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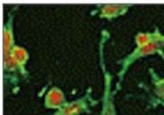
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Neonates need tailored drug formulations

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Abstract

Drugs are very strong tools used to improve outcome in neonates. Despite this fact and in contrast to tailored perfusion equipment, incubators or ventilators for neonates, we still commonly use drug formulations initially developed for adults. We would like to make the point that drug formulations given to neonates need to be tailored for this age group. Besides the obvious need to search for active compounds that take the pathophysiology of the newborn into account, this includes the dosage and formulation. The dosage or concentration should facilitate the administration of low amounts and be flexible since clearance is lower in neonates with additional extensive between-individual variability. Formulations need to be tailored for dosage variability in the low ranges and also to the clinical characteristics of neonates. A specific focus of interest during neonatal drug development therefore is a need to quantify and limit excipient exposure based on the available knowledge of their safety or toxicity. Until such tailored vials and formulations become available, compounding practices for drug formulations in neonates should be evaluated to guarantee the correct dosing, product stability and safety.

INTRODUCTION

Extensive variability is the essence of neonatal clinical pharmacology. This mainly is due to both weight related differences and maturational changes, while non-maturational covariates also further contribute to this within and in between variability, including disease severity, comorbidity or enzyme polymorphisms^[1-4]. Neonates admitted to neonatal intensive care have a weight between below 500 g and up to 5000 g, already resulting in at least one log value of variability in weight between patients. The impact of maturational changes on drug absorption, distribution, metabolism and excretion (ADME, pharmacokinetics) relate to changes in body composition (*e.g.*, body water and fat content, protein binding characteristics), organ weight and also function (*e.g.*, renal maturation, hepatic maturation)^[1-4]. Since these processes do not mature linearly or simultaneously, standardized dosing (*e.g.*, mg/kg) is inadequate in neonates. In addition to these anticipated developmental changes in early infancy, there are other, non-maturational contributors (*e.g.*, co-morbidities like renal failure or hepatic failure, co-medication with interactions) to this extensive between individual variability in drug dosing^[1-4]. The clinical translation of this extensive variability in drug dosing needed in neonates is the obvious need for tailored drug formulations in neonates.

Obviously, "tailoring for neonates" does not mean that the general basic concepts of drug formulation should be neglected, such as including valid data on product stability, palatability and compatibility^[5-10]. Neonates, and also children, are still commonly treated with medicines that have not been designed, developed or

evaluated in the relevant pediatric age groups. As a consequence, this approach puts them at risk of unpredictable or suboptimal (too low, too high or too variable) dosing and side effects from potentially toxic ingredients, including excipients^[5-10].

This need for dosing variability is reflected in the use of extemporaneous formulations or drug manipulations. Both professional and non-professional caregivers are forced to split and divide adult formulations and mix them with food or a liquid in order to deliver an appropriate dose for an individual child. For intravenous formulations with “high” concentrations, this may mean that consecutive dilutions are needed^[6]. All these manipulations introduce additional dosing inaccuracies. Sometimes, “extemporaneous” formulations will be provided by a pharmacist based on a medical prescription for an individual patient. Although this likely results in somewhat improved reproducibility, this is still a long way from having fully tested formulations ready for use. Moreover, practices and guidelines for extemporaneous formulations differ among different pharmacists or regions, introducing the risk of additional uncertainties or errors^[5,6,11]. The need for validation of commonly applied compounding practices has been recently described, based on the evaluation of different paediatric oral formulations with a low proportion of hydrochlorothiazide, assumed to be suitable for use in neonates. Santoveña *et al*^[12] observed that following the evaluation of 5 suspensions of hydrochlorothiazide (2 mg/mL) at present applied by pharmacists, only one guaranteed the correct administered dose and stability after 3 wk of storage at 5 °C and light protection.

To a certain extent, formulation science aims to catch up with the legislative environment for formulations and pediatric pharmacological evaluation. European legislation and similar legal initiatives in other parts of the world made companies develop pediatric formulations for new compounds coming on to the market that could potentially be used in children as part of the drug registration process. Similarly, regulatory agencies became aware that guidelines on issues, like excipients or sub-population specific, preferred formulations having to undergo revision because of newly emerging information, conflicting opinions or unfeasible requests^[13,14].

The need for an appropriate balance between dose, volume, drug manipulations and dose flexibility in neonates calls for dedicated, tailored formulations. We will first discuss issues related to dosage forms for neonates. A second focus of interest is excipients, *i.e.*, the solvents and additives, needed as co-solvents, surfactants, preservatives, colorants and/or sweeteners that are part of the formulation. During formulation development, there is an obvious need to quantify and limit excipient exposure based on the currently available knowledge on their safety or toxicity. Until such tailored formulations become available, compounding practices for drug formulations should be evaluated to guarantee correct dosing, product stability and safety.

DOSAGE FORMS TAILORED FOR USE IN NEONATES

A formulation allows an active pharmaceutical ingredient to be combined with other ingredients in a dosage form according to standardized practices, with the aim to result in predictable and safe exposure. When applied to age-appropriate dosage forms for neonates, commonly administered formulations are intravenous formulations and oral liquid (*e.g.*, drops, suspension or syrup) formulations^[5,13,15]. The rectal route is only rarely used because of variability in bio-availability.

Intravenous formulations

During intravenous administration, volume overload should be avoided. However, administering very low volumes may also result in additional dose inaccuracy. These conflicting issues related to concentration need a balanced approach since serial dilutions in order to achieve the required dose should be avoided if all possible. It has repeatedly been documented that serial dilutions are prone to errors, while such errors can be avoided by providing appropriate concentrations based on a population specific dedicated formulation. Serial dilution also results in additional dose inaccuracy. The impact of a “pediatric vial” on dose inaccuracy has been quantified in neonates^[16,17]. Using population pharmacokinetics in a cohort of 254 preterm neonates, the unexplained variability in amikacin clearance in neonates is in part related to the vial used. A pediatric vial (50 mg/mL, 2 mL) resulted in a relevant reduction (8%) in unexplained variability when compared to an adult vial (250 mg/mL, 2 mL)^[17].

Nunn *et al*^[16] reported on the clinical practice to manipulate medicines to provide accurate doses, including in neonates. Over a 5-d period, 5375 drug administration events were recorded in neonatal and pediatric patients in one regional children’s hospital. Despite this specific regional children’s hospital setting, 10% of the prescriptions were judged to require manipulation or needed a small volume (< 0.2 mL). Measured doses below 0.1 mL (oral or intravenous) accounted for 25% of the manipulations, most commonly (60%) in the neonatal intensive care unit^[16]. To further illustrate the practice and the need for sequential dilutions, reference doses (mg or mg/kg) in preterm and a term neonate (1.5 and 3 kg) were compared to intravenous formulations available on the Belgian market (Table 1).

Formulations suited for the enteral route

Enteral administration can be achieved by different types of formulations. Because of the specific characteristics of neonates (*e.g.*, inability to swallow solid unit dosage formulations) and the need for dose flexibility, oral liquid formulations (*e.g.*, syrup, drops, suspension) are preferred in neonates and young infants^[5,13,15]. Specific aspects of relevance in (pre)term neonates that remain commonly underexplored are the potential interactions with (human) milk and issues related to the use of feeding tubes (*e.g.*,

Table 1 Reference doses (mg/kg) compared to intravenous formulations to illustrate the need for sequential dilutions in neonates

Active agent	Available concentration	Reference doses	Preterm, 1.5 kg	Term, 3 kg
Amikacin, adult vial	500 mg/2 mL	15-20 mg/kg	130 mg, 0.12 mL	50 mg, 0.2 mL
Amikacin, pediatric vial	100 mg/2 mL	15-20 mg/kg	30 mg, 0.6 mL	50 mg, 1.0 mL
Enoxaparin	40 mg/0.4 mL	1 mg/kg	11.5 mg, 0.015 mL	13 mg, 0.03 mL
Erythromycin	1000 mg/20 mL	5-10 mg/kg	12 mg, 0.24 mL	25 mg, 0.5 mL
Fentanyl ¹	100 µg/2 mL	1-3 µg/kg	13 µg, 0.06 mL	16 µg, 0.12 mL
Insulin	300 U/3 mL	0.1-1 U/kg per hour	10.3 U, 0.03 mL	10.6 U, 0.06 mL
Midazolam	15 mg/3 mL	0.1 mg/kg	10.15 mg, 0.03 mL	10.3 mg, 0.06 mL
Paracetamol	500 mg/50 mL	10 mg/kg	15 mg, 1.5 mL	30 mg, 3 mL
Phenobarbital	200 mg/1 mL	5 mg/kg	17.5 mg, 0.0375 mL	115 mg, 0.075 mL
Propofol	200 mg/20 mL	1-3 mg/kg	2 mg, 0.2 mL	4.5 mg, 0.45 mL
Ranitidine	50 mg/2 mL	0.5-1 mg/kg	11.5 mg, 0.06 mL	13 mg, 0.12 mL

Formulations were sorted alphabetically and reported as available in Belgium, not necessary reflecting the setting in another country. A dose in a 1.5 and 3 kg newborn has been used for illustrative purposes. ¹Initial volumes ≤ 0.2 mL.

particle size, viscosity, volume, osmolarity, compatibility with the plastic of the feeding tube)^[5,13,15].

EXCIPIENTS IN NEONATAL FORMULATIONS: NEVER PRESCRIBED, COMMONLY ADMINISTERED

Excipients are commonly added to a drug formulation, *e.g.*, to ensure stability over a given shelf life, to improve palatability or to facilitate solubility or to bulk up formulations that otherwise contain highly potent active ingredients, and are referred to as preservatives, sweeteners, fillers and solvents, coating materials or coloring agents^[18-20]. Examples of excipients are lactose, aspartame, ethanol, propylene glycol, benzyl alcohol, sorbitol, xylitol, mannitol and poly-ethylene glycol. Some of these excipients cause specific harms in specific, rare diseases. Examples include lactose in the setting of lactase-deficiency, aspartame in patients suffering from phenylketonuria or fructose containing formulations in the setting of fructose intolerance. More recently, the concept of “functionality” has been introduced by adding excipients to enhance product performance^[18-20]. Illustrations of such a “functionality” approach or relevance in early neonatal life are liposomal amphotericin, to reduce exposure of renal tubular cell and the subsequent toxicity, or the use of an oil-in-water emulsion as an adjuvant to improve the efficacy of influenza vaccines in infants.

Although medicines are formulated with excipients that are Generally Regarded As Safe (“GRAS” status), such a “GRAS” status does not consider the population specific aspects and neither are such claims based on well-validated prospective studies in neonates. History provides us with different case observations on the deleterious effects of excipient exposure in neonates. Excipients can be harmful to neonates, since benzyl alcohol, propylene glycol and polysorbate 80 co-administration resulted in different toxicological syndromes in neonates^[21-24].

Fatal benzyl alcohol related poisoning has been described following co-administration of this compound as a

bacteriostatic with normal saline in preterm neonates^[21,22]. Following at least a minimal exposure to 130 mg/kg per day of benzyl alcohol, neonates developed metabolic acidosis and a raised anion gap from the second day of exposure onwards. This was followed by progressive bradycardia, gasping and clinical seizures^[21]. Similarly, toxicity to propylene glycol has also been reported following exposure of up to 3000 mg/d for at least 5 consecutive days^[22,23]. Such a significant exposure was due to high concentrations of propylene glycol as a co-solvent in parenteral nutrition solutions. The toxicity was both biochemical (*e.g.*, hyperosmolarity, lactic acidosis, plasma creatinine, bilirubin) and clinical (seizures). Finally, E-ferol containing high concentrations of vitamin E and high concentrations of Polysorbate 80 resulted in another clinical syndrome and was reported shortly after its introduction^[24].

Unfortunately, the side effects of excipients still do not receive sufficient consideration in contemporary neonatal pharmaceutical care and are not just historical events. To illustrate this, the United States Food and Drug Administration notified healthcare professionals in March 2011 of serious health problems that had been reported in premature babies treated with Kaletra (lopinavir/ritonavir) oral solution. This oral solution contains relevant amounts of ethanol and propylene glycol and a link was made between these excipients and the toxicity observed^[23]. Moreover, recent observations on contemporary exposure to potential toxic excipients (*e.g.*, propylene glycol, ethanol, benzyl alcohol) have confirmed the almost uniform exposure to such excipients in the United Kingdom and Estonian cohorts of neonates admitted in neonatal intensive care units^[25,26]. In our opinion, collaborative research projects on excipients are urgently needed and some initiatives are already ongoing. In addition to improving knowledge on the clinical pharmacology of active compounds, there is a similar need to optimize the knowledge on clinical pharmacology of excipients in neonates^[14]. Illustrations of such initiatives are the Safety and Toxicity of Excipients for Pediatrics (STEP) database and the European Study of Neonatal Excipient Exposure (ESNEE) research initiative^[27,28].

The STEP database aims to improve the availability and access to published information on excipients, including information on excipient toxicity and tolerance in neonates^[27]. The ESNEE research initiative aims to develop a platform for the systematic assessment of excipients in neonates^[28]. The first step of this program is to establish which excipients are in use and how much of each excipient is included in medicines given to neonates. The second step of the ESNEE program is to determine what is known about the effects of excipients in neonates and juvenile animals. The third step of the program is to measure systemic concentrations of key excipients in neonates using dry blood spots and plasma samples. The final step is to integrate the work into a systematic assessment of safety for each excipient. A generic framework for the assessment of excipient safety in neonates will be developed, with the aim to illustrate how this can be applied by prescribers, pharmacists, manufacturers and regulators. Based on the Leuven propylene glycol research project, we recently illustrated that such studies are indeed feasible and of clinical relevance^[23].

NEONATES ARE IN NEED OF TAILORED DRUG DEVELOPMENT

Although the principles of drug disposition also apply in neonates, their specific characteristics warrant focussed assessment. As a consequence, tailored drug development for neonates and clinical research should therefore focus on both new and already existing compounds. Adequate prescription involves assurance that the drug administered is of sufficient pharmaceutical quality, that an appropriate formulation is used, and that there is sufficient knowledge on pharmacokinetics/dynamics and safety of compounds administered.

We aimed to stress that tailored, personalized clinical pharmacology for neonates also needs to consider to neonatal formulations^[5,10,11,13]. We paid particular attention to excipients with different case series on toxicity^[21-24]. Further progress can be made in collaborative efforts between industry, caregivers, academia and regulatory agencies^[27,28]. These efforts need to focus on product availability (tailored formulations), integration and dissemination of currently available information about existing age-appropriate formulations, an evidence-based approach to risk assessment of excipients, and the validation of procedures and practices on compounding with dissemination of validated procedures^[6,15,18].

A roadmap to further improve the current setting includes: (1) a more appropriate balance between dose, volume and drug manipulations; (2) the quantification and limitation of excipient exposure; (3) focussed studies on the clinical pharmacology of excipients in neonates; and (4) the validation of compounding practices for drug formulations in neonates.

We should be aware that drugs are very strong tools used to improve outcome in neonates. In contrast to tai-

lored perfusion equipment, incubators or ventilators for neonates, we still commonly use drug formulations initially developed for adults. At the least, there is still a lot of potential for further product improvement in neonatal drug development and formulation related issues should be part of such a product improvement approach.

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REVIEW

- 6 Indoor air pollution and respiratory health of children in the developing world
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Indoor air pollution and respiratory health of children in the developing world

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Abstract

Indoor air pollution (IAP) is a key contributor to the global burden of disease mainly in developing countries. The use of solid fuel for cooking and heating is the main source of IAP in developing countries, accounting for an estimated 3.5 million deaths and 4.5% of Disability-Adjusted Life Years in 2010. Other sources of IAP include indoor smoking, infiltration of pollutants from outdoor sources and substances emitted from an array of human utilities and biological materials. Children are among the most vulnerable groups for adverse effects of IAP. The respiratory system is a primary target of air pollutants resulting in a wide range of acute and chronic effects. The spectrum of respiratory adverse effects ranges from mild subclinical changes and mild symptoms to life threatening conditions and even death. However, IAP is a modifiable risk factor having potential mitigating interventions. Possible interventions range from simple

behavior change to structural changes and from shifting of unclean cooking fuel to clean cooking fuel. Shifting from use of solid fuel to clean fuel invariably reduces household air pollution in developing countries, but such a change is challenging. This review aims to summarize the available information on IAP exposure during childhood and its effects on respiratory health in developing countries. It specifically discusses the common sources of IAP, susceptibility of children to air pollution, mechanisms of action, common respiratory conditions, preventive and mitigating strategies.

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Key words: Indoor air pollution; Air pollution; Respiratory health; Children; Developing countries

Core tip: Indoor air pollution (IAP) is a key contributor to the burden of disease in developing countries; use of solid fuel for cooking and heating is the main source of IAP. Children are among the most vulnerable groups for adverse effects of IAP. The respiratory system is a primary target of air pollutants resulting in a wide range of acute and chronic effects. The spectrum of respiratory adverse effects ranges from mild subclinical changes and mild symptoms to life threatening conditions, and even death. This review summarizes the available information on IAP exposure during childhood and its effects on respiratory health in developing countries.

Nandasena S, Wickremasinghe AR, Sathiakumar N. Indoor air pollution and respiratory health of children in the developing world. *World J Clin Pediatr* 2013; 2(2): 6-15 Available from: URL: <http://www.wjgnet.com/2219-2808/full/v2/i2/6.htm> DOI: <http://dx.doi.org/10.5409/wjcp.v2.i2.6>

INTRODUCTION

Indoor air pollution (IAP) is a key contributor to the

global burden of disease^[1,2]. There is convincing evidence of the adverse effects of IAP on human health, children being one of the most vulnerable groups^[3-5]. The respiratory system is a primary target for air pollutants resulting in a wide range of acute and chronic effects^[6].

Several reasons are attributed to children's high susceptibility to the harmful effects of air pollution; the growth of airways and alveoli of the respiratory system are guided through a complex chemical pathway and air pollutants are known to interfere with these pathways^[4]. The airway epithelium of growing children is more permeable to air pollutants and the lung defence system is not adequately evolved. Children have a differential ability to metabolize, detoxify, and excrete environmental agents thereby making them prone to more harm^[7]. A higher resting metabolic rate of oxygen consumption per unit body weight in children due to the larger surface area per unit body weight and rapid growth, as compared to adults, makes them more vulnerable. Further, children engage in more physical activity than adults which leads to a higher intake of air relative to body size^[4,8].

This article is an overview of the major sources of IAP exposure in households and associated respiratory health effects in children in developing countries; we do not discuss specific pollutants and their associated effects *per se*.

SOURCES AND CONSTITUENTS OF INDOOR AIR POLLUTION

The sources, pollutant types and extent of IAP are a result of complex interactions between structures, building systems, source strength, removal and deposition rates, indoor mixing and chemical reactions, furnishings, the outdoor environment and source strength, and practices and behaviours of the inhabitants^[9-12]. Thus, variations are seen at different levels (*i.e.*, developed *vs* developing countries; between developing countries; between cities/regions of a country; and between households).

Solid fuel

Fuels used for cooking and heating purposes may be solid or non-solid. Solid fuel can be biomass or coal; biomass fuel includes wood (unprocessed and charcoal), dung or crop residues^[13]. The types of solid fuels used vary by country. For example, coal is commonly used in China^[14], but not in Sri Lanka^[15]. Solid fuels emit a complex mixture of pollutants with more than 200 chemicals and compound groups^[13]. The composition differs by type of solid fuel. Common pollutants include inorganic gases (*e.g.*, CO, NO₂, O₃ *etc.*), particulate matter (PM), hydrocarbons (*e.g.*, polycyclic aromatic, monoaromatic such as benzene *etc.*), oxygenated organic compounds (*e.g.*, aldehydes, phenols *etc.*), chlorinated organic compounds (*e.g.*, methylene chloride *etc.*) and free radicals^[16,17].

Solid fuel use for cooking and heating is the main source of IAP in developing countries^[17]. PM less than 2.5 µm in diameter (PM_{2.5}) is one of the most hazardous pol-

lutants emitted by solid fuels; its concentration increases to milligrams per cubic meter inside kitchens during cooking^[18-20] well above the World Health Organization (WHO) guidelines of 25 µg/m³ (24 h average)^[21].

The use of solid fuel varies across WHO regions: 77%, 74%, 74% and 36% of households use solid fuels in Sub-Saharan Africa, in South-East Asia, in the Western Pacific Region and in the Eastern Mediterranean Region, respectively, as compared to 16% of households in Latin America, Caribbean, and Central and Eastern Europe^[22].

Secondhand smoke

Indoor tobacco smoking is another important source of IAP which exposes non-smokers to tobacco smoke. Secondhand smoke (SHS) comprises a mixture of mainstream smoke (smoke first inhaled by an active smoker and then exhaled) and side-stream smoke (smoke emitted between puffs). Side-stream smoke accounts for about 85% of total SHS, the rest comprising mainstream smoke^[23]. SHS is a complex mixture of more than 4000 chemicals, of which, more than 40 are identified carcinogens in vapor and particle phases^[24]. The vapor-phase compounds include benzene, vinyl chloride, acrolein *etc.* The particulate-phase chemicals include alkaloids, nicotine and its derivatives, aromatic amines, polycyclic aromatic hydrocarbons, *etc.*^[25]. SHS can be more carcinogenic than mainstream smoke inhaled by active smokers^[23].

Outdoor sources

With rapid urbanization and industrialization, ambient air pollutant concentrations in many cities in developing countries far exceed current WHO air quality guidelines^[26]. Globally, 32% of the population live in areas exceeding WHO Level 1 interim threshold of 35 µg/m³ largely encompassing cities in South and East Asia^[27]. Most primary pollutants typically have steep decreasing gradients with distance from roads. In general, the highest exposures are found within the first 50-100 m from roadways, and exposures often fall to background levels by 300-500 m^[28,29]. Interconnected high traffic roads in an area would have a high background pollution level. Pollutants with high outdoor concentrations infiltrate indoors. Infiltration and trapping of pollutants vary depending on the local topography and the configuration of buildings^[29]. A study in urban and rural areas of Bangladesh found that mean concentrations of CO, CO₂, dust particles, and major volatile organic compounds (VOC) were significantly higher in urban biomass fuel using kitchens as compared to rural counterparts^[30]. In Sri Lanka, indoor NO₂, SO₂ and PM_{2.5} levels were high in living rooms of houses in a traffic congested urban area as compared to living rooms of houses using clean fuels in a semi-urban area^[31].

Other sources

A spectrum of biological pollutants is released from dust mites, molds, fungi, bacteria, pests (cockroaches, mice, rats) and also from byproducts of men and pets.

These pollutants may release microbial products such as endotoxins, microbial fragments, peptidoglycans and various types of allergens^[10,32,33]. Several common products used in households may also release pollutants. Such products include personal care products, household products such as finishes, rug and oven cleaners, paints and lacquers, paint strippers, pesticides, mosquito repellants, dry-cleaning fluids, building materials, and home furnishings *etc.* Kerosene is considered as a cleaner alternative for solid fuels, biomass and coal^[34], though it still is low in the energy ladder^[35]. Kerosene use for cooking and lighting remains widespread in developing countries^[34] especially in urban settings where biomass is not freely accessible^[15]. It has been shown that the kerosene stoves and devices emit substantial amounts of PM_{2.5}, CO, NO₂ and SO₂^[34]. Uranium-bearing soil releases radon which may aggregate in poorly ventilated or closed indoor air environments^[36,37]. Very fine asbestos fibers may be released into the air when asbestos containing material such as roof sheets, insulation for heating systems *etc.* are used.

RESPIRATORY HEALTH

Mechanisms

Air pollutants act on one or more host defence mechanisms against pathogens in the respiratory tract. Many pollutants act together in a cascade of partly interrelated biological mechanisms. The biological mechanisms include triggering oxidative stress, both local and systemic inflammation, reduction of the mucociliary clearance, increased reactivity of the respiratory epithelia, reduction of the macrophage responses to microorganisms, increased epithelial permeability and adhesion to microorganisms, bronchial irritation *etc.*^[38-41]. These mechanisms differ by type of pollutant and extent of exposure^[17,42]. PM₁₀ emitted from a mixture of fossil and biomass fuels have been reported to enhance the capacity of pneumococci to adhere to human lower airway cells *in vitro*^[43]. Several animal studies provide evidence of biological mechanisms of adverse respiratory health effects of air pollution. Hsu *et al.*^[44] reported that guinea pigs produce broncho-constriction when they were exposed to wood smoke, and the response increased with subsequent exposures. Rats intermittently exposed to wood smoke for 75 min daily for 15 d showed signs of mononuclear bronchiolitis and mild emphysema, while more severe conditions were reported when the exposure was extended to 30 d and 45 d^[45]. Exposure of mice to cigarette smoke and subsequent infection with *Streptococcus pneumoniae*, increased numbers of bacteria in lung tissue and more severe clinical signs of sepsis were reported as compared to unexposed mice^[46]. Instillation of fossil-fuel-derived PM into the lower airways of mice impaired phagocytosis and clearance of *Streptococcus pneumoniae*^[47]. Harrod *et al.*^[48] exposed the mice to diesel engine emissions and subsequently infected them with *Pseudomonas aeruginosa*. The number of both ciliated and non-ciliated airway epithelial cells was reduced during the infection in

a concentration-dependent manner with increased lung pathogenesis. Although some of these studies were not based on pollutants originating from indoor sources, it is reasonable to believe that a similar pathology exists for IAP sources as well.

Burden of disease

IAP in developing countries is disproportionately high as compared to developed countries due to differences in sources of IAP, the main difference being the use of solid fuels for cooking and heating purposes by the majority of the population in developing countries. In fact, 76% of all global PM pollution occurs indoors in the developing world^[49]. IAP from combustion of solid fuels for cooking and space heating is one of the ten most important contributors to the global burden of disease; it is estimated to have resulted in 3.5 million deaths and 4.5% of Disability-Adjusted Life Years (DALYs) in 2010^[2]. It is estimated that about 3 billion of the world's population and up to 90% of rural households in developing countries use solid fuel as the main source of energy for cooking and heating^[1,50].

Almost 152 million new episodes of "clinical pneumonia" are reported from developing countries while only 4 million episodes are reported from developed countries. In this estimation, "clinical pneumonia" included episodes of pneumonia, bronchiolitis and reactive air way diseases associated with respiratory tract infections^[51]. The South-East Asia Region reports the highest number of episodes of clinical pneumonia (0.36 episodes per child-year). More than half of the new pneumonia episodes are reported from India (43 million), China (21 million) and Pakistan (10 million) and 6 million each from Bangladesh, Indonesia and Nigeria^[51,52].

SHS is a common source of IAP in both the developed and the developing world^[53]. Children are exposed more to SHS than other age-groups as they are unable to avoid the exposure specifically when their close relatives are smoking at home. About 40% of children are exposed to SHS worldwide and an estimated 165000 children under 5 years die each year from lower respiratory infections caused by exposure to SHS. Two-thirds of these deaths occur in developing countries of Africa and South Asia^[54]. The largest contribution to DALYs due to SHS exposure is from children^[54].

Epidemiological evidence

Acute respiratory infections: Acute respiratory infections (ARI) can be classified into acute upper respiratory infections (AURI) and acute lower respiratory infections (ALRI) depending on the area of the respiratory tract that is affected. The upper respiratory tract consists of the airways from the nostrils to the vocal cords in the larynx, including the paranasal sinuses and the middle ear. The lower respiratory tract includes the continuation of the airways from the trachea and bronchi to the bronchioles and the alveoli^[55]. AURIs are usually mild in nature and often caused by viruses, though some cases of sinusitis and otitis media may be caused by virulent bacteria.

The majority of ARI deaths and severe illness episodes are due to ALRIs, consisting mainly of pneumonia^[55]. ARIs do not confine themselves to the respiratory tract and may have systemic effects due to the possible extension of infection or microbial toxins to other organs, inflammation, and reduced lung function^[55]. Risk factors for ALRIs include malnutrition, low birth weight (≤ 2500 g), non-exclusive breast feeding (during the first 4 mo of life), non-immunized for measles within the first 12 mo of life, IAP and crowding^[55].

A large number of epidemiological studies have demonstrated the association between ARI and IAP, though the estimations of associations vary in magnitude and consistency across individual studies^[56,57] and meta-analyses^[58-60]. This heterogeneity may be due to differences in the definition of ARIs, duration of exposure measurements, relative distribution of varying household characteristics including ventilation, stove types, kitchen amenities, country and/or climate, age group studied and relative extent of exposure to other sources of IAP such as indoor smoking *etc*^[61].

A recent meta-analysis of eight studies found that children were more than three times likely to have ARIs when exposed to solid biomass fuel smoke as compared to non-exposed children (OR = 3.52; 95%CI: 1.94-6.43)^[59]. Based on 24 studies, the overall pooled odds ratio was 1.78 (95%CI: 1.45-2.18) for pneumonia among the under-five children exposed to IAP due to solid fuel use as compared to non-exposed children of the same age group^[60]. Based on eight studies conducted prior to year 2000, the estimated risk of IAP due to solid biomass fuel use for ALRI of under-five children was 2.3 (95%CI: 1.9-2.7) as compared to non-exposed children^[62]. Another recent review based on sixteen studies reported significantly elevated odds ratios ranging from 1.38-6.00 for ALRI due to exposure to IAP^[58]. The strongest association of exposure to IAP and respiratory health is found in the youngest age groups^[60]. This finding is consistent with the fact that younger the child the more susceptible they are to air pollutants due to their physical characteristics as well as their likelihood to stay indoors and stay with their mothers during cooking. Preschool children spend more time in the kitchens as compared to their school going elder siblings^[63]. As children grow, they become more independent. School children spend a considerable time away from the house and in outdoors during peak concentration levels of air pollutants indoors. In rural China, younger children living in households that use solid fuel for cooking and heating purposes had higher 24-hour average exposures of PM_{2.5} levels; 5-8 year old, 9-11 year old and 12-14 year old children had exposures of 70 $\mu\text{g}/\text{m}^3$ (95%CI: 60-80 $\mu\text{g}/\text{m}^3$), 46 $\mu\text{g}/\text{m}^3$ (95%CI: 40-49 $\mu\text{g}/\text{m}^3$) and 40 $\mu\text{g}/\text{m}^3$ (95%CI: 37-40 $\mu\text{g}/\text{m}^3$), respectively^[64]. Among 1397 school children (aged 7-14 years), no definitive associations were observed between wheezing and wood/coal use (OR = 1.05; 95%CI: 0.27-4.05) or kerosene use (OR = 0.57; 95%CI: 0.1 -2.12) as compared to children living in households that use gas for cooking^[65].

