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AIM AND SCOPE

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INDEXING/ABSTRACTING

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DIAGNOSTIC ADVANCES

Cardiovascular magnetic resonance: Diagnostic utility and specific considerations in the pediatric population

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Abstract

Cardiovascular magnetic resonance is a non-invasive imaging modality which is emerging as important tool for the investigation and management of pediatric cardiovascular disease. In this review we describe the key technical and practical differences between scanning children and adults, and highlight some important considerations that must be taken into account for this patient population. Using case examples commonly seen in clinical practice, we discuss the important clinical applications of cardiovascular magnetic resonance, and briefly highlight key future developments in this field.

Key words: Cardiology; Pediatrics; Imaging; Diagnosis; Cardiovascular magnetic resonance

Core tip: Cardiovascular magnetic resonance is playing an increasingly important role in the investigation and management of pediatric cardiovascular disease. However, imaging this patient population brings its own unique set of challenges. This article describes some of the key differences between scanning children and adults, discusses the important clinical applications of cardiovascular magnetic resonance in pediatrics, and highlights some of the key future developments in this field.

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INTRODUCTION

Cardiovascular magnetic resonance (CMR) is a non-invasive imaging technique that uses magnetic resonance imaging (MRI) to provide clear delineation of cardiovascular anatomy, detailed tissue characterization, and a comprehensive evaluation of cardiac function. In recent decades there has been a significant increase in use of CMR for a variety of purposes in both congenital and acquired heart disease in children.

Overall, the basic sequences and imaging strategies used in children are similar to those used in adults. Adolescents with normal intellectual and emotional development can usually be successfully imaged using adult techniques[1]. However, younger children may not be able to comply with breath-holding during image acquisition and their faster heart and respiratory rates provide technological challenges. Additionally, their anatomy is smaller and, in cases of congenital heart disease, often unique and complex. Pediatric imaging is therefore more demanding in terms of sequence optimization and each scan requires an individualized approach[2,3].

Despite these challenges, CMR remains a useful tool to assist with investigation and management of a wide range of cardiovascular pathology in children. It can be used for diagnostic and screening purposes to define anatomy and assess function, to monitor disease progression as part of serial follow-up, and to plan and evaluate the outcomes of surgery and other therapeutic interventions. This article provides an overview of the main applications of CMR in children and discusses some of the specific considerations for this patient population.

HOW DOES CMR WORK?

CMR uses magnetic fields and radiofrequency energy to produce tomographic images of the human body. It is based on the phenomenon of "nuclear magnetic resonance" - the ability of some atomic nuclei to selectively absorb then later re-emit radiofrequency energy. The emitted energy can then be captured and transformed into an image. Only nuclei with an odd number of protons and neutrons (thus possessing a "net magnetic moment") are capable of exhibiting this phenomenon[4]. Several examples of such nuclei are present in biological tissues[5], however the hydrogen (^{1}H) atom is the primary choice for clinical imaging due to its abundance in water and fat.

Under normal conditions, hydrogen nuclei in tissues behave as tiny bar magnets randomly oriented in space such that the net magnetization of the tissue is zero. When placed in a strong magnetic field (created by the large superconducting magnet of the scanner), the nuclei align in the direction of the magnetic field creating a net tissue magnetization oriented along the axis of the scanner. The nuclei spin (precess) around the direction of the magnetic field at a frequency specific to the magnetic field strength[4]. The field strengths of clinical scanners can vary from 0.15 to 7 tesla (T) although CMR is typically performed at 1.5 T, approximately 20000 times the magnetic field strength of the earth[6].

External radiofrequency transmitter coils are used to apply radiofrequency energy to the tissue at a specific "resonance" frequency. Hydrogen nuclei absorb this energy and flip their orientation within the magnetic field, going from a stable low energy state to an unstable high energy state. When the radiofrequency transmission ceases, the nuclei relax back to the lower energy state and re-emit the absorbed energy, which is detected by a receiver coil as radiofrequency signals. The signals are electronically amplified by a computer and the intensity of each signal is plotted on a grey-scale in order to build up a cross-sectional image of the tissue. The resulting image is a representation of the spatially-resolved signals[4,5].

In order to localize the part of the tissue from which these emitted radiofrequency signals originate, gradient coils driven by pulses of electricity are used to produce small field gradients in multiple planes within the wider magnetic field of the scanner magnet. These gradients cause a predictable variation in both the magnetic field strength and resonant frequency in different parts of the patient. By varying the times at which gradient fields are switched on and off in relation to the application of the radiofrequency pulses, then analyzing the properties of the emitted signal (in terms of frequency and phase), the computer is able to reconstruct an image of the patient[6].

The hydrogen nucleus relaxes back to the lower energy state by two main processes: Longitudinal relaxation with relaxation time T1, and transverse relaxation with relaxation time T2[7]. The relative proportions of T1 and T2 relaxation times vary between different tissues. By altering the timing of radiofrequency pulses, strength of the gradient fields, and through use of contrast agents and magnetization preparation pulses (such as inversion recovery, saturation recovery, fat-suppression and blood-nulling sequences), the differences in T1 and T2 values between tissues can be exploited to enable detailed tissue characterization, producing images that highlight the tissue of interest[8].

CMR *VS* OTHER IMAGING MODALITIES IN PEDIATRICS

Echocardiography is the mainstay of cardiovascular imaging in children. It is cheap, quick, accessible, non-invasive and particularly informative in neonates and infants for whom it is possible to achieve good acoustic windows. However, it is operator dependent and provides only limited views of extra-cardiac vascular

structures[9,10]. Cardiac catheterization provides useful hemodynamic information and permits concurrent therapeutic intervention. However, it is invasive with rare but potentially fatal complications, involves exposure to ionizing radiation and is dependent on the use of iodine-based contrast agents[11-13]. CMR on the other hand, is non-invasive and radiation-free. This is particularly relevant to children, for whom the risk of risk of radiation-induced malignancy is significantly higher than in adults[14]. Thus CMR is amenable to being used for serial assessments, such as pre- and post-procedure or for ongoing follow-up to monitor disease progression. CMR reduces the requirement for invasive study in certain cases and enables the assessment of anatomy and function where echocardiographic views are sub-optimal. Non-contrast imaging provides excellent soft tissue contrast resolution permitting detailed tissue characterization, and superior structural and functional information, including the determination of extra-cardiac anatomy and hemodynamic parameters[15].

However, CMR scanners are rarely mobile and availability is limited compared to echocardiography. Even in a centre that offers conventional MRI, CMR requires significant software, training and expertise[16]. The data acquisition time is long, typically 20-50 min depending on what information is required, and the space within the magnet is limited. It can be claustrophobic, and for children unable to co-operate with the scanning procedure, general anesthesia may be required. It is also less suitable for clinically unstable patients requiring intensive monitoring, and in the event of a cardiopulmonary arrest, the patient must be removed from the magnet environment of the CMR scanner before advanced life support can commence. All monitoring equipment used during a CMR scan must be MRI compatible, requiring a switch to compatible pumps before the patient enters the scanner.

Computerized tomography (CT) is often used as an alternative to CMR - it permits acquisition of a high resolution data set in a much shorter time period and is therefore useful for children and unstable patients unable to tolerate a lengthy CMR scan. In the pediatric population, it is considered superior to CMR when evaluating airway anatomy in cases of vascular rings, when assessing pulmonary vasculature (particularly in thromboembolic disease of the pulmonary arteries, where breath-holding capability is often compromised), and when determining the presence or absence of any major aorto-pulmonary collateral arteries (MAPCAs)[17,18]. CT can also be used as an alternative where CMR is contraindicated due to the presence of implanted devices, foreign bodies or claustrophobia. However, it is less detailed in terms of tissue characterization and exposes patients to high doses of ionizing radiation - particularly in the case of serial imaging where the cumulative radiation dose is significant[19].

Despite its shortcomings, CMR remains a versatile tool with distinct advantages over other modalities. However, outcomes are most successful when it is used in conjunction with other imaging technologies in a directed manner to obtain an answer to a specific clinical question.

BASIC CMR PULSE SEQUENCES

CMR pulse sequences represent the co-ordinated actions of turning on and off the gradient coils and transmitted radiofrequency pulses in order to highlight specific features of the tissue being imaged[15]. The basic principles of these sequences are similar for adult and pediatric CMR. As with adult imaging, sequences used in the pediatric setting must be carefully selected in order to best answer the clinical question. However, in children specific adaptations must be made in order to accommodate the smaller patient size (demanding a higher spatial resolution), and faster heart rates (demanding a higher temporal resolution)[20]. Also, in cases of congenital heart disease where the anatomy is complex, a more individualized approach to the scan is required[2].

Spin echo
Spin echo pulse sequences produce images that are acquired during one fixed point of the cardiac cycle (Figure 1A and B). They are static images in which blood appears black and the surrounding stationary tissue appears in shades of grey[16]. These images are useful for providing anatomical information[21] and they permit excellent tissue characterization (particularly when magnetization preparation pulses are used), achieving good visualization of pathology for conditions such as myocarditis, pericarditis, cardiomyopathies, vasculitis and cardiac tumours[22]. Acquisition time is long, and although faster variants exist, they result in poorer spatial and temporal resolution[20].

Gradient echo cine
Gradient echo cine imaging enables the generation of short "movies" depicting motion of the heart throughout the cardiac cycle. This is achieved by dividing the cardiac cycle into multiple segments (frames) to produce a series of 2D images that can then be laced together into a cinematic display. Blood appears bright and the resulting "cines" are useful for assessing the dynamic function of the heart - such as blood flow, valvular function, ventricular volumes, ventricular mass, ejection fraction and motion of the ventricular walls. CMR volume measurements are considered more accurate and reproducible compared to echocardiographic measurements[15,23,24], and normal values for both atrial and ventricular volumes have been widely published in children[25-28] with studies demonstrating good reproducibility[29].

Gradient echo cine imaging can be performed using a standard spoiled gradient echo pulse sequence, or the more recent steady state free precession sequence (SSFP) (Figure 1C and D). SSFP has generally surpassed the use of spoiled gradient echo for cine imaging as it is faster and provides superior contrast between blood
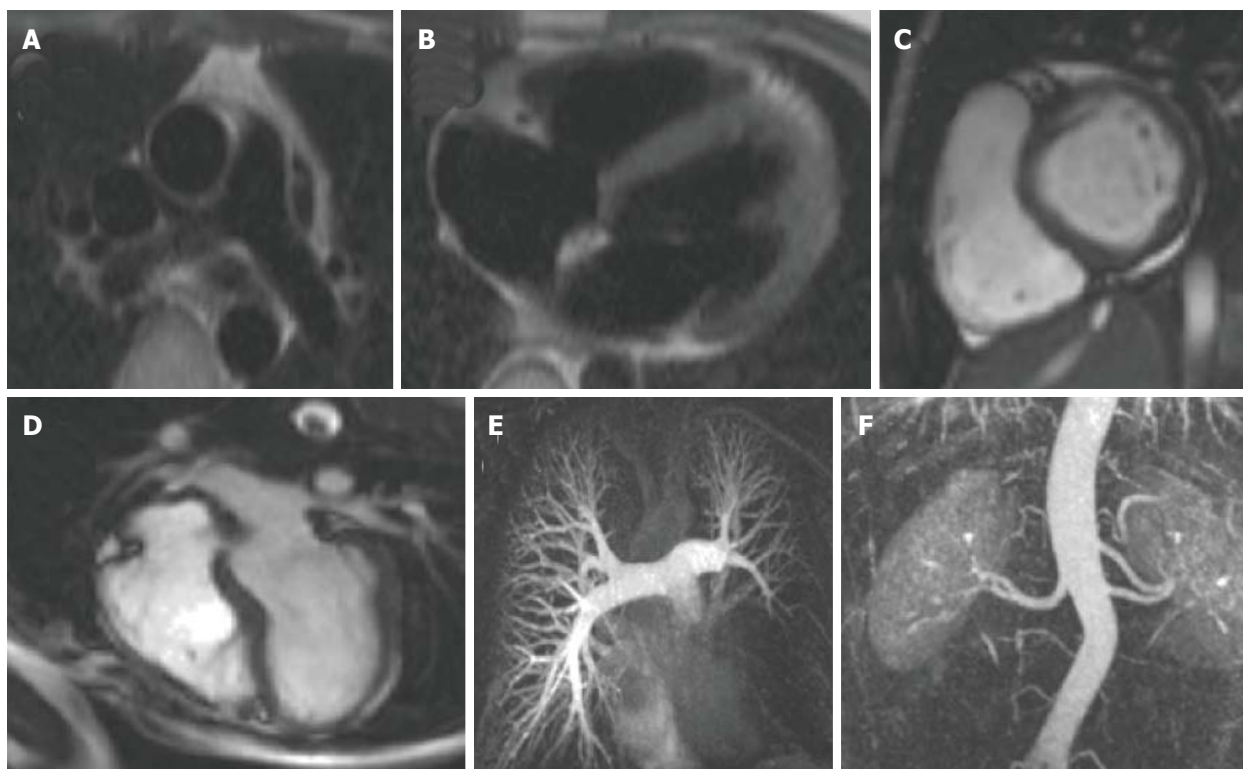
Figure 1 Examples of images produced by individual cardiovascular magnetic resonance pulse sequences. A and B are black blood spin echo images showing the ascending and descending aorta at the main pulmonary artery level A and the 4 cardiac chambers B; C and D are bright blood SSFP cine images showing short axis and 4 chamber views respectively, while E and F are examples of 3D contrast-enhanced MRA; E is a contrast-enhanced MRA of the pulmonary tree in a patient with a large sarcoma. There is no opacification of the arterial supply of the left lower lobe, indicating complete occlusion of the lower branch of the left pulmonary artery; F is a contrast-enhanced MRA of the descending aorta for assessment of renal anatomy, demonstrating an accessory renal artery to left kidney (a common normal variant). SSFP: Steady state free precession; MRA: Magnetic resonance angiography.

and myocardium[20]. It can also be adapted for 3D imaging, enabling acquisition of a high resolution 3D anatomical dataset of the heart and thoracic vasculature without the requirement for intravenous contrast[30-32]. However, SSFP is more prone to artefact when there is inhomogeneity in the magnetic field[20], so both types of sequence still form the basis of multiple cardiac imaging applications. Spoiled gradient echo sequences are still widely used for late gadolinium enhancement (LGE) imaging to detect myocardial fibrosis, 3D contrast-enhanced magnetic resonance angiography (MRA), velocity encoded phase contrast imaging for assessment of *in-vivo* blood flow, and first-pass perfusion imaging for evaluation of myocardial perfusion.

LGE imaging: Gadolinium-based contrast agents (GBCAs) distribute in greater volumes in fibrosed myocardium and demonstrate slower washout times compared to normal myocardium. Using spoiled gradient echo sequences, it is possible to demonstrate the abnormal deposition of contrast late after contrast injection as focal regions of fibrosis become hyperenhanced[16]. Validation studies have strongly correlated the finding of LGE with the presence and extent of myocardial fibrosis[33,34]. In children, LGE is typically seen along areas of reconstruction post-surgically following repair of congenital cardiac lesions[35-38]. The presence of LGE is known to be associated with arrhythmias and poorer ventricular function and studies

in adults have demonstrated that it is associated with a poorer prognosis in dilated cardiomyopathy[39], hypertrophic cardiomyopathy[40,41] and valvular heart disease[42].

