S, Rego S, Shet A. Scrub typhus and spotted fever among hospitalised children in South India: Clinical profile and serological epidemiology. *Indian J Med Microbiol* 2016; **34**: 293-298 [PMID: 27514949 DOI: 10.4103/0255-0857.188315]
13 **World Health Organization (WHO)**. Department of child and adolescent health and development. (CAH). Integrated management of childhood illness (IMCI) Technical seminar acute respiratory infections. 2016; Available from: URL: http://www.who.int/maternal_child_adolescent/documents/pdfs/cah_01_10_ts_ari.pdf
14 **Gupta S**, Shamsundar R, Shet A, Chawan R, Srinivasa H. Prevalence of respiratory syncytial virus infection among hospitalized children presenting with acute lower respiratory tract infections. *Indian J Pediatr* 2011; **78**: 1495-1497 [PMID: 21660398 DOI: 10.1007/s12098-011-0491-0]
15 **Pinzón-Rondón ÁM**, Aguilera-Otalvaro P, Zárate-Ardila C, Hoyos-Martínez A. Acute respiratory infection in children from developing nations: a multi-level study. *Paediatr Int Child Health* 2016; **36**: 84-90 [PMID: 25936959 DOI: 10.1179/2046905515Y.0000000021]
16 **Krishnan A**, Amarchand R, Gupta V, Lafond KE, Suliankatchi RA, Saha S, Rai S, Misra P, Purakayastha DR, Wahi A, Sreenivas V, Kapil A, Dawood F, Pandav CS, Broor S, Kapoor SK, Lal R, Widdowson MA. Epidemiology of acute respiratory infections in children - preliminary results of a cohort in a rural north Indian community. *BMC Infect Dis* 2015; **15**: 462 [PMID: 26502931 DOI: 10.1186/s12879-015-1188-1]
17 **Bharaj P**, Sullender WM, Kabra SK, Mani K, Cherian J, Tyagi V, Chahar HS, Kaushik S, Dar L, Broor S. Respiratory viral infections detected by multiplex PCR among pediatric patients with lower respiratory tract infections seen at an urban hospital in Delhi from 2005 to 2007. *Virol J* 2009; **6**: 89 [PMID: 19558656 DOI: 10.1186/1743-422X-6-89]
18 **Singh AK**, Jain A, Jain B, Singh KP, Dangi T, Mohan M, Dwivedi M, Kumar R, Kushwaha RA, Singh JV, Mishra AC, Chhaddha MS. Viral aetiology of acute lower respiratory tract illness in hospitalised paediatric patients of a tertiary hospital: one year prospective study. *Indian J Med Microbiol* 2014; **32**: 13-18 [PMID: 24399381 DOI: 10.4103/0255-0857.124288]
19 **Saxena S**, Singh D, Zia A, Umrao J, Srivastava N, Pandey A, Singh S, Bhattacharya P, Kumari R, Kushwaha R, Dhole TN. Clinical characterization of influenza A and human respiratory syncytial virus among patients with influenza like illness. *J Med Virol* 2017; **89**: 49-54 [PMID: 27329816 DOI: 10.1002/jmv.24607]
20 **Yeolekar LR**, Damle RG, Kamat AN, Khude MR, Simha V, Pandit AN. Respiratory viruses in acute respiratory tract infections in Western India. *Indian J Pediatr* 2008; **75**: 341-345 [PMID: 18536887 DOI: 10.1007/s12098-008-0035-4]
21 **Fall A**, Dia N, Cisse el HA, Kiori DE, Sarr FD, Sy S, Goudiaby D, Richard V, Niang MN. Epidemiology and Molecular Characterization of Human Respiratory Syncytial Virus in Senegal after Four Consecutive Years of Surveillance, 2012-2015. *PLoS One* 2016; **11**: e0157163 [PMID: 27315120 DOI: 10.1371/journal.pone.0157163]
22 **Mishra P**, Nayak L, Das RR, Dwibedi B, Singh A. Viral Agents Causing Acute Respiratory Infections in Children under Five: A Study from Eastern India. *Int J Pediatr* 2016; **2016**: 7235482 [PMID: 28018433 DOI: 10.1155/2016/7235482]
23 **Simões EA**, DeVincenzo JP, Boeckh M, Bont L, Crowe JE, Griffiths P, Hayden FG, Hodinka RL, Smyth RL, Spencer K, Thirstrup S, Walsh EE, Whitley RJ. Challenges and opportunities in developing respiratory syncytial virus therapeutics. *J Infect Dis* 2015; **211** Suppl 1: S1-S20 [PMID: 25713060 DOI: 10.1093/infdis/jiu828]
24 **Popow-Kraupp T**, Aberle JH. Diagnosis of respiratory syncytial virus infection. *Open Microbiol J* 2011; **5**: 128-134 [PMID: 22262985 DOI: 10.2174/1874285801105010128]
25 **Anestad G**. Surveillance of respiratory viral infections by rapid immunofluorescence diagnosis, with emphasis on virus interference. *Epidemiol Infect* 1987; **99**: 523-531 [PMID: 2824225 DOI: 10.1017/s0950268800068023]
26 **Farley R**, Spurling GK, Eriksson L, Del Mar CB. Antibiotics for bronchiolitis in children under two years of age. *Cochrane Database Syst Rev* 2014; CD005189 [PMID: 25300167 DOI: 10.1002/14651858.CD005189.pub4]
27 **Vereen S**, Gebretsadik T, Hartert TV, Minton P, Woodward K, Liu Z, Carroll KN. Association between breast-feeding and severity of acute viral respiratory tract infection. *Pediatr Infect Dis J* 2014; **33**: 986-988 [PMID: 24751863 DOI: 10.1097/INF.0000000000000364]
28 **Celedón JC**, Litonjua AA, Ryan L, Platts-Mills T, Weiss ST, Gold DR. Exposure to cat allergen, maternal history of asthma, and wheezing in first 5 years of life. *Lancet* 2002; **360**: 781-782 [PMID: 12241839 DOI: 10.1016/S0140-6736(02)09906-3]
29 **Stein RT**, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, Wright AL, Martinez FD. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999; **354**: 541-545 [PMID: 10470697 DOI: 10.1016/S0140-6736(98)10321-5]
30 **El Kholy AA**, Mostafa NA, El-Sherbini SA, Ali AA, Ismail RI, Magdy RI, Hamdy MS, Soliman MS. Morbidity and outcome of severe respiratory syncytial virus infection. *Pediatr Int* 2013; **55**: 283-288 [PMID: 23316763 DOI: 10.1111/ped.12051]

Published By Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk:https://www.f6publishing.com/helpdesk
https://www.wjgnet.com