Parental smoking has a significant effect on respiratory health of children as they may smoke at home and exposure to SHS is inevitable in many scenarios^[66]. In Nepal, it has been shown that respiratory health effects among children were high (adjusted OR = 1.41; 95%CI: 1.02-1.96) when mothers smoked^[67]. Based on findings of 361021 rural and urban families in Indonesia, paternal smoking was associated with increased infant mortality (rural: OR = 1.30; 95%CI: 1.24-1.35. urban: OR = 1.10; 95%CI: 1.01-1.20), and under-5 child mortality (rural: OR = 1.32; 95%CI: 1.26-1.37. urban: OR = 1.14; 95%CI: 1.05 -1.23)^[68].

Studies on middle ear infections (otitis media) due to solid fuel exposure in developing countries are limited. Although otitis media is rarely fatal, it may lead to complications including deafness and mastoiditis^[17]. Exposure to SHS is strongly associated with the occurrence of middle ear infections in children. A recent meta-analysis based on 61 epidemiological studies reported that maternal postnatal smoking was associated with a 1.62 (95%CI: 1.33-1.97) risk and living with a smoker was associated with a 1.37 (95%CI: 1.25-1.50) risk of middle ear infections in children. Maternal postnatal smoking and paternal smoking were associated with a 1.86 (95%CI: 1.31-2.63) and 1.83 (95%CI: 1.61-2.07) risk of surgery for middle ear infection, respectively, as compared to not having a smoker in the household^[69].

Although the evidence from the developing countries is scarce, European studies show that chemicals released from other sources (*i.e.*, indoor sources other than cooking fuel and smoking) are associated with respiratory health of children. Redecoration of apartments with paints *etc.* had a significant influence on the occurrence of obstructive bronchitis in the first (OR = 4.1; 95%CI: 1.4-11.9) and in the second year of life (OR = 4.2; 95%CI: 1.4-12.9) among children living in apartments in Germany^[70].

Asthma: Studies on solid fuel use and asthma have revealed contradictory findings^[17]. Use of wood fuel was associated with an increased risk for asthma among adolescents in rural Belgium (OR = 2.2; 95%CI: 1.1-4.4)^[71]. The pooled OR of four studies on asthma among children exposed to biomass fuels was 0.50 (95%CI: 0.12-1.98)^[59]. However, wheezing, a clinical expression of asthma^[72], was associated with exposure to solid fuel smoke in children. Wheezing is a clinical symptom of lower respiratory tract infections, pneumonia, *etc*^[72]. A study conducted in two settings with different outdoor air pollution levels in Sri Lanka reported that indoor cooking with unclean fuels was a risk factor for wheezing among children 7-10 years of age (adjusted OR = 2.02; 95%CI: 1.13-3.59) independent of the area of residence (adjusted OR = 1.57; 95%CI: 1.01-2.46)^[73].

A recent meta-analysis estimated that the asthma is 1.33 (95%CI: 1.14-1.56) times higher among children exposed to SHS and that the duration of exposure is an important factor in the induction of asthma. The same study showed that "ever having asthma" is 1.48 (95%CI:

1.32-1.65) times higher among children exposed to SHS as compared to children not exposed to SHS^[74].

Exposure to molds increases the susceptibility to asthma^[75]. Although evidence from developing countries is limited, many epidemiological studies in developed countries suggest that asthma is associated with residential dampness and molds. The pooled OR of mold exposure and asthma in studies conducted in Russia, North America and 10 countries in Eastern and Western Europe was 1.35 (95%CI: 1.20-1.51)^[76]. In Taiwan, the presence of visible mold (adjusted OR = 1.76; 95%CI: 1.18-2.62) was shown to be an independent determinant of incident asthma^[77]. Residential concentrations of propylene glycol and glycol ethers, a class of VOC, were associated with a 1.5 fold greater likelihood of asthma (95%CI: 1.0-2.3) in Sweden^[78]. However, evidence of the association between asthma and microbial agents is inconclusive^[79].

Lung functions: Factors that affect development of lung function in children are potentially important in determining the lung functions of adults^[80]. Lung function is a strong predictor of mortality in adults. A review of data from China has shown that reductions in children's forced expiratory volume in the first second (FEV1), forced vital capacity (FVC) and peak flow are associated with domestic coal use^[81]. In Ecuador, children living in homes that use biomass fuel and children exposed to environmental tobacco smoke had lower FVC and lower FEV1 ($P < 0.05$)^[82]. Among 200 school children in north India, FVC and FEV1 were lowest in boys whose households used biomass fuel ($P < 0.05$). The same study reported that all parameters were lower, though not statistically significant, in passive smokers irrespective of the type of fuel used^[83]. In China, FEV1 and FVC growth of children living in households that used coal as fuel was 16.5 mL/year ($P < 0.001$) and 20.5 mL/year ($P < 0.001$), respectively, lower than in children living in households that used clean fuels^[84]. In a prospective study of Chinese school children ($n = 1718$, aged 10.05 ± 0.86 years), lower growth rates in forced expiratory flow (FEF_{25%-75%}) ($P = 0.020$) during the 18 mo of follow up were reported among children exposed to SHS (> 5 cigarettes/d)^[85].

Other respiratory conditions: SHS exposure during childhood is associated with increased lung cancer risk among never smoking adults (OR = 2.25; 95%CI: 1.04-4.90)^[86]. A considerable number of studies has reported that coal and biomass are associated with hypopharyngeal, laryngeal and lung cancers in later life with long time exposure^[10,87-90]. These studies suggest that these cancers in later life may be attributable to exposures in young ages.

FUTURE DIRECTIONS IN PREVENTION

It is evident that IAP is a major risk factor of respiratory ill health in children in developing countries. Multiple synergistic strategies are required to overcome the

problem. Unclean cooking fuels, the major source of IAP, become cleaner, and more convenient, efficient and costly as people move up the "energy ladder" from animal dung, the lowest in the ladder to electricity at the top of the ladder^[35]. People generally move up the ladder as socio-economic conditions improve^[17]. Poverty is a major constraint to move up the energy ladder as clean fuels are more costly^[91,92]. Poverty is associated with poor housing conditions resulting in poor ventilation, inability to partition the kitchen from other microenvironments of houses, and lack of kitchen amenities such as chimneys that increases the exposure to IAP. Poverty also affects other factors such as nutrition which increase the susceptibility of children to ARIs^[92]. There may be other factors involved in shifting towards clean cooking fuels such as their relative availability, cultural practices and attitudes^[15,93]. As a consequence, shifting from unclean to clean cooking fuels in many developing countries is slow and is likely to continue for many more decades^[14].

Potential interventions to reduce IAP may target sources of pollution, improvements in living environments, and change of behaviors of persons who cook and households members^[94]. Introduction of tailor made cook stoves is the intervention of choice to control IAP when shifting of fuel type to cleaner ones is slow. Many limited scale cook stove interventions have been successfully implemented in developing countries and many innovations are on the way^[95-98]. However, penetration of the concept to a larger proportion of the population is a major challenge^[99]. Much commitment is needed to design and distribute improved cook stoves with greater community acceptance, high energy efficiency and marked reduction of emissions to needy communities. A formal evaluation of successful improved stove programs will provide opportunities to understand the reasons for success^[100]; this should be an important component of ongoing and future cook stove interventions. Behavior change activities to reduce the IAP can be focused on four main areas, namely (1) improving ventilation in the kitchen during cooking (e.g., opening of windows and doors during the cooking operations); (2) keeping children away from fires; (3) improving stove maintenance; and (4) reducing the duration of burn^[101,102]. Interventions should attempt to address all possible activities as a package. Further, the change of behavior and practices of communities is an essential element for the sustainability of improved cook stove interventions; audience targeted communication packages should be intertwined in such programs. Essentially, the "threat" of IAP to children's health should be emphasized among traditional stove users through appropriate media and language to target the most rural communities. Improving awareness of adverse health effects of IAP among health administrators, physicians and primary health care workers would complement prevention approaches.

The public health burden of tobacco use has shifted from the developed to the developing world; the tobacco industry is using innovative marketing strategies in the

developing world^[103]. Legislature has been enacted in many countries including some developing countries banning smoking in public places, thus reducing exposure to SHS^[104]. However, these laws are not practically important at household level, perhaps where the most important source of exposure of children to SHS exists^[66]. Thus, extensive awareness campaigns targeting change of indoor smoking habits in households is important, despite the fact that such change of habits is difficult to achieve. Studies have shown that as a country's income level increases, cigarettes become more engineered with reductions in emission levels^[103]. Cigarette design and their adverse effects may be more harmful resulting in a higher disease burden in many developing countries^[103]. This concept needs to be explored and understood at country level to implement standards for cigarettes. In addition, the implementation of the recommended smoking prevention strategies of WHO will certainly reduce exposure to SHS^[53]. Studies on health effects of IAP sources other than from solid fuel and SHS are scarce in developing countries. This may reflect a lack of expertise and capacity to conduct scientifically sound epidemiological studies in such countries. However, "modernization" of households and household practices has introduced a spectrum of hitherto unknown chemical products, the adverse effects of which are yet to be elucidated. Hence, it is important to quantify the epidemiological risk associated with these substances as an initial step in designing mitigating strategies.

CONCLUSION

IAP disproportionately affects children's respiratory health due to their physiological susceptibility and spending more time indoors. Exposure of children in developing countries to IAP has contributed significantly to the global burden of disease. IAP is a modifiable risk factor having known interventions to mitigate its effects. Other than solid fuel and SHS exposure, pollutants from other sources, yet to be explored, may play an important role in impacting on the respiratory health of children in developing countries.

Successful prevention strategies need robust information pertaining to the problem generated from diverse settings. Future research is needed in several areas. Some key areas include quantification of different air pollutants, robust estimation of associations between indoor pollutants and adverse respiratory health effects, genetic susceptibility to indoor pollutants and their carcinogenic effects, impact on lung growth and development, characteristics and assessment of successful IAP reduction interventions (*e.g.*, cook stove interventions), cultural practices and behaviors that lead to a reduction or an increase in IAP and its exposure.

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Potential for treatment of severe autism in tuberous sclerosis complex

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sis complex (TSC)-everolimus and vigabatrin. However, these treatments have not been systematically studied in individuals with TSC and severe autism. The aim of this review is to identify the clinical features of severe autism in TSC, applicable preclinical models, and potential barriers that may warrant strategic planning in the design phase of clinical trial development. A comprehensive search strategy was formed and searched across PubMed, Embase and SCOPUS from their inception to 2/21/12, 3/16/12, and 3/12/12 respectively. After the final search date, relevant, updated articles were selected from PubMed abstracts generated electronically and emailed daily from PubMed. The references of selected articles were searched, and relevant articles were selected. A search of clinicaltrials.gov was completed using the search term "TSC" and "tuberous sclerosis complex". Autism has been reported in as many as 60% of individuals with TSC; however, review of the literature revealed few data to support clear classification of the severity of autism in TSC. Variability was identified in the diagnostic approach, assessment of cognition, and functional outcome among the reviewed studies and case reports. Objective outcome measures were not used in many early studies; however, diffusion tensor imaging of white matter, neurophysiologic variability in infantile spasms, and cortical tuber subcategories were examined in recent studies and may be useful for objective classification of TSC in future studies. Mechanism-based treatments for TSC are currently available. However, this literature review revealed two potential barriers to successful design and implementation of clinical trials in individuals with severe autism-an unclear definition of the population and lack of validated outcome measures. Recent studies of objective outcome measures in TSC and further study of applicable preclinical models present an opportunity to overcome these barriers.

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Key words: Autism; Self-injury; Aggression; Tuberous

Abstract

The Food and Drug Administration (FDA) has approved two mechanism-based treatments for tuberous sclero-

sclerosis complex; Intellectual disability

Core tip: Children with severe behaviors and cognitive impairment may benefit from newly available mechanism-based treatments; however, several factors warrant consideration in clinical trial design and implementation.

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INTRODUCTION

Tuberous sclerosis complex (TSC) is a leading cause of syndromic autism characterized by multi-system hamartomas. Clinically, the diagnosis is classified as definite, probable, or possible TSC based on the presence of a specific combination of major and minor features (Table 1)^[1] or genetically by mutations in TSC1 or TSC2. Although autism has been reported in as many as 61% of individuals with TSC^[2,3], severity is not frequently reported. Existing neurologic comorbidities include epilepsy in 60%-90%^[4-6], intellectual disability in 45%^[7], self-injury in 10%^[8], and severe aggression in 13%^[9]. The contribution of these neurologic comorbidities or the features of TSC to the severity of autism in this population is unknown.

The neurobiology underlying this condition has been established. TSC1 and TSC2 encode hamartin and tuberlin respectively, which indirectly inhibit mammalian target of rapamycin (mTOR). This enzyme is an essential component of two complexes, mTORC1 and mTORC2, which have distinct, wide-ranging effects on gene transcription, protein translation and cell proliferation. Excessive mTOR activity results from a mutation in either TSC1 or TSC2. Disrupted synaptic plasticity, characterized by excessive glutamate activity, may occur through downstream effects of this excessive mTOR activity on ribosomal s6 and EIF4E. Targeted drug development based on this neurobiology has resulted in FDA approval of the mTOR inhibitor, everolimus, and the irreversible inhibitor of GABA transaminase, vigabatrin, for individuals with TSC. While everolimus targets the underlying mechanism of TSC and may have wide-ranging effects, vigabatrin, in addition to anti-seizure effects, may also have an impact on glutamatergic mechanisms important in brain development, synaptic plasticity and learning.

TSC is considered a cause of syndromic autism; however, causality and determinants of severity are unknown. Additionally, the risk/benefit profile of approved treatments in this population has not been tested in a clinical trial. The aim of this review is to identify the clinical features of severe autism in TSC, applicable preclinical models, and potential barriers that may warrant strategic planning in the design phase of clinical trial development.

In order to identify key research, a comprehensive search strategy was formed and then searched across PubMed, Embase and SCOPUS from their inception to 2/21/12, 3/16/12, and 3/12/12 respectively. Database-specific controlled vocabulary terms were combined with keyword terms and phrases for each concept. These terms and phrases were then combined and translated for use in each database. The results were limited to studies conducted on humans and to those written in English. References from key papers were also reviewed and key studies, including preclinical studies were included.

AUTISM

Autism is a syndrome clinically defined by the presence of stereotyped, repetitive behavior and impairments in language and social interaction with onset prior to the age of 3^[10]. TSC, the first identified cause of autism, is now considered a leading cause of syndromic autism^[2,3,11,12]. The prevalence of autism in TSC is 26%-61% with an average prevalence of 32%^[2,3,9,13-23]. Variability in operational definitions of autism and study design, in particular the approach to individuals with intellectual disability, may contribute to variation in these estimates. We did not discover any studies of the severity of autism in TSC. However, self-injury was observed in 10% of a large clinical population. Self-injury, one of the main parental concerns in TSC, may signal a severe form of autism in this subgroup^[24].

Operational definitions of autism in TSC

The Diagnostic Statistical Manual and the International Classification of Disease have provided checklist criteria for defining autism. The study with the highest reported prevalence of autism and 3 additional studies used these checklists^[3,10,19-21,25]. Other instruments have been developed to assist professionals in identifying individuals with autism, and combined use of the Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview (ADI, ADI-R) has been widely accepted as the gold standard. However, the original sample used in developing these instruments did not include individuals with profound intellectual disability^[26,27]. The Social Responsiveness Scale (SRS) and the Social Communication Questionnaire (SCQ) are screening instruments for autism; however, the presence of intellectual disability decreases the specificity of SCQ from 80% to 67%^[28,29]. The Hunt and Dennis Questionnaire is a screening instrument designed for use in individuals with TSC, behavioral difficulties, and profound intellectual disability. Autism is evaluated by 13 out of 321 questions; however, psychometric properties have not been reported to date^[9].

Prevalence study designs

The diagnostic approach used in each of the 19 reviewed studies is indicated in Table 2. Multiple sequenced evaluations were used in 3 studies. The highest reported preva-

Table 1 Criteria for clinical diagnosis of tuberous sclerosis complex

Major features	Minor features
Cortical tubers	Dental enamel pits
Subependymal nodules	Hamartomatous rectal polyps
Subependymal giant cell astrocytoma	Bone cysts
Hypomelanotic macules (3 or more)	Cerebral white matter radial migration lines
Shagreen patch	Gingival fibromas
Facial angiofibromas or forehead plaque	Nonrenal hamartoma
Multiple renal nodular hamartomas	Retinal achromatic patches
Nontraumatic ungual or periungual fibromas	"Confetti" skin lesions
Cardiac rhabdomyoma, 1 or >	Multiple renal cysts
Pulmonary	
Lymphangiomyomatosis and/or renal angiomyolipomas	

Tuberous sclerosis complex (TSC) can be clinically diagnosed as definite, probable, or possible based on the presence or absence of a specific combination of major and minor features. Definite TSC: two major features or one major and two minor features; Probable TSC: one major and one minor feature; Possible TSC: one major feature or two or more minor features.

lence of autism in TSC was determined using 3 structured interviews, Childhood Autism Rating Scale (CARS), Autistic Behavior Checklist, and review of DSM III-R criteria^[3]. In addition to variation in diagnostic approach, inclusion of children with severe or profound intellectual disability varied among these studies and may contribute to variability in prevalence estimates.

Risk factors

Potential risk factors and clinical associations of autism in TSC include epilepsy, structural or neurophysiological abnormalities, and/or joint effects of these categories, but causality has not been established. Intellectual disability and autism were not uniformly characterized among the studies, making it difficult to compare studies focused on risk factors associated with autism or ASD to those focused on risk factors associated with cognitive impairment. Among studies using standard measures to assess cognition, the choice of measure was often based on the assessed functional level or age of the individual. However, this approach was not routinely applied to assessments for autism or ASD. Therefore, we included studies of risk factors for autism and/or intellectual disability in TSC.

Epilepsy

Epilepsy affects 60%-90% of individuals with TSC^[2,6,30-38]. Age of epilepsy onset, seizure type and/or severity have been evaluated as potential risk factors for autism and/or intellectual disability in children with TSC. Early age of seizure onset^[2,33,34,36-40] and infantile spasms were most commonly identified as risk factors^[2,14,17,34,34-42,41-46].

Epilepsy intractability and/or severity was character-

ized using measures such as development of multiple seizure types after infantile spasms, time to epilepsy control, duration of seizure control, and/or EEG features and was reported as a potential risk factor in 6 of 8 studies that characterized severity^[14,37,38,41,46,47].

Neuropathology

Early studies focused solely on lesion location^[34,39,40,43,45,48] as a potential risk factor for autism in TSC. Temporal lobe location was linked to poor neurodevelopmental outcomes, including severe language impairment, autism and intellectual disability in these studies; however, this approach did not fully explain the neurodevelopmental phenotype of TSC. Newer studies, illuminating the neurobiology of classic brain lesions in TSC through detailed structural and functional analysis, have expanded knowledge regarding the potential impact of these lesions on the neurodevelopmental phenotype in TSC^[21,49-51].

Different approaches to analysis of brain lesions in TSC have identified features associated with autism. Cortical tuber and/or white matter lesion load has been identified as a potential risk factor for poor neurodevelopmental outcomes^[14,33,36,42,50]. Cortical lesions seem to worsen the neurological phenotype. The mechanism by which this occurs is unclear, but may be associated with epilepsy and/or circuit disruption or reorganization. T1, T2 and FLAIR imaging have been used to identify specific characteristics of white matter that are associated with autism^[51]. White matter abnormalities have also been detected using diffusion tensor imaging fractional anisotropy (FA), a measure of white matter integrity. Lower FA values indicate loss of the typically restricted diffusion found in normal white matter, and are thought to represent deficits in white matter. Autism spectrum disorder in 12 individuals with TSC was associated with lower average FA when compared to 28 individuals with TSC without autism spectrum disorder and 29 age-matched controls^[52]. A subsequent study compared the FA of the arcuate fasciculus, which interconnects language areas in the temporal and frontal lobes, among individuals with TSC, with or without autism, to typical controls. FA in this key pathway for language was lowest in those individuals with TSC and autism^[53].

Functional analysis has been carried out using EEG, PET, and diffusion tensor imaging (DTI). Across all of these studies, temporal lobe abnormalities were most commonly identified as a risk factor for poor neurodevelopmental outcome. In a retrospective study of 19 individuals, temporal lobe epileptiform activity and seizure onset within the first 36 mo of life were independently associated with autism and PDD^[39]. Interictal temporal lobe spikes increased the likelihood of autism spectrum disorder by a factor of 15 in another group^[20]. Poor neurodevelopmental outcome was also associated with a subtype of hypsarrhythmia^[47]. PET studies revealed reduced glucose metabolism (a measure of neuronal activity) in temporal lobe of individuals with autism and TSC, but increased glucose metabolism in deep cerebellar nuclei

Table 2 Prevalence studies for autism in tuberous sclerosis complex

Instrument	Administration method(time)	Children with severe or profound ID included (Y/N)	Measured prevalence of autism in TSC (N)	Ref.
ADOS	Observation schedule (20-30 min)	N	29% (28) +ADI 15-33% (4 age-based groups of 12-15)	[18]
ADI	Structured Interview (2-3 h)	Y-severe	54% (13) 20% (20)	[15,16]
SRS	65-item Screening Questionnaire (15-20 min)	N (37 w/IQ data)	52% (21)	[22]
SCQ	40-item Screening Questionnaire (15-20 min)	Y-severe	43% (21)	[22]
Hunt and Dennis Questionnaire	321 item interview/13-item subset for autism (1-4 h)	Y-estimates only	50% (90) 26% (23) 24% (21) 5% (131)	[2,9,14,23]
DSM III-R	Checklist (10-20 min)	Y-severe	61% (28)	[3]

These are reviewed studies reporting epidemiology of autism in tuberous sclerosis complex (TSC). ADI: Autism diagnostic interview; DSM: Diagnostic and statistical manual of mental disorders; ADOS: Autism diagnostic observation schedule; SRS: Social responsiveness scale; SCQ: Social communication questionnaire; Y: Yes; N: No.

that was associated with intellectual disability, stereotypical behavior, communication impairments, and impaired social interaction^[43]. Subsequent PET studies examined tubers in cerebellar cortex and found decreased glucose metabolism associated with symptoms of autism^[54]. Output from cerebellar cortex normally inhibits the deep cerebellar nuclei, and decreased activity in cerebellar cortex would be expected to result in increased activity in the deep cerebellar nuclei, as reported in the earlier study. The effect of tuber location within the cerebellum has also been examined, and children with right cerebellar tubers showed increased social isolation and deficits in communication and development, compared to children with left cerebellar tubers^[54]. The output of the right cerebellum influences activity in the left cerebral cortex, where language areas are located in most individuals, and taken together these findings support a role for cerebellar deficits in TSC-associated autism.

Joint effects: neuropathology and epilepsy

Almost half of the reviewed studies simultaneously investigated features of both epilepsy and neuropathology as potential risk factors. In the majority of those studies, autism was associated with joint effects of these two risk factors^[33,34,36,39,40,42,43,45,47,48]. Thus, multiple factors should be considered in a risk assessment for autism in individuals with TSC. Development of a standard, generalizable approach to assessing these complex risk factors would be informative for clinical trial design in this population.

NEUROBIOLOGY OF TSC RELATED TO DEVELOPMENTAL DISABILITIES

An excellent and comprehensive review recently outlined effects of TSC proteins and the mTOR pathway in the nervous system^[55]. A working knowledge of basic neuroscience research as it pertains to TSC is critical in considering therapeutic targets for humans. Highlighted here are discoveries most relevant to patients with severe

manifestations of TSC: central nervous system effects of TSC, animal models with phenotypes that mimic particular manifestations of TSC, and effects of rapamycin in these models. Autism, epilepsy and cognitive impairment have all been modeled preclinically, and treatment with inhibitors of mammalian target of rapamycin, such as rapamycin and everolimus, has demonstrated potentially beneficial effects in some models (Table 3).

TSC is an autosomal dominant condition that occurs when there is a mutation in either TSC1, which is located on chromosome 9q34 and encodes the protein hamartin, or TSC2, which is located on chromosome 16 and encodes the protein tuberlin. Normally, TSC1 (hamartin) and TSC2 (tuberlin) form a complex in which TSC1 stabilizes TSC2 by blocking its ubiquitination and degradation. The TSC1/TSC2 complex inactivates Rheb, which otherwise stimulates mammalian target of rapamycin (mTOR) activity. Mutations in TSC1/TSC2 lead to increased mTOR activity and dysregulation of gene transcription, metabolism, and cell proliferation. Although most cells in individuals with TSC have a single germline mutation in TSC1 or TSC2, recent studies discovered a second somatic mutation limited to giant cells within cortical tubers or to subependymal giant cell astrocytomas (SEGAs). These cells with mutations in both alleles of TSC1 or TSC2 were characterized by hyperactivation of mTOR^[56,57]. These studies suggest that a two-hit mechanism may underlie the formation of cortical tubers or SEGAs, but the contribution of this mechanism to development of autism/ASD remains unclear.

Animal models have been developed to investigate the effects of heterozygous mutation of TSC1 or TSC2, or of conditional deletion of one or both alleles in different populations of neurons or in astrocytes. The characteristics and major findings obtained with selected models are summarized in Table 3.

Heterozygous Tsc1 and Tsc2 models

Mouse lines that are *Tsc1*^{+/-} or *Tsc2*^{+/-} and a rat strain with a spontaneous mutation in *Tsc2* (Eker rat) have been

Table 3 Selected preclinical models of tuberous sclerosis complex

Model	Genetic manipulation	Behavioral effects	Seizures	Autism	Synaptic plasticity	Neuropathology	Rapamycin	Ref.
<i>Tsc1</i> ^{-/-} mice	Heterozygous <i>Tsc1</i> deletion, exons 6-8	↓Hippocampal dependent learning	None	↓Social interaction	-	None	-	[58]
<i>Tsc2</i> ^{+/-} Eker rat	Spontaneous autosomal dominant (Heterozygous)	-	-	-	↓LTP, ↓LTD, ↑PPF	-	-	[61]
<i>Tsc2</i> ^{+/-} Eker rat	Spontaneous autosomal dominant (Heterozygous)	No learning and memory deficits	No spontaneous	↓Social interaction, ↓↓ after seizure induction	-	-	-	[59]
<i>Tsc2</i> ^{+/-}	Heterozygous disruption in second exon	↓Hippocampal dependent learning	-	-	↓Threshold for L-LTP	-	Reversed all	[93]
<i>Tsc2</i> ^{+/-} or WT pups from <i>Tsc2</i> ^{+/-} or WT dams	Heterozygous <i>Tsc2</i> deletion	↑Maternal care by <i>Tsc2</i> ^{+/-} dams	-	↑Vocalization in WT and <i>Tsc2</i> ^{+/-} pups of <i>Tsc2</i> ^{+/-} dams	-	-	-	[60]
<i>Tsc2</i> ^{+/-}	Heterozygous <i>Tsc2</i> deletion	-	-	-	↓mGluR-LTD, ↓Arc synthesis	-	Reverses deficits in protein-synthesis-dependent mGluR-LTD	[62]
<i>Tsc1</i> ^{fl/fl} Nestin- <i>rtTA</i> (+) <i>TetO</i> - <i>cre</i> (+)	Mosaic homozygous <i>Tsc1</i> deletion in cortical neural progenitors	-	Yes ⁺	-	-	Heterotopias with enlarged, pS6+ neurons	↑Survival, ↓seizures, ↓neuropath [94]	[94]
<i>Tsc1</i> ^{fl/fl} - <i>Syn1</i> -Cre	Homozygous <i>Tsc1</i> deletion in neurons from mid-gestation; (heterozygous in all other cells)	-	Yes ⁺	-	-	White matter nodules	↑Survival, ↑myelination, ↓body weight, ↓neurologic impairment [95,96]	[95,96]
<i>Tsc1</i> ^{fl/fl} Emx1-Cre	Homozygous <i>Tsc1</i> deletion in early embryonic neural progenitors	-	Yes ⁺	-	-	↓Cortical organization	↓Seizures, ↑survival, ↓glial abnl, ↑weight, ↓brain size [97]	[97]
<i>Tsc1</i> ^{fl/fl} Nestin-Cre	Homozygous <i>Tsc1</i> deletion in differentiating neurons	-	-	-	↑EPSCs ↑AMPA	↑Cell size ↑Spine width ↑Spine length ↓Spine density	↓Spine width ↑spine length [98]	[98]
<i>Tsc1</i> ^{fl/fl} <i>Syn1</i> -Cre	Homozygous <i>Tsc1</i> deletion in neurons	-	No spontaneous Epileptiform discharges	-	↑EPSCs ↑AMPA	No gross abnormalities	-	[99]
<i>Tsc1</i> ^{fl/fl} GFAP-Cre	Homozygous <i>Tsc1</i> inactivation in glial-fibrillary acidic protein (GFAP)+ cells	-	Yes ⁺ , ↓Glt1	-	-	↑Astrocytes ↑Brain size ↓Hippocampal organization	↑Glt1, ↑survival ↓neuropath Early: prevented epilepsy, Late: decreased seizure frequency [100]	[100]
<i>Tsc2</i> ^{fl/fl} GFAP-Cre	Homozygous <i>Tsc2</i> inactivation in GFAP+ cells	-	Yes ⁺⁺ , ↓Glt1	-	-	↑Astrocytes ↑Brain size ↓Hippocampal organization	↑Survival, ↓seizures, ↓neuropath [102]	[102]
<i>Tsc1</i> ^{fl/+} <i>L7-Cre</i> , or <i>Tsc1</i> ^{fl/fl} <i>L7-Cre</i>	Heterozygous or homozygous <i>Tsc1</i> deletion limited to cerebellar Purkinje cells	Normal acquisition, ↓ reversal of spatial learning in homozygous mutants	-	↓social interaction in both genotypes ↑ grooming, vocalization	PC ↓excitability in heterozygous, ↓↓ in homozygous	PC loss in homozygous, ↑PC dendritic spine density in both heterozygous and homozygous	Reversed pathological and behavioral abnormalities [64]	[64]
<i>Tsc2</i> ^{fl/+} , or <i>Tsc2</i> ^{fl/fl} <i>Pcp2-Cre</i>	Heterozygous <i>Tsc2</i> deletion (global), or homozygous <i>Tsc2</i> deletion in cerebellar Purkinje cells; heterozygous in other cells	-	-	↑repetitive behavior in homozygous, ↓social interaction in both genotypes	-	PC loss in homozygous, Reversed social deficits	Reversed social deficits [63]	[63]

These are existing preclinical models designed to re-capitulate neurologic features of tuberous sclerosis complex (TSC). - Not examined; +: Mild seizure severity; ++: Moderate seizure severity.

characterized in a number of studies. Although there are no neuropathologic findings and no spontaneous seizures reported in these heterozygous mutants, behavioral abnormalities include social interaction deficits^[58,59], abnormal vocalization^[60] and learning deficits that may be related to altered synaptic plasticity^[58,61]. Both synaptic and behavioral learning deficits were reversed by acute rapamycin treatment. One study found that social interaction deficits in the Eker rat were exacerbated by seizure induction in the postnatal period^[59]. Thus, in animal models with heterozygous mutations in *Tsc1* or *Tsc2*, social interaction deficits and altered maternal-pup interactions that model some aspects of autism occur in the absence of cortical lesions or epilepsy, but can be exacerbated by seizures induced early in postnatal brain development. The latter findings support intervention to control seizures in infants with TSC.