3D contrast-enhanced MRA: Running a spoiled gradient echo pulse sequence during administration of intravenous GBCAs enables detailed, high resolution vascular imaging (Figure 1E and F). Gadolinium reduces the T1 relaxation time of blood, enhancing the contrast between blood and the surrounding tissue[43]. By varying the time delay between contrast administration and the pulse sequence, it is possible to alter the portion of the thoracic vasculature imaged. In this manner, clear images of the aorta and its branches, the pulmonary vessels, systemic veins, collateral vessels and any shunts, conduits or vascular grafts can be obtained[44-49]. The collected data can be formatted to generate 2D slices in any orientation, or volume-rendered into a 3D image, often negating the requirement for invasive diagnostic catheterization.

Velocity encoded phase contrast imaging: When hydrogen nuclei (such as in blood) flow through specially designed magnetic field gradients, the signal they emit accumulates a phase shift relative to the signal from the surrounding tissue that is proportional to their velocity. Velocity encoded phase contrast sequences capture

data encoding this velocity information in addition to data encoding information about the surrounding tissue. Software can then be used to obtain measurements of flow rates within individual vessels by contouring the vessel in a cross-sectional plane and calculating volume of blood passing through the plane as a product of velocity and cross-sectional area[50-52]. Using this technique, it is possible to assess flow in large and small arteries and the systemic and pulmonary veins. It is also possible to quantify cardiac output and intra- and extra-cardiac shunts, measure pressure gradients across areas of stenosis and calculate valvular regurgitant fractions[53-56].

First-pass perfusion imaging: Performed using spoiled gradient echo or SSFP sequences, this technique involves the administration of a GBCA followed by dynamic imaging of the passage of contrast through the myocardium in order to detect zones of decreased perfusion[57]. Normally perfused myocardium gives a bright signal and areas of poor perfusion appear darker. Images are typically acquired both at rest and under pharmacological stress (induced *via* administration of a coronary artery vasodilator such as adenosine) in order to accentuate the difference between the perfusion of myocardium supplied by normal coronary arteries compared to myocardium supplied by abnormal vessels[16,20]. Examples of uses in children include the investigation of chest pain, congenital heart disease with anomalous coronary artery origins, post-surgery involving coronary artery re-implantation and in acquired abnormalities of the coronaries such as aneurysms in Kawasaki disease[17,58-60]. CMR offers distinct advantages compared to the traditional nuclear perfusion imaging in terms of improved spatial resolution and lack of ionizing radiation[61-64]. Dobutamine instead of adenosine is also used for stress imaging in certain circumstances[65,66].

SPECIFIC CONSIDERATIONS IN CHILDREN

In addition to the technological challenges with regards to performing CMR in children, there are a number of specific practical considerations to take into account. These include the strategies employed in order to minimize both generalized and cardio-respiratory motion artefact (both accentuated in children due their reduced ability to co-operate with the scanning procedure, and their elevated heart rate and respiratory rate in comparison with adult patients), the equipment used, and some of the additional preparation steps that can be taken with children in order to facilitate the scanning process.

In terms of generalized motion artefact, older children (typically greater than 7 years of age) with normal development are often capable of lying still and following instructions such that adequate quality images can be obtained. However, for neonates, infants, younger children and patients with developmental delay, specific

strategies must be employed in order to minimize motion artefact. Approaches will vary depending on the age of the child, their clinical condition, and the expertise and resources available. For infants less than 6 mo, it may be possible to perform the scan during natural sleep after feeding[67], however early awakening will likely compromise the scan. Deep sedation using sedative medications is an option[68], but is avoided where possible due to risks of hypoventilation and aspiration. Thus, for children unable to breath-hold, the preferred approach is endotracheal intubation and mechanical ventilation under general anesthesia (GA). CMR under GA is resource intensive, requiring a pediatric anesthetist with cardiac experience and CMR compatible equipment. It is also challenging since intensive monitoring is required despite limited access to the child during the scan. However, with trained personnel, good communication and a comprehensive emergency plan in place, it has an excellent safety profile[69-71]. Additionally, it presents the opportunity to perform other invasive investigations during a single GA, for example trans-oesophageal echocardiography and endoscopic procedures. However, the decision for a child to undergo CMR under GA is not taken lightly and is usually made in discussion with the wider multi-disciplinary team. There should be careful consideration of the age and maturity of the child, the parents' perception of the child's ability to co-operate with a non-GA procedure, their clinical condition, relevant past experiences, the length of the scanning protocol, the risks of anesthesia and the benefits of the scan in terms of diagnosis and patient management[2,72].

In order to minimize the effect of cardio-respiratory motion on image quality, specific strategies are employed. For cardiac motion, the techniques used are broadly similar for adults and children. To obtain images of acceptable quality, CMR data is acquired over multiple heart beats, synchronizing the data acquisition to a particular time point in the cardiac cycle. MRI compatible electrodes and leads are applied to the patient's chest and specific software detects the ECG trace, synchronizing the CMR pulse sequence (and thus data acquisition) to the R wave. In this manner, with each cardiac cycle there is a new repetition of the pulse sequence. Images can be obtained either at a single time point in the cardiac cycle for still imaging, or at multiple time points for cine imaging and the resulting images are laced together in a cinematic display. Two main ECG synchronization techniques exist: Prospective triggering and retrospective gating. Typically for still imaging using spin echo sequences, prospective triggering is used, whereas for cine imaging using gradient echo sequences, either technique can be used[6,73]. For respiratory motion, most pulse sequences enable data acquisition to be completed within a single breath-hold, thus older children can be taught to breath-hold with practice. Breath-holding can also be achieved under general anesthetic, with the anesthetist strategically pausing the ventilator at specific times. In sedated infants and small children, breathing tends to be shallow and regular so a technique

Table 1 Tips for a successful pediatric cardiovascular magnetic resonance scan
Before the scan
Begin preparation for the scan well in advance of the appointment
If multiple children from the same family require scans (such as when screening for hereditary conditions), where possible arrange for all children to be scanned at a similar time (ideally on the same day) so they can prepare together
Discuss the procedure with the child in an age-appropriate manner and provide parents with a detailed description of the procedure so they can be of assistance
Play therapists can help prepare the child by talking them through pictures of the scan, and using dummy scanners to practice lying still and breath-holding
Arranging a pre-scan visit to the CMR department and allowing the child to see the scanner before their scheduled appointment may help reduce anxiety
Perform a full metal screen on parents so that they can demonstrate going into the scanner if the child is anxious, and so that they can remain in the room for the duration of the scan to reassure the child if necessary
Some modern scanners have MRI compatible audio-visual equipment, where this is available allow the child to pre-select their own music or movie to play during the scan (ideally bringing a favourite one from home) – this may help them tolerate longer scanning times
Within reason, allocate a lengthier appointment for the scan to give the child time to get accustomed to the magnet, coils, ear protection and breath-holding instructions
During the scan
Be patient and flexible
Minimize the time the child must spend in the scanner by only running sequences that will directly answer the relevant clinical questions
Run the most essential sequences first bearing in mind that the child may not tolerate the whole scan
For a breath-holding child, use short sequences only as they may struggle with a long breath-hold
An inspiratory breath-hold is easier for a child to understand and achieve compared to an end-expiratory breath-hold
For stress perfusion studies provide the child with a stress ball that can be repeatedly squeezed during administration of the stress agent to minimize side effects (61)
After the scan
Praise and reward the child with stickers and certificates even if the scan was not entirely successful, bearing in mind that for many conditions repeat scanning may be required in future so all attempts to alleviate bad experiences should be made

employing multiple signal averages can be used to average out respiratory motion artefact at the expense of reduced spatial resolution. Alternatively, respiratory gating strategies can be employed to synchronize data acquisition to the respiratory cycle such as using a navigator beam to track the motion of the diaphragm and gating data acquisition to a point in end expiration when the diaphragm is relatively still[73,74].

For pediatric CMR, the use of smaller coils placed directly on top of or underneath the child significantly improves image quality. Adolescent children can be imaged using a standard adult surface coil, while for younger children, infants and neonates, better image quality can be obtained with a smaller surface coil. Specific pediatric coils are commercially available for imaging the brain and spine, and while pediatric thoracic coils are becoming increasingly available, often adult coils designed for other applications (such as adult orthopedic extremity coils) are used for this purpose[75].

On a practical level, better outcomes are achieved with children when there is thorough planning and preparation prior to the scan. Some practical considerations and tips for achieving a successful outcome are described in Table 1.

CMR SAFETY CONSIDERATIONS

It is essential that all patients and any accompanying persons (such as parents) undergo thorough screening for the presence any implanted medical devices or foreign bodies - these include pacemakers, implantable defibrillators, neurostimulators, stents, cerebro-spinal fluid shunts, cerebrovascular clips and coils, cochlear implants, orthopedic devices, shrapnel, bullets and metal fragments[76]. Where the history is unreliable, plain radiographs can be used to aid the screening process. The strong magnetic fields of the scanner may disrupt the function of some electrically, magnetically or mechanically activated devices, and ferromagnetic objects risk becoming dislodged during the scan causing local tissue damage[77]. Many modern devices are designed to be MRI compatible - they may cause artefact but are not ferromagnetic and will not overheat or fail in the presence of the magnetic field. Older devices, on the other hand, are less likely to be MRI compatible, therefore it is essential to thoroughly check the safety information for each specific device and follow all recommendations made by the manufacturer.

The use of GBCAs can also raise issues. Although the incidence of complications relating to the use of these agents is low, children are susceptible to all the adverse effects experienced by adults. These include feelings of coldness or warmth on injection, nausea, vomiting, headache, paresthesia, dizziness, itching, extravasation of contrast agent and allergic reactions ranging from a simple rash to anaphylaxis[78-80]. A serious complication of GBCAs is nephrogenic systemic fibrosis (NSF), a progressive, incurable and often fatal condition that involves widespread fibrosis of the skin, subcutaneous tissue, joints, skeletal muscles, and organs such as the eyes, lungs, heart and liver. It typically occurs in the context of renal dysfunction when GFR is less than 30 mL/min per 1.73 m^2, additional risk factors include the requirement for renal replacement therapy, concurrent hepatic disease and a pro-inflammatory state[81,82]. NSF is exceedingly rare, and even more so in children compared to adults[83]. This is surprising given the immature renal function of
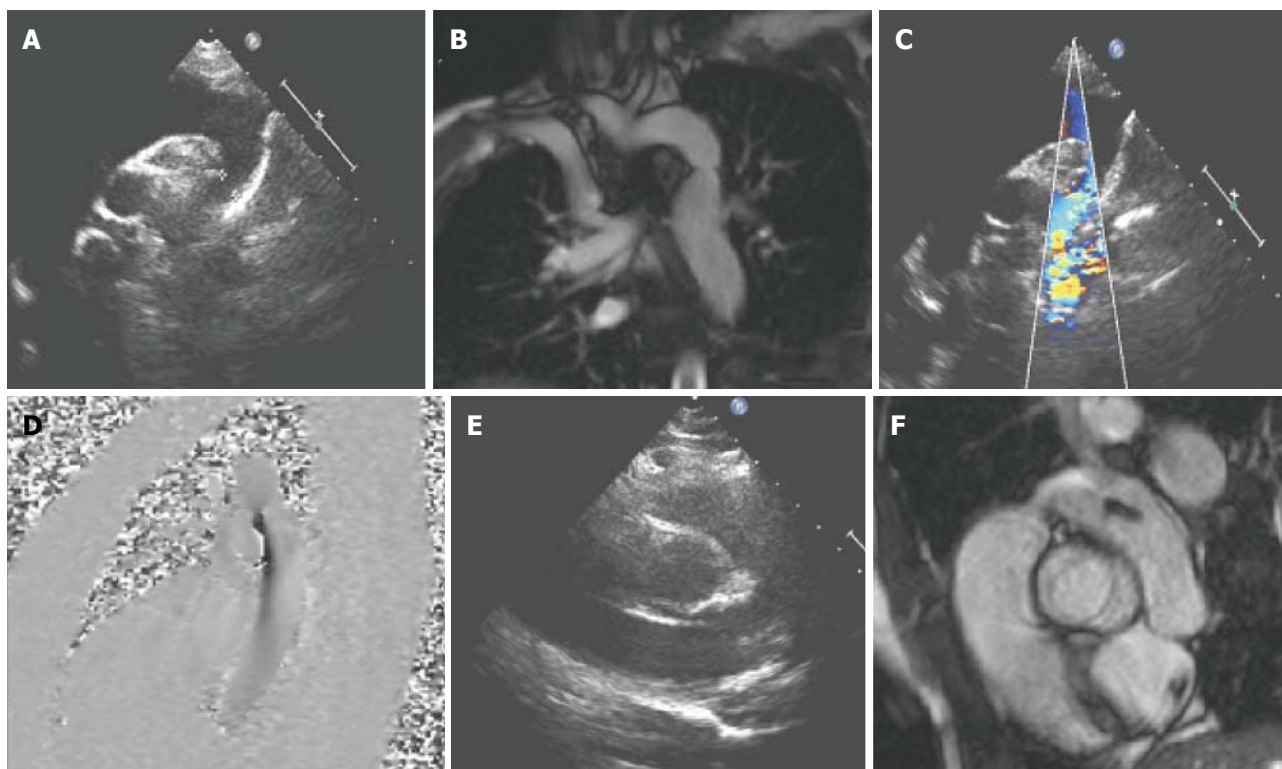
Figure 2 A 17-year-old girl with coarctation of the descending aorta, imaged by transthoracic echocardiography (A,C,E) and cardiovascular magnetic resonance (B,D,F). Imaging of the aortic arch using TTE can be challenging, depending on the suprasternal image quality A and especially in older children with poorer acoustic windows. CMR allows accurate measurement of the dimensions of the area of stenosis B and assessment of the remainder of the thoracic aorta and head and neck vessels. Flow velocity mapping D, similar to Doppler echocardiography C is used to measure peak velocity through the stenosed area and assess for a diastolic tail. In this case, the bicuspid aortic valve was not well visualised on TTE E but was clearly seen on CMR F. CMR: Cardiovascular magnetic resonance; TTE: Transthoracic echocardiography.

neonates and infants. Nevertheless, all patients should be screened for risk factors and renal function should be checked before GBCAs are administered[84]. If renal dysfunction is identified, local or national guidelines should be consulted and steps should be taken to minimize relevant risk factors with contrast-enhanced scanning only proceeding after careful consideration of the risks and benefits[85]. In light of this screening process, and the introduction of safer agents bound to a cyclic chelate[83], the incidence of GBCA-related NSF has fallen significantly in recent years[86].

Additional CMR safety considerations, especially with relation to neonates and infants, include the use of ear protection in order to prevent hearing damage from the acoustic noise of the scanner[87], and the requirement for close monitoring of body temperature. Scanning rooms are deliberately kept cool to reduce overheating of the electrical equipment however local heating of the coils in close proximity to the patient can still occur. Thus, small children, infants and neonates with reduced ability to control their body temperature are at risk of both hypothermia and hyperthermia during CMR[88].

CLINICAL APPLICATIONS IN CHILDREN

Disease of the aorta
Conditions amenable to assessment with CMR include coarctation, interrupted aortic arch, vascular rings and congenital connective tissue diseases[2]. Echocardiography is usually sufficient for diagnostic purposes for these conditions in neonates and infants, however CMR can be a useful adjunct in older children with poor acoustic windows, especially with regards to planning surgical or catheter intervention (Figure 2). CMR is also used first-line for post-intervention follow-up[89-91]. The use of black blood sequences along with contrast-enhanced 3D MRA and non-contrast 3D SSFP allows delineation of arch geometry and morphology, evaluation of the presence of collaterals, the site and size of areas of stenosis, the extent of any aneurysm formation, and characterization of coarctation stents[92-97]. SSFP cine sequences are also useful for assessing aortic valve morphology (often bicuspid), and left ventricular function. Velocity encoded phase contrast imaging can be used to quantify collateral flow[98]. CMR can also be used for monitoring aortic dimensions, aortic root dilation and aortic regurgitation in cases of connective tissue disease such as Marfan's in order determine optimum time for intervention[99,100].