TSC and Fragile X syndrome may share neurobiology related to intellectual disability and autism, since these conditions have been found to result in opposite effects on metabotropic glutamate receptor (mGluR)-mediated protein synthesis in a recent preclinical study. In *Tsc2*^{+/-} mice, mGluR-mediated long term depression was abnormally decreased, but in *Fmr1*^{-/-} mice it was increased. These alterations were rescued by up or down modulation of mGluR5 activity, respectively, and by rapamycin treatment in *Tsc2*^{+/-} mice. Investigators also crossed the two mouse lines and observed reversal of memory deficits in offspring expressing both mutations^[62].

Conditional homozygous deletion of *Tsc1* or *Tsc2*

Mice with conditional deletion of both alleles of *Tsc1* or *Tsc2* in cortical neurons exhibit a variety of structural abnormalities such as increased brain and cell size, dysplastic neurons and deficits in cortical organization; mosaic homozygous inactivation of *Tsc1* produced heterotopias and white matter nodules that resemble some cortical lesions in individuals with TSC. Spontaneous epileptiform discharges, seizures and increased excitatory synaptic activity have also been reported in these mice. Conditional homozygous deletion of *Tsc1* in GFAP-expressing cells, which include astrocytes and possibly adult neural progenitors in neurogenic niches such as the hippocampal subgranular zone, decreased the expression of the astrocyte transporter for glutamate, Glt1. These mice exhibited spontaneous seizures, altered hippocampal organization, as well as increased astrocyte number and brain size. Thus, homozygous deletion of *Tsc1* or *Tsc2* produced more severe effects on brain structure and function and also reduced survival; rapamycin treatment reversed or ameliorated glial and neuronal abnormalities and increased survival. In the mice with homozygous deletion of *Tsc1* in astrocytes and GFAP-positive neural progenitors, early rapamycin treatment prevented seizures and later treatment reduced seizure frequency. None of these studies of mice with homozygous deletion in cortical neurons or astrocytes examined social behavior.

Two recent studies examined the behavioral effects of

conditional loss of one or both alleles of *Tsc1* or *Tsc2* that was limited to cerebellar Purkinje cells (PCs)^[63,64]. Homozygous deletion caused a progressive loss of PCs, and both heterozygous and homozygous deletion mutants exhibited a striking increase in PC dendritic spine density and reduced PC excitability. Autistic-like behaviors were observed in both heterozygous and homozygous mutants, including deficits in social approach, response to social novelty, altered vocalization during a limited postnatal period, and increased grooming. Chronic rapamycin treatment initiated at about 1 wk of age reversed the neuropathologic and behavioral effects in these mice. These animal models provide important insights into the role of the cerebellum in autism caused by TSC, which was initially proposed based on human neuropathologic and PET imaging studies.

These animal models of TSC exhibit the neuropathological, neurophysiological and behavioral abnormalities characteristic of TSC. They have been used to evaluate therapies based on mTOR inhibition, antiepileptic medications, and positive modulation of mGluR5 activity, and provide essential platforms for developing and preclinical testing of new therapies.

IMPLICATIONS FOR TREATMENT OF AUTISM

Currently available behavioral and pharmacological treatment options for children with TSC and autism do not differ from treatments available to children without TSC^[65,66]. Mutations in *TSC1* or *TSC2* lead to increased activity of mTOR. Two mechanism-based treatments have been FDA-approved for use in individuals with TSC, everolimus for tumors (SEGAs and renal angiomyolipomas) and vigabatrin for infantile spasms. It is unknown whether either treatment would be effective for severe autism.

The protein kinase mTOR is incorporated into two protein complexes, mTORC1 and mTORC2, which have distinct downstream effects on gene transcription, metabolism, cell proliferation, and synaptic plasticity. Everolimus targets excess mTOR activity in the protein complex mTORC1 (mTORC2 is unaffected). After 12-18 mo of treatment, everolimus has been shown to increase abnormally low FA in an open-label study of 20 individuals with TSC and SEGAs^[67], indicating an improvement in white matter structure. A clinical trial of everolimus for neurocognition in high-functioning individuals with TSC is ongoing and may include individuals with autism.

Vigabatrin, an irreversible inhibitor of γ -aminobutyric acid (GABA) transaminase, targets excessive levels of glutamate (the brain's most common excitatory neurotransmitter) by increasing levels of GABA (the brain's inhibitory neurotransmitter). To date, there are no active clinical trials of vigabatrin for neurocognition or autism. Directly targeting the neurobiology of TSC may be therapeutic for children with severe autism; however, definitive clinical trials are warranted. Successful design

and implementation of these trials will require careful planning. In this review, we identified 4 areas of need: (1) validation of severe autism as an endophenotype in TSC; (2) natural history of severe autism in TSC; (3) selection of reliable, valid outcome measures in this population; and (4) assessment of recruitment and protocol compliance (feasibility factors relevant to trial implementation).

DISCUSSION

To date, discovery of mechanism-based treatments for TSC has not impacted the clinical and educational intervention offered to children with autism and TSC. A severe form of autism characterized by self-injurious behavior and/or aggression may exist in some individuals with TSC; however, few data are available about this potential endophenotype. Level of parental concern, danger to self and others, decreased quality of life, and lack of full participation in the community provide a rationale for prioritizing inclusion of this population in clinical trials. Clearly, improved treatment options for individuals with extreme behavioral manifestations of TSC are needed. Targeting the neurobiology directly may result in greater improvement than non-specific treatments.

This structured review of TSC literature revealed important clinical and preclinical features of TSC, including autism, intellectual disability, self-injury, and/or aggression in individuals with TSC. Several clinical associations were without clear causation, such as location of cortical tubers and autism. However, clinical trials demonstrating the efficacy of vigabatrin in individuals with TSC and infantile spasms^[68] and of everolimus for non-surgically resectable SEGAs^[69] have led to FDA approval for these indications. A comprehensive phenotypic analysis of patients with TSC can be achieved using measures that provide an assessment of neural activity, such as EEG and PET scans, newer MRI methods that detect subtle structural abnormalities, and appropriate behavioral assessments. This approach may also prove useful for evaluating the efficacy of mechanism-based treatments.

Review of the basic science literature highlighted the biological pathways and downstream effects of increased mTOR activity in TSC, alterations in neurochemistry in TSC, effects of pharmacological agents that directly impact the mTOR pathway, and described several animal models that recapitulate important features of TSC in humans. Although an animal model that exhibits all of the severe manifestations of this disorder does not currently exist, further study of the current models will continue to lead to important discoveries.

FUTURE DIRECTIONS

Shared deficits in neurobiology related to neurodevelopmental disabilities may represent an opportunity to generalize findings from research in TSC to individuals affected by related conditions. Abnormalities in mTOR signaling and associated pathways, as seen in TSC, have

been identified in focal cortical dysplasia type II, hemimegalencephaly^[70], phosphatase and tensin homologue hamartoma syndromes, such as autism associated with mutations in PTEN^[71-73], neurofibromatosis-1^[74-76], and autism associated with mutations in EIF4E (eukaryotic translation initiation factor 4E)^[77-79]. Targeting pathophysiological mechanisms has resulted in phenotypic rescue in animal models of several other single gene disorders, such as Lhermitte-Duclos^[80], Fragile X syndrome^[81], Angelman syndrome^[82], Rett syndrome^[83], Neurofibromatosis I^[84,85], Down syndrome^[86,87], and Rubinstein-Taybi^[88-90].

Although usually considered to be chronic, life-long conditions, there is hope for children and adults affected by TSC and other diseases that are associated with severe neurological manifestations, such as self-injurious behavior, aggression, intellectual disability, autism and seizures. Rescue of the synaptic plasticity deficits that underlie these manifestations may eventually be achieved with comprehensive treatment by pairing available mechanism-based treatments with evidence-based educational and behavioral interventions^[91,92].

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Acute flaccid paralysis in a patient with sacral dimple

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Abstract

Sacral dimples are the most common cutaneous anomaly detected during neonatal spinal examination. Congenital dermal sinus tract, a rare type of spinal dysraphism, occurs along the midline neuraxis from occiput down to the sacral region. It is often diagnosed in the presence of a sacral dimple together with skin signs, local infection, meningitis, abscess, or abnormal neurological examination. We report a case of acute flaccid paralysis with sensory level in a 4 mo old female infant with sacral dimple, diagnosed by magnetic resonance imaging to be a paraspinal subdural abscess. Surgical exploration revealed a congenital dermal sinus tract extending from the subdural abscess down to the sacral dimple and open to the exterior with a minute opening.

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Key words: Paralysis; Paraplegia; Spinal dysraphism; Spinal cord compression; Spinal epidural abscess

Core tip: Acute flaccid paralysis with sensory level ne-

cessitates a detailed examination of the back and magnetic resonance imaging spine. Sacral dimple of more than 5 mm in diameter, lying 2.5 cm above the anus, covered by hair tufts or hemangioma, has a non visualized base, or associated with abnormal neurological examination should be further evaluated by radiology for a hidden sinus that could be a source of infection.

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INTRODUCTION

Acute flaccid paralysis (AFP) is a clinical syndrome characterized by rapid onset of weakness that involves the voluntary muscles and frequently includes respiratory and bulbar muscles^[1]. It represents an initial manifestation of upper motor neuron lesions involving the central nervous system and spinal cord or a persistent manifestation of lower motor neuron lesions at the level of the anterior horn cell, roots and nerves, neuro-muscular junction, and muscles. Variable etiologies for acute flaccid paralysis in infants and children are listed in Table 1.

The urgency to arrive at the diagnosis and the choice of the initial investigations require a thorough patient history and a detailed clinical examination to reach the probable neuro-anatomical level of the lesion and the accounting etiology. Accordingly, a step wise and judicious use of investigations helps to reach the diagnosis with the minimum use of resources. Suggested approaches for diagnosis of AFP in children recommend starting by identifying evidence of spinal cord lesion through the presence of sensory level on examination, which then necessitates magnetic resonance imaging (MRI) evaluation of the spine to differentiate between compressive and non-compressive myelopathies^[1,2]. We report a case

Table 1 Common causes of acute flaccid paralysis in infants and children

Type of lesion	Common presenting features	Site of Lesion	Causes	Clues for diagnosis
UMNL	AFP and hypotonia are initial presentations for UMNL AFP occurs on the opposite side of the central nervous system lesion Spasticity develops later with hyper-reflexia and positive Babinski sign	Cerebral cortex	Intracranial hemorrhage	History of trauma Signs of increased intra-cranial tension Signs of lateralization
			Brain tumor	History of known brain tumor Signs of increased intra-cranial tension Todd paralysis following seizure activity
			Seizure	History of perinatal asphyxia
		Spinal cord	Hypoxic ischemic encephalopathy	Trauma Sensory loss/level Early bowel/bladder involvement Spinal tenderness, painful spine movement
			Spinal cord trauma	Sensory loss/level Early bowel/bladder involvement Spinal tenderness, painful spine movement
			Spinal cord tumor	Sensory loss/level Early bowel/bladder involvement Spinal tenderness, painful spine movement
LMNL	AFP and hypotonia are persistent presentations for LMNL Muscle weakness Hypotonia Fasciculations Decreased spinal cord reflexes	Anterior horn cell disease	Paraspinal infection or inflammation	Fever Sensory loss/level Early bowel/bladder involvement Spinal tenderness, painful spine movement
			Transverse myelitis	Fever Sensory loss/level Early bowel/bladder involvement Neck stiffness
			Poliomyelitis	Fever Preceding intramuscular injection Neck stiffness
			Non Poliomyelitis Enteroviruses	Fever Hand-foot-mouth disease with enterovirus type 71 and Cocksackie infections
			Enterovirus type 71	Myocarditis and/or hepatitis
			Cocksackie viruses	Parotitis
		Peripheral Nerve	Echoviruses	
			Mumps virus	
			Guillain-Barré syndrome	Preceding infectious prodrome/vaccination Prominent autonomic signs/symptoms Ascending weakness Facial weakness Myalgia Neck stiffness
			Peripheral nerve toxins	Exposure
			Acute intermittent porphyria	Intermittent nature Abdominal pain Peripheral neuropathies Central nervous system signs (seizures, mental status changes, cortical blindness, and coma)
			Neuromuscular junction disorders	Exposure Constipation Descending weakness Facial weakness
			Botulism	Prominent and early ptosis Facial weakness Fluctuating symptoms, fatigability
			Myasthenia gravis	

	Muscle	Organophosphate poisoning	Exposure Myosis muscle fasciculation
		Neurotoxic snake envenomation	Exposure Site of bite
Others		Rhabdomyolysis	Muscle tenderness
		Myositis	Fever Muscle tenderness
		Familial periodic paralysis	Preserved muscle stretch reflexes
		Electrolyte disturbance	Diarrhea
		Drug-related	Exposure

AFP: Acute flaccid paralysis; UMNL: Upper motor neuron lesions; LMNL: Lower motor neuron lesions.

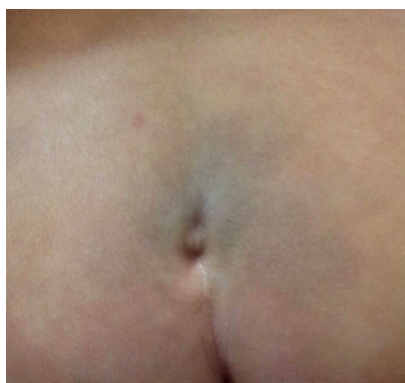


Figure 1 Sacral dimple.

of acute flaccid paralysis with sensory level in a female infant with sacral dimple, diagnosed by MRI to be a compressive myelopathy secondary to paraspinous abscess.

CASE REPORT

A 4 mo old female infant presented with acute flaccid paralysis of both lower limbs following 15 d of high grade fever, rhinorrhea, cough, and irritability. She received antibiotic for 10 d with no response. There was no associated weakness of the upper limbs, no respiratory distress, no constipation, but decreased urine output.

General examination revealed normal growth parameters and normal consciousness level. Examination of the back showed a sacral dimple of 7 mm in diameter, lying 4 cm above the anus, not covered by hair tufts or hemangioma and with an apparently intact base (Figure 1). Neurological examination was significant for atonia and areflexia of both lower limbs, loss of sensation below the umbilicus, absent abdominal and anal scratch reflexes together with urine retention. Tone, reflexes and sensation were intact in the upper half of the body.

Her laboratory investigations were significant for leucocytosis with prominent neutrophilic count, high erythrocyte sedimentation rate and positive C-reactive protein. Magnetic resonance imaging of the spine revealed a subdural abscess extending from dorsal 4 to sacral 1 and syringohydromyelia at the same level (Figure 2).

Surgical exploration, for evacuation of the subdural

abscess, showed a congenital dermal sinus tract extending from the subdural abscess down to the sacral dimple and open to the exterior with a pin point hall. Surgical excision of the abscess and the sinus was done with culture of drained purulent fluid. This revealed a heavy growth of *Candida* infection. Following surgical excision and antifungal therapy, the patient regained full range of movements, tone and sensation in her lower limbs as well as normal voiding acts and no further urine retention.

DISCUSSION

Sacral dimples, also known as sacrococcygeal or coccygeal dimples or pits, are the most common cutaneous anomaly detected at neonatal spinal examination^[3]. Sacral dimple may be associated with a congenital dermal sinus which is a tract lined by stratified squamous epithelium^[4]. The most widely accepted theory regarding the embryogenesis of congenital dermal sinus tracts proposes that they arise through faulty disjunction of the neuroectoderm from the overlying cutaneous ectoderm between the third and eighth week of gestation^[5,6]. They have been previously reported along the midline neuroaxis from the nasion and occipital area down to the lumbar and sacral regions^[4], with the highest frequency (75%) in the lumbar and lumbosacral region^[6]. The majority of these tracts terminate in a dermoid or epidermoid cyst, however some tracts incorporate a cutaneous ectoderm from the dorsal midline skin that extends for a variable distance into the underlying mesenchymal tissue and in many instances penetrates the dura to end within the thecal sac adjacent to or continuous with the neural tube^[5].

Literature evidence for the association between sacral dimple and congenital dermal sinus is controversial. The association between sacral dimples and occult spinal dysraphism was failed to be proven in five different case series including over 1300 children^[3,7-10]. However, Kriss *et al*^[7] examined 207 neonates with 216 cutaneous stigmata; the most common of which was midline dimple and found that simple midline dimples indicate low risk for spinal dysraphism. They reported atypical dimples particularly those that are large (> 5 mm), high on the back (> 2.5 cm from the anus), or appear in combination with other lesions to be associated with a high risk for spinal dysraphism. Accordingly, authors recommended

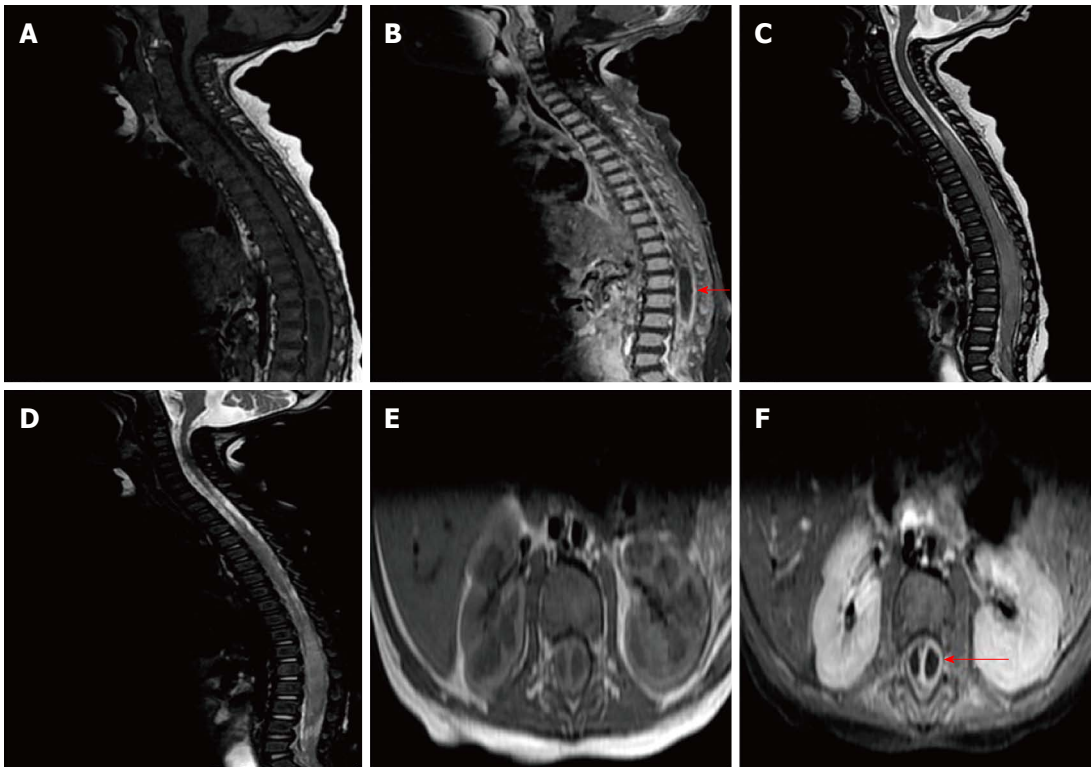


Figure 2 Magnetic resonance imaging spine. An intra-spinal and extra-dural cystic lesion of low signal intensity non-contrast sagittal T1-weighted imaging (T1WI) (A), non-contrast axial T1WI (E). It shows an intense marginal enhancement post-contrast sagittal T1WI (B, red arrow) and post-contrast axial T1WI (F, red arrow). The lesion extends up to the cervical spine sagittal T2WI (C) and lateral to the left side of the spinal canal sagittal T2WI (D).

that dimples of large size, located at a higher spinal level, or associated with other cutaneous stigmata should be investigated.

A wide spectrum of clinical manifestations can occur, ranging from asymptomatic dermal sinus to serious complications. There is no apparent timeframe for an asymptomatic lesion to later become symptomatic. Symptomatic sinuses may present clinically with varying degrees of drainage from their cutaneous openings, recurrent bouts of septic or aseptic meningitis, or mass effect on the cerebrospinal fluid pathways secondary to subdural abscesses and consequent hydrocephalus^[11]. The slow growth rate of these abscesses often masks their presentation for years, although there are some patients which present with acute neurological deterioration^[4,12]. Our case represents an example of the acute neurological manifestations of sacral sinus tracts. Similar to our case, Kurisu *et al*^[13] reported a case of repeated fever and rapidly progressive paraplegia in a one year old male infant with lumbo-sacral dimple proved by MRI to be an intra-spinal and intramedullary abscess where surgical exploration revealed an underlying sinus tract.

Ultrasonography is the screening modality of choice for the infant with atypical sacral dimples. The presence of an intra-spinal mass, abnormal position or shape of the conus, or a thick filum necessitates MRI study^[14]. Magnetic resonance imaging has become the reference study technique because of its ability to accurately depict the extent of the sinus tract and associated lesions^[12].

Magnetic resonance imaging accurately differentiate between compressive and non compressive diseases of the spinal cord. Chen *et al*^[15] investigated the MRI features of enterovirus 71 related acute flaccid paralysis in patients with hand-foot-mouth disease and found that MRI could efficiently show a characteristic pattern of affection to the anterior horn regions and ventral root of the cervical spinal cord and spinal cord below T9 level. Therapy is almost always surgical, aiming for obliteration of the tract with elimination of the communication between the skin and the neural structures. The earlier the lesion is detected and corrected, the less likely any long-term morbidity may develop.

In conclusion, acute flaccid paralysis with sensory level necessitates a detailed examination of the back and spinal MRI. Sacral dimple of more than 5 mm in diameter, lying 2.5 cm above the anus, covered by hair tufts or hemangioma, has a non visualized base, or associated with abnormal neurological examination should be further evaluated by radiology for a hidden sinus that could be a source of infection.

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Indoor smoke and prenatal and childhood growth: The role of (gestational) age

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Abstract

Growth at birth and during infancy predicts several outcomes in the immediate future as well as in the long term. Weight and height are commonly used surrogates of growth, however, infants and young children are constantly growing unlike adults. Hence, weight and height alone are insufficient measures of growth if the time component is not associated with them. Recent studies have investigated the relationship between indoor air pollution and growth using height and weight. In this commentary, I have argued using a directed acyclic graph, that a causal association between indoor pollution exposure and growth at birth cannot be established unless birth weight is adjusted for gestational age. Furthermore, to make any causal inference between growth during the first few years of life and indoor exposure, in addition to age standardization, studies must also account for fetal growth to discount any continuation of prenatal effects, which may be in the causal pathway. A careful consideration is warranted from future studies investigating these relationships.

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Key words: Biofuel; Coal smoke; Wood smoke; Birth weight; Fetal growth; Height

Core tip: Prenatal and early childhood estimators of growth, such as birth weight, height *etc.* by themselves are inadequate measures for inter-individual comparison, unless accompanied by gestational or chronologic age. The existing evidence points toward an association between indoor air pollution and growth, however few considered age. In order to establish a causal relation-ship it is imperative to consider age adjusted growth.

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INTRODUCTION

Reduced growth prenatally and after birth, which is associated with childhood mortality and morbidity^[1] and also with chronic diseases during adulthood^[2], is considered a major problem in many developing countries. Coincidentally, indoor air pollution is also high in these countries^[3]. More than half of the world population, predominantly from developing countries use some form of biofuel. A few studies have presented evidence that indoor air pollution may be one of the hitherto unknown factors associated with reduced prenatal and early childhood growth. This is a commentary on the studies mostly from the last decade, focusing on residual confounding that may arise if (gestational) age is not accounted for. There are many other sources of bias that pose similar threats to internal validity, which are not the focus of this commentary.

PRENATAL GROWTH

Prenatal exposure to indoor biofuel smoke and birth weight has now been investigated in several populations.

A Zimbabwean study^[4] reported maternal exposure to combustion smoke from wood, dung or straw was associated with infants born with 175 g ($P < 0.01$) lower mean birth weight than those who resided in homes that used gas or electricity for cooking. In spite of the significant association, owing to the cross-sectional nature, it is difficult to establish if exposure preceded outcome. Furthermore, the association with birth weight may not be construed to be with prenatal growth, as it was not adjusted for gestational age.

A study on Guatemalan infants^[5] reported 63 g (95%CI: 1-126) reduction in birth weight for those exposed to wood smoke compared to infants from households that used electricity or gas. This cross-sectional study accounted for some important variables but not gestational age and the possibility of exposure misclassification exists. This was one of the earliest studies and the magnitude of the point estimate is on the lower side compared to the estimates of other studies conducted subsequently.

Another Guatemalan study^[6] reported 89 g (95%CI: -27-+204) higher birth weight and the association with low birth weight (LBW) was 0.74 (95%CI: 0.33-1.66) for infants born in families that used a chimney stove compared to infants born in families that used open fire. The study, though not sufficiently powered, provides higher quality of evidence owing to the longitudinal design but did not adjust for gestational age.

A population-based longitudinal study^[7] from India, designed from a randomized trial reported a 105 g (95%CI: 140-69) reduction of birth weight and a RR of 1.49 (95%CI: 1.25-1.77) for LBW amongst those prenatally exposed to wood or dung smoke compared to those unexposed. The estimate was adjusted for a range of covariates including secondhand tobacco smoke (SHS) and a surrogate of socioeconomic status (SES). This study provides stronger evidence because of the design, availability of information on and adjustment for potential confounders as well as for the power to detect a significant difference in the exposed and unexposed group.

Another population based retrospective cohort study^[8] from Pakistan reported adjusted OR of 1.64 (95%CI: 1.10-2.35) for LBW and -82 g (95%CI: -170-+9) reduction in birth weight (continuous) among those exposed to wood smoke compared to natural gas combustion exposure. The study accounted for a wide range of potential confounders directly, including tobacco use, maternal BMI *etc.* and others indirectly, using propensity scores. The association with LBW was significant, while the association with birth weight was not, even though, birth weight as a continuous variable would have more variations (than the dichotomy of LBW), and hence lend more power to the model, assuming both models had same number of observations. There was a degree of misclassification in growth when LBW (without gestational age) was considered because all LBW infants were not due to growth retardation. This dichotomy and

hence the misclassification was absent in the continuous birth weight model and may have been the cause for the statistically non-significant result. Interestingly, the study from India^[7] reported a RR of 1.21 (95%CI: 1.11-1.31) for small for gestational age (SGA, < 10 percentile of birth weight-for-gestational age), which was less than half of the LBW estimate (RR = 1.49) from the same study. SGA reflects retarded prenatal growth more appropriately than LBW or birth weight because it takes into account gestational age. It is appreciated that accurate gestational age measurement is a challenge in settings in which these studies were conducted.

A meta-analysis^[9] pooled all the estimates from the above studies (and one more), to summarize information and increase power. However, is it prudent to pool together estimates from cross-sectional and longitudinal studies? Perhaps, a non-significant test of heterogeneity is an argument in favor. If one digs deeper, even the two longitudinal studies, the models from which the LBW/birth weight estimates were used for pooling^[6,7], did not have a single covariate in common. Furthermore, it included two estimates from the Indian cohort study^[7] one for term LBW and the other for preterm LBW. The original article did not anywhere associate gestational age with the LBW estimate (RR = 1.49); presumably the authors performed additional stratified analyses but the estimates (term LBW in the meta-analysis and all LBW in the original paper) are strikingly similar. The comparison in the meta-analysis is essentially between term and preterm LBW-while the former is certainly due to intrauterine growth retardation, the latter is due to early parturition and may or may not be from growth retardation? A careful approach with particular attention to study characteristics and their differences, beyond any statistical test, should be considered before choosing the meta-analytic approach, to avoid spurious results.