Conotruncal anomalies
For tetralogy of Fallot (ToF) CMR is usually only performed pre-operatively if there are associated situs and aortic arch anomalies, however it is the preferred tool for the post-operative serial follow-up of these patients[101]. Pulmonary regurgitation, right ventricular outflow tract (RVOT) obstruction and pulmonary artery stenosis are common
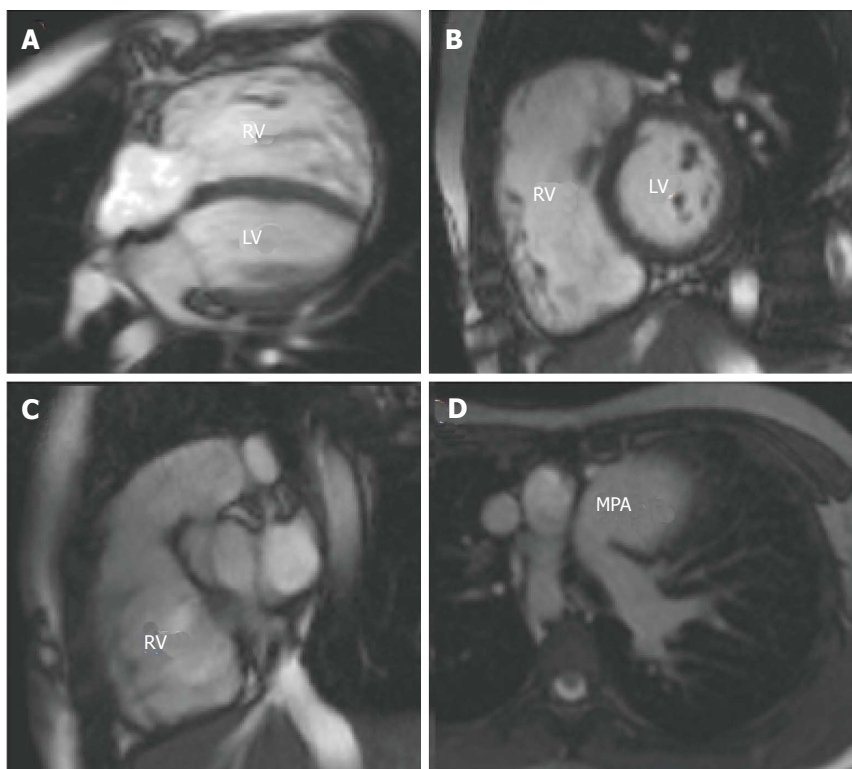
Figure 3 A 10-year-old girl with tetralogy of Fallot repaired at 1 year of age. She had a 1 year history of increasing exertional breathlessness and reduced exercise capacity. Her CMR showed near-free pulmonary regurgitation with a dilated right ventricle and a reduced right ventricular ejection fraction. In view of these findings, she was offered a pulmonary valve replacement. A shows a 4 chamber SSFP cine image; B a short axis through the mid ventricle; C a right ventricular outflow tract view and D a transverse section through the thorax at main pulmonary artery level showing the dilated main pulmonary artery. RV: Right ventricle; LV: Left ventricle; MPA: Main pulmonary artery; SSFP: Steady state free precession; CMR: Cardiovascular magnetic resonance.

post-operatively and lead to right ventricular volume and pressure overload. This is initially well tolerated in childhood and adolescence, but may ultimately cause right ventricular dysfunction, arrhythmias and premature death[102,103]. Intervention is required in most cases, either with a surgical pulmonary valve replacement or percutaneous pulmonary valve implantation (PPVI) and the decision about when to intervene is controversial[104]. CMR can assist the decision making process[105,106]. LGE CMR contributes to risk stratification of these patients, SSFP cines enable accurate right ventricular volumetric and functional analysis (Figure 3), and velocity encoded phase contrast sequences can be used for flow assessment in relation to the pulmonary regurgitation and stenosis. Contrast-enhanced 3D MRA and 3D SSFP can also be used to delineate RVOT anatomy for intervention planning[107]. Similarly, with transposition of the great arteries (TGA), echocardiography is usually sufficient to define anatomy pre-operatively and the predominant role of CMR is for the investigation of late post-operative complications. These will vary depending on the corrective procedure performed, however for the most commonly performed arterial switch operation (ASO), complications include RVOT stenosis, supravalvular pulmonary artery stenosis, branch pulmonary artery stenosis, coronary ostial stenosis, dilatation of the neo-aortic root and neo-aortic valve regurgitation[108-110]. Spin echo, gradient echo and phase contrast sequences are used for assessment of anatomy, stenosis and valvular function and 3D MRA and 3D SSFP and first-pass perfusion sequences are useful for assessing the patency of the re-implanted coronary arteries[111-113].

Complex congenital heart disease

CMR is considered the first-line imaging modality for complex congenital heart disease (CHD) as it achieves superior delineation of anatomy and enables a concurrent hemodynamic assessment[2,101]. Specific congenital anomalies amenable to assessment with CMR include viscero-atrial situs anomalies, abnormal atrio-ventricular and/or ventriculo-arterial connections, septal defects, outflow tract malformations, abnormal extra-cardiac thoracic vessels and any associated tracheo-bronchial anomalies. CMR can also play a key role in the staged palliation of univentricular hearts[114]. Initially, it can help determine the approach required particularly in borderline cases where there is debate over whether to perform a single ventricular or bi-ventricular repair[115], and subsequently CMR can be used in conjunction with echocardiography and catheterization to perform an anatomical and functional assessment at each stage of the palliation process for evaluating outcomes and planning subsequent interventions[116-119].

Assessment of valvular disease, pulmonary vessels and shunts

Although echocardiography remains the gold-standard modality for valvular morphological assessment, CMR can play a complementary role when acoustic windows are poor. SSFP cine imaging can be used to determine the functional consequences of valvular lesions, particularly in terms of the effect on ventricular volumes and myocardial mass, and velocity encoded phase contrast imaging permits visualization and quantification of regurgitant and stenotic jets[15]. In terms of the pulmonary vasculature, contrast-enhanced 3D MRA and 3D SSFP sequences provide good visualization of the morphology and dimensions of the pulmonary arteries and veins, revealing anomalous connections and areas of stenosis, and
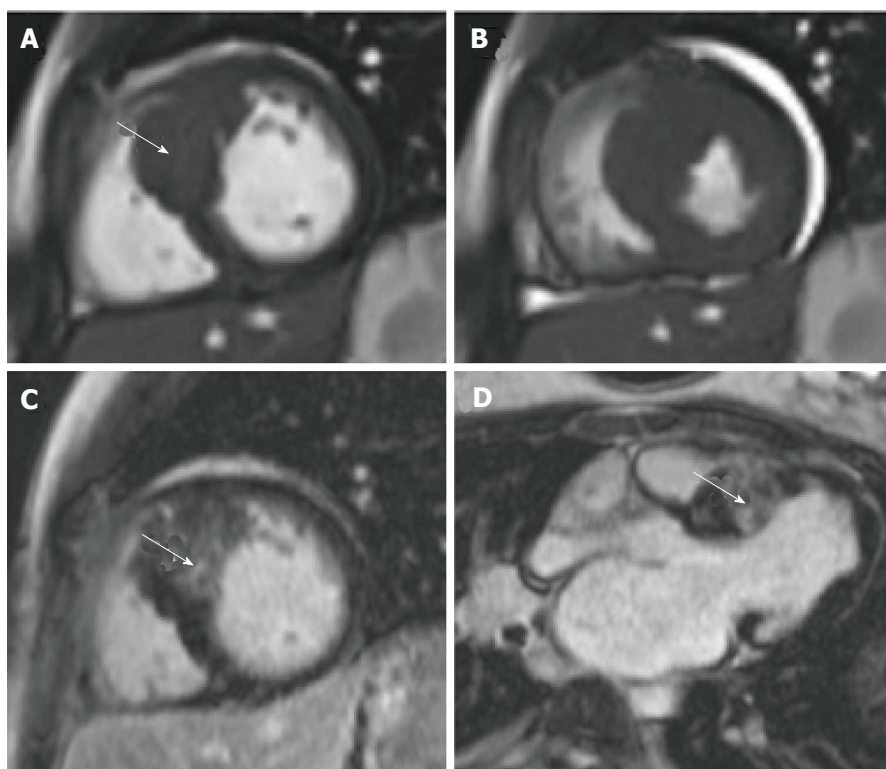
Figure 4 A new diagnosis of hypertrophic cardiomyopathy in a 12-year-old girl who presented as an out of hospital arrest with documented ventricular fibrillation. A shows a short axis view of the left ventricle at end systole at basal level. There is asymmetrical septal hypertrophy (arrowed) with a maximal wall thickness of 26 mm; B shows the same ventricular position in end systole; C and D are late gadolinium enhancement images demonstrating extensive patchy fibrosis of the hypertrophied septum (arrowed).

velocity encoded phase contrast cines enable quantitative measurements of blood flow within these vessels[2]. CMR also permits evaluation cardiac shunts in terms of their location, flow direction, magnitude and functional consequences such as volume loading of any of the cardiac chambers whilst providing detailed anatomical information. Shunt quantification is performed using velocity encoded phase contrast cines to assess the ratio of pulmonary (Qp) to systemic flow (Qs), and this important hemodynamic parameter is often used as a determining factor when planning surgical or interventional management[120].

Assessment of coronary arteries

Imaging the coronary vessels is challenging due to small vessel size and an increased susceptibility to cardio-respiratory motion artefact. Indications for coronary artery imaging in children include presence of congenital anomalous coronary arteries, vasculitis (in particular Kawasaki disease), before any surgery or interventional procedure close to the proximal course of the coronary arteries, and post-operatively for procedures involving transfer and re-implantation of the coronary arteries[2]. Although cardiac catheterization is considered gold standard for assessment of the coronary arteries, 3D SSFP CMR sequences can being increasingly used for this purpose[121-125]. First-pass perfusion and LGE imaging are also useful for the assessment of myocardial viability in the context of coronary artery pathology, and cine imaging can provide information about the functional consequences of myocardial ischemia where this is suspected.

Cardiomyopathy

CMR permits detailed *in vivo* myocardial tissue charact-

erization and therefore can play a key role in the diagnosis, risk-stratification and ongoing management of these patients[126]. Dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) are the most commonly encountered cardiomyopathies in children[127]. In DCM, the extent of both left and right ventricular dilation can be easily visualized and quantified using CMR. LGE imaging can help distinguish between DCM of ischemic and non-ischemic etiology[126], and SSFP cine sequences can be used to provide valuable information about global ventricular function especially in terms of contractility, relaxation impairment and wall motion anomalies. DCM in children is also known to be associated with anomalous coronary arteries, the presence of which can be determined using CMR[121]. Where myocarditis is suspected, spin echo sequences are useful in revealing the extent of inflammatory change[128]. Since left ventricular hypertrophy is generally considered an independent risk factor for cardiac events[129], accurate assessment of the magnitude and distribution of hypertrophy is essential in order to appropriately risk stratify and manage affected patients. Echocardiography is the most commonly used modality for the diagnosis and follow-up of HCM, however it has been shown that CMR can detect hypertrophy missed by echocardiography[130,131]. Ventricular mass and function in HCM can also be well characterized using CMR (Figure 4) and it has been demonstrated that with LGE imaging in HCM, the extent of myocardial fibrosis is closely correlated with the development of left ventricular dilatation and failure, and the risk of sudden cardiac death[40,132,133]. Where myocardial hypertrophy is related to a metabolic defect such as in Pompe's disease or Fabry's disease, CMR can play a useful role in the diagnosis of these conditions and in monitoring response to enzyme replacement therapies[134,135]. CMR

can also be used in the assessment of iron overload cardiomyopathy. This predominantly affects children with inherited severe anemias such as thalassemia and sickle cell disease, where the excess iron load from regular blood transfusions deposits in the tissues of organs such as the heart and liver. In the myocardium, this causes fibrosis, systolic impairment ultimately cardiac failure. The measurement of T2*, a CMR relaxation parameter derived from local magnetic field inhomogeneities[6], has been shown to accurately reflect tissue iron load[136,137], thus CMR can be used as a means of evaluating the requirement for and response to chelation regimens[138-140].

Cardiac tumours

The most common cardiac tumours in children are benign fibromas or rhabdomyomas. Malignant secondaries from leukemia, lymphoma, neuroblastoma and nephroblastoma are rare, and malignant primaries, typically cardiac sarcomas, are rarer still[141]. Using spin echo, SSFP cine, first-pass perfusion and LGE sequences it is possible to assess the size, site and malignant potential of the tumour through detailed tissue characterization, and assess hemodynamic relevance in terms of how any obstructive mass effects may impair myocardial or valvular function[142-144].

FUTURE DIRECTIONS

Technological advances in CMR hardware and software are continually occurring, resulting in faster sequences with shorter acquisition times, and improved image quality with greater spatial and temporal resolution. Such advances have permitted the development of real-time imaging, involving rapid and continuous data acquisition with nearly instantaneous image reconstruction and a reduced requirement for cardio-respiratory motion compensation - which is particularly advantageous in pediatrics[145-147]. Real-time imaging has also paved the way for the growing field of interventional CMR, whereby CMR performed using open magnets can be used to guide cardiac catheterization procedures, thus avoiding exposure to ionizing radiation[148]. However, although the concept of purely CMR guided interventional procedures is promising, a number of obstacles still exist that prevent it translating into routine clinical practice[149-151]. Thus, interventional CMR in current practice falls into the realm of hybrid CMR/X-ray cardiac catheter (XMR) laboratories. In these laboratories both modalities are present in the same room and the patient can be rapidly moved between them during the imaging process permitting cross-modality image integration. Additional emerging techniques include time-resolved 3D MRA permitting direct visualization of complex flow dynamics in vessels[152] and time-resolved 3D (4D) velocity encoded phase contrast imaging allowing quantification of flow parameters in multiple planes[153]. Higher field strength 3T scanners also exist that yield a higher signal-to-noise ratio and better spatial resolution. This is particularly beneficial when imaging small children. However, these scanners have their own limitations and are not yet compatible with all CMR sequences[154].

CONCLUSION

CMR is emerging as helpful imaging tool in pediatric cardiology and is becoming increasingly available for a wide range of range of both congenital and acquired cardiac disease. Its non-invasiveness and lack of exposure to ionizing radiation are particular advantages with regards to the pediatric population, and CMR is well tolerated in children of all ages with an excellent safety profile when performed in specialist centres by experienced personnel. Outcomes are most successful when scans are undertaken using an individualized approach specifically tailored to the clinical question. In these circumstances, CMR is capable of providing detailed anatomical and functional information, and it is well suited to serial imaging for long-term follow-up and as a means of planning and evaluating surgical and interventional management. In light of all the technological developments currently taking place in the field of CMR, it will be interesting to see what the future holds for this modality in the world of pediatric cardiology.