POSTNATAL GROWTH

A few studies also investigated exposure to indoor smoke and early childhood growth. About two decades ago the first study^[10] provided evidence of an adverse association between exposure to smoke produced from gas burning during cooking and height at 10 years, a 3.3 cm reduction ($P < 0.03$) in the exposed compared to the unexposed. The cross-sectional study on Kuwaiti and European children was a modest attempt to explore the relationship by accounting for important determinants like SHS, SES, ethnicity *etc.* It acknowledged that there may have been selection bias because fewer Kuwaiti families participated and amongst those who did, a larger proportion used gas. The authors did not standardize height, which made the comparison amongst participants untenable unless they were all of 10 years when height was measured.

A seven country study^[11] using data from national surveys investigated exposure to biofuel smoke (wood/straw/dung *vs* electricity/gas/biogas/kerosene) and child's height-

for-age and reported -0.13 (95%CI: -0.19--0.07) SD units reduction in a multilevel analyses. The analytical strategy and the wide range of covariates (including population differences) make this important evidence except the fact that owing to the cross-sectional nature temporal precedence of exposure cannot be established. In a categorical analysis it reported an OR of 1.27 (95%CI: 1.02-1.59) for severe stunting, defined as $-3 \text{ SD} \leq Z < -2 \text{ SD}$, amongst exposed *vs* unexposed. Another study^[12] using data from Indian national family health survey reported larger association for severe stunting, OR of 1.90 (95%CI: 1.49-2.42) for those using biofuels compared to those who used cleaner fuels (definition similar to the previous one). Amongst the strength was the large number of covariates used but it did not account for multistage clustered sampling and there may be some unaccounted variance in the models affecting the significance.

Two longitudinal studies provide evidence of the association between exposure to biofuel generated smoke and reduced height-for-age. The study from India^[7] investigated growth at 6 mo and reported RR of 1.45 (95%CI: 1.20-1.75) for underweight ($< -2 \text{ SD}$, weight-for-age) for those exposed to wood and dung smoke compared to those unexposed and RR of 1.30 (95%CI: 1.06-1.60) for stunting ($< -2 \text{ SD}$, height-for-age) in the same comparison groups. As stated above, this is one of the high quality evidence we have so far. A second longitudinal study^[13] measured continuous height-for-age of children 36 mo old in Caucasian children from the Czech Republic and reported 1.3 cm reduction in height of 36 mo old children exposed to coal smoke compared to those who were unexposed. Retarded growth (from biofuels exposure or something else) may be initiated prenatally; such a condition needs to be accounted if we are to estimate the magnitude of a true causal association with early childhood growth. The Czech study adjusted for growth at birth using birth weight-for-gestational age-and-sex thus discounting for any such conditions initiated prenatally.

The currently available studies are heterogeneous-one longitudinal study^[13] reported continuous Z-scores while another^[7] reported both continuous Z-scores and dichotomous stunting. Furthermore, the definition of stunting was different from those used in the cross-sectional studies.

CONCLUSION

The underlying question is - can exposure to indoor biofuel smoke impede normal growth before and after birth? Residual confounding is an important issue that needs to be addressed to answer this question. In case of prenatal growth without the adjustment for gestational age none of the evidence can be concluded as causal. Additionally, maternal tobacco smoking^[14] and household ETS^[15] exposure are also established confounders. ETS adjustment may appear to be conservative because some of the constituents of tobacco smoke and indoor biofuel smoke are similar (*e.g.*, PAH, nitrogen oxides, PM_{2.5}), it is still a robust approach to eliminate the possibility of type

1 error and to estimate the true magnitude of the effect. The converse argument against the idea could be that this may lead to over-adjustment, masking or attenuating a true association.

Childhood height and weight change relatively rapidly with inherent age and sex differences. Standardization for age and sex using reference populations is therefore necessary to make the results generalizable. Additionally, in the developing countries malnourishment is an important factor for retarded childhood growth and studies investigating indoor air pollution and postnatal growth in these settings should also account for factors (*viz.* SES and morbidity) that cause malnourishment. Interestingly, all but one childhood growth study used age-standardized measures^[10], which is pertinent. If standardization by age is imperative for growth after birth, why is it not for prenatal growth, when it is well known that a week or even days of gestational age in the third trimester make a difference to birth weight? It will be inappropriate to establish causality between indoor air pollution exposure and prenatal growth, using birth weight (or LBW) without gestational age, which, at best, is a crude surrogate of growth.

Another key issue for studies on childhood growth is accounting for growth at birth. It is critical to differentiate reduction in growth due to the exogenous exposures (secondhand smoke or indoor air pollution) during childhood from that which is simply a continuation of a retarded trajectory initiated prenatally. A retarded trajectory initiated prenatally may be due to similar exposures accrued over the prenatal period or may be from other causes, *e.g.*, malnourished mother. Either way, it is important to make this distinction for an appropriate assessment of the magnitude of the association between indoor air pollution exposure and postnatal growth.

The key issues presented in the two paragraphs above can also be described using a Directed Acyclic Graph (DAG) shown in Figure 1. Age and gestational age are determinants of pre and postnatal growth, respectively and according to the definition of confounder these two variables need to be connected to exposure and outcome either through a directed or a backdoor path. Aside from the fact that higher age prolongs exposure, age or gestational age is not connected to indoor air pollution and the outcomes through any directed or unblocked backdoor paths in the DAG (Figure 1). However, age is absolutely necessary to measure growth when weight or height is used as the outcome. For example, an infant with 2800 g birth weight born at 42 wk does not have the same growth as another infant with the same weight but born at 37 wk. Therefore, it should not be mistaken that absence of any directed or backdoor path between age and exposure/outcome justifies its exclusion from consideration in the relationship. On the contrary, weight or height as an outcome is an insufficient measure of growth without age. Additionally, the DAG shows that postnatal growth has a backdoor path connecting prenatal growth and indoor air pollution, which suggests adjustment of prenatal growth is imperative in any model

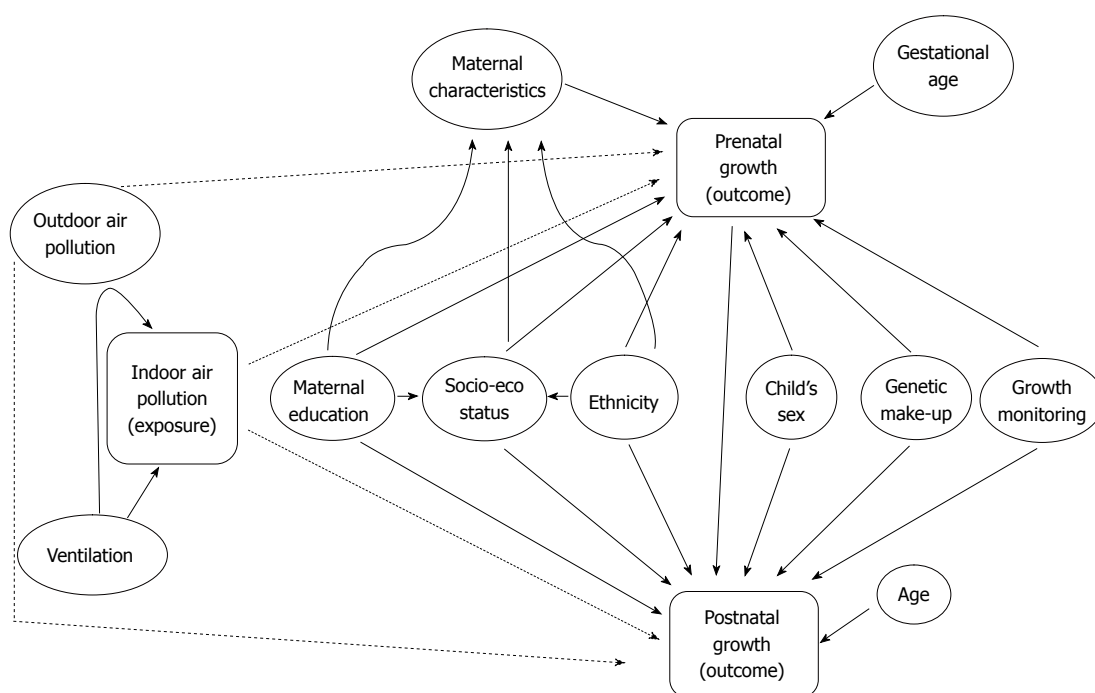


Figure 1 A directed acyclic graph depicting the relationship between indoor air and prenatal and early childhood growth.

associating indoor air pollution with postnatal growth.

The other variable that deserves a mention from the DAG (Figure 1) is outdoor air pollution. The causality of the relationship between outdoor air pollution and growth is yet to be ascertained. Meanwhile, outdoor air pollution will influence indoor air and vice versa if the windows and doors are kept open for long duration. This would apparently suggest that outdoor air pollution should be adjusted while investigating the relationship between indoor air pollution and growth. However, if outdoor and indoor air has the same pollutants and under the assumption of causal relationships, adjustment for one to investigate the relationship with the other, would be taking out the very association one is interested to find.

To conclude, the evidence point towards a potential adverse association between indoor air pollution exposure and growth, and encourages further well-designed investigation with adequate power, addressing the limitations of the current ones, to estimate the true magnitude. The last trimester and early childhood is marked by steady growth, it is therefore important to adjust for gestational age or chronologic age, respectively, to eliminate any differences in growth due to age before an adverse impact can be assessed. An exercise of procuring these datasets to perform a standardized analysis can be the next step forward.

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Echocardiography in children with Down syndrome

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Echocardiographic examination is recommended for all neonates with DS in the first month of life, before any cardiac surgery, to follow up after cardiac surgery and for serial evaluation of pulmonary hypertension. It is also indicated before involvement in non-cardiac major surgery and before involvement in physical exercise.

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Abstract

Congenital heart disease is a common problem in children with Down syndrome (DS). Echocardiography plays an important role in the detection of both structural and functional abnormalities in this group of patients. Fetal echocardiography can help in the early recognition of DS by detecting soft markers of DS, but its main role is to define the exact nature of the suspected cardiac problem in the fetus. Postnatal echocardiography is mandatory in the first month of life for all neonates with DS. It is also indicated before any cardiac surgery and for serial follow-up after cardiac surgery. In this article, we discuss the types and mechanism of cardiac abnormalities in DS children and the role of both fetal and postnatal echocardiography in the detection of these abnormalities.

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Key words: Fetal; Echocardiography; Congenital heart disease; Down syndrome

Core tip: Cardiac affection is a common issue in the Down syndrome (DS) population, in the form of both congenital and acquired heart disorders. Echocardiography plays an important role in the detection of such disorders. Fetal echocardiography can detect cardiac disorders as early as 10-12 wk of gestation.

INTRODUCTION

Down syndrome (DS) is an autosomal trisomy 21 and is one of the most frequently occurring chromosomal abnormalities. DS occurs once in every 600 to 800 live births and is frequently associated with congenital heart disease (CHD). The incidence of CHDs increases from 0.8% in the general population to approximately 40%-65% in patients with DS. At the same time, children with DS comprise approximately 10% of all children with CHD. Such malformations include all structural and functional cardiac defects present at birth, even if discovered later in life. These malformations can be single or multiple and usually lead to significant implications for the children and their families. These children may develop congestive heart failure, pulmonary vascular disease, pneumonia, or failure to thrive. CHDs are the most common cause of death in children with DS during the first two years of life^[1,2].

Atrioventricular septal defects (AVSD; with or without other CHD) and ventricular septal defects (VSD; with or without other CHD) have both been reported as the most common congenital heart defects and make up approximately 45% and 35% of CHD associated with DS, respectively. Additionally, isolated secundum atrial septal defect (ASD), isolated persistent patent ductus arteriosus (PDA), and an isolated Fallot of Tetralogy

(TOF) individually comprise approximately 8%, 7%, and 4% of CHD associated with DS, respectively. The remaining 1% of CHDs found in patients with DS includes arch abnormalities (aortic coarctation, right aortic arch, aberrant right subclavian artery). DS tends to be associated with the more severe forms of endocardial cushion defect, while the inlet VSD is common in trisomy 21. Several cardiac lesions seen in the non-DS population are rarely if ever found in individuals with DS, *e.g.*, heterotaxy, aortic coarctation or transposition of the great arteries. These differences between the types of CHD in DS and non-DS populations could suggest the impact of a third copy of a gene or genes on chromosome 21 on only specific developmental points^[3,4].

There is an increased prevalence of persistent pulmonary hypertension of the neonate (PPHN) in children with DS. These children have an increased risk of developing PPHN even in the absence of structural heart disease and should be followed until resolution of the pulmonary hypertension^[5]. Weijerman *et al*^[6], 2010 observed a significantly increased and elevated incidence of PPHN in neonates with DS (5.2%) compared to the general population. Another rare presentation in neonates with DS is the association of hypertrophic cardiomyopathy with complete atrioventricular canal defect in an infant with trisomy 21^[7].

While a defect of the atrioventricular canal remains the most common heart malformation in children with DS, the type of associated CHD may be affected by various factors. For example, the parents' consanguinity status could affect the pattern of CHDs. Al-Jarallah^[8], 2009 documented a slightly higher frequency of CHD in a sample of DS children from a Saudi population with a high consanguineous marriage rate. That study found that VSD was the most frequently detected CHD with the predominance of left-right shunt lesions and the relative rarity of cyanotic and complex CHD in this DS population. However, Chéhab *et al*^[9] previously showed that the risk of congenital cardiac anomalies in children with DS was not associated with the parents' consanguinity; instead, having a maternal age above 32 years was more associated with a lesser occurrence of congenital cardiac anomalies in children with DS.

Ethnicity and sex are additional factors that appear related to the type and frequency of CHD in the DS population. In a study conducted in the United States of America, Freeman *et al*^[10] showed that atrioventricular septal defects had the most significant sex and ethnic differences, with twice as many females affected and with twice as many blacks and half as many Hispanics affected compared to whites. In the Saudi population with DS, VSD was the most common (33.3%) followed by AVSD (22.8%), ASD (21.1%), patent ductus arteriosus (14%) and tetralogy of Fallot (11%). In a Turkish sample, the most common single defect was AVSD (34.2%), followed by second ASD (16.7%) and VSD (16.5%)^[11,12]. On the other hand, PDA was the most common cardiac malformation observed in

Guatemalan children with DS, followed by VSD, ASD and then AVSD^[13]. The most common cardiac malformations in Mexican children with DS were ASD, VSD and PDA, while the AVSDs were less common than the other malformations^[14].

Children with DS may also have dysfunctional autonomic cardiac regulation even in the absence of concomitant congenital heart disease, which may be manifested mainly in a reduced heart rate response to excitatory stimuli, including arousal from sleep. This is especially noted if accompanied by sleep-disordered breathing (SDB). O'Driscoll *et al*^[15] described a compromised acute cardio-respiratory response and dampened sympathetic response to SDB in children with DS and SDB compared to typically developed children with SDB. This altered response may be due to inadequate sympathetic activation or blunted vagal withdrawal and could reflect autonomic dysfunction in children with DS that may place them at increased risk for cardiovascular complications, such as pulmonary hypertension.

DS children with CHD have a greater predisposition to develop irreversible pulmonary arterial hypertension (PAH). The increased incidence of pulmonary hypertension in DS children could be a result of additional related problems, such as an upper airway obstruction, pulmonary hypoplasia, structural lung disease, thinner media of the pulmonary arterioles, abnormal pulmonary vasculature growth, alveolar hypoventilation, recurrent pulmonary infection or gastro-esophageal reflux^[16]. It has been suggested that PAH may develop earlier and may have a more violent course in patients with Down's syndrome, carrying with it a high risk of morbidity in a relatively young patient^[17,18].

MECHANISM OF CHD IN CHILDREN WITH DS

Heart development in humans is complex and starts very early, from the third to eight weeks of gestation. Development begins with a primitive tube that beats at 25 d of gestation and ends in the four-chamber heart. Many steps occur after the formation of the primitive heart tube, including looping, cell migration, cell transition, and septation events^[19]. The development of CHD is a multifactorial condition and is affected by a series of molecular signaling pathways and morphological events. In children with DS, it is postulated that variations in gene dosage of chromosome 21, environmental factors and genetic modifications not linked to chromosome 21 contribute to the development of CHD^[20].

Down's syndrome is most commonly caused by the presence of an extra copy of all or part of human chromosome 21 (Hsa21). The extra set of the approximately 200-300 genes on the chromosome leads to the many abnormalities associated with this condition. Due to triplication of Hsa21, there is a 1.5-fold increase in the expression of some, if not all, of these genes present in the

extra copy. However, all of these genes do not necessarily exhibit a straightforward 1.5-fold increase in expression, and only 30% of *Hsa21* genes are significantly over-expressed. Gene expression may be regulated by dosage compensation, which means that only a subset of these genes will exhibit the expected 50% increase in expression^[21]. Genetic imbalance caused by the presence of an extra copy of chromosome 21 will seriously disrupt one or more developmental pathways. This imbalance could also induce also an interaction between *Hsa21* genes and other disomic genes with relatively subtle or massively disruptive effects on genes located on chromosomes other than 21^[22]. These effects could be mediated through modulation of transcription factors, chromatin remodeling proteins, and related molecules or other targets. Thus, the imbalance-induced dysregulation of pathways involved in heart development may cause the cardiac defects observed in DS^[23].

The DS critical region (DSCR) is located on the long (q) arm of chromosome 21 and contains a number of genes that are thought to be responsible for some, if not all, of the features of DS. These genes may include the DS critical region 1 gene, or DSCR1, on chromosome 21q11.2; DSCR2 on chromosome 21q22.3; DSCR3 on chromosome 21q22.2; DSCR4 on chromosome 21q22.2; and DSCR5 in the chromosome region 21q22.1-q22.2. DS critical region 1, also known as Calcipressin-1, Adapt78, myocyte-enriched calcineurin-interacting protein 1 or regulator of calcineurin 1, is a 252 amino acid protein that belongs to the RCAN family (regulators of calcineurin) and exists as 4 alternatively spliced isoforms. DSCR1 is abundantly expressed in skeletal muscle, as well as in the brain and heart, and is thought to influence cardiac and nervous system development^[24]. DSCR1 is possibly part of a signal transduction pathway involving both the heart and brain and is implicated in cardiac valve formation and in the inhibition of cardiac hypertrophy^[25]. Overexpression of DSCR1 may be involved in the pathogenesis of DS, in particular mental retardation or cardiac defects^[26]. Barlow *et al*^[27] had previously mapped the DS-CHD region in humans to a 5.27-Mb chromosomal segment containing 82 genes, and Korbelt *et al*^[28] had narrowed this segment to a 2.82-Mb critical region likely involved in a DS-CHD endocardial cushion defect.

The presence of specific gene variants or modifiers could, in addition to trisomy 21, further increase the susceptibility to cardiac defects. One such genetic modifier is cysteine-rich with epidermal growth factor (EGF)-like domains (CRELD1), initially identified as a candidate for the AVSD2 locus. CRELD1 belongs to an epidermal growth factor-like family and encodes a cell surface protein that likely functions as a cell adhesion molecule. CRELD1 encodes a novel cell adhesion molecule that is expressed during cardiac cushion development. Missense mutations in CRELD1 have been found in DS patients with AVSD. CRELD1 (3p25.1) is one of the well-studied non-chromosome 21 loci variations that may predispose one to a heart defect. Other genetic modifiers have also

been shown to affect heart development. For example, somatic mutations in basic helix-loop-helix (bHLH) transcription factor *HEY2* (gridlock) have been identified in CHD in people with DS but not in euploid populations with heart defects^[29-32].

Environmental factors interact with the trisomic genome and may modify the occurrence of associated CHD in children with DS. Maternal cigarette smoking, for instance, has been associated with ASVD, TOF, and ASD but not with VSD^[33]. However, Khoury and Erickson observed an inverse relationship between maternal alcohol use and the presence of VSD in children with DS. Maternal folic acid supplementation may be associated with a reduced risk for CHD. Individuals with DS are at a high risk for CHD and have been shown to have abnormal folate metabolism^[34]. Bean *et al*^[35] found that a lack of maternal folic acid supplementation was more frequent among infants with DS and AVSD or ASD than among infants with DS and no heart defect or with VSD.

FETAL ECHOCARDIOGRAPHY

Fetal echocardiography is considered in cases of suspected DS. Such instances include the observation of a fetal nuchal translucency measurement of 3.5 mm or greater in the first trimester, the presence of a single umbilical artery or following an abnormal or incomplete cardiac evaluation on an anatomic scan, 4-chamber study. Fetal echocardiography can identify fetal cardiac structures as early as 10-12 wk of gestation using vaginal probes with high-resolution transducers, while conventional trans-abdominal echocardiography can detect fetal cardiac anomalies by 16-18 wk of gestation. The optimum period in which to perform a screening examination is at 20-22 wk because, at that time, the fetal cardiac structures can be defined more clearly with ultrasound screening in more than 90% of cases. Fetal echocardiographic examination can be difficult because of fetal physiology, the effect on flow across defects and valves, the inability to see the fetus for orientation reference, and an inability to examine the fetus for clinical findings. Likewise, a detailed heart evaluation can be very difficult during the third trimester because of acoustic shadowing, as in cases of maternal obesity or prone fetal position^[36].

Fetal echocardiography can assist in the early recognition of DS by detecting soft syndrome markers, but its main role is to define the exact nature of the cardiac anomaly suspected in the fetus. Such an assessment helps both the parents and the treating physician make the most informed decisions. Fetal echocardiography can provide the possibility of pregnancy termination in cases of severe malformations and of treating in utero the potentially treatable and less severe disorders, *e.g.*, fetal supraventricular tachycardia^[37]. In addition, fetal echocardiography can identify babies with complex congenital heart diseases that need delivery in a special tertiary care level center equipped with a Neonatal Intensive Care Unit so that during the transition from pre- to post-natal

life, the baby does not face periods of hypoxia or acidosis and can be given immediate care^[38].

Soft syndrome markers are ultrasound findings that are considered abnormal and whose presence increases the risk for underlying fetal aneuploidy and congenital heart diseases. These markers are nonspecific and could also present in fetuses without abnormalities. They are often transient and can be readily detected during the second trimester^[39].

Echogenic intracardiac foci (EICF) are examples of these soft markers that have been associated with DS, as well as with trisomy 13. They are a common finding seen in approximately 4% of obstetric sonograms, and their incidence can vary with ethnicity. The lowest rates of EICF are seen in black populations, while the highest rates occur among Asian patients. These foci are of no hemodynamic or other short or long term clinical or functional significance, but their importance arises from being a possible marker of a chromosomal abnormality^[40]. These foci are discrete areas of increased echogenicity in the region of papillary muscles. They may be single or multiple and usually present in the left ventricle (88% of cases) but occasionally present in the right ventricle (5%) and may be bilateral in approximately 7% of cases. The right-sided, biventricular, multiple, or significantly obvious EICF are associated with a higher risk for fetal aneuploidy compared with the more common single, left ventricular EICF^[41,42].

These foci may result from the aggregation of chordal tissues that have failed to fenestrate completely, the enhancement of abnormal tissue, or from a collection of fibrous tissue with increased echogenicity. They may also represent true microcalcifications within the cardiac muscle^[43]. The larger size of the left ventricular papillary muscle and the larger masses of chordae tissue, the more likely is to see echogenic foci in this area^[44].

A finding of EICF is subjective. Its detection depends on a variety of factors, including the resolution of the sonographic equipment, technique, thoroughness of the examination, and the sonographer's experience^[45]. For proper visualization and grading of EICF, an appropriate transducer frequency (≤ 5 MHz) and an appropriate gain setting should be used. These foci can be diagnosed on the standard 4-chamber view of the fetal heart. Fetal position is also important because intracardiac foci are best visualized when the cardiac apex is oriented toward the transducer. The foci echogenicities are graded according to their comparison to the surrounding bones, especially the thoracic spine. In grade 1, the echogenicity is less than that of the thoracic spine and the EICF image will be lost before that of the thoracic spine. Grade 2 suggests that the echogenicity is representative of bone and that the EICF and thoracic spine images disappear at the same gain setting. In grade 3, the echogenicity of the EICF is greater than that of bone, and the thoracic spine image will be lost before that of the EICF^[46].

The foci should be differentiated from other cardiac

conditions that can be misdiagnosed with these foci. These conditions include intra-cardiac tumors (rhabdomyomas, teratomas, fibromas, hemangiomas), ventricular thrombi (especially if adherent to the papillary muscles in the left ventricle), dystrophic valves, air in the chambers from fetal demise, endocardial fibroelastosis that is usually multiple and along the endocardial surface, idiopathic infantile arterial calcification, viral infections or metabolic disorders^[47].

The presence of an aberrant right subclavian artery (ARSA) is another potential new soft marker that is more commonly seen in fetuses with trisomy 21 and other chromosomal defects than in normal fetuses. An ARSA has been found postnatally in 1%-2% of normal individuals (from neonates to adulthood) at autopsy, but its incidence is increased in cases of DS, with figures ranging between 2.9% and 37.0%^[48]. Although it can be considered a weak marker, the second trimester diagnosis of an ARSA should prompt a detailed search for additional "soft markers" and potential structural defects^[49].

Once DS is suspected, fetal echocardiography should be performed to detect associated structural cardiac abnormalities. A cardiac anomaly can be classified according to its detectability by fetal echocardiography and its severity. With regard to detectability, the structural cardiac abnormalities are classified as detectable or undetectable. Detectable cardiac anomalies are those that can be identified during routine antenatal assessment incorporating a four-chamber view of the heart at approximately eighteen week' gestation. These abnormalities usually produce marked cardiomegaly, an obvious abnormality of the atrioventricular connection, or a disparity between the sizes of the atria or ventricles or both. In undetectable abnormalities, the four-chamber view fails to identify them as types of major malformations affecting the left or right heart outflows. Ventricular septal defect, pulmonary stenosis, aortic stenosis, and coarctation of the aorta are considered among the possible undetectable abnormalities^[57].

With regard to severity, structural cardiac abnormalities are classified into "complex," "significant," and "minor" heart disease. Complex heart disease occurs when there is an atretic or hypoplastic valve or cardiac chamber, which may include a hypoplastic left heart, Truncus arteriosus, pulmonary atresia with ventricular septal defect, or a double outlet ventricle. Heart diseases are considered significant when four chambers of normal or increased size and four valves are present but require intervention, *e.g.*, Ebstein/tricuspid valve dysplasia, significant complete AVSD, large VSD, partial AVSD, ASD, PDA, severe aortic stenosis, severe pulmonary stenosis (PS), transposition of the great arteries (TGA), coarctation of the aorta, total anomalous pulmonary venous connection, or TOF. Cardiac abnormalities are considered minor when no treatment is required, such as in cases with small VSD or relatively mild aortic or pulmonary valve stenosis. If there are multiple cardiac abnormalities, the disorder is

classified according to the most severe diagnosis. There are two exceptions from this classification: the endocardial fibroelastosis, which is classified as “complex”, and complete AVSD, which is classified as “significant”^[50].

There is a further classification according to severity suggested by Choi *et al*^[51], who identified 5 classes of fetal echocardiography results: normal, minor abnormalities, simple cardiac anomalies, moderate cardiac anomalies, and complex cardiac anomalies. Minor abnormalities are those that do not require any interference, such as PFO, isolate peripheral PS, abnormally looking aortic arch, simple right aortic arch, tortuous ductus without obstruction, and mild right ventricular dilation with or without tricuspid regurgitation (at most mild regurgitation). Simple cardiac anomalies are defined as a simple defect or a defect completely correctable by medical treatment, such as VSD, ASD, and possibly CoA. Moderate cardiac anomalies include defects that are surgically correctable with a low risk for reoperation, such as TOF, CoA, AVSD, and complete TGA. Complex cardiac anomalies are defined as defects correctable with surgery but that carry a high risk for sequelae or defects potentially suitable for a Fontan procedure, such as a double outlet right ventricle, TGA with PS, critical PS, and other Fontan candidates (pulmonary atresia with intact ventricular septum, functional single ventricle, hypoplastic left heart syndrome). There is an additional classification according to surgical risk that has the disadvantages of having great variability between institutions and the need to change the risk stratification in accordance with future advancements in surgical treatment^[51,52].

For optimal views of the heart, it is best to direct the fetal cardiac apex toward the anterior maternal wall. Optimization of the sonographic images could be achieved by appropriate adjustment of technical settings, such as acoustic focus, frequency selection, signal gain, image magnification, temporal resolution, harmonic imaging, and Doppler-related parameters. Accurate interpretation of obtained echocardiographic images will depend on a firm understanding of the anatomy of the fetal heart, either to diagnose a congenital abnormality or to confirm normality. According to guidelines from the American Institute for Ultrasound in Medicine, fetal echocardiography should include imaging of the aortic arch, ductal arch, four-chamber view, inferior vena cava, left ventricular outflow tract (LVOT), right ventricular outflow tract (RVOT), short-axis views (“low” for ventricles and “high” for outflow tracts), superior vena cava, and three-vessel and trachea views^[53].

For fetuses with major CHD, a full diagnosis requires a sequential segmental approach, similar to postnatal practice^[54]. The first step taken in studying the fetal heart is to definitely recognize the “Situs.” *In situs solitus*, the inferior vena cava (IVC) is anterior and to the right of the aorta, the abdominal aorta is posterior and to the left of the spinal cord, the gastric air bubble is on the left side and the liver is on the right. *In situs inversus*, there is a mirror image pattern, with the aorta to the right of

the IVC, and in *situs ambiguous*, the aorta and IVC are located on the same side of the spine in right isomerism, while the aorta is centrally located with an interrupted IVC in left isomerism^[55]. For determination of the cardiac position and axis, the heart is normally located within the left chest, with the apex pointing to the left (levocardia). In dextrocardia, the heart is located within the right chest with the apex pointing to the right, while in mesocardia the heart is centrally located with the apex pointing anteriorly. Dextroposition should be distinguished from dextrocardia by the normal left-sided axis of the heart despite being displaced to the right due to extracardiac reasons (for example, diaphragmatic hernia, congenital pulmonary cystic adenomatoid malformation, pleural effusion, *etc.*)^[38,56].