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Synthetic cannabinoids 2015: An update for pediatricians in clinical practice

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Abstract

Synthetic cannabinoids are a group of substances in the world of designer drugs that have become increasingly popular over the past few years. Synthetic cannabinoids are a chemically diverse group of compounds functionally similar to THC. Since first appearing on the world market a few years ago these compounds have evolved rapidly. Newer more potent analogues have been developed. Identifying youth who abuse these substances can be difficult. Newer forms of consumption have also evolved. These products are now manufactured in products that look like natural cannabis resin and in liquid cartridges used in electronic cigarettes. Synthetic cannabinoids appear to be associated with potentially dangerous health effects that are more severe than that of marijuana. Some synthetic cannabinoid compounds have been associated with serious physical consequences, such as, seizures, myocardial infarction and renal damage. In addition, psychoactive effects, such as aggression, confusion, anxiety and psychosis have also been reported. The diagnosis remains primarily clinical with toxicological confirmation difficult due to manufacturers constantly developing new analogues to avoid detection. Pediatricians are urged to familiarize themselves with these drugs and the typical presentations of patients who use them.

Key words: Synthetic; Cannabinoids; Youth; Children; Adolescents

Core tip: Synthetic cannabinoids are a group of substances that are typically much more potent than natural cannabis. These substances have been increasingly abused by youth over the past few years. A number of published reports have emerged documenting the serious health consequences associated with use of these products. Seizures, myocardial infarction and renal damage are some of the significant physical consequences associated with their use. With current limitations of toxicological analyses pediatricians are urged to familiarize themselves with these drugs and the typical presentations of patients who use them.

INTRODUCTION

Synthetic cannabinoids (SCs), also known as cannabimimetics, were originally synthesized in the 1960's, but emerged as recreational drugs in Europe in about 2004. They became popular as a recreational drug in Germany in 2008, and have been increasingly available worldwide since then[1-3]. Over 130 different synthetic cannabinoids have been detected in recent years. Most of these substances appear to be manufactured in China. After being shipped in powder form to Europe, the chemicals are typically added to plant material, packaged for sale as "legal high" products and often misleadingly labelled "not for human consumption".

SCs are usually smoked. Recently several countries have also reported finding the substances in products that look like cannabis resin either in branded "legal high" products or simply misrepresented as cannabis resin on the illicit market. This development is likely to be a response to the popularity of cannabis resin in many countries. Another recent development has been the manufacturing of synthetic cannabinoids in the liquid cartridges used in electronic cigarettes. The use of SCs in electronic cigarettes likely reflects manufacturers' opportunistically taking advantage of the recent popularity of "vaping" among young people. Synthetic cannabinoids have also been detected in mixtures containing other psychoactive substances such as stimulants, hallucinogens and sedatives and in a small number of cases, they have been detected in what are sold as ecstasy tablets[4].

Users often perceive these preparations as a natural way of getting high that is also legal and undetectable. The preparations are far from natural: They consist of unknown mixtures of plant products that are sprayed with a liquid form of the SCs, containing many unidentified chemical structures[5]. New preparations are constantly being synthesized, leading to the difficulties developing reliable methods of detection. Several identified forms of synthetic cannabinoids have been banned, and are therefore illegal, but newly synthesized SCs are designed to circumvent any laws or regulations that ban them[6].

SCs are sold commercially under a variety of names, such as Mr. Nice Guy, Green Buddha, Blonde, Summit, Standard, Blaze, Red Dawn X, Citron, Green Giant, Smacked, Wicked-X, AK-47, Spice, Special K, K2, Kronic, Barely Legal, and Fake Weed[7]. The cost of synthetic marijuana preparations is comparable to the cost of cannabis. These drugs have become increasingly popular among adolescents and young adults[8]. SCs are the second-most widely used illicit drug in high school seniors

in the United States. In a 2012 national survey of 8th, 10th and 12th grade students, 4.4% of the 8th graders, 8.8% of the 10th graders and 11.3% of the 12th graders admitted using synthetic marijuana. The rise in use of SCs among younger individuals is particularly alarming. Among 8th graders SCs are the third highest category of illicit drug being used after marijuana and inhalants[9]. A nationally representative sample of nearly 12000 high school seniors revealed 10% of students reported using SCs in the previous 12 mo, and 3.2% reported "frequent use" (at least 6 times) in the previous 12 mo. Females were significantly less likely than males to use SCs in this study. The odds of using SCs was significantly increased if the teenagers endorsed a history of using alcohol, cannabis, or cigarettes and was directly related to the number of evenings per week the teenagers went out "for fun". The strongest correlation was with a history of cannabis use. Indeed, only 0.5% of non-marijuana users in this study reported the use of SCs[10]. Correlates from this important study of high school seniors can be used by pediatricians in the evaluation of youth who are suspected of using SCs.

In a study of college students, eight to 14% of participants in the study reported the use of SCs, starting at an average age of 18 years[11]. The attractiveness of these SCs for young people include the lack of readily available methods of detection, the perception that these drugs are legal or "harmless," and availability in shops that sell paraphernalia for marijuana and tobacco users (head shops), in gas stations or convenience stores, or sometimes over the internet. Studies have demonstrated that the motivation for use of these products were not only to "get high" but also to avoid detection[12,13]. Those individuals who have used both marijuana and SCs describe a "better high" from the natural cannabinoids (fewer negative subjective mood experiences), but may gravitate toward SCs for the reasons described above, especially the difficulties in detection with routine urine toxicology screenings. In a study of patients admitted to outpatient treatment for SC use, those who were on probation reported the main reason for using SC instead of marijuana was to avoid detection[14].

In a recent study of 5947 athletes in the United States, 4.5% tested positive for SCs (using specialized detection methods unavailable in routine urine toxicology screening). This rate is much higher than the rate found in earlier studies of athletes, making it imperative for physicians to be aware of the possibility of synthetic cannabinoid use in this population. While the ages of the athletes in this study were not specified, pediatricians should be aware of the possibility of synthetic cannabinoid use in middle school, high school, and college athletes[15].

Young people may perceive these "natural" preparations of SCs as safe. On the contrary, many instances of dangerous reactions to the SCs have been reported. These include seizures, kidney failure, rhabdomyolysis, aggression and psychosis. Calls to poison control centers regarding human exposures to synthetic marijuana have increased.

Poison control centers have already registered 4377 calls in the first 6 mo of 2015 compared with 3680 for the entire year of 2014[16]. In 2010, more than 11000 emergency department (ED) visits in the United States involved a synthetic cannabinoid: Three-fourths of these visits involved young patients aged 12 to 29[17]. The following year the number of ED visits involving SCs increased significantly, totaling 28531. Pediatricians should be aware that the number of ED visits involving SCs for patients aged 12 to 17 years doubled from 3780 visits in 2010 to 8212 visits in 2011. Males accounted for nearly 80% of ED visits, but a threefold increase in synthetic cannabinoid-related ED visits for females has been observed[18]. While it is true that youth who use SCs also frequently use other substances[10,13,14,19,20], only one third of the synthetic cannabinoid-related emergency department visits revealed use of other substances at the time of the encounter[18]. These statistics should serve to highlight the frequency and severity of ED presentations of synthetic cannabinoid-related visits in the pediatric population.

Many clinicians are unaware of the prevalence and severity of physical and psychoactive symptoms, and the potentially serious consequences related to the use of SCs. A study of clinically active emergency physicians at a large, urban emergency department revealed that knowledge of SCs came mostly from nonmedical sources, and that most emergency physicians have only a general familiarity with SCs[21]. Because of the prevalence of the use of SCs in adolescents, and the potential serious consequences of their use, it is imperative that pediatricians and other physicians become educated in the identification, evaluation, and treatment of youth who are using these substances. We will present information below on the identification and evaluation of young patients in the emergency department setting and outpatient office setting who may be using SCs.

PHARMACOLOGY

Cannabinoids may be classified as phytocannabinoids, endocannabinoids, or SCs, based on their origin. THC is the phytocannabinoid found in cannabis plants, and is responsible for the "high" associated with smoking natural cannabis or marijuana. Endocannabinoids are endogenous molecules involved in nervous system and immune system function. SCs are a chemically diverse group of molecules functionally similar to THC. Cannabinoids exert their effects on the nervous system *via* the CB1 receptor, found in the brain and peripheral nervous system. Within the brain, CB1 receptors are located in the cerebral cortex, basal ganglia, and hippocampus. The desired effect, or "high" associated with smoking marijuana or SCs occurs when THC or SCs bind to the CB1 receptor in the brain. SCs are a full agonist at this receptor; THC is a partial agonist. Accordingly, SCs can bind to the CB1 receptor with an affinity up to one hundred times as great as THC[22,23].

Interestingly, synthetic cannabinoid preparations do not contain cannabidiol, a substance found in marijuana that has anxiolytic and antipsychotic properties. In addition to their higher affinity for the CB1 receptor, SCs also have a longer half-life than naturally-occurring cannabinoids[24]. The potentially longer duration impacts both the desired effects and the adverse psychoactive and physical effects of SCs. The absence of cannabidiol, together with a higher affinity for CB1 receptors and longer half-life compared to marijuana, may account for the increased potential of SCs to produce adverse psychiatric and physical symptoms[25].

SCs are metabolized in the liver *via* conjugation and oxidation pathways. The complex pharmacokinetics are poorly understood, but it is clear that active metabolites exist, and that cytochrome P450 pathways can be involved in adverse drug-drug interactions in patients who mix SCs with prescription medications[26].

CLINICAL EFFECTS

Acute effects of synthetic cannabinoid use typically last from 30 to 120 min but symptoms may last until the next day ("Hangover" feelings). Users report effects can be similar to cannabis use with the "rush" being similar to the one from cannabis, but shorter and more intense[13,27]. Frequently, users report other effects not typical of cannabis use that are more serious in nature[13,27-29]. Reactions are generally reported to be experienced on a sliding scale of intensity. Users mention inexperience with SC use leading to excessive dosing and type/generation or potency of SCs as influencing factors[30]. A systematic review was conducted of literature regarding synthetic cannabinoid use in Medline, PubMed, review of abstracts from professional meetings and conferences and government reports and alerts. Our knowledge base of the clinical effects regarding SCs has grown over the past few years but many of the publications reviewed lacked toxicological confirmation. To date there are no randomized controlled studies on the clinical effects of SCs. Most of our current knowledge is based on case series and reports, admissions to emergency services, reports to poison control centers and internet forums.

The adverse effects associated with SCs appear related to both the intrinsic properties of the substances and to the way the products are produced. There have been numerous reports of non-fatal intoxications and a smaller number of deaths associated with their use. Some of these compounds are very potent; the potential for toxic effects is high, even for the experienced user. The process by which synthetic cannabinoid products are manufactured has been associated with uneven distribution of the substances within the herbal material, which may result in some products containing doses that are higher than intended[4].

Synthetic cannabinoid use has been associated with both physical and psychoactive effects.

Physical effects
Cardiovascular: The most common cardiovascular

side effects are tachycardia and elevated blood pressure. Individuals can also present with palpitations, chest discomfort or tightness, or dysrhythmias[14,27-29,31-42]. Zimmermann *et al*[43] reported on two persons who presented with ischemic stroke after the use of synthetic cannabinoids. More serious cardiovascular consequences of SC use have also been noted. Several studies have documented chest pain, and cardiac ischemia after SC use[28,29,35,40,44-54]. Evidence that SC use is associated with myocardial infarction also exists[55,56]. Anecdotal reports describe two adolescents who died in the United States after ingestion of a SC product called "K2", one due to a coronary ischemic event[57]. At least four case reports now exist of pediatric patients who have been diagnosed with myocardial infarction (MI) associated with smoking SCs[58,59].

Gastrointestinal: Gastrointestinal effects, such as nausea, vomiting, and gagging, are also common after consumption of SC products[14,25,28,29,35,42,44,46,47,51,60,61]. SC use can also induce "cotton" mouth or xerostomia typical of marijuana use[13,31,35,36,46,60,62].

Neurological: A number of motor neurological effects of SCs have been reported including tremors, ataxia, fasciculations, hypertonicity, hyperflexion, and hyper-extension[24,37-39,45-48,62]. Musshoff *et al*[63] and the study[64] describe several case examples of youngsters who displayed impairment of fine motor skills associated with difficulty operating a motor vehicle. Sensory changes, such as numbness, have been reported[14,47]. Other neurological symptoms associated with SC use include headaches[37,44,52] and dizziness[37,51].

There are several instances of SC use being associated with more serious neurological effects such as seizures[28,29,34,35,40,47,59,64-67], loss of consciousness[51] and coma[47,59].

Renal: Over the past two years acute kidney injury has been added to the list of toxicities associated with use of SCs. Recently, over 20 cases of acute renal failure with associated acute tubular necrosis after SC use have been reported[68-72]. While the precise cause of renal damage in these patients is unclear, one specific synthetic cannabinoid may be implicated[68,69].

Metabolic: Similar to marijuana, SC products have been reported to stimulate appetite[13,14,27-29,46,59]. However, Buser *et al*[73] discovered in a large global sample that users of SCs reported having less appetite-stimulating properties than marijuana. SCs can also induce other metabolic effects, such as hypokalemia, hyperglycemia, acidosis[25,28,29,36] and diaphoresis[37,44,48].

Ophthalmologic: Conjunctival injection or redness of the eyes, typical of marijuana use has also been frequently observed after SC use. Other ocular effects include pupillary changes, such as miosis and mydriasis, blurry vision and light sensitivity[25,31,35-37,40,44,51].

Pulmonary: A handful of case reports have emerged describing respiratory symptoms and complications after SC use. Hyperventilation[37,40,52], apnea[51], alveolar infiltrates[74,75] and pneumonia have been reported[76].

Other physical effects: Other physical symptoms reported with SC use include hyperthermia, rhabdomyolysis, symptoms suggestive of anticholinergic effects, and tinnitus[27-29,44,61]. Insomnia[14,77], hair loss and unspecified "skin problems"[14] have also been reported.

Psychoactive effects
Cognitive: The most common cognitive effects of SC use are impairments in attention, concentration and memory[13,27,51,52,63]. Difficulty thinking clearly (not associated with psychosis)[43] and confusion have also been reported[4,36,37,40,41,43,44,50,78].

Affective: Although synthetic cannabinoid users frequently experience euphoria with use of these products, negative emotions are also commonly reported. Anxiety and panic are frequently associated with SC use[13,37,40,48,77,79,80]. Since panic symptoms frequently accompany palpitations, it can be difficult to differentiate to what extent these symptoms are due to anxiety. To a lesser extent, irritability is less frequently reported by users[13,44,81].

Speech: Dysarthric[37], pressured[37], slowed[37,48] and disorganized speech[51] as well as inappropriate laughter[27,40], have been observed with and reported by SC users.

Behavioral: Restlessness[13,37] and agitation during acute intoxication has been described in several scientific reports[32,37,40,41,44,46,47,52,79]. Reports of users of SCs exhibiting violent and aggressive behavior have dominated the lay and scientific literature. Many of the subjects displayed symptoms consistent with psychosis and altered mental status[8,37,77,79,82,83].

Psychosis and perceptual distortions: Researchers have increasingly described the mental status changes associated with SC use and intoxication. Perceptual changes such as, "alteration of perception"[31] and seeing "things not actually there"[13,37] have been described. Psychotic symptoms, such as hallucinations, disorganization of thoughts and delusions in subjects with and without a previous history of psychosis have been reported[14,27-29,32,35,39,40,46-48,80,84-86].

Suicide: Non-fatal, self-mutilatory behaviors secondary to SC use appear rare, with just two cases described in the literature[52,87]. Multiple reports connecting SC use and suicidal behaviors can be found in the media and throughout the internet[88,89]. Our review of the scientific literature discovered ten subjects in six different studies describing suicidal ideation[40,52,77,80,90].

Tolerance, dependence and withdrawal: Case series

and reports have indicated that use of SCs can produce effects beyond acute intoxication, with tolerance and withdrawal symptoms described following prolonged use. These preliminary reports suggest dependency may be associated with chronic SC use. A few reports indicating dependence are noted throughout the literature. Banerji *et al*[44], Nacca *et al*[91] and Rominger *et al*[92] each described one to two person case reports of persons exhibiting significant craving and acute withdrawal, presumably due to prolonged synthetic cannabinoid use. Bozkurt *et al*[14] examined one hundred fifty-eight patients enrolled in an outpatient substance abuse clinic. Seventy percent of these individuals had unsuccessful attempts to stop SC usage and/or symptoms of dependence. At the present state of knowledge it appears that the withdrawal syndrome from synthetic cannabimimetics is similar to but more severe than that from marijuana[14,92].

EVALUATION

In an emergency department (ED) setting, adolescent or young adult patients often present in a state of acute intoxication with SCs (either alone, or in combination with other substances)[93]. Physical consequences of SC intoxication can affect any system of the body. The pediatrician should always be aware of the potential serious medical sequelae of SC intoxication, such as myocardial infarction, seizures and acute kidney injury. In light of current limitations of toxicological testing in the emergency department, a diagnosis requires a high index of suspicion and knowledge of the typical history of users and possible symptoms. Evaluation of the patient should therefore include a thorough medical history, physical examination with documentation of vital signs, and laboratory studies to evaluate kidney function, electrolytes, and hepatic function. An EKG is recommended, and other cardiac testing as appropriate depending on the clinical presentation (including serial cardiac enzymes if chest pain is present)[94].

Acute intoxication with SCs can produce alterations in mental status, behavioral disturbances, changes in mood and affect, and psychotic symptoms. In an acute emergency setting a typical presentation may include confusion, hallucinations, anxiety and panic, agitation and aggression as well as suicidal behaviors. Evaluation of the youngster should include a mental status examination, with particular attention to the suicide risk assessment, and also an assessment of the risk of aggressive behavior toward others[37]. Given the fact that the patient may present with confusion or agitation, gathering collateral information from other informants such as family members or friends is important. Obtaining information about pre-existing psychiatric conditions will help guide the treatment of the patient. Information from paramedics or others who transport the youngster to the ED about the possible ingestion of substances, a history of substance use or the presence of drug paraphernalia, may be very helpful.

The clinician must suspect intoxication with SCs in any young person who presents with the sudden onset of otherwise unexplained psychosis. Psychotic symptoms frequently include paranoid thoughts, disorganized thoughts, flat or inappropriate affect, visual misperceptions, and auditory and/or visual hallucinations. Psychotic symptoms may represent the direct effects of the SCs, *vs* exacerbation of a pre-existing psychotic disorder such as schizophrenia. Patients may sometimes present to the ED in a state of withdrawal from SCs, with symptoms of insomnia, anxiety, nausea, and lack of appetite[91]. Since SCs are not detectable in routine urine toxicology screening, the pediatrician should be familiar with the characteristic symptoms and signs of intoxication and have a high index of suspicion to help make the diagnosis of SC intoxication. Alcohol and drug use are not rare in teenagers. Seventy percent of 12th graders in the United States are reported to have at least tried alcohol. Marijuana is by far the most widely used illicit drug used by youth in the United States. The most commonly used illicit drugs by 12th graders (lifetime) include marijuana (45%), ecstasy (7.2%) and cocaine (4.9)[9]. Synthetic cannabinoid intoxication should be strongly suspected in an adolescent who is known to use other substances such as marijuana and/or alcohol, is in a setting where he or she is undergoing periodic urine toxicology screening, and presents to the ED with the characteristic symptoms and signs of synthetic cannabinoid intoxication described here[95]. Urine toxicology screening may be helpful in that a positive screening test for marijuana, together with the characteristic presentation, greatly increases the likelihood of synthetic cannabinoid use/intoxication[14,20,34].

Adolescents rarely present to the pediatrician's office in a state of acute SC intoxication. In the office setting, the diagnosis of SC use is based more on the clinical history than on the mental status examination. Some persistent symptoms and signs resulting from SC use can, however, be identified on examination in the pediatrician's office. These include fatigue, persistent psychotic symptoms, and conjunctival injection[95]. Routine urine toxicology screening in the office setting may be helpful in identifying other substances the patient may be using, such as marijuana. If the diagnosis of SC use is made in the outpatient setting, laboratory screening for liver function and kidney function, as well as an evaluation of cardiac function, would be appropriate.

Some general principles involved in the evaluation and diagnosis apply in both the ED and office settings. The discovery of paraphernalia used in the consumption of SC products (pipes, rolling papers, electronic cigarettes) increases the likelihood that the patient is using these substances[4]. There is no characteristic odor of SCs, but the presence of the characteristic odor of marijuana may be present in a youngster who is using both substances. Manufacturers are constantly producing new synthetic cannabinoid compounds. Toxicological exams that screen for routine drugs of abuse may not detect most synthetic cannabinoid compounds. Unfortunately, many clinical laboratories do not routinely test for these recreational drugs because of financial

Table 1 Symptoms and history supportive of synthetic cannabinoid use

Synthetic cannabinoid use should be strongly suspected if a youngster presents with
A history of marijuana or other drug use
Symptoms and signs consistent with cannabis use
Unexplained sudden onset of psychotic symptoms
Unexplained sudden-onset renal, neurological, and/or cardiovascular problems is in a situation in which his or her urine is being routinely monitored for illicit substance use has had negative routine urine toxicology screens

constraints, analytical capabilities, and time limitations. Long turnaround times for the sophisticated laboratory examinations necessary to detect SCs greatly diminish the usefulness of these tests in the acute ED setting. The newest SC compounds on the street are chemically different than the earlier generation compounds. For this reason many SC compounds are invisible to older designer drug screens and traditional drug tests. This diversity of new products make detection by emerging enzyme-linked immunosorbent assay (ELISA) tests difficult. If possible, healthcare professionals are urged to utilize a laboratory experienced in testing for emerging drugs that uses comprehensive mass spectrometry testing[40,96-98].

Synthetic cannabinoid use should be strongly suspected if a youngster presents with: (1) a history of marijuana or other drug use; (2) symptoms and signs consistent with cannabis use; (3) otherwise unexplained sudden onset of psychotic symptoms; (4) otherwise unexplained sudden-onset renal, neurological, and/or cardiovascular problems; (5) is in a situation in which his or her urine is being routinely monitored for illicit substance use; and/or (6) has had negative routine urine toxicology screens (Table 1).

TREATMENT

A thorough clinical history, knowledge of clinical effects of synthetic cannabinoids and high index of suspicion are necessary for the diagnosis. Interventions for acute intoxication with all designer drugs target the presenting symptoms. No medications are currently available to treat synthetic cannabinoid intoxication per se. Symptoms of SC intoxication may be self-limited and resolve spontaneously, generally within 4-14 h[28,29]. In EDs, intravenous hydration, electrolyte replenishment and monitoring may suffice for youngsters who present with mild to moderate signs and symptoms of intoxication. Treatment of any particular renal (*e.g.,* acute tubular necrosis), neurological (*e.g.,* seizures) or cardiovascular (*e.g.,* cardiac ischemia) morbidities should be implemented promptly. Patients who present with symptoms of anxiety, panic, agitation, and arousal after SC exposure may benefit from a benzodiazepine. Lorazepam administered intravenously or intramuscularly, is the benzodiazepine most often utilized by practitioners[28,29,37,58,99]. An antipsychotic medication may be indicated when a patient presents with symptoms of psychosis, particularly when the psychosis is associated with behavioral disturbances (*e.g.,* agitation, aggression);

the patient has a history of a psychotic disorder; or the psychotic symptoms do not appear to be remitting spontaneously or with supportive measures[28,29,99].

Our review of the literature did not identify any studies that have addressed formal treatment of SC use. Synthetic cannabinoids can be more psychologically addictive than marijuana. Outpatient services are a viable option for less severe cases, especially if synthetic cannabinoids are the only drugs being used and the youngster is displaying little or no symptoms of withdrawal. Inpatient or residential treatment centers offer intensive care that can help youth get through the early stages of withdrawal in a prompt manner. The length of inpatient or residential synthetic cannabinoids treatment depends on the severity of the use and/or addiction, whether the youngster is also abusing other substances and varies from patient to patient.

Because substance abuse and addiction are multi-dimensional and disrupt so many aspects of a person's life, treatment is complex. Parents and other family members should be engaged to ensure appropriate linkage and follow-up with a qualified substance abuse professional and/or program. Intensive therapy helps the youngster apply new behavioral skills to daily life. Effective substance use treatments are typically comprehensive and incorporate various components, each targeting a particular aspect of the illness.

CONCLUSION

Synthetic cannabinoids are a group of substances in the world of designer drugs that present potentially dangerous health effects. These compounds have evolved rapidly since first appearing on the world market a few years ago. Identifying youth who abuse these drugs can be difficult. Since the safety profile of synthetic cannabinoid compounds is largely unknown, the ability to perform human studies to determine their effects presents an ethical challenge. As more research continue to emerge our understanding of both the extent of use and the associated harms will continue to develop. Our review of the literature suggests that synthetic cannabinoids may have adverse effects that are more severe than that of marijuana. In addition to the psychoactive effects, some SC compounds have been associated with more serious physical consequences, such as, seizures, myocardial infarction and renal damage. Clinicians are urged to familiarize themselves with these drugs and the typical presentations of patients who use them. Synthetic cannabinoid use should be strongly suspected if a youngster presents

with a history of marijuana use, symptoms and signs consistent with cannabis use, unexplained sudden onset of mental status changes and/or renal, neurological, or cardiovascular problems, and is in a situation in which his or her urine is being routinely monitored for illicit substance use.

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REVIEW

Novel insights in the management of sickle cell disease in childhood

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Abstract

Sickle cell disease (SCD) is a life-threatening genetic disorder characterized by chronic hemolytic anemia, vascular injury and multiorgan dysfunctions. Over the last few decades, there have been significant improvements in SCD management in Western countries, especially in pediatric population. An early onset of prophylaxis with Penicillin and a proper treatment of the infections have increased the overall survival in childhood. Nevertheless, management of painful episodes and prevention of organ damage are still challenging and more efforts are needed to better understand the mechanisms behind the development of chronic organ damages. Hydroxyurea (Hydroxycarbamide, HU), the only medication approved as a disease-modifying agent by the United States Food and Drug Administration and the European Medicines Agency, is usually under-used, especially in developing countries. Currently, hematopoietic stem-cell transplantation is considered the only curative option, although its use is limited by lack of donors and transplant-related toxicity. SCD symptoms are similar in children and adults, but complications and systemic organ damages increase with age, leading to early mortality worldwide. Experts in comprehensive care of young patients with SCD, especially those approaching the transition age to adulthood, are missing, leading people to rely on urgent care, increasing health care utilization costs and inappropriate treatments. It would be important to establish programs of comprehensive healthcare for patients with SCD from birth to adulthood, to improve their quality and expectancy of life.

Key words: Clinical management; Hydroxyurea; Sickle cell disease; Children; Hematopoietic stem-cell transplantation

Core tip: The correct management of sickle cell disease (SCD) requires a comprehensive medical care. Both a wider use of hydroxyurea and an early treatment of pain in children are needed to improve long-term outcomes. Moreover, we report in details the possibility offered by hematopoietic stem cell transplantation as a future curative option for SCD patients.

INTRODUCTION

Sickle cell disease (SCD) is an inherited red blood cell disorder, characterized by chronic haemolysis, vaso-occlusive complications and progressive multi-organ damage, with a major impact on patients' life expectancy and quality of life[1,2]. The incidence is estimated as more than 300000 new cases worldwide per year, mostly from sub-Saharan Africa, Arabian Peninsula and India[3]. Migration of affected populations from their native countries makes SCD a global disease.

SCD results from a single-point mutation (replacement of glutamic acid with valine in position 6) on the β-globin subunit of hemoglobin, creating the sickle hemoglobin (HbS)[4]. Homozygous (HbSS) patients inherit two copies of the HbS mutation and present clinical symptoms and complications of the disease while heterozygous carries (HbAS) do not exhibit clinical manifestations except in extremely rare cases.

Other sickle-related hemoglobinopathies occur when HbS is inherited in heterozygosis with other β globin chain mutation (HbSC) or quantitative defects in β-globin production (HbSβ^0 and HbSβ$^+$ thalassemia). HbSS and HbSβ^0 patients have the most severe clinical course, while patients with HbSC and HbSβ$^+$ have milder phenotypes[5]. Hypoxia, acidity and cellular dehydration influence the polymerization of HbS within erythrocytes and their deformation into the characteristic sickle shape.

Interaction between vascular endothelium and sickle red blood cells leads to episodic microvascular occlusion, with consequent tissue ischemia and further reperfusion; these processes are characterized by severe vascular and inflammatory stress due to increased expression of vascular oxidase, inflammatory cytokines and adhesion molecules[6].

Severe SCD-related complications may begin during early infancy, but thanks to the current multidisciplinary care, almost all SCD children have a chance to achieve adulthood. The improvement in survival rate results from important interventions including newborn screening, prophylaxis with penicillin, immunization against Heamophilus influenzae type B and Streptococcus pneumoniae, advances in supportive care and increased use of disease modifying treatments.

The aim of the review is to provide an overview of SCD management in childhood, focusing on common complications, current standard treatments, implementation of neonatal screening and comprehensive care programs.

NEWBORN SCREENING AND INFECTIONS PROPHYLAXIS

Bacterial infection is the primary cause of death in childhood; infants and children younger than 3 years of age are at risk of mortality and morbidity from sepsis.

In the 1970s, SCD had a very poor prognosis with high mortality in the first 5 years of life[7]. More recently, the mortality rate has been significantly reduced, and the number of children with SCD able to achieve adulthood is continuously growing[8-11]. The main cornerstones that have influenced SCD prognosis improvement are the introduction of neonatal screening and the antibacterial prophylaxis. In fact, neonatal screening leads to precocious identification of SCD subjects and provides the opportunity for early initiation of antibiotic prophylaxis, coupled with early immunization against *Streptococcus pneumoniae* and *Haemophilus influenzae*. Thirty years ago the PROPS Study showed the effectiveness of daily penicillin prophylaxis (starting before two months of age at 125 mg twice/day *per os*) in reducing incidence of pneumococcal infections in children with SCD[12]. A recent review confirmed that prophylactic penicillin significantly reduces the risk of pneumococcal infections in children with homozygous SCD, and is associated with minimal adverse reactions. However, further clinical trials are needed to determine the ideal age to safely discontinue penicillin[13].

Unfortunately, neonatal screening programs are still insufficient: Each newborn affected by SCD should rapidly achieve a definitive diagnosis and appropriate intervention[14] to avoid complications and reach the optimal development.

The implementation of newborn screening programs in Western countries (especially in Europe) is recommended by WHO and European Community, seen the large diffusion of a wide spectrum of hemoglobinopathies, caused by high migration rate from Middle East, South East Asia and malaria-endemic countries.

VASO-OCCLUSIVE CRISIS AND PAIN MANAGEMENT

Painful vaso-occlusive crisis (VOC) is the most common debilitating manifestation of SCD and the first cause of hospitalization for both children and adults[15]. Patients with SCD averagely refer pain more than the half of the days of the year[16] and approximately 60% of them have at least one episode of severe pain per year. Subjects with highest pain rates have an increased risk of early death compared to those with the lowest rates[17]. The sites more affected are extremities, chest and back although first episodes, that may occur as early as 6 mo of age, often present as dactylitis. For healthcare professionals, pain management in SCD is challenging and often complicated by the subjective nature of pain and the lack of standard care[18]. The treatment of painful VOC consists of non-opioid and opioid analgesics and intravenous hydration[19,20]. Early opioids administration, within 30-60 min from pain onset, improves VOC outcomes together with the use of an adequate starting dose, and frequent repeat doses until pain is reduced[21]. Parenteral administrations should be scheduled according

to an individualized protocol agreed by patients and clinicians or, when not available, guided by an institutional SCD-specific protocol[22].