The four-chamber view (Figure 1A and B) is a key view of the fetal heart. It is effective in prenatal cardiac screening and can detect 43%-96% of fetal anomalies. It has the advantage of using fetal ribs as external reference points to ensure that the sonographer has “cut” the thorax in the appropriate plane. In a correct four-chamber view, there should be the appearance of a single rib around the fetal thorax. The four-chamber view is situated in a more horizontal plane than in the postnatal period because of the large liver. It can show the two atria and ventricles along with atrioventricular (AV) valves (mitral and tricuspid) and septa (interventricular and interatrial)^[57]. The detectability of CHDs by fetal echocardiography increases if the outflow tracts are examined as well as the four-chamber view, but appreciation of abnormalities in the outflow tracts is more challenging than in the four-chamber view^[58]. “Extended basic views” of LVOT and RVOT increase the sensitivity of anomaly detection. Alternatively, a comprehensive set of five short-axis projections can be acquired. The LVOT view can be obtained by tilting the transducer 45° from the four-chamber view perpendicular to the septum. This view can show the aorta originating from the LVOT with its main great vessels of the head and neck appearing distally. The RVOT view can be obtained by further rotation in the same direction and by gentle rocking the transducer from the LVOT view. In this view, the pulmonary artery can be seen rising from the RV and dividing into its main branches. The left pulmonary artery and ductus arteriosus can be observed, and the ascending aorta is seen centrally, wrapped by the RV and PA. A comprehensive set of five short-axis projections can also be obtained by cranial or caudal angulation of the ultrasound probe from the four-chamber view with the ventricular septum parallel to the ultrasound beam and the fetal spine^[54]. The pulmonary artery and RVOT can be visualized in the most cephalad short-axis view as they course around the aorta. Despite being small, the fetal pulmonary branches bifurcation can still be identified. The ductus arteriosus can likewise be identified and traced into the descending aorta. With caudal and more horizontal angulation from this plane, the ventricles and their respective AV valves can be observed. These views are better for the detection of conotruncal

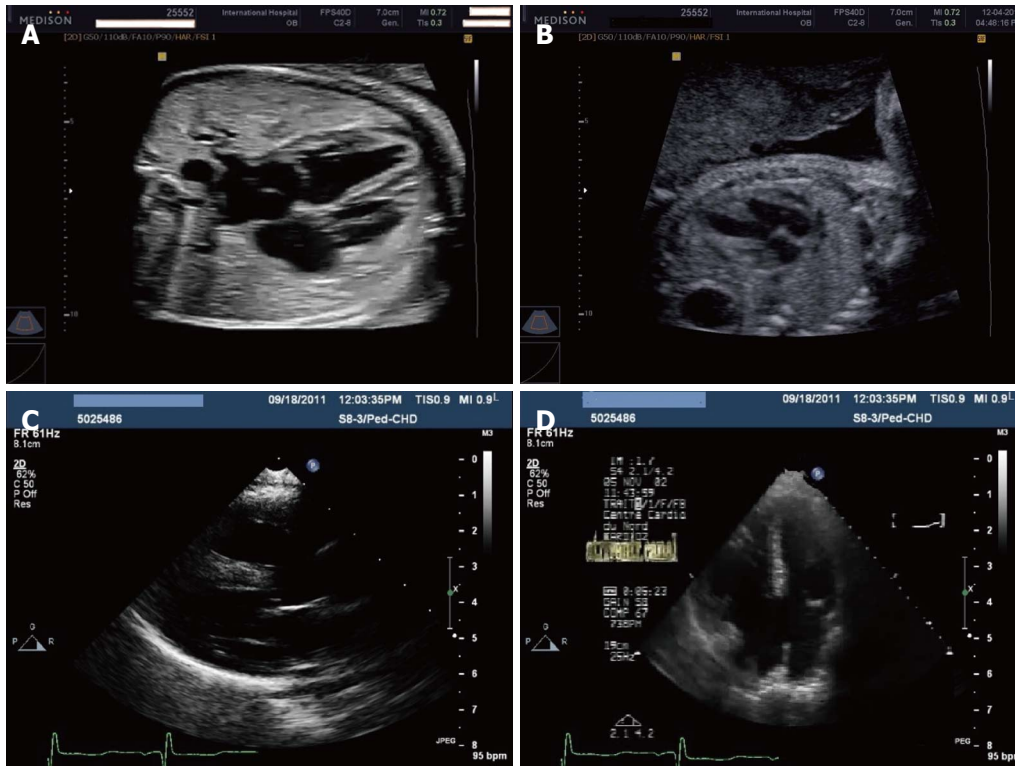


Figure 1 Ultrasonography. A: Normal 4-chamber view by fetal echocardiography at 26 wk gestation; B: Four-chamber view by fetal echocardiography at 22 wk gestation showing ventricular septal defects; C: Left-parasternal long-axis view in an infant with Down syndrome and complete atrioventricular (AV) canal and pulmonary hypertension; D: Apical 4-chamber view in an infant with Down syndrome and complete AV canal and pulmonary hypertension.

abnormalities that could otherwise be missed in more routine views. However, a specific diagnosis should not be made from a single plane.

The left and right fetal ventricles are nearly the same size, and the diameter of the pulmonary artery is typically larger than the aorta by approximately 10%. Ventricular volumes can be measured in 2-D mode using the Simpson rule. Other important measures include the RV/LV ratio, LV wall thickness, septal wall thickness, left atrial dimension, PA diameter, and aortic root diameter. These measures should be plotted against gestational age, which can be determined by measurement of the biparietal diameter or fetal length. These measures are helpful in the diagnosis of ventricular hypoplasia (either left or right) and cardiomegaly due to various congenital abnormalities, including pericardial effusion, aneurysms, cardiomyopathies, or tumors. Fetal heart motion, heart rate, wall thickness, chamber size, and motion of the valves or myocardium can be easily traced by M-mode, which can provide information on wall thickness and ventricular shortening fraction. The fetal long axis function may provide additional insight into endocardial function, which is most usefully in the detection of early ischemic changes before the development of sonographically detectable endocardial fibroelastosis. Moreover, color Doppler can be used to detect vascular flow through cardiac chambers, vascular structures, and septal defects^[59-63].

Clur *et al*^[64] showed that cardiac functions in trisomy

21 fetuses were abnormal irrespective of the presence of CHD. Evidence for cardiac loading (increased preload and afterload) and LV systolic (in the first trimester) and later diastolic dysfunction was observed. The authors showed significant reduction of tricuspid valve (TV) A-wave velocity and aortic valve peak velocity in trisomy 21 compared with normal fetuses. In addition, they also identified significant increases in the TV-E/A ratio and the ductus venosus-pulsatility index for veins and decreased pulmonary valve peak velocity. Moreover, the authors observed some evidence of left ventricular (LV) systolic dysfunction, such as a reduction of stroke volume (SV) and an increased myocardial performance index. They also found significant reduction of the mitral valve A-wave peak velocity and E/TVI ratio after 14 wk of gestation in the trisomy 21 fetuses with normal hearts compared to the controls with increased nuchal translucency thickness.

A complete AV canal can be more easily visualized in a 4-chamber view than an ASD primum, so that diagnosis of ASD primum type should be considered whenever a defect is noted in the portion of the atrial septum near the AV valves (septum primum). Opening the AV valve during diastole makes a large complete AV canal more obvious on the 4-chamber view, while during systole, the atrial and ventricular septal defects can be clearly seen above and below the closed AV valves, rendering its diagnosis possible as early as the late first trimester. Color

flow Doppler can show mixing of flow in the area of the septum primum defect, the dysplastic AV valves, and the ventricular septal defect. It can also show a lack of separation between the right and left ventricular inflow tracts in diastole, producing a classic “H” configuration. Color flow Doppler is also helpful in detecting and evaluating the degree of dysplastic AV insufficiency that may be severe enough to produce fetal heart failure with ascites^[65].

TRANSTHORACIC ECHOCARDIOGRAPHY

Although it has been recommended that infants with trisomy should have an echocardiogram in the first month of life, the value of routine neonatal echocardiography in this population is still in debate. Nevertheless, physical examination alone is not sufficient to identify cardiovascular anomalies in neonates with DS. In the newborn with DS, the potential benefits of early diagnosis, in the context of physical examination findings, should be considered in determining whether an echocardiogram should be performed during the neonatal period. Echocardiography should be obtained in all children with DS before proceeding with surgery^[66,67].

Echocardiographic examination can provide thorough real-time, non-invasive anatomic and functional information at relatively low cost. In neonates, the echocardiographic windows are more easily obtained and clearer than at any other age because of the reduced interference by lung tissue, which is impenetrable to ultrasound, and because the heart and the great vessels are nearer the probe. Echocardiographic examination should be conducted systematically with the classic standard views [left parasternal, apical, subcostal and suprasternal (Figure 1C and D)] and completed with Doppler ultrasound (color Doppler, pulsed Doppler and continuous wave Doppler). Trans-thoracic echocardiographic examination can usually detect cardiac defects in most cases. It can also determine the level of intra-cardiac shunting, along with its degree and direction, which can be confirmed by saline contrast injection. One mL of saline/blood mixture (which provides a greater intensity and more prolonged effect than the use of agitated saline only) is rapidly injected into a peripheral vein while capturing a four-chamber view of the heart. The simultaneous appearance of bright echoes from air bubbles in the fluid in the right ventricle and left atrium documents right-to-left atrial shunting as the air bubbles produced by hand agitation are too large to cross the pulmonary vascular bed, thereby predominantly aiding visualization of the right heart. The injection of the fluids into a vein that drains into the inferior vena cava could produce better results^[68,69].

Echocardiography can also evaluate cardiac functions. For example, left ventricular systolic function can be evaluated by measuring left ventricular ejection fraction, fraction shortening, SV, stroke index, cardiac output and cardiac index. Left ventricular diastolic function can be evaluated by measuring the left ventricular inflow velocity pattern or

trans-mitral flow velocity pattern [the early diastolic filling velocity (E-wave) is normally higher than the peak atrial filling velocity (A-wave)], the pulmonary venous flow velocity pattern (pulmonary venous flow velocity pattern usually consists of the antegrade flow during ventricular systole (S-waves: S and S'), antegrade flow during early ventricular diastole (D wave), and retrograde flow), the flow propagation velocity (Vp) during the rapid filling period and the peak early diastolic velocity of the mitral annulus (Ea, E'). The global function of LV can be measured using the Tei index. The Tei index is the first comprehensive index of cardiac functions that covers both systolic and diastolic functions, and it deteriorates and improves with either systolic or diastolic dysfunction^[70].

The quantitative assessment of RV size and function is often difficult because of the complex geometric anatomy, anatomical position under the sternum and the heavily trabeculated chamber with poor endocardial definition. However, 2-D echocardiography can easily obtain valuable information about RV size and function. Right ventricular dilatation, compared to the LV size, is a sign of RV dysfunction. Additionally, abnormal motion of the interventricular septum and the eccentricity index estimate RV pressure overload. The Tei index and the tricuspid annular plane systolic excursion both correlate well with RV function^[71].

Echocardiography can also evaluate the presence and severity of pulmonary arterial hypertension, which is relatively more common in DS neonates than in non-DS neonates. Doppler echocardiography allows estimation of both systolic and diastolic pulmonary artery pressure (PAP). The systolic PAP can be estimated by measuring the tricuspid regurgitation jet that represents the right ventricular-right atrial pressure gradient. Both PAP and RV systolic pressure are nearly equal in the absence of stenosis of the RV outflow tract, which is the reason that systolic PAP is commonly estimated by techniques that measure RV systolic pressure^[72].

Additionally, echocardiography is a useful tool for following up cases and for evaluating treatment outcome. Likewise, it has a further role even in the absence of congenital heart disease. Echocardiography can detect the presence of pericardial effusion in children with DS and hypothyroidism. A number of case studies have described the presence of pericardial effusion in this group of patients. Anah *et al*^[73], for instance, described the presence of a complex association of DS-hypothyroidism-pericardial effusion.

Echocardiography may also reveal impaired cardiac function even in the absence of congenital or structural cardiac defects. A number of studies have documented impaired cardiac functions in patients with DS. They recommended echocardiographic examination before involving patients with DS in surgery or physical exercise, even in the absence of structural cardiac diseases^[74,75].

Recommendation

Echocardiographic examination is recommended in chil-

dren with DS in the following situations: (1) in the first month of life for all neonates with DS; (2) before any cardiac surgery; (3) follow-up after cardiac surgery; (4) serial evaluation of pulmonary hypertension; (5) before involvement in major non-cardiac surgery; and (6) before involvement in physical exercise.

CONCLUSION

Echocardiography plays an important role in the detection of both structural and functional abnormalities in children with DS. Fetal echocardiography can help in the early recognition of DS by detecting soft markers of DS; however, its main role is to define the exact nature of the cardiac problem suspected in the fetus. Postnatal echocardiography is recommended in the first month of life for all neonates with DS. It is also indicated before any cardiac surgery and for serial follow-up after cardiac surgery.

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Bacterial colonization and intestinal mucosal barrier development

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Abstract

The intestinal tract is colonized soon after birth with a variety of ingested environmental and maternal microflora. This process is influenced by many factors including mode of delivery, diet, environment, and the use of antibiotics. Normal intestinal microflora provides protection against infection, ensures tolerance to foods, and contributes to nutrient digestion and energy harvest. In addition, enteral feeding and colonization with the normal commensal flora are necessary for the maintenance of intestinal barrier function and play a vital role in the regulation of intestinal barrier function. Intestinal commensal microorganisms also provide signals that foster normal immune system development and influence the ensuing immune responses. There is increasingly recognition that alterations of the microbial gut flora and associated changes in intestinal barrier function may be related to certain diseases of the gastrointestinal tract. This review summarizes recent advances in un-

derstanding the complex ecosystem of intestinal microbiota and its role in regulating intestinal barrier function and a few common pediatric diseases. Disruption in the establishment of a stable normal gut microflora may contribute to the pathogenesis of diseases including inflammatory bowel disease, nosocomial infection, and neonatal necrotizing enterocolitis.

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Key words: Bacterial colonization; Intestinal barrier; Intestinal microflora; Microbiota; Neonatal necrotizing enterocolitis; Nosocomial infection; Premature infants; Short chain fatty acids

Core tip: This review summarizes recent advances in understanding the complex ecosystem of intestinal microbiota and its role in regulating intestinal barrier function and a few common pediatric diseases. There is increasingly recognition that the stimulation of initial intestinal microbial colonization is important for proper maturation of the innate immune system and continued regulation and maintenance of intestinal barrier function. Disruption of the establishment of a stable normal gut microflora may contribute to the pathogenesis of diseases including inflammatory bowel disease, nosocomial infection, and neonatal necrotizing enterocolitis in premature infants.

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INTRODUCTION

The human gut is home to a large collection of micro-

organisms, the composition of which varies along the intestinal tract. The important role of normal microbial intestinal colonization in human health has gained increased recognition over the past several decades. The gut microflora, which is composed of approximately 10^{14} bacteria, or approximately 10 times the number of body cells, is now considered as a functional human organ^[1]. New studies such as the Human Microbiome Project are revealing how the gut microflora manipulates and complements physiology in ways that are important for the host^[2-4]. The normal gut microflora provides protection against infection, educates the immune system, ensures tolerance to foods, and contributes to nutrient digestion and energy harvest. In addition to these important functions, normal microbial colonization of the intestine is important in the induction of the host innate response and plays a vital role in the regulation and maintenance of intestinal barrier function. Disruption in the establishment of a stable normal gut microflora may be associated with or even contribute to the pathogenesis of diseases including inflammatory bowel disease (IBD), nosocomial infection, and neonatal necrotizing enterocolitis (NEC)^[5]. This review summarizes recent advances in understanding the complex ecosystem of the gut microflora and the roles of gut microflora in regulating intestinal barrier function as well as a few common pediatric diseases which may be related to an altered gut microbiota.

ESTABLISHMENT OF NORMAL INTESTINAL MICROBIAL COLONIZATION IS ESSENTIAL FOR THE POSTNATAL INTESTINAL BARRIER MATURATION

Before birth, the intestine is sterile. The intestinal tract becomes colonized soon after birth with a variety of ingested environmental and maternal microorganisms. This process is influenced by many factors including mode of delivery, diet, environment, and the use of antibiotics^[6,7]. For example, a breast-fed full-term infant normally has an intestinal microbiota in which bifidobacteria predominate over potentially harmful bacteria, whereas in formula-fed infants, enterococci, bacteroides and clostridia predominate^[8]. In premature infants, the immature intestinal mucosa is even more sensitive to gut colonizing bacteria. Host defenses can be improved by feeding the breast milk which helps the immature intestinal mucosal immune system to develop and respond appropriately to the highly variable bacterial colonization^[8].

The dense communities of bacteria in the intestine are separated from body tissues by a monolayer of intestinal epithelial cells. Therefore, normal intestinal function depends on the establishment and maintenance of the mucosal epithelial barrier which prevents the invasion of host tissues by resident bacteria. The assembly of the multiple components of the intestinal barrier is initiated during fetal development and continues during early postnatal life; thus the intestinal barrier has not completely developed

soon after birth, particularly in preterm infants^[9]. Several studies indicate that the normal bacterial colonization process may be important for postnatal intestinal barrier development. By using a newborn piglet model, Kansagra *et al.*^[10] demonstrated that intestinal barrier function was significantly less developed in full term newborn piglets receiving total parental nutrition compared to those receiving enteral nutrition. Even in the mature intestine, lack of enteral nutrition is associated with loss of intestinal epithelial barrier function which can lead to bacterial translocation and subsequent sepsis^[11]. In a rodent model, replacing enteral nutrition with parenteral nutrition can lead to bacterial translocation from the gut^[11,12]. Total parenteral nutrition significantly increases intestinal permeability, which can be ameliorated by enteral feeding and especially with a fiber enhanced diet^[13]. All of these results suggest that enteral feeding and colonization with the normal commensal flora are necessary for the maintenance of intestinal barrier function.

Recent studies have demonstrated that certain commensal bacteria increase intestinal epithelial cell survival by inhibiting the activation of the epithelial cell proapoptotic pathway associated with pathogenic bacteria^[14]. The intestinal commensal flora is also involved in maintenance of barrier function by inducing increased epithelial cell proliferation and enhancing intestinal epithelial integrity, through translocation of the tight junction proteins and up-regulation of genes involved in desmosome maintenance^[15,16]. Fermentation products of commensal bacteria have been shown to enhance the intestinal barrier function by facilitating the assembly of tight junctions through the activation of AMP-activated protein kinase^[17]. On the other hand, the deletion of all detectable commensal flora in the gut by a four-week course of orally administration of vancomycin, neomycin, metronidazole, and ampicillin leads to a more severe intestinal mucosal injury in a dextran sulfate sodium induced mouse colitis model^[18]. The importance of normal bacterial colonization in the development and maintenance of the intestinal barrier is further supported by the observations that the gastrointestinal tract gene expression profile and intestinal barrier development are altered by early treatment with broad-spectrum antibiotics or withholding enteral feeding^[19].

INTESTINAL MICROFLORA STIMULATES THE MATURATION OF THE MUCOSAL INNATE IMMUNE SYSTEM

The colonization with normal gut microflora protects against infection from pathogenic bacteria. This long-known but poorly understood protection provided by commensal flora against pathogens is commonly referred to as colonization resistance^[20-24]. Several mechanisms have been proposed to explain colonization resistance including a direct competition for nutrients, prevention of access to adherence sites, limitation of pathogen proliferation

through production of inhibitory substances or conditions, and stimulation of host natural immune defenses^[21,25].

Before birth, the fetus is protected from microbial exposure, and postnatal stimulation by initial intestinal microbial colonization is important for the proper maturation of the innate immune system. Exposure to immunostimulatory microbial constituents may trigger activation of the infant's mucosal innate immune system as shown recently in the gnotobiotic (germ-free) mouse model^[26-28]. For example, when compared to mice with normal intestinal microbial colonization, gnotobiotic mice need 30% more calories to maintain their body weight, exhibit an enhanced susceptibility to infection with enteropathogenic bacteria, and have an immature immune system^[28].

Remarkably, intestinal mucosal homeostasis is maintained despite the large surface area continuously being exposed to different bacterial species^[29,30]. To face the menace represented by intimate contact with a huge concentration of bacteria, the intestinal epithelium has evolved into a highly regulated barrier that can recruit immune cells of hematopoietic origin and produce mucus and a diverse arsenal of antimicrobial proteins that directly kill or inhibit the growth of microorganisms^[30,31]. The intestinal epithelium comprises several cell lineages. Enterocytes constitute the most abundant epithelial cell type, and secrete several antimicrobial proteins. Paneth cells are unique to the small intestine and secrete abundant quantities of antimicrobial proteins, such as α -defensins. Finally, goblet cells secrete mucin glycoproteins and trefoil factors that assemble to form a thick mucus layer overlying the epithelium^[32].

The development of the gut immune system is initiated before birth by a genetic program that drives the formation of Peyer's patches and mesenteric lymph nodes, but its postnatal maturation depends on the establishment of a balanced indigenous microbiota^[28]. Intestinal commensal microorganisms provide signals that foster normal immune system development and influence the ensuing immune responses^[33]. Signals delivered by these commensal microorganisms drive the development of isolated lymphoid follicles, stimulate maturation of Peyer's patches and initiate the migration of IgA producing plasma cells and mature T lymphocytes into the mucosa^[33-35]. Among the first colonizing organisms evident in the intestine of newborn infants are strains of *Escherichia coli* (*E. coli*) derived from the maternal gastrointestinal tract^[36-38]. It has been demonstrated in animal models that commensal *E. coli* strains can inhibit invasive *E. coli* O157:H7 growth in the intestine^[39]. These studies prove that the gut commensal microflora clearly have important effects on the development of normal immunity.

INTERACTION BETWEEN INTESTINAL MICROFLORA AND IMMUNE HOMEOSTASIS

The intestinal microflora regulates immune homeostasis

through many different mechanisms. Gut epithelia actively sense enteric bacteria and play an essential role in maintaining host-microbial homeostasis at the mucosal interface. Many innate immune responses are regulated by Toll-like receptors (TLRs), a conserved family of innate immune receptors that recognize microbial-derived molecules, including lipopolysaccharide, lipoprotein, RNA, and methylated DNA. Experiments in mice demonstrate that the beneficial effects of commensal bacteria are mediated *via* TLRs^[40]. Intestinal epithelial cells and mucosal immune cells express pattern-recognition-receptors such as TLRs, enabling them to respond to distinctive microbial-associated molecular patterns^[41]. TLRs are therefore critical for the specific detection of microbe-associated patterns, allowing differentially regulated responses to commensal versus pathogenic flora. The recognition of commensal bacterial-derived molecules by TLRs represents a critical component of the symbiosis between the host and indigenous microflora and is important for protection against gut injury and associated mortality^[42]. Deregulated interactions between commensal bacteria and TLRs have been reported to promote chronic inflammation and tissue damage, such as that seen in IBD^[43]. TLRs may also direct expression of the MyD88-dependent antimicrobial response. Paneth cell-intrinsic MyD88 activation limits translocation and dissemination of microbes across the mucosal barrier, while having little impact on luminal microbial numbers^[44]. These results highlight the essential role of TLR-dependent pathways in compartmentalization of enteric commensal bacteria^[45].

TLR-dependent signals mediate important regenerative signals to maintain intestinal mucosal integrity^[46]. TLR-mediated protection may work through both constitutive and damage-induced production of protective factors by TLRs expressed on colonic epithelium^[40]. Stimulation of TLRs results in activation of multiple signaling cascades that control expression of a wide range of innate immune response genes^[47,48]. Recent evidence indicates a role for CpG DNA, the bacterial agonist of TLR9, in mediating some of the beneficial effects of probiotics in the intestine, and more generally, to modulate the immuno-physiological status of the gut^[49]. Furthermore, TLR2 has been demonstrated to be responsible for the effects of *Bacteroides fragilis*. This bacterium possesses an unusual capsular polysaccharide A that exerts potent immunoregulatory effects and can dampen intestinal inflammation in several models of colitis^[50,51]. Systemic administration of flagellin, a bacterial protein that stimulates TLR5 protects mice from infection with vancomycin-resistant enterococcus (VRE)^[52].

Another immune regulatory effect of commensal bacteria involves the inhibition of the nuclear factor- κ B (NF- κ B) pathway through the stabilization of I κ B α ^[53]. I κ B α is a central inhibitor of the NF- κ B pathway, which acts by retaining the inactive NF- κ B dimers in the cytosol. Most pro-inflammatory signals trigger the NF- κ B pathway by inducing the phosphorylation of I κ B α , which targets the molecule for degradation by the

ubiquitin/proteasome system^[54]. Incubation of epithelial cells with nonpathogenic *Salmonella* has been shown to induce the accumulation of IyB α through the down-regulation of the protein's ubiquitination^[53]. Similarly, it has been recently reported that the probiotic bacteria *Lactobacillus casei* also inhibits the NF- κ B pathway by targeting the degradation of IyB α ^[55].

The specialized intestinal epithelial cells are capable of secreting proteins into the lumen of the intestinal tract which enhance epithelial barrier function and/or interact with the bacterial flora resident in the intestine. Goblet cells are highly polarized exocrine epithelia that secrete proteins apically into the lumen of the small and large intestine through the release of secretory granules. One particular class of glycoproteins produced by goblet cells, known as mucins, forms a viscoelastic protective gel that covers the intestinal epithelium^[56]. Another class of secretory peptides, now designated as trefoil factors, is also normally produced by goblet cells and is important for the maintenance and repair of the gut mucosal barrier^[57]. Mucins interact with trefoil factors and perform a defensive role during establishment of the intestinal barrier. Mucin oligosaccharides influence the bacterial milieu of the intestinal tract by enhancing the ability of certain bacteria to colonize the intestinal tract while inhibiting the adherence of others^[58]. Mucins are also a direct source of carbohydrates and peptides that can promote the growth of bacteria^[59]. To further enhance the symbiotic relationship between gut bacteria on the host, bacteria can alter mucus synthesis, secretion, and chemical composition^[60]. Changes in mucin profile in response to bacterial colonization suggest a potential role as a protective mechanism against pathogenic invasion of the intestinal mucosa during early development^[61].

The mucosal barrier is also reinforced by secretory immunoglobulin A (sIgA) and sIgM generated through external translocation of locally produced dimeric IgA and pentameric IgM. The dimeric IgA and pentameric IgM, containing a small polypeptide called joining chain to form sIgA and sIgM, can be actively exported by secretory epithelia. This external transport is mediated by the polymeric Ig receptor (pIgR), also known as membrane secretory component (SC)^[62]. Notably, pIgR/SC knock-out mice that lack secretory IgA and IgM antibodies exhibit reduced epithelial barrier function with aberrant mucosal leakiness^[63]. sIgA and sIgM form the first line of defense against pathogens as well as other potentially dangerous agents. Therefore, secretory immunity is of great importance for the intestinal epithelial barrier. In newborn infants, only a few IgA-secreting cells circulate in the blood. However this number is remarkably increased after 1 mo of age mainly due to the progressive microbial stimulation of gut-associated lymphoid tissues^[64]. A much faster establishment of secretory immunity is often seen in infants from developing countries where there is exposure to a heavy microbial load, and an associated lower incidence of atopy^[65]. Altogether, the secretory immune system is critical for the mucosal barrier

function and the intestinal epithelial barrier maturation depends on exposure to microbial factors from the environment and the indigenous microbiota.

DISEASES ASSOCIATED WITH AN ALTERED INTESTINAL MICROFLORA AND ABNORMAL BARRIER FUNCTION

Alterations of the microbial gut flora and changes in intestinal barrier function are associated with certain diseases of the gastrointestinal tract. There is growing evidence that changes of the intestinal flora composition may play a pathogenic role in IBD, nosocomial infection, and NEC^[66-68]. It has been proposed that a genetic predisposition causes IBD patients to have a deregulated immune response against harmless antigens derived from intestinal commensal bacteria and changes of the intestinal flora composition have been described in patients with IBD^[66]. Several studies found an enhanced number of Proteobacteria and Actinobacteria but decreased numbers of Firmicutes (particularly the species *Faecalibacterium prausnitzii*) in stool samples of IBD patients as compared to healthy controls^[69,70]. In biopsies of pediatric IBD patients, higher numbers of mucosa-associated aerobic and facultative-anaerobic bacteria were found, whereas bacterial species of the normal anaerobic intestinal flora such as *Bifidobacteria* were reduced^[71,72].

It is well recognized that nosocomial infection is frequently a consequence of gut derived organisms. The infections with highly antibiotic-resistant bacteria are usually acquired during hospitalizations. Destruction of the normal flora by antibiotic administration disinhibits antibiotic-resistant members of these bacterial families, leading to their expansion to very high densities^[73]. Reintroduction of a diverse intestinal microbiota to densely VRE-colonized mice eliminates VRE from the intestinal tract^[74]. Characterization of the fecal microbiota of patients undergoing allogeneic hematopoietic stem cell transplantation demonstrated that intestinal colonization with *Barnesiella* confers resistance to intestinal domination and bloodstream infections with VRE^[74]. Furthermore, there is an increased incidence of septic complications in patients receiving parenteral as opposed to enteral nutrition and this, in some cases, is due to alterations in intestinal barrier function predisposing to bacterial translocation^[75].

In premature infants, colonization with abnormal gut flora increases the risk of hospital acquired infection or late-onset sepsis (LOS)^[76]. Prolonged use of broad spectrum antibiotics, reduced bowel motility, immature epithelial host defenses, lack of enteral feeding, and parenteral nutrition are common risk factors for an altered microbial gut flora and abnormal mucosal barrier function. The possible subsequent bacterial translocation from the gastrointestinal tract may be an important pathway initiating LOS in premature infants^[76]. Mai *et al.*^[77] analyzed stool samples that had been prospectively

collected from ten preterm infants with LOS and from 18 matched controls. A 16S rRNA based approach was utilized to compare microbiota diversity and identify specific bacterial signatures that differed in their prevalence between cases and controls. They found that the types and distributions of bacteria that initially colonize the intestine in premature infants differ in those with LOS compared to uninfected control babies. Therefore, it was proposed that a distortion in normal microbiota composition, and not an enrichment of potential pathogens, is associated with LOS in preterm infants. This may suggest that administration of probiotics may protect high-risk neonates and infants from developing sepsis. However, currently there is no clinical evidence regarding the usefulness of probiotics or prebiotics for the prevention of nosocomial sepsis in preterm infants^[78].

Failure of the postnatal developmental of the intestinal barrier in the immature intestine plays an important role in the pathogenesis of NEC, a devastating disease seen mainly in preterm infants^[68,79,80]. A major component of the pathophysiology of NEC is the nature of the interaction of bacteria with the premature gut. The pattern of bacterial colonization in the intestine of the premature neonate is quite different from that of the healthy full-term infant. Infants requiring intensive care acquire intestinal organisms slowly, and the establishment of bifidobacteria flora is retarded. A delayed bacterial colonization of the gut with a limited number of bacterial species tends to be virulent. Indeed, several clinical observational studies have shown that the duration of antibiotic exposure including prenatal exposure to antibiotics is associated with an increased risk of NEC in preterm infants^[81-83]. Therefore, an aberrant colonization of the bowel of the premature infant has been proposed to contribute to the development of NEC^[84]. By using non-culture-based microbial analyses of feces, Wang *et al.*^[85] studied fecal samples of ten preterm infants with NEC and ten matched controls and found that patients with NEC had less bacterial diversity and an increased abundance of γ -proteobacteria in the stools. Similar findings were presented in another study by Mai *et al.*^[86], in which one of the bacterial signatures detected more frequently in NEC patients matched closest to γ -proteobacteria. These observations suggest that abnormal patterns of microbiota contribute to the cause of NEC. However, a study by Mshildadze *et al.*^[87] using the same technology demonstrated that the overall microbial profiles in patients with NEC were not different from those of control infants. Thus to date, molecular methods have not clarified the bacterial pathogenesis of NEC.

None of the clinical studies to date has been able to fulfill Koch's postulate linking NEC to a particular pathogen. Nevertheless, we proposed a hypothesis that excessive production/accumulation of short chain fatty acids (SCFAs) due to bacterial fermentation of undigested formula or abnormal bacterial colonization contributes to the pathogenesis of NEC^[88,89]. There is substantial indirect clinical evidence to support the theory that bacte-

rial fermentation is involved in the development of NEC in premature infants^[90]. Further, in two separate studies, increased breath hydrogen excretion (an indicator of bacterial fermentation and an indirect measurement of SCFAs production) was found in NEC patients even prior to the onset of clinical symptoms^[91,92]. The well-known finding of pneumatosis intestinalis (gas in the bowel wall) in NEC patients is also thought to be secondary to hydrogen gas produced by bacterial fermentation^[93]. Recent reports of several cases of premature infants who developed NEC after they were fed SimplyThick®, a xanthan gum-based thickener used in the management of dysphagia, is another example^[94,95]. Increased production of hydrogen and SCFAs as the consequence of accumulation of luminal carbohydrates and fecal bacteria fermentation of xanthan gum were proposed as the main mechanisms for NEC^[94]. Both probiotics and prebiotics have been proposed to promote a healthy gut microbiota in human, and oral probiotics have been proven to be effective in reducing the incidence of NEC in premature infants in several clinical trials^[96-98]. On the other hand, there is no evidence showing that prebiotics can effectively reduce the incidence of infection or NEC in premature infants. Therefore, there is insufficient evidence to recommend the use of oligosaccharides as prebiotics in formula for premature infants since these may prove to be unsafe.

In summary, this review summarizes recent advances in understanding the complex ecosystem of intestinal microbiota and its role in regulating intestinal barrier function and a few common pediatric diseases. There is increasingly recognition that the stimulation of initial intestinal microbial colonization is important for proper maturation of the innate immune system and continued regulation and maintenance of intestinal barrier function. Disruption of the establishment of a stable normal gut microflora may contribute to the pathogenesis of diseases including IBD, nosocomial infection, and NEC in premature infants.

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Imaging evaluation of hemoptysis in children

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Abstract

Hemoptysis is an uncommon but distressing symptom in children. It poses a diagnostic challenge as it is difficult to elicit a clear history and perform thorough physical examination in a child. The cause of hemoptysis in children can vary with the child's age. It can range from infection, milk protein allergy and congenital heart disease in early childhood, to vasculitis, bronchial tumor and bronchiectasis in older children. Acute lower respiratory tract infections are the most common cause of pediatric hemoptysis. The objective of imaging is to identify the source of bleeding, underlying primary cause, and serve as a roadmap for invasive procedures. Hemoptysis originates primarily from the bronchial arteries. The imaging modalities available for the diagnostic evaluation of hemoptysis include chest radiography, multi-detector computed tomography (MDCT), magnetic resonance imaging (MRI) and catheter angiography. Chest radiography is the initial screening tool. It can help in lateralizing the bleeding with high degree of accuracy and can detect several parenchymal and pleural abnormalities. However, it may be normal in up to 30% cases. MDCT is a rapid, non-invasive multiplanar imaging modality. It aids in evaluation of hemoptysis by depiction of underlying disease, assessment of

consequences of hemorrhage and provides panoramic view of the thoracic vasculature. The various structures which need to be assessed carefully include the pulmonary parenchyma, tracheobronchial tree, pulmonary arteries, bronchial arteries and non-bronchial systemic arteries. Since the use of MDCT entails radiation exposure, optimal low dose protocols should be used so as to keep radiation dose as low as reasonably achievable. MRI and catheter angiography have limited application.

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Key words: Hemoptysis; Lower respiratory tract infection; Bronchiectasis; Cystic fibrosis; Foreign body

Core tip: Hemoptysis is a cause of immense concern to the child, the family and the pediatrician. Thorough history and physical examination is necessary to ascertain its presence, which is particularly challenging in the pediatric population. Imaging has an important role in identifying the source of bleeding and its underlying cause. Acute lower respiratory tract infections are the most common cause of pediatric hemoptysis. The imaging modalities include chest radiography, multi-detector computed tomography (MDCT), magnetic resonance imaging (MRI) and catheter angiography. MDCT is a rapid multiplanar imaging modality which should be used judiciously to keep radiation dose to a minimum. MRI and catheter angiography have selected application.

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INTRODUCTION

Hemoptysis is defined as expectoration of blood or blood tinged-sputum due to bleeding from the respiratory tract^[1]. Massive hemoptysis is termed as blood loss > 8

Table 1 Causes of hemoptysis in children

Acute lower respiratory tract infections
Bacterial
Viral
Fungal
Parasitic
Bronchiectasis
Aspiration
Cystic fibrosis
Ciliary dyskinesia
Post-infectious
Congenital heart diseases
Eisenmenger syndrome
Aplasia/hypoplasia of pulmonary artery or veins
Primary pulmonary hypertension
Pulmonary artery narrowing
Infectious
Inflammatory
Pulmonary thromboembolism
Pulmonary arteriovenous malformation
Alveolar hemorrhage syndrome
Idiopathic
Associated with rheumatologic disease
Pulmonary-renal syndrome
Neoplasms
Bronchial carcinoid
Bronchial adenoma
Metastatic
Foreign body
Trauma
Cryptogenic

mL/kg in 24 h^[2]. The life-threatening element in massive hemoptysis is asphyxiation due to flooding of the airways by blood. Hence, securing the airway needs immediate attention. Hemoptysis in lesser amounts poses a diagnostic challenge in pediatric patients as it may initially remain unnoticed because children tend to swallow their sputum and are unable to provide a clear history or undergo thorough physical examination. It is a cause of immense concern to the child, the parents and the pediatrician. After confirming the presence of hemoptysis, the next step is to establish the cause, so that an appropriate treatment regimen can be adopted. The spectrum of causes of hemoptysis in children is considerably different from that of the adults. Imaging has a pivotal role in evaluation of hemoptysis. There are various modalities which can be resorted to, namely, conventional radiography, multi-detector computed tomography (MDCT), magnetic resonance imaging (MRI) and in certain cases, catheter angiography which can also fulfill a therapeutic purpose. The advent of MDCT has paved the way for non-invasive multi-dimensional visualization of the thoracic vasculature, tracheobronchial tree and pulmonary parenchyma. This is of tremendous value as it can obviate the need for invasive bronchoscopic procedure with its attendant complications. The following sections illustrate the etiology, pathogenesis and role of imaging in hemoptysis.

ETIOPATHOGENESIS

There are several causes of hemoptysis in children (Table 1).

The common causes are acute lower respiratory tract infections, bronchiectasis (due to cystic fibrosis, aspiration, ciliary dyskinesias, post infectious), congenital heart disease and foreign body aspiration. Of these, acute lower respiratory tract infections may constitute upto 40% of cases^[3]. The etiology also varies with the child's age. Sim *et al*^[4] observed that infection, Heiner syndrome (milk protein allergy) and congenital heart disease were the major causes in early childhood; while during late childhood, vasculitis, bronchial tumor and bronchiectasis were far more prevalent.

The lungs receive dual blood supply; one from the high pressure bronchial arteries, the other from the pulmonary arteries with relatively lower pressure. The pulmonary arteries account for 99% of the arterial blood supply to the lungs and take part in gas exchange while the bronchial arteries provide nourishment to the supporting structures of the airways and form the vasa vasa of the pulmonary arteries. The bronchial vasculature is in close proximity to the pulmonary arteries at the level of the vasa vasorum where the two systems are connected by thin-walled anastomoses between the systemic and pulmonary capillaries^[5,6]. Pulmonary vascular obstructive disorders (congenital heart disease, vasculitis, embolism) open up these anastomoses in regions of the lung that are deprived of their pulmonary arterial blood flow. This subjects these fragile vessels to increased systemic arterial pressure and can cause hemoptysis by rupturing into the alveoli or bronchial airways.

In the setting of tracheobronchial infection, there is inflammation of the airways. As a result, they become congested and friable, which increases their susceptibility to bleed. Chronic inflammation (as in bronchiectasis) can lead to increase in systemic arterial flow due to release of angiogenic growth factors which lead to neovascularization and formation of "leaky" vessels prone to rupture. Approximately 5% of patients with cystic fibrosis can present with massive hemoptysis^[7]. This is due to hypertrophy of bronchial arteries along with the presence of multiple bronchopulmonary anastomoses. Foreign body aspiration causes hemoptysis due to mechanical trauma or due to associated intense inflammation incited by organic matter. Pulmonary hemosiderosis is an uncommon but significant cause of hemoptysis in children. It is mostly idiopathic; however, it may be associated with an allergy to cow's milk (Heiner syndrome)^[8]. Although rare in children, endobronchial or pulmonary parenchymal tumors (carcinoid, bronchial adenoma) may cause significant hemorrhage.

Imaging evaluation of hemoptysis

The aim of initial diagnostic evaluation is to identify the immediate source of bleeding along with determination of the primary cause of hemoptysis. Traditionally, the diagnostic algorithms in acute setting have been based on various combinations of conventional radiography, chest computed tomography (CT) and thoracic aortography. MDCT has now made comprehensive visualization of

thoracic anatomy possible. It provides high-resolution images of the thoracic and upper abdominal vasculature which aids in diagnosis and also provides a roadmap prior to any intervention. CT findings can forewarn the endoscopist about the presence of peribronchial or endoluminal aneurysms. MRI does not have a role in the acute setting. However, it may serve as a problem-solving tool in certain situations.

Conventional radiography

Chest radiography serves as a valuable screening modality. It can help in lateralizing the bleeding with high degree of accuracy and can detect several parenchymal and pleural abnormalities. The commonly used views include frontal and, in some cases, lateral. The utility of lateral radiographs is in case of presence of a radio-opaque foreign body on frontal view, when it can determine if it is in the trachea or esophagus. Lynch *et al*^[9] observed that addition of a lateral radiograph in children with pneumonia did not improve diagnostic accuracy. Common findings include presence of focal infiltrates which may suggest infection. Unilateral air-trapping with hyperinflation can give a clue towards presence of an unsuspected tracheobronchial foreign body. A radio-opaque foreign body may be seen. Ancillary findings include “tram-track” appearance of bronchiectasis; pulmonary nodules, lymphadenopathy, pleural effusion; cardiomegaly; and vascular redistribution due to pulmonary venous obstruction. Approximately one-third of chest radiographs may be normal in children with hemoptysis. A tracheobronchial source of bleed may eventually be identified in about half of these cases^[10]. Therefore, additional follow-up testing is recommended in patients with hemoptysis in whom the underlying cause is not detected by initial radiography.

MDCT

The role of MDCT in evaluation of hemoptysis includes: (1) depiction of underlying disease; (2) assessment of consequences of hemorrhage which may be a cause of clinical concern or may conceal the underlying abnormalities; and (3) panoramic visualization of the thoracic vasculature by various reconstruction techniques.

Technique

CT technique involves acquisition of multiple sections from the base of the neck to the level of the renal arteries (L2 level). Optimal enhancement of the pulmonary and systemic arteries is achieved by administration of 2 mL/kg body weight of iodinated non-ionic contrast media containing 300 mg I/mL at a rate of 4 mL/s *via* a wide gauge cannula. The scan should be started during the phase of peak systemic arterial enhancement (scanning delay of 18 s or a threshold of 100 HU in the descending aorta with bolus tracking). Images should be acquired with thin collimation and with the table movement adjusted to allow wide volume coverage during a single breath-hold. Radiation exposure is a significant

concern in the pediatric population. Hence, the exposure parameters and kilovoltage need to be adjusted according to the patient's weight so as to minimize radiation dose with optimal image quality.

Data processing and interpretation

Since a large volume of data is acquired, the images are best interpreted at the scanner console or remote workstation. The soft-tissue structures and lung parenchyma can be assessed adequately in axial sections of 5 mm thickness with mediastinal and lung window settings, respectively (Figure 1A and B). High resolution CT images allow detailed evaluation of the pulmonary parenchyma. The tracheobronchial airway can be evaluated on thinner sections and reformatted images.

Two-dimensional maximum intensity projection images (Figure 1C) in the coronal/oblique and sagittal planes can demonstrate the tortuous course of the bronchial arteries from the descending thoracic aorta to the lungs. Intercostal and internal mammary arteries are best visualized in the coronal planes while the inferior phrenic arteries and celiac axis branches are demonstrated well in axial images. Three-dimensional volumetric and shaded-surface-display images not only depict the abnormal vessel, but also illustrate its relationship with the surrounding structures, thus providing a preview of the internal anatomy.

Minimum Intensity Projection can be used to generate images of the central airways (Figure 1D) and demonstrate areas of air-trapping within the lungs. These can provide valuable perspective in defining a lesion prior to any intervention. Therefore, a host of reconstructed images need to be analyzed for a thorough CT assessment of hemoptysis.

STEPWISE STRUCTURAL ASSESSMENT

Lung parenchyma

The pulmonary parenchyma should be evaluated for presence of bronchiectasis, consolidation and ground-glass opacity. The site of hemorrhage can be localized on the basis of presence of fluid density material in the segmental and lobar bronchi and ground-glass opacity with hazy consolidation which represents alveolar hemorrhage. Acute hemorrhage can mask the underlying pathology. Blood clots can also simulate more ominous entities like masses.

Tracheobronchial tree

This should be evaluated for presence of any stenosis which may be due to intraluminal (foreign body, neoplasm) or extraluminal (lymphadenopathy, fibrosing mediastinitis) causes. Multiplanar reformatted (MPR) images are accurate in detection of lesions, depiction of degree of narrowing, distal visualization and calculation of distance of the lesion from the carina in selected locations^[11].

Pulmonary arteries

The pulmonary arteries should be analyzed for any narrowing due to extrinsic or intrinsic causes. The presence

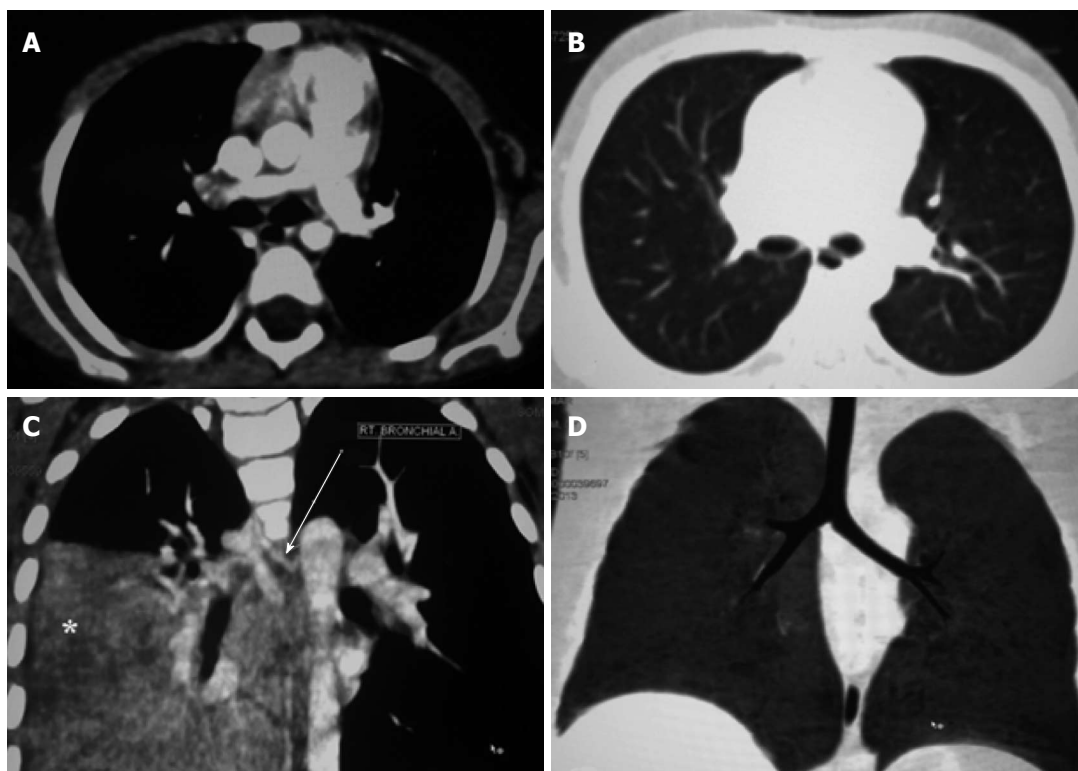


Figure 1 Multi-detector computed tomography image interpretation. Axial computed tomography image showing mediastinal window (A) and lung window (B). Coronal maximum intensity projection image (C) demonstrating the origin and proximal portion of the right bronchial artery (arrow). There is consolidation in the right lower lobe (asterisk). Coronal Minimum Intensity Projection image (D) delineating the central airways.

of accompanying subpleural areas of enhancement can represent lung infarcts. The pulmonary arteries can also show dilatation (Rasmussen aneurysm) and pulmonary arteriovenous malformations.

Bronchial arteries

Hemoptysis originates from bronchial arteries in 95% of cases^[12]. A bronchial artery diameter of more than 2 mm is considered abnormal^[13]. In 70% of individuals, bronchial arteries arise from the descending thoracic aorta between T5 and T6 levels. There are usually one or two bronchial arteries supplying each lung, arising independently or from a common trunk. They are visualized as a cluster of enhancing nodules in the posterior mediastinum just below the level of the aortic arch on axial images. Active bleeding can rarely be detected on CT. Anomalous bronchial arteries are defined as arteries which originate outside the T5-T6 level. Their most common site of origin is the concavity of the aortic arch^[14].

Non-bronchial systemic arteries

Non-bronchial systemic arteries can arise from the branches of brachiocephalic arteries, subclavian arteries, axillary, internal mammary and infradiaphragmatic branches from the inferior phrenic artery and celiac axis^[15,16]. On CT, these are seen as dilated tortuous arteries that are not parallel to the bronchi. The presence of pleural thickening greater than 3 mm with enhancing arteries within the extrapleural fat is a pointer of presence of these vessels^[17].

MRI

MRI does not have any utility in imaging evaluation of acute hemoptysis. Since it has superior soft-tissue resolution, it is excellent in the evaluation of the mediastinum and hilum in the non-emergent setting. It provides less information about the lung parenchyma. It may be used to demonstrate arteriovenous malformations and congenital anomalies of the pulmonary arteries and delineate the nature of mediastinal soft-tissue in fibrosing mediastinitis. With the introduction of hyperpolarized nuclei like ^3He and ^{129}Xe , the horizon of MRI is likely to expand from limited utility in evaluating the pulmonary parenchyma to evaluation of pulmonary structure, function and metabolism with a high sensitivity^[18]. Ventilation and dynamic imaging in patients with asthma and cystic fibrosis have shown regional patterns of obstruction and ventilation defects in these individuals. Further knowledge can go a long way in the early diagnosis, monitoring disease progression and evaluation of response to treatment^[19-21].

COMMON CAUSES OF PEDIATRIC HEMOPTYSIS

Acute lower respiratory tract infections

Tracheobronchitis, pneumonia and lung abscess can lead to hemoptysis. The infective process may be bacterial, viral, fungal or parasitic in origin. Although tuberculosis is a significant cause of adult hemoptysis, very few cases have been reported in the pediatric literature^[22]. Chest radio-

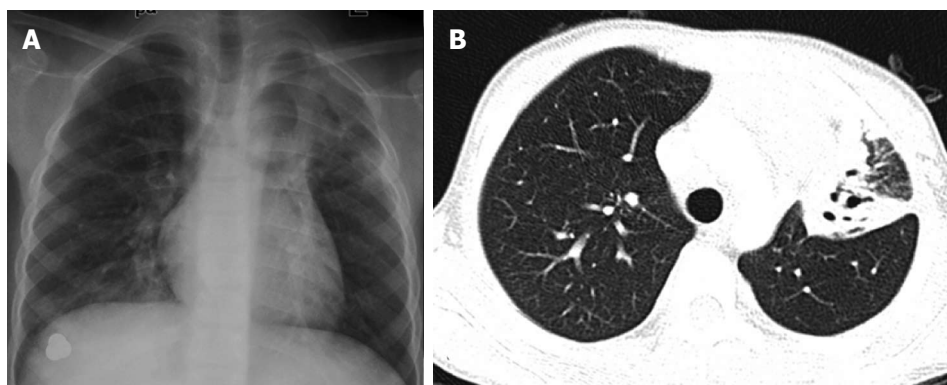


Figure 2 Chest radiograph (A) and axial computed tomography image (B) showing consolidation with cavitation in the left upper lobe.

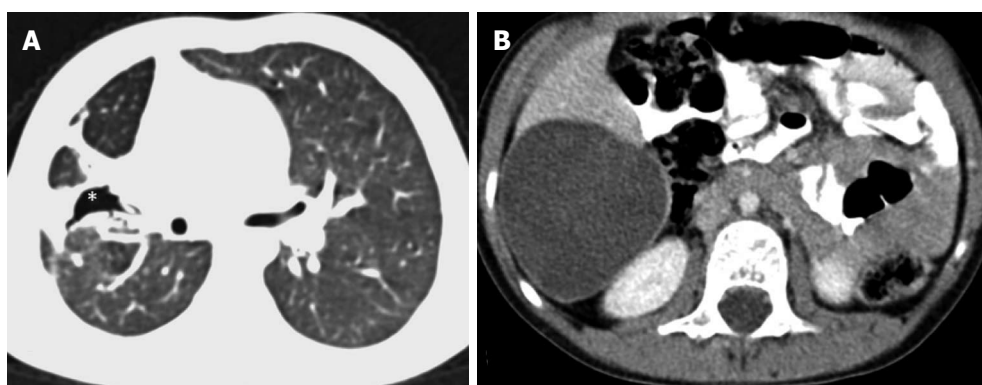


Figure 3 A seven-year-old girl with ruptured pulmonary hydatid cyst. Axial computed tomography image (A) showing the ruptured cyst with air (asterisk) in the right upper lobe along with surrounding consolidation. Axial section of the abdomen (B) shows an unruptured cyst in the segment VI of the liver.

graphs can show pulmonary infiltrates, hyperinflation and cavity with or without air-fluid level (Figure 2A). There may be associated pleural effusion and lymphadenopathy. CT findings can be in the form of consolidation, ground-glass opacity, interstitial thickening, air-trapping, cavity with shaggy walls and air-fluid level, pleural effusion and mediastinal or hilar lymphadenopathy (Figure 2B). CT can also demonstrate complications like empyema (thick enhancing visceral and parietal pleura, “split pleura” sign), bronchopleural fistula, *etc.*

Parasitic cysts (*Echinococcus*) can cause hemoptysis by rupturing into the airway. These may be seen as fluid density lesions with a smooth wall and air foci due to communication with the adjacent bronchus (Figure 3A). Detached membranes and daughter cysts can be visualized within the cyst. Concomitant cysts may be seen in other organs, most commonly in the liver (Figure 3B).

The most commonly implicated fungus is *Aspergillus*. It can have a varied spectrum of presentation, namely aspergilloma, allergic bronchopulmonary aspergillosis (ABPA), semi-invasive aspergillosis, airway or angioinvasive aspergillosis^[23,24]. Aspergilloma is the saprophytic colonization of a pre-existing cavity by the fungus and is typically seen as an opacity within a cavity producing the “air-crescent” sign. It is mobile and can show postural change in position. ABPA is a manifestation of type I and III hypersensitivity reaction to the organ-

ism and presents as central bronchiectasis with mucous plugged bronchi producing ‘finger-in-glove’ appearance with upper lobe preponderance on radiograph. The mucous plugs have a high-density on CT (Figure 4). There may be centrilobular nodules with “tree-in-bud” appearance. Some patients can also have associated allergic fungal sinonasal disease. Invasive aspergillosis is encountered in immunocompromised patients. Invasive airway disease presents as peribronchial areas of consolidation and multiple branching centrilobular nodules on CT^[25]. Nodules with surrounding ground-glass opacity (halo sign) or pleural-based, wedge shaped areas of consolidation (Figure 5) are the hallmark of angioinvasive aspergillosis^[26].

Bronchiectasis

Bronchiectasis can occur secondary to aspiration, infections, cystic fibrosis and ciliary dyskinesias. On chest radiographs, it manifests as “tram-track”, parallel line opacities, ring opacities and tubular structures (Figure 6A). However, chest radiographs are insensitive for detecting mild to moderate disease. CT (Figure 6B) has a higher sensitivity and on CT imaging, bronchiectasis is characterized by the absence of normal bronchial tapering, the presence of visible bronchi in the peripheral 1 cm of the lung and a bronchoarterial ratio more than 1 (signet ring sign). The etiology can be narrowed by considering the anatomic location and distribution of pathology.

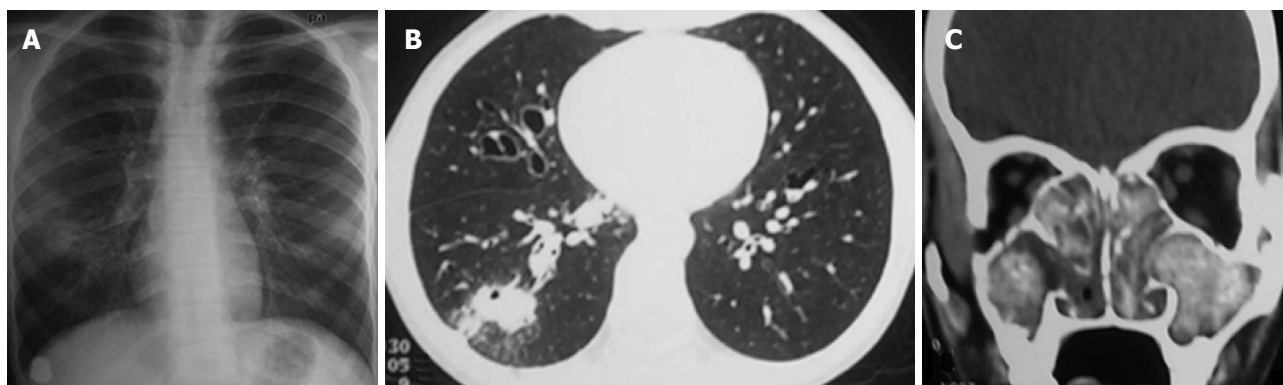


Figure 4 A 12-year-old girl with allergic bronchopulmonary aspergillosis. Frontal chest radiograph (A) and axial computed tomography (CT) image (B) showing tubular opacities with consolidation in the right lung suggestive of mucocoeles along with cystic bronchiectasis in bilateral lungs. Coronal CT image (C) of the patient showing evidence of bilateral allergic fungal rhinosinusitis.