The management of VOC has not significantly changed during the last decades. Recent studies about novel agents including inhaled nitric oxide and purified poloxamer-188, have demonstrated little benefits[23,24]. Among other emerging treatments for the management of vaso-occlusive events, pan-selectin inhibitor (GMI-1070)[25], anti-platelet therapies (*e.g.*, prasugrel), antioxidants (*e.g.*, N-acetyl cysteine), and anti-inflammatory medications (*e.g.*, regadenoson) have been tested in early-phase clinical trials and may represent future therapeutic opportunities[26].

Patients with SCD and acute pain crises may be wrongly identified as those with drug seeking or addiction. SCD patients are not drug- but care-seekers, seen the lack of psychosocial support, poor coping skills and inappropriate therapeutic expectations[27].

ABDOMINAL AND GENITO-URINARY COMPLICATIONS

Children with SCD often present vaso-occlusive pain as abdominal pain. The differential diagnosis of abdominal pain is broad in any child, and more complicated in patients with SCD. The most urgent cause of abdominal pain is splenic sequestration. It is defined as an acute enlargement of the spleen with a drop in hemoglobin of at least 2 g/dL from baseline associated with normal or increased reticulocyte count. In severe cases it may result in hypovolemic shock and death[28]. Early transfusion can be life saving, the starting volume is usually 5 mL/kg. Splenic sequestration can occur as early as 3 mo of age but is rarely seen beyond the age of 6 years. Hepatic sequestration is rare, caused by the obstruction of hepatic sinusoidal blood flow and characterized by painful hepatomegaly, anemia and reticulocytosis. Severe abdominal pain in patients with sickle cell disease often unresponsive to analgesia and associated with intestinal ileus and acute ischemic colitis is "Girdle syndrome" owing the circumferential distribution of pain.

Even if early implementation of supportive therapy may prevent irreversible ischemic damage to the gut, some authors have reported abdominal perforation requiring emergency surgery. Although most of these cases have been reported in adult patients, this syndrome should be considered in pediatric patients with abdominal pain.

Other more common causes of abdominal pain to be considered in children with SCD are: Cholelithiasis due to gallstones derived by unconjugated bilirubin and constipation. In children with SCD urinary tract impairment is frequent and include: Renal infarction, urinary tract infections/pyelonephritis[29].

A typical complication that occurs in male with SCD is priapism defined as unwanted painful erection of the penis. It can occur as young as 3 years of age and 90% of males with HbSS will have at least one episode by 20 years. Usually at the onset of priapism urinate, drink water, take a warm shower promote detumescence. Oral analgesic for pain and pseudoephedrine can be given at home and usually terminates priapism. Prolonged episode (> 4 h) represent a urological emergency[28,30].

ACUTE CHEST SYNDROME

Acute chest syndrome (ACS), an acute illness characterized by fever and/or respiratory symptoms associated to pulmonary infiltrate on chest X-ray[31], is a life-threatening complication of SCD with at least one episode during life in 30% of patients[32,33].

ACS usually originates from a lung injury due to pulmonary infection, fat embolism and/or pulmonary infarction. Consequently, alveolar oxygenation tension falls causing the HbS polymerization[34] and the ischemia-reperfusion process in lung vessels leads to the respiratory impairment[35].

Infective triggers are more common in children than adults and are frequently associated with viral infections especially in patients under 10 years of age. Multicenter studies, in particular in United States, demonstrate that the most common virus isolated is the respiratory syncytial virus while the most common bacteria are the Chlamydia pneumoniae in adults and the Mycoplasma pneumoniae in children[34].

Symptoms vary depending on age. Young patients often present with fever, cough and wheezing while adults show more painful episodes and impaired clinical conditions due to severe hypoxia, higher requirement for transfusion and higher mortality.

The optimal management of ACS requires an aggressive and comprehensive approach. Vital signs (pulse rate, systemic blood pressure, respiratory rate), oxygen saturation, frequent assessment of symptoms, should be monitored at least every four hours. Chest X-ray, full blood count, basic biochemistry tests, blood cultures, infectious respiratory disease testing, and blood group and screen are also needed. Oxygen administration or more aggressive respiratory supports, as Bi-level positive airway pressure or mechanical ventilation, should be used to maintain $SpO_2 \geqslant 95\%$, while intravenous fluids and opioids should be carefully managed to avoid risks of acute pulmonary edema and alveolar hypoventilation.

Other therapeutic tools include: Incentive spirometry, chest physiotherapy, antibiotic, and for those patients with progressive hypoxia or clinical deterioration, simple or exchanged red blood cell transfusion[36,37].

Although it is not confirmed by randomized controlled trials, it has been reported that blood transfusion can produce rapid improvements in clinical and radiological parameters in ACS[34,38]. Both "top up" and exchange transfusions increase oxygenation but exchange blood transfusion may have additional benefits in terms of reduction of circulating sickle cells and it is more indicated in patients with severe disease or with higher hemoglobin

concentration (> 90 g/L)[36].

It is important to bear in mind that approximately 50% of ACS occurs in patients hospitalized for other causes such as VOC or surgical intervention. This percentage could be reduced by incentive spirometry during hospitalization or by preoperative transfusion[22]. ACS consequences are scarring, pulmonary fibrosis and chronic sickle lung disease: Prevention of infections with antibiotic prophylaxis, administration of annual influenza vaccination, and avoidance of smoking may decrease the risk of ACS. Furthermore, children recovered from an episode of ACS should be offered therapy with hydroxyurea[39], at least to those with homozygous sickle cell anemia.

NEUROLOGICAL IMPAIRMENT

Neurological complications in SCD include silent cerebral infarct, stroke, and intracranial bleeding. Sickle cell anemia (SCA; HbSS and HbSβ0) is associated with a high prevalence (4.01%) and incidence (0.61/100 patient years) of cerebrovascular accidents[40] and is higher still in the absence of primary prevention. Overt stroke has been reported in about 10% of children with HbSS with a peak incidence between 2 and 5 years of age[41]. Prior transient ischemic attack, low steady-state hemoglobin concentration, recent episode of ACS, and elevated systolic blood pressure are considered risk factors for ischemic stroke. Despite limited available evidences to guide best practice in the acute management of ischemic stroke in SCD, initial supportive strategies including co-operation of a multidisciplinary team of specialists (hematologist, neurologist, neuroradiologist, and transfusion medicine specialist) and exchange transfusions are recommended[42]. The risk of stroke recurrence, in the absence of secondary preventative measures, has been reported to be as high as 60%-90%. Chronic transfusions are currently considered the standard for care of secondary stroke prevention. Moreover, the use of Hydroxyurea, although not as effective as regular blood transfusions, represents a reasonable therapeutic alternative and recently it has also been showed that hematopoietic stem-cell transplantation (HSCT) reduces rate of stroke recurrency when compared with regular blood transfusions in the following five years[43-46].

Children with HbSS and HbSβ0 should be routinely monitored by Transcranial Doppler (TCD) from the age of 2 until 16[47,48]. The STOP trial (Stroke Prevention Trial in Sickle Cell Anemia) showed that an abnormal TCD flow velocity exceeding 200 cm/s is associated with a 40% increased risk of stroke within 3 years. In patients with abnormal velocity, the introduction of regular transfusions resulted in a reduction in stroke incidence by almost 95%[49]. Although long-term transfusions have major long-term side effects, the STOP 2 trial showed that cessation of transfusions after the normalization of TCD resulted in a recurrence of abnormal blood flow velocity on TCD and increased risk of stroke[50]. The role of hydroxyurea for prevention of primary and secondary

stroke has also been investigated. An ongoing phase III trial, TWiTCH (Transcranial Doppler with Transfusions Changing to Hydroxyurea) (ClinicalTrials.gov identifier number NCT01425307) intends to compare hydroxyurea *vs* transfusions for pediatric patients with SCA and abnormally high TCD velocities, who currently receive chronic transfusions to reduce the risk of primary stroke. The SWiTCH study (Stroke with Transfusions Changing to Hydroxyurea) was designed to compare alternative therapy of hydroxyurea and phlebotomy with standard therapy (transfusions and iron chelation) for the prevention of secondary stroke. However, this study was stopped, as alternative therapy was associated with a higher stroke rate[51]. Lifelong transfusion remains the standard of care for secondary stroke prevention, and in those with high flow velocity on TCD (primary prevention)[37]. However, hydroxyurea is a reasonable alternative in patients with complications of transfusions, with poor compliance, or in countries with limited blood supplies.

Silent cerebral infarcts

Silent cerebral infarcts (SCI) are characterized by an abnormal brain MRI in the absence of history or physical findings of an overt stroke. SCI occur in approximately one-quarter of the children with SCA before six years of age and in one-third of those younger than fourteen. Risk factors include male sex, lower baseline hemoglobin concentration, higher baseline systolic blood pressure and previous seizures[52]. While overt strokes are typically located in both cortex and white matter, SCI usually occur in deep white matter of the frontal, parietal lobes or, less frequently, in basal ganglia, thalamus and temporal lobes[53]. Children with SCI have lower cognitive test scores compared with the general population, and have additional specific functional impairment, impacting such executive functions as selective attention, card sorting, working memory and processing speed, visual motor speed and coordination, vocabulary, visual memory and abstract reasoning and verbal comprehension[54-59]. Children with SCD and SCI have twice the chance of academic difficulties than those without SCI[60]. The presence of SCI is a risk factor for additional neurologic injury, with a higher risk of both clinical stroke (14-fold), and progressive silent infarction. It has been showed that approximately 25% of children with SCI, have new and/or enlarging lesions on follow-up MRI scan[53,61]. Despite the high prevalence of SCI in patients with SCD, no established therapy is available for primary or secondary prevention. The STOP Trial showed that the presence of SCI in the setting of an abnormal TCD measurement is associated with increased risk of stroke compared to those with no SCI (52% *vs* 21%). In the same group of patients the stroke risk decreased with blood transfusion therapy compared to those with only elevated TCD measurement (0% *vs* 5%, respectively)[49]. These results provided the preliminary evidence suggesting that regular blood transfusion therapy may be effective in

preventing neurologic injury. At the end of STOP2 study, when children with elevated TCD measurements were randomized to continue or stop transfusions, 8% of those who continued transfusions developed new brain MRI lesions compared with the 28% of those who stopped[50]. Similarly, the SIT trial showed that children with SCA had a relative risk reduction of infarcts recurrence of 58%, if they were receiving regular blood transfusions. The optimal timing to detect SCI is still unclear but most data support commencing MRI from around 5 years or school entry[62]. Developmental delay or declining school performance may be the only clinical signs of SCI and therefore in this instance an early neurocognitive evaluation should be offered in patients with SCD.

DISEASE-MODIFYING AGENTS

Hydroxyurea (HU) is an inhibitor of ribonucleotide reductase that increases fetal hemoglobin (HbF) in red blood cells, rising cellular size and deformability. HU also impairs leukocytes and reticulocytes production and the expression of adhesion molecules, reducing vascular occlusion. In addition, when metabolized, HU releases nitric oxide, contributing to local vasodilation[63,64]. HU is the only medication approved for treatment of SCD by the United States Food and Drug administration (1998) and by the European Medicines Agency (2007)[65]. A multicenter, randomized, double-blind, placebo-controlled clinical trial in infants from 9-18 mo of age with SCA (BABY-HUG) demonstrated that treatment with HU was associated with statistically significant lower rates of initial and recurrent episode of pain, dactylitis, ACS, and hospitalization compared to placebo group[66]. Similar results have been also showed by the Multicenter Study of Hydroxyurea, with positive effects of HU on painful vaso-occlusive events at all ages[67]. HU treatment seems to be associated with decreased mortality[68,69] and it is generally well tolerated in both children and infants, with no influence on growth and development[70]. Leukopenia, neutropenia and thrombocytopenia are the most frequently reported side effects, but they are generally mild and reversible with discontinuation or with dose decreasing[66,71]. However, several concerns about the HU long-term side effects have been expressed especially in pediatric population. Even though HU does not appear to increase the risk of malignancy in SCD patients[63], potential effects on fertility and teratogenicity have been described[72-75]. Considering the benefit of HU in preventing end organ damage and improving survival, it seems reasonable to recommend hydroxyurea to SCD patients with HbSS or HbSβ^0 genotypes, regardless of their disease severity. Recently, it was suggested to offer HU therapy in children starting at 9 mo of age, including those who are asymptomatic[22]. When considering the use of hydroxyurea for patients with SCD, it is important to balance its well-established benefits with its hypothetical long-term side effects, in particular in asymptomatic children[37].

HEMATOPOIETIC STEM CELL TRANSPLANTATION AND GENE-THERAPY

Despite the development of supportive care including HU and transfusion programs, hematopoietic stem cell transplantation (HSCT) remains the only curative treatment for patients affected by severe SCD. The first successful pediatric HSCT was performed in 1984[76], and since there, several hundreds of patients with SCD have been transplanted, mainly from an HLA-identical sibling donor. Results of major clinical studies on allogeneic HSCT from an HLA-identical sibling donor in children with SCD, in the past decade, reported an overall survival rate (OS) greater than 90% and an event-free survival rate (EFS) greater than 80%[44,46,77-81]. Recent studies on HLA-identical sibling HSCT are summarized in Table 1[82-87]. Of interest, 37/38 subjects, treated with HU before HSCT survived free of SCD, with an estimate EFS of 8-year, significantly higher compared to those who did not receive hydroxyurea before HSCT ($P < 0.001$)[85]. HSCT from matched-sibling related donor offer the best outcomes of transplantation and it seems to be curative in 90%-95% of pediatric recipients with severe SCD following a conventional conditioning regimen[88]. Transplants outcomes are best in young people (< 16 years) before long-term transfusions become necessary, in absence of comorbidities and organ damages[46]. With the achievement of such survival rates, the risk of transplant related morbidity has become a major concern and clinical focus has recently shifted to the minimization of the regimen-related toxicity. A recent Italian study[89] showed that a conditioning regimen with treosulfan/thiotepa/fludarabine for HLA-matched sibling and unrelated donor HSCT was well tolerated with no case of grade Ⅲ and Ⅳ regimen-related toxicity. The 7-year OS and DFS for the whole cohort were 100% and 93%, respectively.