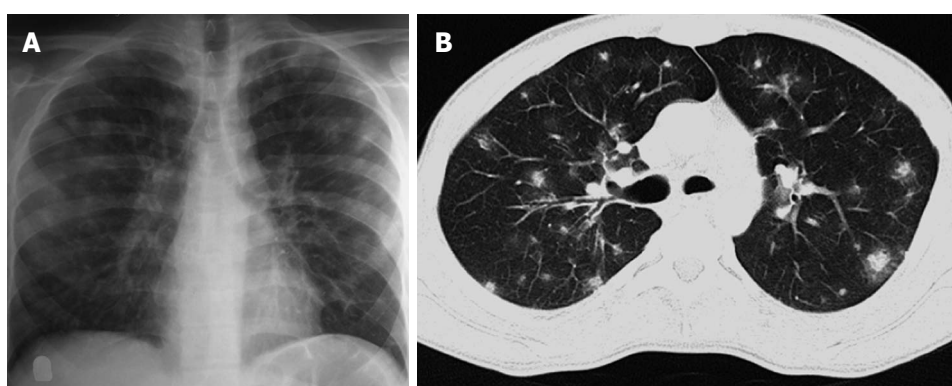


Figure 5 A 17-year-old boy with acute lymphocytic leukemia along with angioinvasive aspergillosis. Chest radiograph (A) showing multiple fluffy nodules in bilateral lung fields. High resolution CT image (B) of the same patient shows multiple nodules with surrounding ground glass opacity (halo sign).

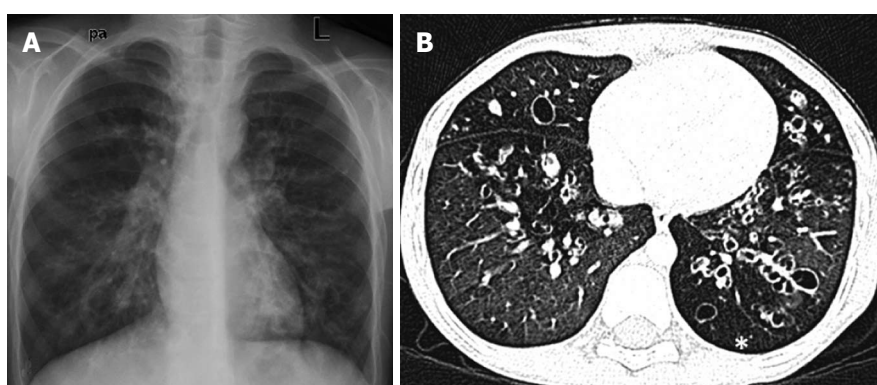


Figure 6 A 10-year-old boy with post-infectious bronchiectasis. Frontal chest radiograph (A) showing multiple cystic lucencies and tubular opacities in both lungs. Chest high resolution CT image (B) shows multiple areas of cystic bronchiectasis with associated air trapping (asterisk).

Aspiration tends to involve the lower lobes (right > left). Cystic fibrosis shows lung hyperinflation and interstitial infiltrates with upper lobe preponderance (Figure 7). Bronchiectasis due to ciliary dyskinesias has a lower lobe predisposition^[27].

Congenital heart disease

Hemoptysis can occur in patients with congenital heart diseases associated with pulmonary artery or venous stenosis or atresia. This is attributed to hemorrhage from enlarged, tortuous aorto-pulmonary collateral arteries and thrombotic

lesions in the small pulmonary arteries^[28]. Chest radiography may show cardiomegaly with abnormality in cardiac silhouette and a small hilum. An abnormal vascular channel parallel to the right cardiac border (scimitar vein) can be seen in pulmonary venolobar hypoplasia^[29]. There may be associated pulmonary volume loss. MDCT is the modality of choice to demonstrate the site and extent of pulmonary artery narrowing and delineate anomalous pulmonary venous drainage (Figure 8). It exquisitely depicts the various aorto-pulmonary collaterals. Other associated cardiac anomalies can also be evaluated^[30].

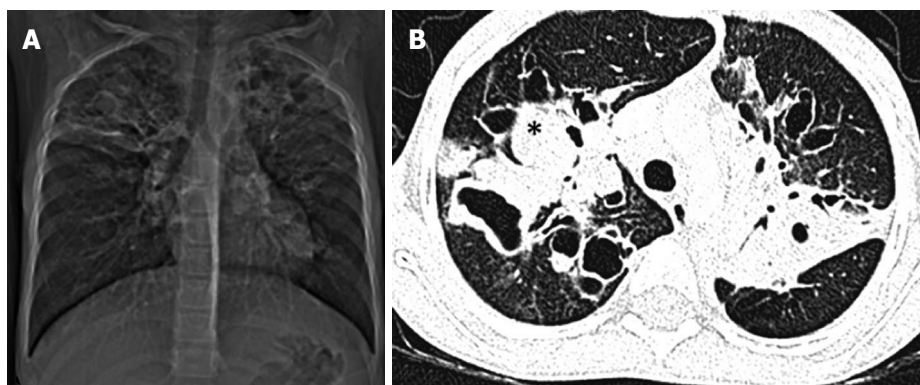


Figure 7 A 7-year-old boy with cystic fibrosis. Computed tomography (CT) scout image (A) and axial CT chest image (B) showing bilateral upper lobe bronchiectasis with bronchocele formation (asterisk) due to mucous plugging and sparing of lower zones.

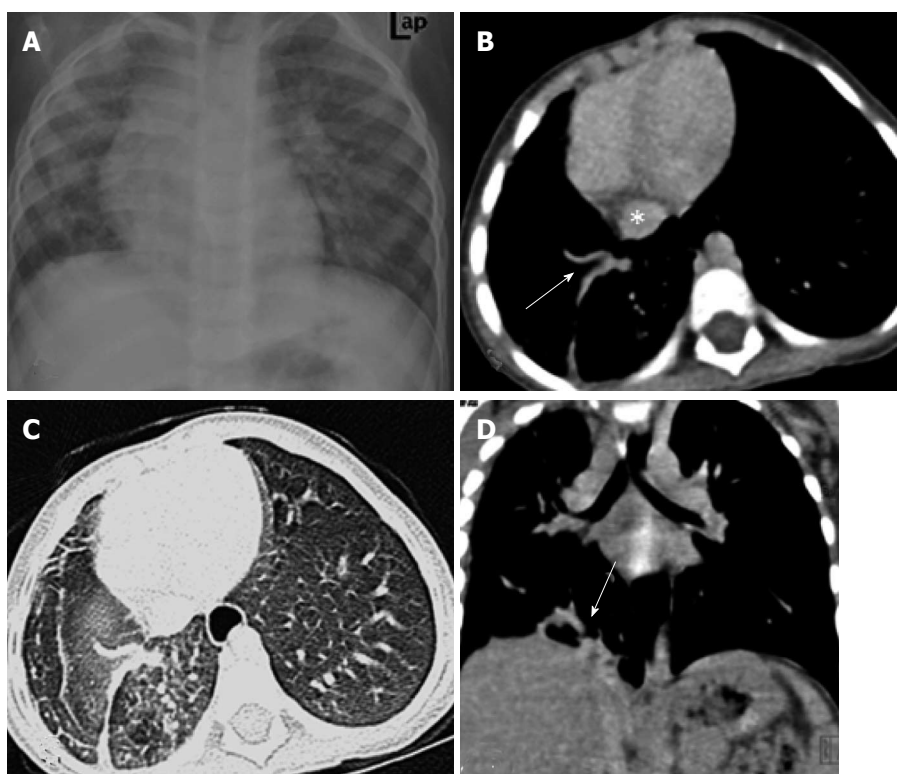


Figure 8 Pulmonary venolobar hypoplasia. Chest radiograph (A) shows volume loss of the right hemithorax with ipsilateral mediastinal shift. Contrast-enhanced computed tomography images (B-D) showing anomalous right inferior pulmonary vein (arrows) coursing inferiorly towards the inferior vena cava (asterisk).

Pulmonary artery narrowing

Chronic pulmonary artery narrowing can occur due to a variety of causes like infections, inflammation and thromboembolism^[31]. Infections are the most common cause. Narrowing of the pulmonary artery can be caused in the setting of infection by mediastinal lymphadenopathy or fibrosis. Fibrosis may be focal or diffuse. CT finding of focal fibrosis is a calcified soft-tissue mass in the paratracheal and hilar location. It can occur secondary to tuberculosis in the developing countries and histoplasmosis in United States. The diffuse form manifests as an infiltrative, non-calcified soft-tissue mass extending into multiple mediastinal compartments. It can be associated with autoimmune disorders, drugs, or be idiopathic^[32]. Pulmonary artery narrowing in these cases leads to pulmonary hypoperfusion and consequent bronchial artery hypertrophy while can lead to hemoptysis of varying severity. CT pulmonary angiography is the investigation of choice in this condition as it elucidates pulmonary artery

narrowing and bronchial/systemic artery hypertrophy^[33].

Pulmonary arteriovenous malformations

Pulmonary arteriovenous malformations (PAVM) are direct communication between the branches of the pulmonary artery and veins without capillary bed. There is a strong association between PAVM and hereditary hemorrhagic telangiectasia^[34]. Chest radiography is an important tool for diagnosis and follow-up. Classic findings of PAVM are a round or oval well-defined mass, frequently lobulated, ranging in size from 1-5 cm. Two-thirds of these are located in the lower lobe. A connecting vessel may be seen radiating from the hilum. MDCT can identify the PAVM and connecting vessels more accurately (Figure 9). PAVMs have rapid blood flow and hence produce a low intensity signal on MRI. Catheter angiography remains the gold standard in diagnosis of PAVM as it defines the angio-architecture which is necessary before therapeutic embolization or surgical resection.

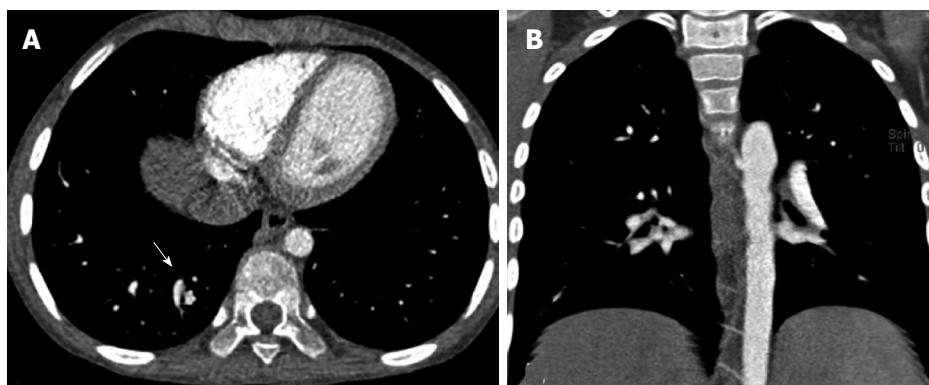


Figure 9 Pulmonary arteriovenous malformation. Axial (A) and coronal (B) computed tomography images showing abnormal communication between branches of the pulmonary artery and vein in right lower lobe (arrows).

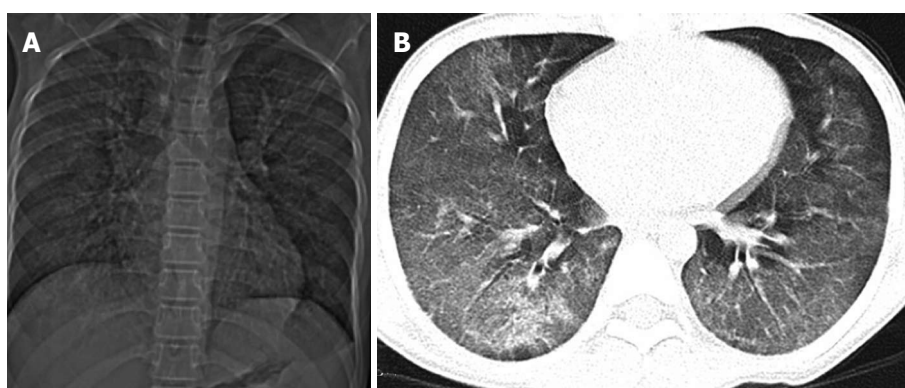


Figure 10 A 15-year-old boy with idiopathic pulmonary hemosiderosis. Scout computed tomography (CT) image (A) and axial CT image (B) showing diffuse ground glass opacity in bilateral lungs.

Idiopathic pulmonary hemosiderosis

Idiopathic pulmonary hemosiderosis (IPH) is a rare pulmonary disorder which manifests as a triad of hemoptysis, anemia and diffuse parenchymal infiltrates on chest radiographs^[35]. Diagnosis is confirmed by detection of hemosiderin-laden macrophages in broncho-alveolar lavage fluid, sputum or gastric aspirate. Secondary hemosiderosis is associated with systemic vasculitis, bleeding disorders and cardiac disease. Imaging findings are non-specific and need to be correlated with clinical and laboratory data to arrive at a diagnosis of IPH. Chest radiographs may reveal symmetric diffuse or patchy alveolar shadows sparing lung apices, which can clear on follow-up imaging. CT can show diffuse or patchy ground-glass opacity (Figure 10). There can be interstitial thickening in some cases^[36].

Foreign body

Foreign body aspiration can be a cause of hemoptysis primarily in patients less than 3 years of age. The aspirated foreign body can be visualized on a radiograph if it is radio-opaque. Associated radiographic findings include non-specific infiltrates, atelectasis, areas of hyperinflation, parenchymal consolidation or bronchiectasis (Figure 11A). Chest radiographs can be normal in 30% cases.

MPR and endoluminal virtual bronchoscopic images derived from MDCT can delineate the shape, location and volume of a foreign body. It can reveal associated pulmonary parenchymal changes (Figure 11B and C). Thus, imaging can help the surgeon plan the bronchoscopy for safe removal of foreign body^[37].

Neoplasm

Bronchial neoplasms are a rare cause of hemoptysis (Figure 12). Bronchial carcinoid tumors are the most frequent primary pulmonary neoplasms of childhood. The lesion can be central or peripheral. Radiological findings include hilar or perihilar masses with lobulated margins and associated obstructive changes (atelectasis, consolidation, bronchocele or hyperinflation)^[38]. On CT, carcinoid is seen as a well-defined, centrally located mass that narrows or deforms the airway and contains diffuse or punctuate calcification. It shows intense homogenous contrast enhancement. However, all carcinoids do not enhance. There can be associated pulmonary obstructive changes and mediastinal/hilar lymphadenopathy^[39].

CONCLUSION

Hemoptysis is a distressing symptom for the child, the



Figure 11 Foreign body aspiration. Chest radiograph (A) shows a radio-opaque foreign body in the left main bronchus (arrow) with hyperinflation of the left lung. Axial computed tomography images (B, C) delineate the morphology of the foreign body in the left main bronchus causing luminal compromise. There is associated air trapping in the left lung with patchy consolidation in the apical segment of the lower lobe.



Figure 12 Bronchial carcinoid. Scout computed tomography (CT) image (A) revealing non-visualization of the right main bronchus with volume loss and opacification of the right hemithorax along with bronchiectasis in right lower zone. Axial CT image (B) shows a mass in the right lung with mediastinal infiltration. Coronal Minimum Intensity Projection image (C) shows the outline of the mass projecting in the right main bronchus along with bronchiectasis in the right lower lobe.

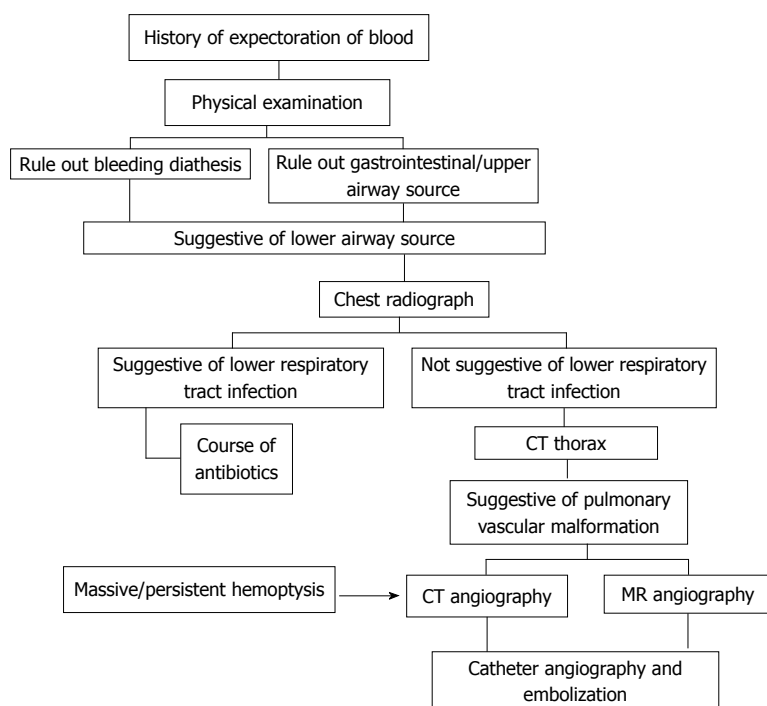


Figure 13 Flowchart depicting approach to a child presenting with hemoptysis. MR: Magnetic resonance; CT: Computed tomography.

family and the pediatrician. It poses a diagnostic challenge. Once the presence of hemoptysis has been ascertained, one needs to identify the source of bleeding and primary underlying cause. Acute lower respiratory tract infections are the most common cause of pediatric hemoptysis. The imaging modalities available for the work-up of hemoptysis include chest radiography, MDCT, MRI and catheter angiography. Chest radiographs may be normal in 30% cases. MDCT is a rapid, non-invasive multiplanar imaging modality which should be tailored to keep radiation dose to a minimum for optimal evaluation of hemoptysis in pediatric patients. MRI and catheter angiography have selected application. The use of the various imaging tools available is determined by the clinical presentation and the possible etiology (Figure 13). Maximum diagnostic and therapeutic benefit can be attained by the judicious use of imaging modalities in a child presenting with hemoptysis.

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Tramadol use in pediatric sickle cell disease patients with vaso-occlusive crisis

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Abstract

AIM: To evaluate whether the addition of scheduled oral tramadol to intravenous morphine and intravenous ketorolac reduces morphine requirements.

METHODS: This single-centered, Institutional Review Board-approved, retrospective study at Moses Cone Memorial Hospital included pediatric patients who were ≥ 2 years old with vaso-occlusive crisis (VOC) caused by sickle cell disease (SCD), were on morphine patient-controlled analgesia (PCA), and had scheduled oral tramadol added to their standard pain regimen. The study population was admitted between March 2008 and March 2011. The data was collected from electronic records and included age, weight, morphine use, tramadol use, hemoglobin, pain scores, number of days on PCA, length of hospital stay, respiratory rate, and polyethylene glycol use. Thirty patients were analyzed as independent admissions and seven patients as paired admissions.

RESULTS: Eighteen pediatric SCD patients with VOC received morphine PCA and intravenous ketorolac and

twelve patients received morphine PCA and intravenous ketorolac and scheduled oral tramadol. Baseline characteristics were similar between both groups with the exception of the average weight, which was greater in the tramadol group than in the morphine group. The average morphine requirements in patients with and without the use of tramadol were similar, both for the independent admissions [0.58 mg/kg per day vs 0.65 mg/kg per day ($P = 0.31$)] and the paired admissions [0.71 mg/kg per day vs 0.77 mg/kg per day ($P = 0.5$)]. The daily polyethylene glycol requirement was less in the tramadol group for both the independent [0.5 g/kg per day vs 0.6 g/kg per day ($P = 0.64$)] and paired admissions analyses [and 0.41 g/kg per day vs 0.55 g/kg per day ($P = 0.67$)].

CONCLUSION: The addition of scheduled tramadol in patients receiving concomitant morphine and ketorolac demonstrates a trend toward decreased morphine and polyethylene glycol use.

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Key words: Pediatrics; Sickle cell; Tramadol; Morphine; Vaso-occlusive crisis

Core tip: A small clinical study has shown that balanced analgesia using intravenous morphine, intravenous ketorolac, and intravenous tramadol followed by erythrocytapheresis was effective, as shown by pain relief and significant improvement in mood and sleep, in seven sickle cell disease patients aged three to twenty-eight years who presented with vaso-occlusive crisis. The objective of this study is to evaluate whether the addition of scheduled oral tramadol to intravenous morphine plus intravenous ketorolac provides adequate pain relief, and reduces morphine requirements, adverse effects, length of patient-controlled analgesia therapy, and length of hospital stay.

Borgerding MP, Absher RK, So TY. Tramadol use in pediatric

sickle cell disease patients with vaso-occlusive crisis. *World J Clin Pediatr* 2013; 2(4): 65-69 Available from: URL: <http://www.wjgnet.com/2219-2808/full/v2/i4/65.htm> DOI: <http://dx.doi.org/10.5409/wjcp.v2.i4.65>

INTRODUCTION

One of the major causes of hospitalization for patients with sickle cell disease (SCD) is vaso-occlusive crises (VOC). The hallmark characteristics of VOC include organ damage and pain due to the presence of dense red blood cells. The pain is generated through multiple pathways including somatic, neuropathic, and vascular mechanisms^[1-4].

Balanced analgesia is a strategy based on the co-administration of drugs with different pharmacological mechanisms in order to control pain at different origins, improve the efficacy of treatment, and reduce adverse effects of each drug^[5]. The administration of ketorolac and tramadol has been proven as a form of effective balanced analgesia, particularly for post-operative pain and pain caused by trauma^[6]. Tramadol and its active metabolite (M1) work by binding to the mu-opioid receptors in the central nervous system (CNS) and inhibiting the reuptake of norepinephrine and serotonin, causing inhibition of the ascending pain pathway and altering the perception of and response to pain^[7]. Tramadol is clinically known to have a better safety profile than the other major opioids, causing less respiratory depression and constipation.

In 2005, de Franceschi *et al.*^[8] published a study evaluating balanced analgesia using intravenous (*iv*) morphine, *iv* ketorolac, and *iv* tramadol followed by erythrocytapheresis in seven SCD patients aged three to twenty-eight years who presented with VOC. The co-administration of tramadol and ketorolac was effective in all VOC, as shown by pain relief and significant improvement in mood and sleep. The use of erythrocytapheresis, which is not available at our hospital, Moses Cone Memorial Hospital (MCMH), likely contributed to pain relief in this study.

At MCMH pediatric patients who presented prior to August 2010 with VOC caused by SCD were routinely prescribed *iv* morphine and *iv* ketorolac for pain control. However, because of the high morphine requirement in this patient population, severe respiratory depression and constipation can occur. After August 2010, pediatric physicians began adding scheduled oral (*po*) tramadol to the standard regimen of *iv* morphine patient-controlled analgesia (PCA) and *iv* ketorolac in an attempt to reduce narcotic-induced side effects. The objective of this retrospective study is to evaluate whether the addition of scheduled *po* tramadol to *iv* morphine and *iv* ketorolac reduces morphine requirements, provides adequate pain relief, decreases length of hospital stay, and reduces severe respiratory depression, severe constipation, and length of PCA therapy.

MATERIALS AND METHODS

This single-centered, Institutional Review Board (IRB)-approved, retrospective study included pediatric patients who were ≥ 2 years old with VOC caused by SCD, were on PCA morphine and *iv* ketorolac and had scheduled *po* tramadol added to their regimen. Tramadol was dosed at 1-2 mg/kg per dose *po* every four to 6 h (max: 400 mg/d and 100 mg/dose) and ketorolac at 0.5 mg/kg per dose *iv* every 6 h (Max: 30 mg/dose). Morphine PCA orders included a basal rate, intermittent dose, lockout interval, and a 1-hour and 4-hour limit. Using the International Classification of Diseases (ICD)-9 code for SCD, all patients < 21 years old who were admitted between March 2008 and March 2011 were included in this retrospective review. Patients were excluded from the review if they did not have a diagnosis of VOC or did not receive morphine PCA. The data was collected from electronic records and included age, weight, morphine use, tramadol use, hemoglobin, pain scores, number of days on PCA, length of hospital stay, respiratory rate, and polyethylene glycol (PEG) use. All patients were analyzed as independent admissions. Additionally, patients with multiple admissions during the study period (at least one with morphine only and one with both morphine and tramadol) were analyzed as paired admissions, acting as their own controls.

The primary outcome of this study was average daily morphine requirement. Secondary outcomes included average pain scores, respiratory rate, PEG dose, length of PCA therapy and number of days in the hospital.

All patients were analyzed as independent admissions using the Wilcoxon Rank Sum test. Patients who had multiple admissions, one with tramadol use and one without were also analyzed as paired admissions using the Wilcoxon Signed Rank test. The statistical analysis was completed using Stata, Version 10.1 (Cary, NC).

RESULTS

Between March 2008 and March 2011 eighteen pediatric SCD patients with VOC received morphine PCA and *iv* ketorolac and twelve patients received morphine PCA plus *iv* ketorolac and scheduled *po* tramadol. Baseline characteristics were similar between both groups with the exception of the average weight, which was greater in the tramadol group than in the morphine group because the latter group had a younger sample (Table 1).

Average morphine requirements with and without tramadol were similar in the independent admission analysis [0.58 mg/kg per day and 0.65 mg/kg per day, respectively ($P = 0.31$)]. Average morphine requirements with and without tramadol were also similar in the paired admissions analysis [0.71 mg/kg per day and 0.77 mg/kg per day, respectively ($P = 0.5$)]. Contradictory to what was expected, pain scores were higher when tramadol was added to the pain regimen for both the independent admissions (6.75 *vs* 5) and the paired admissions (6.5 *vs* 5.5). The daily

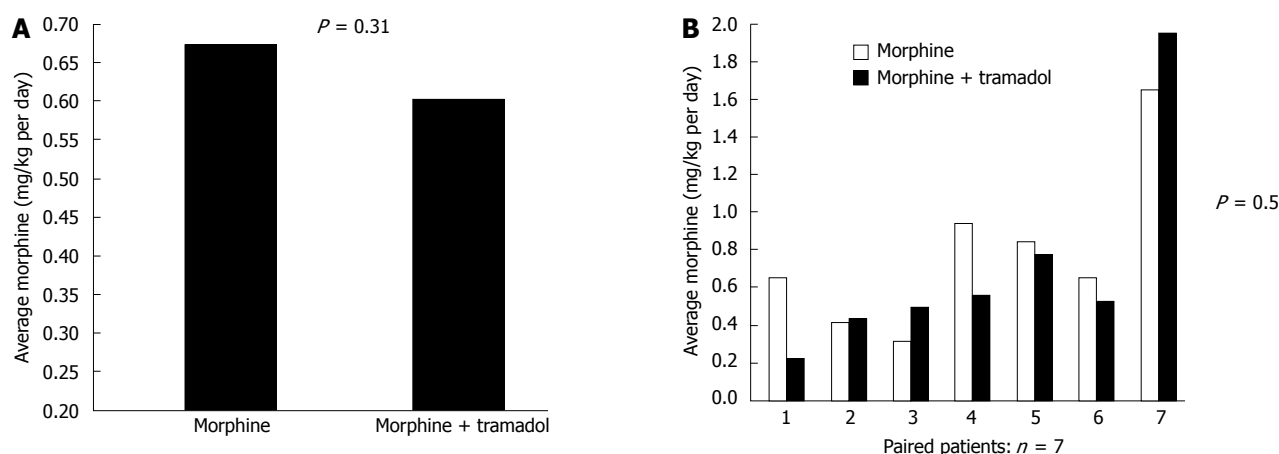


Figure 1 Average morphine requirement for independent admissions (A) and average morphine requirement for paired admissions (B).

Table 1 Baseline characteristics

	Morphine	Morphine + tramadol
Sample size	18	12
Age (yr), Mean (range)	11.5 (3-20)	13.4 (7-20)
Gender		
Male	9	6
Female	9	6
Weight (kg), mean (range)	38.7 (11.6-77.4)	52 (27.0-77.4) ^a
Hemoglobin (mg/dL), mean (range)	9.0 (6.0-11.9)	9.4 (6.5-12.0)

^a $P < 0.05$ vs morphine.

polyethylene glycol requirement was less in the tramadol group for both the independent and paired admissions analyses (0.5 g/kg per day *vs* 0.6 g/kg per day and 0.41 g/kg per day *vs* 0.55 g/kg per day, respectively) but neither difference was statistically significant ($P = 0.64$ and 0.67 , respectively). The paired admissions analysis demonstrated a greater difference in PEG requirements which, while not statistically significant, may provide a more accurate comparison. Furthermore, there was no difference in the length of stay, number of days on PCA, or respiratory rate between groups in either analysis (Tables 2 and 3).

DISCUSSION

The addition of scheduled oral tramadol in patients receiving concomitant morphine and ketorolac did result in numerically lower average daily morphine requirements (Figure 1A) and polyethylene glycol use; however, differences in these endpoints were not statistically significant (Table 2). In the paired admissions analysis, four of the six patients who received less than 1 mg/kg per day of morphine used less morphine when tramadol was added to their regimen (Figure 1B); however, pain scores did not correlate with the decreased morphine requirement.

The lack of correlation between pain scores and morphine requirement may be due to the subjectivity of pain. After completion of the study, a pediatric psychiatrist

spoke with several patients in the study and found that many patients did not understand how to use the visual analogue pain scale or the numeric scale to rate their pain. When asked to rate their pain as red, yellow or green (red corresponding to the most pain and green the least), patients gave more accurate representations of their true pain level. Extensive education on rating pain would be required to provide a more precise representation of pain relief with and without the use of tramadol.

There were several limitations to this study. The retrospective nature of the study forced us to rely on electronic nursing documentation for all of our data collection, which may have resulted in some inaccurately charted data. Frequently, bowel movements were not documented. We therefore measured average daily polyethylene glycol use to assess constipation. Additionally, we were unable to stratify patients based on their basal morphine PCA rate and determine how much of their daily morphine requirement was due to demand dosing, as this information was not documented electronically. Patients with multiple admissions for VOC can develop tolerance to narcotics, resulting in an increased morphine basal rate requirement. Documentation of this may have provided a more accurate assessment of which analgesic regimen provided better pain control and fewer narcotic-induced side effects.

VOC most commonly involves the back, legs, knees, arms, chest and abdomen. The location of the vaso-occlusive crisis has a significant impact on the intensity of pain and the ability to control that pain; however, this study did not stratify patients based on VOC location or disease severity^[9]. Additionally, the disease severity has interpatient and inpatient variability making it more difficult to compare patients. Also, one patient in this study had drug-seeking behavior, which may have skewed the results causing increased average morphine requirement and pain scores.