Umbilical cord blood (UCB)-derived hematopoietic stem cells in pediatric patients with hematological disorders are increasing as alternative source of hematopoietic stem cells, seen the safe technique of hematopoietic collection, the low risk of viral contamination of the graft and the reduced incidence and severity of acute and chronic graft *vs* host disease (GVHD)[90,91]. A recent update of HLA-identical sibling UCB transplantation compared marrow and UCB transplants outcomes of 485 recipient cases with thalassemia major and SCD. The overall 6-year DFS in 160 patients with SCD was 92% ± 2%: 90% ± 5% in 30 patients after UCB transplantation and 92% ± 2% in 130 patients after bone marrow transplantation. None of the patients developed chronic extensive GVHD and none died of GVHD after UCB transplantation[92]. UCB and marrow from HLA-identical donors might be used interchangeably, and UCB from a sibling donor appears to be useful in terms of lower risk of acute and chronic GVHD. For patients with no HLA-identical sibling donor hematopoietic

Table 1	Recent series of human leukocyte antigen-identical transplant for sickle cell disease in children					
Ref.	**Conditioning regimen**	***n***	**Age range in years**	**Deaths**	**Follow-up (yr)**	**Outcome**
Krishnamurti *et al*[82]	BU, Flu, eATG, total lymphoid irradiation	7	6-18	None	2-8.5	All patients alive EFS 86%
McPherson *et al*[83]	BU, CY, ATG	25	3.3-17.4	1	0.1-10	OS and DFS 96% (median survivor follow-4.9 yr)
Lucarelli *et al*[84]	BU, CY, rATG ± Flu	40	2-17	3	1-10	5-yr OS and DFS 91%
Dedeken *et al*[85]	BU, CY, ± rATG, ± HU	50	1.7-15.3	2	0.4-21.3	8-yr EFS 85.6% and OS 94.1%
Bhatia *et al*[86]	BU, Flu, Alem	18	2.3-20.2	None	0.4-7.5	2-yr EFS and OS both 100%
Soni *et al*[87]	BU, CY, rATG	15	1.5-18	None	0.9-7.5	3-yr EFS and OS both 100%

ATG: Antithymocyte globulin; BU: Busulfan; CY: Cyclophosphamide; DFS: Disease free survival; eATG: Equine antithymocyte globulin; EFS: Event free survival; Flu: Fludarabine; HU: Hydroxyurea; OS: Overall survival; rATG: Rabbit antithymocyte globulin; Alem: Alemtuzumab.

cells from an HLA-mismatched related donor could help. The Johns Hopkins group in 2012 reported the largest study of HLA haploidentical bone marrow transplantation in severe SCD. In this pilot investigation, 14 recipients of haploidentical HSCT were treated and all had prompt recovery after HSCT, although 6 of these patients developed graft rejection, all were alive with a median follow-up of 711 d[93]. Despite promising data, allo-HSCT is underutilized[94]. The main barrier is the limited availability of suitable donor. It has been estimated that only 14% of those patients with SCD have a suitable HLA-identical sibling donor and that only 19% have a very well matched unrelated marrow donor in the volunteer registry[95,96]. Nevertheless, conventional treatment itself is a high-risk of mortality procedure and of treatment-related morbidity, due to GVHD, infertility and gonadal failure[88]. Considering all the allogenic HSCT limitations, the gene therapy using autologous stem cells can potentially cure SCD, and could overcome the problems of lack of available donors and immunologic side effects. Inherited hematopoietic disorders are potentially targetable, because hematopoietic stem cells can be readily isolated from bone marrow or mobilized from peripheral blood, manipulated *ex vivo*, and transplanted back using current tools and knowledge of bone marrow transplant technology. Gene therapy has exploited the ability of retrovirus vectors, which are equipped with the machinery to reverse, to transcribe their RNA into complementary DNA and integrate this latter into the host cell genome to deliver therapeutic genes into cells. Seen the success in β-thalassemia gene therapy, similar studies on SCD are started. Both γ-globinbased and modified β-globin-based vectors have been developed for SCD gene therapy[97]. There are still no data available, but clinical trials using lentiviral vectors have begun in France (NCT02151526) and in United States (NCT02140554, NCT02186418, NCT02247843)[98]. The development of gene therapy technologies holds the promise of genetic correction of future hemoglobinopathies.

TRANSITION FROM PEDIATRIC TO ADULT HEALTHCARE

Management programs for pediatric patients with SCD in Western world areas include acute care, routine prevention, monitoring and treatment of complications[99]. The management of SCD in adult patients is more complex, because of the additional co-morbidities, increased multi-organ involvement, chronic pain and psychosocial and socioeconomic factors. Although an increasing number of children with SCD are achieving adulthood, there has not been a corresponding increase in medical experts trained to treat older patients, delaying transition from adolescent to adult care[100]. Epidemiological studies indicate that SCD-related mortality and morbidity are increased in young adults and most patients feel that they are not ready for transition to adult healthcare[101]. These data supported by findings of a recent study of the Dallas Newborn Cohort show that SCD patients are at greatest risk of mortality when they are transition-aged. In this cohort, seven of the most recent patients died were aged 18 years or older, and six of these patients had recently transitioned out of the pediatric care[8]. With no adult SCD care providers, patients become dependent of acute care services and do not receive the necessary coordinated multi-disciplinary care[102]. Identification of a designated adult SCD provider and enhanced early education of all pediatric SCD patients regarding the need to continue comprehensive care in the adult setting is imperative for a successful transition from pediatric to adult care.

CONCLUSION

Sickle cell disease is a global health problem that affects more than 300000 newborns per year, predominantly in sub Saharan Africa. In this area, mortality is estimated to be more than 50% by the age of 5 years for those with homozygous hemoglobin S. With the implementation of neonatal screening programs and new therapeutic approaches, SCD related morbidity in childhood is decreasing, raising the number of patients achieving adulthood. Currently, there are few pharmacological treatments available for SCD, while promising disease-modifying agents, as HU, are still significantly under-utilized. Moreover, the use of curative options as HSCT, is limited because of the lack of matching donors and some concerns regarding long-term toxicity. Although

comprehensive management programs for SCD pediatric patients have started in the last years in United States and Europe, an improvement in transition age care, to reduce early mortality in young adults and hospital costs, is needed.

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Retinopathy of prematurity: Past, present and future

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Abstract

Retinopathy of prematurity (ROP) is a vasoproliferative disorder of the retina occurring principally in new born preterm infants. It is an avoidable cause of childhood blindness. With the increase in the survival of preterm babies, ROP has become the leading cause of preventable childhood blindness throughout the world. A simple screening test done within a few weeks after birth by an ophthalmologist can avoid this preventable blindness. Although screening guidelines and protocols are strictly followed in the developed nations, it lacks in developing economies like India and China, which have the highest number of preterm deliveries in the world. The burden of this blindness in these countries is set to increase tremendously in the future, if corrective steps are not taken immediately. ROP first emerged in 1940s and 1950s, when it was called retrolental fibroplasia. Several epidemics of this disease were and are still occurring in different regions of the world and since then a lot of research has been done on this disease. However, till date very few comprehensive review articles covering all the aspects of ROP are published. This review highlights the past, present and future strategies in managing this disease. It would help the pediatricians to update their current knowledge on ROP.

Key words: Retinopathy of prematurity; Retrolental fibroplasia; Screening guidelines; Oxygen; Classification; Epidemics; Anti vascular endothelial growth factor; Vitrectomy; Laser; Future trends

Core tip: Although literature is full of various articles on retinopathy of prematurity (ROP), there are very few comprehensive review articles on this disease. This article covers ROP from 1940s and 1950s when seen as retrolental fibroplasia, to the current screening and treatment guidelines to the future trends. Our objective is to consolidate the literature on this disease, which will benefit the pediatricians.

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INTRODUCTION

Retinopathy of prematurity (ROP) used to be called as retrolental fibroplasia (RLF) in 1940s. RLF was the term first coined in the year 1942 by Terry[1] and was defined as a progressive disorder seen exclusively in premature infants of low birth weight, where in a fibrous tissue is formed behind the lens, resulting in blindness and severe visual impairment. When it was first described this disease was not commonly seen, and hence had little interest but 10 years later it became a major problem to all paediatricians and ophthalmologists. It now affects thousands of children worldwide[2].

STUDY METHODS

Literature search in PubMed was conducted covering the period 1940-2015 with regards to retinopathy of prematurity, retrolental fibroplasia, classification, treatment, laser, anti vascular endothelial growth factor and recent advances[3-81].

PAST

Role of oxygen - the cause or the treatment

Many studies were conducted worldwide since 1951 to determine the exact mechanism of this disorder. Ophthalmic literature of the past reveals, anoxia in premature babies to be the prime causative factor for the development of RLF and hence in 1952 was called as anoxic retinopathy. A study by Szewczyk[3] revealed that this was the response of immature neural tissue to anoxia. He explained the mechanism would probably be due to low oxygen tension in the fetal blood of premature which causes retinal vessels to dilate initially, and still when the demand is not satisfied it leads to edema, transudation and haemorrhages. Campbell[4] first brought to notice the development of RLF in infants undergoing intensive oxygen therapy. A clinicopathologic study at the Women's hospital Melbourne, Australia by Ryan[5] between 1948 and 1950 revealed 23 cases of RLF. It was noted that no case of RLF was reported prior to the introduction of an oxygen cot. The nursing staffs were giving oxygen liberally to all babies with this oxygen cot and hence there was increase in the incidence. Later on, from October 1950, oxygen was restricted to only babies with cyanosis and since then a fall in number of RLF was seen. With this study it was understood that, the normal human fetus is in a state of cyanosis, because pure arterial blood is not carried by any of the arteries. Since high oxygen concentrations are toxic to adults, similarly normal concentration is toxic to immature tissues. Hence it was concluded that RLF can be prevented by giving oxygen only to those premature babies who require it.

It was observed that anoxia might occur at the cellular level during oxygen therapy even though the environmental oxygen and the blood oxygen levels are increased. This paradoxical situation, which was called as "hypoxic-anoxia" will occur as a result of inactivation of oxidative enzymes from prolonged exposure to high oxygen levels.

Equal importance was also given to the rate of withdrawal from oxygen since it was noted that it minimizes the retinal damage induced by hyperoxia. A controlled nursery study by Bedrossian *et al*[6] reported a significantly higher incidence of RLF in infants who were rapidly withdrawn from an atmosphere of continuous oxygen as compared with a group where oxygen was gradually reduced.

Hence, it became necessary to monitor the oxygen and prior to the availability of arterial oxygen tension to measure, ophthalmoscopic monitoring of retinal vessel caliber was done. The following guidelines for oxygen therapy were recommended: (1) Oxygen should be given to premature infants proved to be hypoxic or strongly suspected; (2) When high concentrations of oxygen are required for significant periods, in addition to measuring the incubator oxygen level, arterial oxygen tension monitoring should also be done; (3) Ophthalmoscopic monitoring of retinal vasoconstriction should be done at regular intervals, and when marked constriction is detected, prompt reduction in the concentration of administered oxygen may prevent retinal damage; and (4) Even for a full term infant, retina is incompletely vascularised temporally, hence oxygen therapy should be cautiously administered and limited to specific indication only.

Pathogenesis

The effect of oxygen on the retina on the immature vasculature was described in two stages: (1) Primary stage or vasoconstrictive phase: This occurs during exposure to hyperoxia and there is also suppression of the normal anterior ward vascularisation of the retina. This mechanism of vasoconstrictive and obliterative effect of oxygen is seen predominantly in the developing retinal vessels. This inturn leads to suppression of vascular endothelial growth factor; and (2) Secondary stage or vasoproliferative phase: This occurs during the shifting from oxygen to room air, and involves dilatation and tortuosity of the existing larger vessels with neovascularisation and proliferation of new vessels into the vitreous. This is mainly due to the sudden surge in vascular endothelial growth factor levels.

Laboratory findings

In the 1950's the kitten model was used in most experiments as its immature retinal vessels showed selective response to oxygen[7,8]. Smith *et al*[9] in the year 1994 demonstrated a good, easily replicable and measurable mouse model of oxygen induced retinopathy. One week old C57BL/6J mice were put in 75% oxygen chambers for 5 d and then brought back to normal atmospheric air. Vascular pattern was assessed using a fluorescein-dextran perfusion method.

The abnormal neovascularization was measured by counting the nuclei of new vessels extending from the retina into the vitreous in 6 μ sagittal cross section[9]. Fluorescein-dextran angiography highlighted the entire retinal vasculature, including the neovascularization. Hyperoxia induced new vessels occurred at the junction between vascularised and avascular retina in the mid periphery[9]. Retinal neovascularisation was seen between day 17 and day 21, postnatally. Thus from this study it was concluded that, neovascularisation was seen after loss of patent vessels in the central retina with hyperoxia exposure. A shift from hyperoxia to room air causes relative ischemia in the non-perfused retina and the development of neovascularisation was seen at the interface of perfused and non-perfused retina.

Epidemics in ROP

A variation in number of cases was seen in different era and in different countries. This was termed as epidemics in ROP. What actually triggered the beginning of first epidemic was unmonitored oxygen supplementation in the late 1940's and 1950's in Europe and North America[10,11]. After this incident, overuse of oxygen was stopped and careful administration of oxygen was recommended. Second epidemic was faced by the developed countries, in premature and low birth weight babies (< 1000 g at birth)[12]. India and the other developing countries come under the third epidemic which is characterized by severe ROP in bigger premature babies[13]. The reason again being lack of proper neonatal care and improper oxygen administration. Hence there is a need for strict guidelines of oxygen administration and monitoring and neonatologist play a major role in this aspect.

PRESENT

Classification

A committee for ROP classification was formed in 1984, which proposed an international classification of ROP (ICROP) by dividing the retina into three zones, extending from posterior to anterior retina and describing the extent of ROP in clock-hours of involvement[14]. However with the advances in retinal imaging techniques, a revised ICROP classification was put forth which described the zones better[15].

Zones

Three concentric zones, centered on the retina define the antero-posterior location of retinopathy.

Zone Ⅰ: With optic disc as the center, and twice the distance from the disc to fovea, the circle formed is zone I. Using a 25 or 28 diopter (D)-condensing lens, when the nasal edge of the optic disc is kept at one edge, the temporal field of view is zone Ⅰ extent.

Zone Ⅱ: It starts from the edge of zone Ⅰ and extends till the ora serrata nasally, with a corresponding area temporally.

Zone Ⅲ: Zone Ⅲ is the remaining crescent of retina temporally.

Extent of retinopathy

The extent of the ROP is documented by the number of clock hours involved. For the observer examining each eye, the temporal side of the right eye is 9 o'clock and that of the left eye is 3 o'clock and *vice versa*.

Stages of ROP

It denotes the degree of vascular changes. There are five stages.

Stage 1 - demarcation line: A demarcation line is seen between the vascular and avascular retina. It is a thin structure that lies in the plane of the retina (Figure 1A).

Stage 2 - ridge: The demarcation line grows to occupy a volume and has a height and width to form a ridge above the plane of retina (Figure 1B). Small tufts of new vessels also called as "popcorn" vessels may be seen posterior to the ridge.

Stage 3 - ridge with extra retinal fibrovascular proliferation: In this stage extraretinal fibrovascular tissue is seen arising from the ridge into the vitreous (Figure 1C). It may be continuous or non-continuous and is posterior to the ridge.

Stage 4 - subtotal retinal detachment: Here a partial detachment of the retina is seen which may be exudative or tractional. It is sub divided into the following: (1) Partial retinal detachment not involving the fovea (stage 4A) (Figure 1D); and (2) Partial retinal detachment involving the fovea (stage 4B) (Figure 2A).

Stage 5 - total retinal detachment: Here a total retinal detachment is seen as child usually presents with leukocoria (white pupillary reflex) (Figure 2B).

Plus disease: It is an indicator of severity of the disease and is defined as venous dilation and arterial tortuosity of the posterior pole vessels (Figure 2C).

Pre-plus disease: It is defined as posterior pole vascular dilation and tortuosity which is more than normal but less than plus disease.