A larger, controlled study would be more likely to determine statistical difference in the primary and secondary endpoints. Pediatric physicians at MCMH no longer routinely prescribe tramadol in this population but con-

Table 2 Independent admissions statistical analysis

	Morphine (<i>n</i> = 18)	Morphine + tramadol (<i>n</i> = 12)	<i>P</i> -value ¹
Morphine requirement (mg/kg per day), mean ± SD	0.65 ± 0.35	0.58 ± 0.48	0.31
Pain score, median (range)	5 (0-8)	6.75 (3-9)	0.07
PCA duration (d), median (range)	4.5 (0-13)	5 (3-14)	0.33
LOS (d), median (range)	7 (4-14)	7.5 (3-17)	0.85
Respiratory rate, mean (range)	21 (14-29)	19 (16-23)	0.28
Polyethylene glycol dose (gm/kg per day), mean (range)	0.6 (0-1.66)	0.5 (0-1.12)	0.64

¹*P*-value calculated using Wilcoxon Rank Sum test. PCA: Patient-controlled analgesia; LOS: Length of stay.

Table 3 Paired admissions statistical analysis (*n* = 7)

	Morphine	Morphine + tramadol	<i>P</i> -value ¹
Morphine requirement (mg/kg per day), mean ± SD	0.77 ± 0.44	0.71 ± 0.57	0.50
Pain score, median (range)	5.5 (2-8)	6.5 (3-8)	0.27
PCA duration (d), median (range)	6 (4-13)	7 (3-10)	0.35
LOS (d), median (range)	7 (5-14)	9 (3-12)	0.61
Respiratory rate, mean (range)	19 (14-20)	19 (17-23)	0.87
Polyethylene glycol dose (gm/kg per day), mean (range)	0.55 (0.28-1.1)	0.41 (0.28-0.98)	0.67

¹*P*-value calculated using Wilcoxon Rank Sum test. PCA: Patient-controlled analgesia; LOS: Length of stay.

tinue to use it in patients who have clinically shown improved pain control with tramadol in the past. Tramadol use is appropriate in this population as it has proven safe, usually causing no additional side effects and potentially providing some benefit in controlling pain and reducing narcotic-induced constipation. If tramadol continues to be clinically beneficial for pain control in other patients in this population, it may be possible to review our primary and secondary endpoints on a larger scale to determine any true differences.

ACKNOWLEDGEMENTS

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COMMENTS

Background

Pain is a hallmark of vaso-occlusive crisis (VOC) caused by sickle-cell disease (SCD). Intravenous (*iv*) morphine plus *iv* ketorolac is generally the combination of choice for VOC in SCD patients at Moses Cone Memorial Hospital (MCMH); however, morphine can cause severe respiratory depression and constipation. The objective of this study is to evaluate whether the addition of scheduled oral tramadol to *iv* morphine plus *iv* ketorolac provides adequate pain relief, and reduces morphine requirements, adverse effects, length of PCA therapy, and length of hospital stay.

Research frontiers

In 2005, de Franceschi *et al* published a study evaluating balanced analgesia using *iv* morphine, *iv* ketorolac, and *iv* tramadol followed by erythrocytapheresis in seven SCD patients aged three to twenty-eight who presented with VOC. The co-administration of tramadol and ketorolac was effective in all VOC, as shown by pain relief and significant improvement in mood and sleep.

Innovations and breakthroughs

This is the first article evaluating the use of oral tramadol in addition to *iv* morphine and ketorolac on whether this combination provides adequate pain relief, and reduces morphine requirements, adverse effects, length of PCA therapy, and length of hospital stay.

Applications

The study results suggest that the addition of scheduled oral tramadol in patients receiving concomitant morphine and ketorolac demonstrates a trend toward decreased morphine and polyethylene glycol use.

Terminology

Erythrocytapheresis is a process in which red blood cells are extracted from the whole blood.

Peer review

This is a very good clinical study in which the authors analyzed the efficacy of adding oral tramadol to usual pain regimen used for sickle cell pain crisis.

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Pediatric vs adult pulmonary tuberculosis: A retrospective computed tomography study

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tion, bronchiectasis and fibrosis and these were noted in 60% of children, 71% of adolescents and 76.9% of adults. These changes were more common in right upper lobe in all age groups. There was no significant difference in the frequency of these changes (except nodules) in different age groups. Centrilobular nodules were seen less commonly in children less than 10 years ($P = 0.028$). Pleural effusion was noted in 28 (18.42%) patients and pericardial effusion in 8 (5.3%) patients. No significant difference in the serosal involvement is seen among children and adults. Mediastinal adenopathy was seen 70% of children, 76.3% adolescents and 76.9% of adults and paratracheal nodes were seen most frequently. Nodes had similar features (except matting) among all age groups. Matting of nodes was seen more commonly in children ($P = 0.014$).

CONCLUSION: Pediatric chest tuberculosis can have severe parenchymal lesions and nodal involvement similar to adults. The destructive lung changes observed in children needs immediate attention in view of the longer life span they have and hence in formulating optimal treatment strategies.

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Abstract

AIM: To compare the manifestations of chest tuberculosis (TB) in pediatric and adult patients based on contrast enhanced computed tomography of chest.

METHODS: This was a retrospective study consisting of 152 patients of chest TB including 48 children and 104 adults who had undergone contrast enhanced computed tomography of chest prior to treatment. The patterns and severity of parenchymal, mediastinal and pleural manifestations were analyzed and compared among different age groups.

RESULTS: Parenchymal changes observed include consolidation, air space nodules, miliary TB, cavita-

Key words: Tuberculosis; Pulmonary; Primary tuberculosis; Children; Computed tomography

Core tip: Primary tuberculosis in children was traditionally thought to be distinct from reactivation tuberculosis in terms of location, pattern and severity. On the contrary, aggressive forms of pulmonary tuberculosis akin to adult forms are increasingly seen in pediatric clinical practice especially in adolescents. Our study revealed that similar to older patients, children with tuberculosis are equally prone to develop significant destructive lung changes with severe sequelae. Having longer life expectancy the impact is much more severe in children.

Moreover, the cavitating lesions with high bacterial load make them highly infective and pose an important threat to community health.

Thotton Veedu P, Bhalla AS, Vishnubhatla S, Kabra SK, Arora A, Singh D, Gupta AK. Pediatric vs adult pulmonary tuberculosis; a retrospective CT study. *World J Clin Pediatr* 2013; 2(4): 70-76 Available from: URL: <http://www.wjgnet.com/2219-2808/full/v2/i4/70.htm> DOI: <http://dx.doi.org/10.5409/wjcp.v2.i4.70>

INTRODUCTION

Pulmonary tuberculosis (TB) is a common lung infection worldwide with higher prevalence in developing countries. It continues to be a major medical and social problem with high morbidity and mortality. TB is second only to human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) as the greatest killer worldwide due to a single infectious agent. In 2011, 8.7 million people contracted tuberculosis out of which 1.4 million died from TB. About half a million children (0-14 years) fell ill with TB, and 64000 children died from the disease in 2011^[1]. The annual risk of tuberculous infection in children in developing countries is 2%-5%. About 8%-20 % of deaths due to tuberculosis occur in children^[2,3].

Tuberculosis in children is mostly related to primary infection and earlier studies stated that it present with various forms of relatively less aggressive primary tuberculosis^[4]. Traditionally it was thought that manifestations of primary tuberculosis are distinct from reactivation tuberculosis in terms of location and pattern^[5,6]. The most common form of pediatric TB, the classical primary complex consists of a focal parenchymal lesion typically in mid-lower zones with enlarged draining hilar/paratracheal node. Other presentations of primary TB include miliary TB^[7], exudative pleuritis and tracheo-bronchial TB^[8,9]. However contrary to the common notion, aggressive forms of pulmonary tuberculosis akin to adult forms are increasingly seen in pediatric clinical practice especially in adolescents^[10-13]. A thorough review of available literature did not reveal any comparative studies with computed tomography (CT) scan in pediatric and adult tuberculosis. Our retrospective study is aimed to compare the pulmonary manifestations of TB in pediatric and adult population.

MATERIALS AND METHODS

In this retrospective study we analyzed CT records of 152 patients of pulmonary tuberculosis who underwent CT scans from November 2010 to January 2013. The diagnosis of pulmonary tuberculosis was based on clinical/and radiographic/and pathologic criteria. Patients were presented with clinical symptoms such as cough for more than two weeks, fever, weight loss, hemoptysis or anorexia. Along with clinical features two out of four of the fol-

lowing criteria had to be met: (1) A positive mantoux test; (2) History of contact with a sputum positive patient of tuberculosis; (3) Radiographic findings of mycobacterium tuberculosis such as primary complex, miliary disease, cavitary lesion, or hilar adenopathy; and (4) Isolation of AFB from sputum, gastric aspirate or broncho-alveolar lavage, lymph node aspirate^[14-19]. Immunocompromised patients were excluded from the study. Informed consent and clearance from the local ethical committee was not required due to the retrospective nature of the study.

CT scans were performed either on Somatom Sensation 40 (Siemens, Erlangen, Germany) or Somatom Definition Flash (Siemens healthcare, Forchheim, Germany). Images were acquired after administering intravenous nonionic iodinated contrast [Iomeron 400 (Iomeprol, Bracco, Milano, Italy), Iohexol 300 (Omnipaque, GE Health care, Ireland)] which was injected by hand with an average delay of 50-70 s and thus providing venous phase images. Adult patients were given 60 mL of contrast and pediatric dose was calculated according to the body weight not exceeding 2 mL/kg.

Scanning was performed in adults with a collimation of 1.2 mm, a pitch of 1.4:1, a 512 × 512 matrix, field view of 38 cm, 120 kVp, and 100 mAs. In children images were acquired with a smaller field of view with 120 kVp and variable mAs (on Somatom Sensation 40) and variable kVp and mAs (on Somatom Definition Flash) according to the body thickness by tube current modulation.

Image reconstruction

After acquisition images were reconstructed in lung and mediastinal windows. Lung window images were reconstructed using sharp kernel (B60f) and a wide window width of 1500 HU with centre at -600 HU. Mediastinal window images are reconstructed using smooth kernel (B30f) and window width of 400 HU with centre at 40 HU. For both lung and mediastinal windows images were reconstructed with a thickness of 5mm in adults and 3 mm in children. HRCT images were reconstructed in lung window settings using ultra-sharp kernel (U80f) with section thickness of 1.5 mm. For HRCT reconstruction interslice gap was 10 mm in adults and 8 mm in children.

Image analysis

Images were reviewed by two radiologists, with 15 years and 7 years of experience in thoracic imaging and interpretations made by consensus. Images were qualitatively analyzed for the presence of parenchymal changes and lymph nodal involvement. Parenchymal changes such as consolidation, centrilobular nodules, miliary nodules, bronchiectasis, cavitation and fibrosis were assessed. For analyzing the zonal predominance and bulkiness of the disease both lungs were divided into upper, mid and lower zones. Lung field from apex to carina as upper zone, carina to the level of inferior pulmonary veins as mid zone and below as lower zone. Distribution of

abnormalities and total number of zones involved as a measurement of bulkiness of the disease were assessed. Mediastinal and hilar lymph nodes were assessed for the size, necrosis, matting and calcification. Other findings like pleural and pericardial effusion were noted.

Statistical analysis

For descriptive statistics the study population was divided into three groups; group A: children (less than or equal to 10 years), group B: adolescents (11-18 years) and Group C: adults (above 18 years). The incidence, pattern and severity of parenchymal changes and nodal involvement were compared among the groups. They were also divided as below and above 10 years; and below and above 18 years for the determination of statistical significance. Statistical analysis was done using stata statistical software (version 12.1). χ^2 test and Fisher's exact test were used for analysis. A *P* value of less than 0.05 was considered as significant.

RESULTS

The study group included 80 males and 72 females patients ranging in age from 3 mo to 96 years (mean 30 years). They were comprised of 10 children, 38 adolescents and 104 adults. Incidence and pattern of parenchymal changes and lymph nodal involvement were analyzed and compared among different groups (Figure 1).

We compared the parenchymal changes among pediatric, adolescent and adult patients. Parenchymal lesions were noted in 6 (60%) children, 27 (71%) adolescents and 80 (76.9%) adults (Table 1). Parenchymal changes were more common in right upper followed by right middle zone in all age groups. Higher incidence of changes noted in upper and middle zones than lower zones. Left lower zone is least commonly involved in patients older than 10 years. Among the patients with parenchymal disease, multiple zones (≥ 3) were seen to be involved in 3 (30%) children, 16 (42.1%) adolescents and 45 (56.25%) adult patients (Table 1). The average number of zones involved in children, adolescent and adult patients were 2.83, 3.33 and 3.32 respectively.

Among the 152 patients we studied, consolidation was found in 54 (35.53%), centrilobular nodules in 93 (61.18%), bronchiectasis in 26 (17.11%), miliary in 4 (2.63%), fibrosis in 22 (14.47%) and cavitation in 36 (23.84%) patients. There was no statistically significant difference in the incidence of consolidation, miliary nodules, bronchiectasis, cavitation and fibrosis among different age groups studied. Centrilobular nodules were seen less commonly in children (*P* = 0.028) (Table 1).

Mediastinal lymphadenopathy was seen in 7 (70%), 29 (76.3%) and 74 (71.2%) respectively in children, adolescents and adult patients (Table 1). Among the mediastinal lymph nodes right paratracheal is the most commonly involved followed by subcarinal in all age groups. Involvement of multiple nodal groups (≥ 2) was seen in 28 (58%) of younger patients (≤ 18 years) and 51 (49%) older pa-

tients. In less than 10 years category all the 7 (70%) children with significant lymphadenopathy had involvement of multiple nodal groups. Involvement of multiple nodes (≥ 2 groups) was more commonly seen in children less than 10 years. The average number of nodal groups involved in children, adolescent and adult patients were 3.57, 2.52 and 2.23 respectively. Lymph node matting was seen more commonly in children (Table 1).

In 4 (40%) patients below 10 years lymphadenopathy was the only finding. Similarly 11 (29 %) patients between 10 to 18 years and 24 (23%) patients above 18 years had only lymphadenopathy.

Pleural effusion was noted in 28 (18.42%) patients and 13 of them showed loculation. Pericardial effusion was present in 8 (5.3%) patients. No significant difference in the serosal involvement is seen among children and adults (Table 1).

DISCUSSION

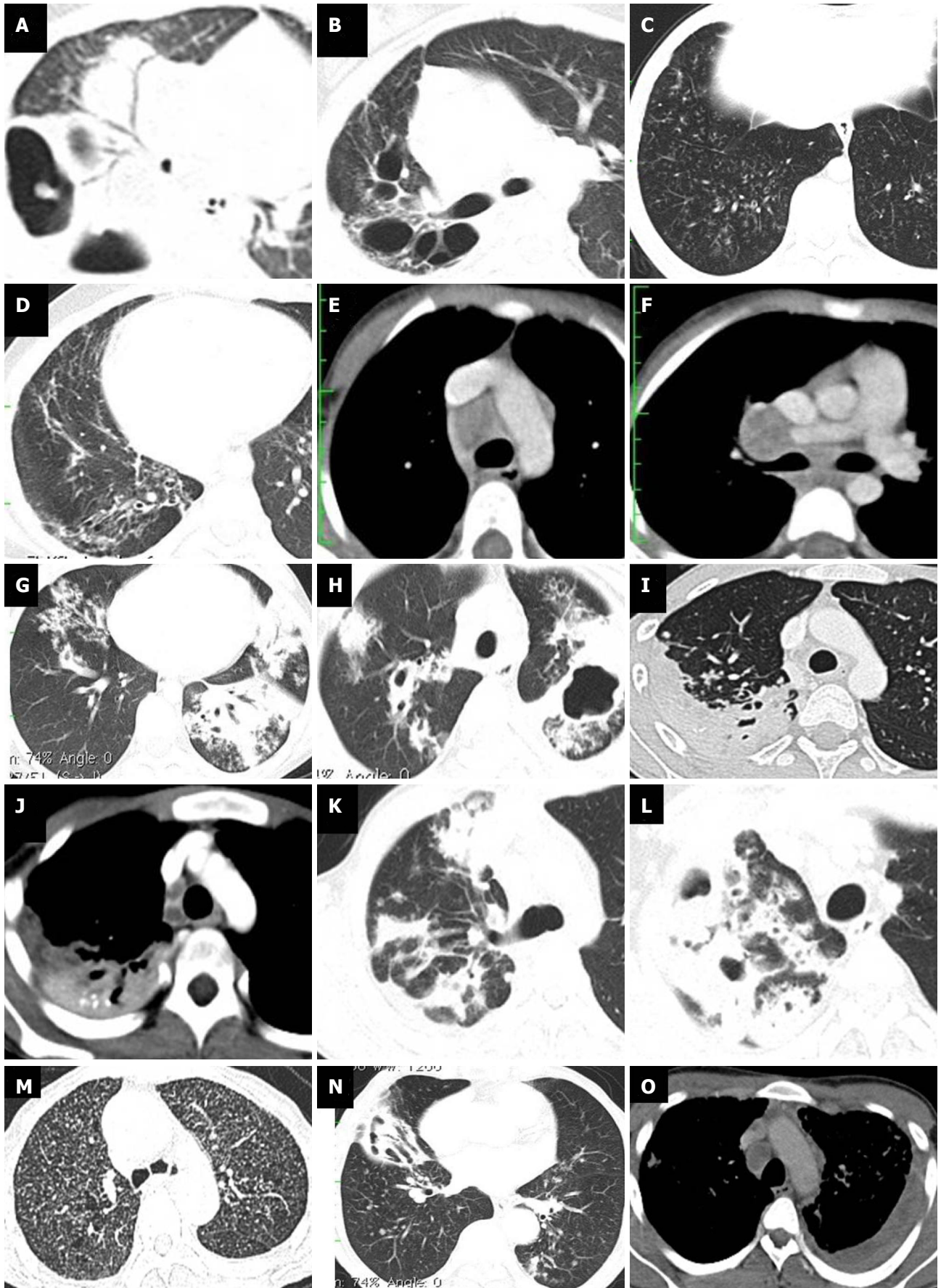
Tuberculosis continues to be a major medical, social and economic problem worldwide with its high morbidity and mortality. Children constitute about 5%-10% of patients suffering from tuberculosis worldwide. World Health Organization (WHO) data shows that in the year 2011 itself about half a million children fell ill with tuberculosis and about 64000 died from the disease^[1].

The pathologic form of pulmonary tuberculosis is classically classified as primary and post primary or secondary tuberculosis and is depended on the sensitivity of infected host. Primary tuberculosis presenting with hilar/paratracheal adenopathy with or without focal parenchymal changes in mid-lower zones is thought to be predominant form of tuberculosis in children. However contrary to the traditional belief incidence of adult form of TB is increasingly seen in pediatric patients in terms of location and severity. Previous authors have already questioned the validity of the terminologies (primary and secondary tuberculosis) in recent literature^[10,11].

Our study included 152 patients including 48 children (below 18 years). Previous studies on pediatric tuberculosis used varying age criteria for children (Table 2). We also have separately analyzed data of children below 10 years and adolescents (11-18 years).

Mediastinal lymph nodes

Mediastinal lymphadenopathy is the predominant finding in primary tuberculosis either alone or in association with lung lesions^[20]. Our study showed mediastinal adenopathy in 7 (70%), 29 (76.3%) and 74 (71.2%) respectively in children, adolescents and adult patients. Compared to previous studies slightly lower incidence of adenopathy is observed in our study (Table 2). Although there was no significant difference in the incidence of adenopathy in different age groups, there was a definite trend in the extensiveness of nodal involvement. Involvement of multiple nodal groups was seen significantly more common in children. Mediastinal adenopathy was the only finding in



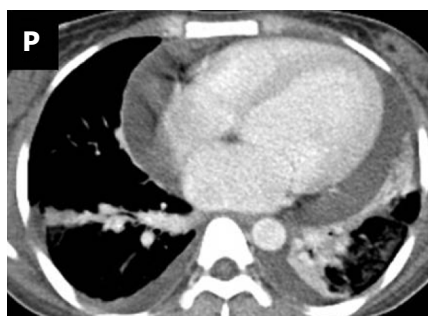


Figure 1 Computed tomography findings in children (less than 10 years), adolescents (10-18 years), and adults (above 18 years) with chest tuberculosis. A-F: Children; A: Parenchymal consolidation in right lung with adjacent thick walled cavities; B: Multiple cavities with adjacent fibrosis in right upper lobe; C: Tiny low density centrilobular nodules seen diffusely in both lungs; D: Fibrobronchiectatic changes in right middle and lower lobes; E-F: Enlarged low attenuating necrotic nodes in right paratracheal, and right hilar location respectively; G-J: Adolescents; G: Parenchymal consolidation with centrilobular nodules in left lung, nodules in right middle lobe; H: Thick walled cavities with surrounding consolidation and air space nodules in both upper lobes; I-J: Pleural based fibrotic changes with calcification in right upper lobe with necrotic right paratracheal node; K-P: Adults; K: Multifocal consolidation in right upper lobe; L: Thick walled cavities in right upper lobe; M: Miliary TB- multiple tiny nodules distributed randomly in both lungs; N: Fibrobronchiectasis in right middle lobe with nodules in left lower lobe; O: Necrotic right paratracheal node with empyema in left side; P: Pericardial and bilateral pleural effusion.

Table 1 Incidence of lung and nodal involvement, Parenchymal changes, Mediastinal lymph nodal involvement and Pleural and pericardial effusion in the patients *n* (%)

		Children (<i>n</i> = 10)	Adolescents (<i>n</i> = 38)	Adults (<i>n</i> = 104)
Incidence of lung and nodal involvement	Parenchymal	6 (60)	27 (71)	80 (76.9)
	Lesions			
Zonal distribution of parenchymal changes	Mediastinal nodes	7 (70)	29 (76.3)	74 (71.2)
	Right upper	5 (50)	19 (50)	58 (55.77)
Pattern of parenchymal changes	Left upper	2 (20)	15 (39.47)	45 (43.27)
	Right middle	4 (40)	19 (50)	54 (51.92)
	Left middle	1 (10)	16 (42.11)	43 (41.35)
	Right lower	3 (30)	12 (31.58)	35 (33.65)
	Left lower	2 (20)	9 (23.68)	30 (28.85)
	Consolidation	3 (30)	16 (42.11)	35 (33.65)
Lymph nodal distribution	Centrilobular nodules ^a	2 (20)	24 (63.16)	67 (64.42)
	Miliary nodules	1 (10)	0 (0.00)	3 (2.88)
	Bronchiectasis	1 (10)	8 (21.05)	17 (16.35)
	Fibrosis	1 (10)	7 (18.42)	14 (13.46)
	Cavitation	3 (30)	10 (26.32)	23 (22.33)
	Paratracheal	7 (70)	22 (57.59)	57 (54.81)
Characteristics of lymph nodes	Precarinal	6 (60)	9 (23.68)	27 (25.96)
	Subcarinal	7 (70)	17 (44.74)	40 (38.46)
	Hilar	5 (50)	13 (34.21)	27 (25.96)
	AP window	0 (0)	9 (23.68)	12 (11.54)
	Lymphadenopathy	7 (70)	21 (55.26)	50 (48.08)
	Necrosis	6 (60)	17 (44.74)	43 (41.35)
Pleural and pericardial effusion in the patients	Matting ^a	5 (50)	11 (28.95)	16 (15.38)
	Calcification	4 (40)	10 (26.32)	25 (24.04)
	Pleural effusion	2 (20)	5 (13.16)	21 (20.19)
	Pleural loculation	1 (10)	2 (5.3)	10 (9.6)
	Pericardial effusion	0 (0)	1 (2.63)	7 (6.73)

^a*P* < 0.05.

31.3% of children (group A and B) against 23% of older patients (group C). This is different from data of Kim *et al*^[21] and Khatami *et al*^[22] who showed isolated nodal involvement in 7% and 10% respectively. Paratracheal followed by subcarinal were the most common locations of nodal involvement in all the groups and is similar to a recent data from Andronikou *et al*^[23] and Mukund *et al*^[24].

Necrotic nodes characterized by inhomogeneous

attenuation or low attenuation centre and enhancing peripheral rim were seen in 48% of children (group A and B) and 41% of adults (group C)^[25]. Necrotic nodes were shown in 65% of children in a study by Mukund *et al*^[24] which is comparable. Matted nodes were seen in 50% of young children (group A) against 19% of older patients (groups B and C) (*P* = 0.02). Nodal calcification was seen in 40%, 26.3% and 24% of children, adolescents and

Table 2 Previous studies on imaging in pediatric chest tuberculosis

Ref.	Age (yr)	Mod	No	Cons	Nodu	Mil	Cavity	Bects	Node	Plefn
Leung <i>et al</i> ^[4]	< 16	X-ray	191	69%	NS	NS	NS	NS	92%	6%
Kim <i>et al</i> ^[21]	< 14	CT	41	49%	29%	17%	7%	NS	83%	17%
Khatami <i>et al</i> ^[22]	< 15	X ray	30	43.30%	NS	NS	NS	NS	90%	6.70%
Koh <i>et al</i> ^[10]	15-19	X-ray	90	25%	96%	NS	45%	0	2%	0
Mukund <i>et al</i> ^[24]	< 17	CT	91	NS	NS	NS	NS	NS	96.70%	NS
Our study	< 18	CT	48	39.60%	54%	2%	27%	18.80%	75%	14.60%

Mod: Imaging modality used; No: Number of cases; Cons: Consolidation; Nodu: Centrilobular nodules; Mil: Miliary nodules; Bects: bronchiectasis; Node: Mediastinal adenopathy; Plefn: Pleural effusion; NS: Not specified.

adults respectively. Nodal calcification was seen in 12 % and 28.4% of children in studies by Kim *et al*^[21] and Mukund *et al*^[24] respectively. Hence apart from higher incidence of matting seen in young children no other significant difference in nodal characteristics is seen in different age groups.

Parenchymal changes

The typical parenchymal change in primary tuberculosis is focal consolidation (Ghon's focus) seen classically in mid and lower zones. Cavitation, fibrosis and bronchiectasis are not commonly seen in primary tuberculosis. In our study, right upper zone was most commonly involved in all age groups and is comparable to the data by Koh *et al*^[10] in which upper zone predominance is seen in 49% of patients. 68.75% of children (group A and B) showed lung parenchymal changes of tuberculosis against 76.9% of older patients (group C). Cavitation was seen in 30% of young children and 26% adolescents. Cavitating pneumonia was seen in 7% of children in the study by Kim *et al*^[21] and 45% of adolescents by Koh *et al*^[10]. The recognition of cavitating disease in children is important because the presence of cavities correlate with organism load, drug resistance, treatment outcome and infectivity^[11]. Bronchiectasis was seen in 18.8 % children (group A and B) against 16.4% in adults (Table 1).

There was no significant statistical difference in the incidence of consolidation, miliary disease, bronchiectasis, fibrosis and cavitation among different age groups. Centrilobular nodules were less commonly seen in children. When we compared the incidence of nodules in children (group A) against older patients (group B and C) significantly lower incidence is noted in young children ($P = 0.014$).

Our study showed comparable frequency of pleural effusion, empyema and pericardial effusion in all age groups. It showed 20% and 13% incidence of pleural effusion in children and adolescents (Table 1). Kim *et al*^[21] observed pleural effusion in 17% of children with tuberculosis.

To conclude, children with pulmonary tuberculosis are equally prone to develop significant destructive changes in the lung with severe sequelae similar to older patients. The impact is much more severe in cases of children because they have longer life expectancy. Moreover,

the cavitating lesions with high bacterial load make these children highly infective and pose an important hazard to community health, than previously thought. The similar location and aggressiveness of parenchymal changes observed in children, blurs the boundary defining primary and reactivation tuberculosis. Hence the need for revision of these terminologies demands urgent attention.

Limitations

First, our study included only those patients who had undergone CT scan at a tertiary institution and hence may not represent the exact patient population at the primary care level. However as all the patients in different age groups had been filtered in the same way the comparisons should remain valid. Secondly, we had only ten patients in below 10 years category and hence further studies including more number of children will help in strengthening the observations.

COMMENTS

Background

Tuberculosis is a contagious lung disease caused by mycobacterium tuberculosis. It is widely prevalent in developing countries and is a major threat to community health. Tuberculosis in children is traditionally believed to be less severe and less contagious as compared to adult disease. However recent evidences suggest that the distinction is far from truth and children are presenting with severe disease in presentation and outcome. This retrospective study is done to compare the disease characteristics based on imaging [computed tomography (CT) of chest] among children and adults who were presented to a tertiary care centre.

Research frontiers

Tuberculosis in children can present in various forms ranging from subclinical infection to destructive parenchymal disease or extensive miliary disease. Early recognition of children having severe disease is essential in the management of disease and in prevention of spread. Although chest radiography is the primary imaging modality in chest tuberculosis, CT is advisable in children presenting with atypical and severe manifestations. Studies on this background are essential in planning and modifying the strategies in the management of pediatric tuberculosis.

Innovations and breakthroughs

Present study has shown that the disease can strike children with similar aggressiveness as in adults and can very much be a source of infection in the community. Hence it is important to take adequate measures to prevent disease spread along with optimal treatment planning.

Applications

Early recognition of severe manifestations of pediatric chest tuberculosis is beneficial in optimal treatment planning. Despite the higher radiation exposure

involved CT of chest is the best investigation to get an accurate estimate of the disease in atypical and complicated cases.

Terminology

CT stands for CT which is a radiological investigation using X-rays to produce tomographic images of the body. In the chest using different post-processing techniques CT provides much greater information than radiography.

Peer review

The study of pediatric vs adult pulmonary tuberculosis is very good. The study highlights the incidence of severe manifestations in pediatric chest tuberculosis, the early detection of which is beneficial in optimal treatment planning.

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