Aggressive posterior ROP: This refers to an uncommon, rapidly progressive, form of ROP previously referred to as "rush disease". It is characterized by a posterior location, severe plus disease, and flat intraretinal neovascularization (Figure 2D). It can progress very fast to stage 5 ROP and blindness, if not intervened early. The flat neovascularization can be quite subtle and can easily confuse less experienced
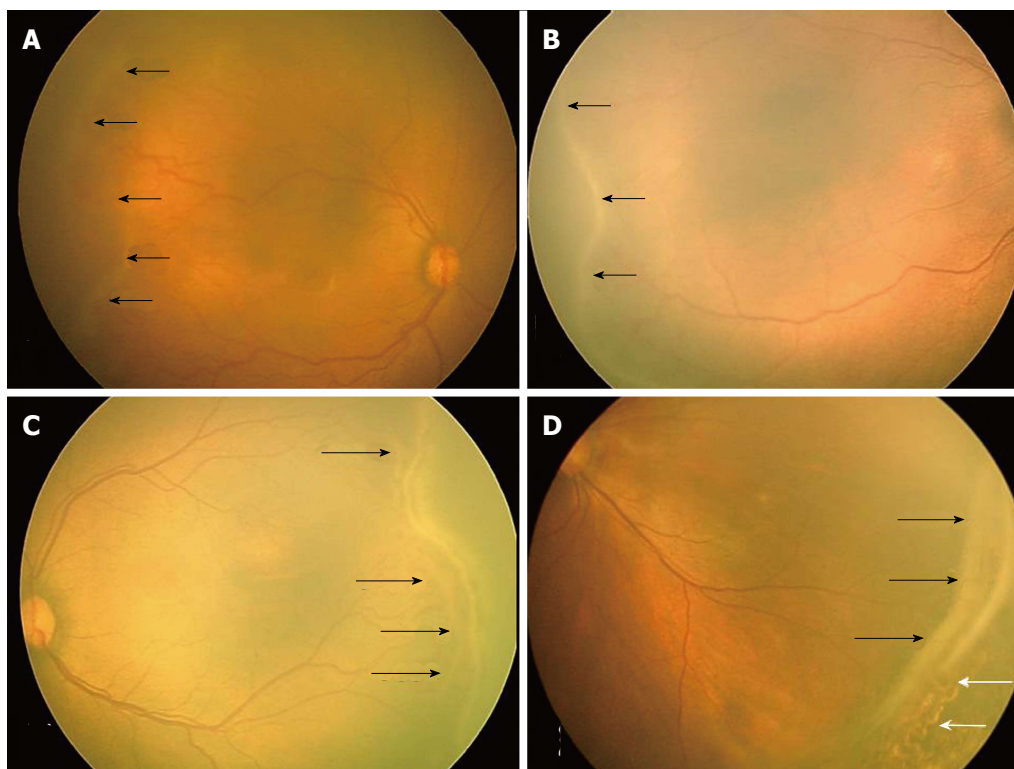
Figure 1 RetCam fundus images showing retinopathy of prematurity stages 1, 2, 3 and 4A. A: Fundus image of right eye showing stage 1 ROP with demarcation line (black arrows); B: Fundus image of right eye showing stage 2 ROP with ridge (black arrows); C: Fundus image of left eye showing stage 3 extra retinal fibrovascular proliferation (black arrows); D: Fundus picture of left eye showing stage 4A partial retinal detachment not involving the fovea (black arrows). Laser scars are shown with white arrows. ROP: Retinopathy of prematurity.

examiners.

Screening for ROP - present concept

Worldwide ROP is amongst the leading causes for childhood blindness[16]. Early detection and timely intervention to reduce this burden of blindness, makes screening an important aspect of ROP.

Screening is a process of identifying disease in the apparently normal subjects who are at risk by applying simple, safe, repeatable, sensitive and valid tests for disease detection. Due to lack of gold standard tests for ROP, the screening process may also be referred as "case detection initiative"[16]. The neonatal care for each country needs to be understood as ROP is diverse in presentation owing to the geographic variations, available infrastructure and altered temporal development of retinopathy in different locations in the retina[17]. Screening ultimately aims at reducing the incidence of ROP, prompt case detection and optimal treatment for ROP, thereby reducing the severity and overall burden of childhood blindness.

Eighty percent of infants with birth weight less than 1500 g born in the United Kingdom survive and the incidence of stage 3 ROP of approximately 8% to 10% has been reported[18]. Thus all babies having gestational age ≤ 31 wk or ≤ 1500 g are screened in United Kingdom[18]. American guidelines given by the American Academy of Pediatrics state that, infants with a birth weight ≤ 1500 g or gestational age of ≤ 30 wk and selected infants with a birth weight between 1500 and 2000 g or gestational age of more than 30 wk with an unstable clinical course, should be screened for ROP[19].

In many developing economies, larger babies with a birth weight between 1500 and 2000 g may also develop ROP[20]. Hence in counties like India, a birth weight ≤ 1750 g and/or gestational age of ≤ 34 wk may be used as a cut-off for ROP screening. Bigger babies with a gestational age of 34 to 36 wk gestation or a birth weight between 1750 and 2000 g should also be screened if child has a stormy neonatal course[20]. New Zealand has reported a reduced incidence in ROP due to progress in the screening and clinical management and recommends screening criteria of < 31 wk' gestation or < 1250 g to be sufficient[21]. Other risk factors for ROP include severe respiratory distress syndrome, anemia, neonatal sepsis, thrombocytopenia, multiple blood transfusions and apnea. If these risk factors are not seriously taken into consideration, affected infants may inadvertently get excluded and hence careful review for risk factors should be taken by the pediatrician.

ROP screening should start by 31 wk postconceptional age or 4 wk after birth, whichever is later[22]. In developing countries some babies may develop early aggressive posterior (AP)-ROP[23,24]. Thus, in developing countries, to enable early identification and treatment of AP-ROP, infants < 28 wk or < 1200 g birth weight should be screened relatively earlier at 2-3 wk of age[25].
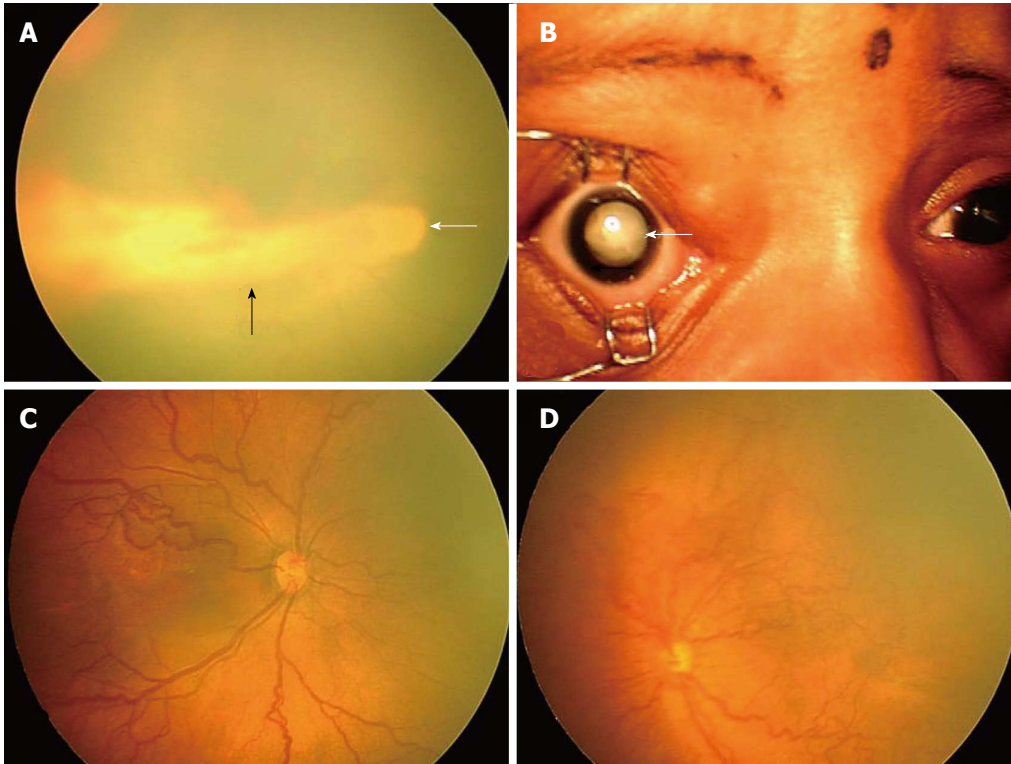
Figure 2 RetCam fundus images showing retinopathy of prematurity stages 4B, 5, plus disease and aggressive posterior-retinopathy of prematurity. A: Fundus picture of right eye showing stage 4B partial retinal detachment involving the fovea (black arrow). Optic disc is shown with a white arrow; B: Anterior segment picture of right eye showing stage 5 ROP with total retinal detachment (white arrow); C: Fundus picture of right eye showing dilated and tortuous vessels suggestive of plus disease; D: Fundus picture of left eye showing aggressive posterior ROP. ROP: Retinopathy of prematurity.

Examination technique: The examination technique traditionally involves two steps namely the dilatation of pupil and indirect ophthalmoscopy preferably with a 28D lens. It is preferred to perform pupillary dilatation 45 min prior to commencement of the screening. Dilating drops used are a mixture of cyclopentolate (0.5%) and phenylephrine (2.5%) drops to be applied two to three times about 10-15 min apart. Alternatively, tropicamide (0.4%) may be used instead of cyclopentolate. Diluted cyclopentolate may also be used to reduce probable systemic adverse effects. Use of atropine is to be avoided. The neonatal nurse should be instructed to wife any excess drops from the eye lid to prevent systemic absorption and complications like tachycardia and hyperthermia. If the pupil is resistant to dilatation, it may indicate presence of persistent iris vessels (tunica vasculosa lentis) and must be confirmed by the ophthalmologist before applying more drops.

The United Kingdom guidelines do not mandate use of eye speculum (*e.g.*, Barraquer, Sauer, Alfonso specula) and scleral depression (*e.g.*, Flynn depressor) with topical anaesthesia. However, meticulous examination, warrants its use.

Present screening tools: ROP screening today follows a telemedicine approach which refers to use of information technology between participants who are geographically separated and offers a possible solution to screening challenges and aids effective management. There are no reports requiring on-site diagnostic examination by an ophthalmologist even if images have not appropriately identified severe retinopathy[26,27]. Retinal examination of infants at risk for ROP using the RetCam digital camera system using wide angle lens with interchangeable high magnification lenses allows photographic documentation permitting remote interpretation of images and is incre-asingly being used for telemedicine world over[28-31]. But this telescreening is advisable only in places where no ophthalmologist is available for bed side screening, as a recent review showed that digital imaging screening cannot replace indirect ophthalmoscopy[32].

Predictive factors for ROP progression which include postnatal weight gain, serum insulin-like growth factor 1 (IGF-1) levels, and quantifiable vessel changes in the retina can reliably be isolated and used to indicate presence or absence of disease. The Weight, IGF-1 levels, Neonatal, ROP (WINROP) study[33] carried out weekly measurements of IGF-1 levels and weekly weight from birth until 36 wk. WINROP correctly identified all infants with a low risk ROP and those requiring laser treatments for proliferative ROP on the basis of predictive factors.

The rapid advances in technologies and increasing knowledge about disease and genetics along with the growing need for efficient, effective, and timely ROP evaluations may completely transform the present diagnostic
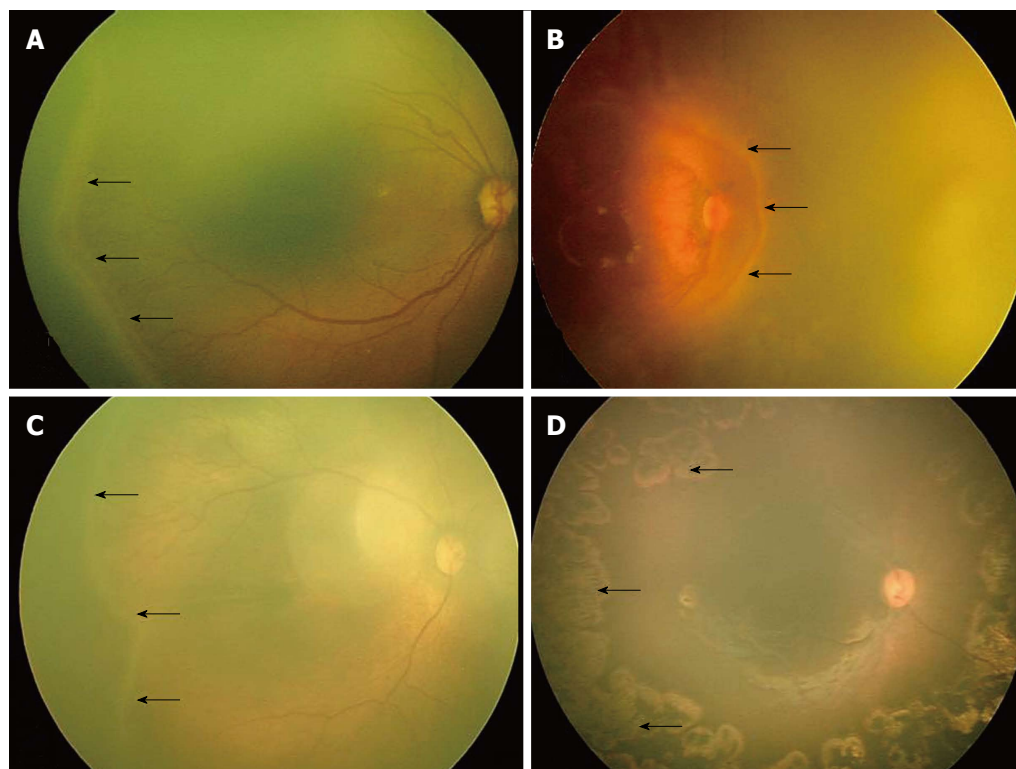
Figure 3 RetCam fundus images showing threshold, type 1, type 2 retinopathy of prematurity and post laser regressed retinopathy of prematurity. A: Fundus image of right eye showing "threshold ROP" (black arrows); B: Fundus image of right eye showing stage 3 ROP in zone 1 (black arrows) with plus disease suggestive of "type 1 ROP"; C: Fundus image of right eye showing stage 2 ROP in zone 2 (black arrows) without plus disease suggestive of "type 2 ROP"; D: Fundus picture of right eye showing laser scars (black arrows). ROP: Retinopathy of prematurity.

approach in near future.

Treatment for ROP - present concept

Although the ICROP classification gave a detailed classi-fication of ROP, it never recommended when to treat ROP. Following are the treatment stages of ROP.

Threshold ROP: The cryotherapy for retinopathy of prematurity (CRYO-ROP) study[34] stated that treatment should be imparted to eyes with threshold disease, defined as stage 3 ROP in zone Ⅰ or Ⅱ, having five contiguous or eight discontiguous clock hours with plus disease (Figure 3A). This was the previous "cut off" for treatment.

Pre-threshold ROP: The early treatment for retinopathy of prematurity (ETROP) study[35] redefined these guidelines. They defined the actively treatable and observational types of pre-threshold ROP as "type 1" (high-risk prethreshold ROP) and "type 2" ROP respectively. "Type 1 ROP" is defined as: (1) Any stage of ROP in zone Ⅰ with plus disease (Figure 3B); or (2) Stage 3 in zone Ⅰ without plus; or (3) Stages 2 or 3 in zone Ⅱ with plus disease. These are the modified guidelines for treatment. "Type 2 ROP" is defined as stages 1 or 2 in zone Ⅰ (Figure 3C) without plus, or stage 3 in zone Ⅱ without plus. These can be observed and watched at one week or less follow-up. Cases having stages 1 or 2 in zone Ⅱ require two weekly follow up, while stages 1 or 2 in zone Ⅲ require three weekly follow-up[19].

Treatment modalities

Cryotherapy: This involves treatment of the avascular retina using of a cryoprobe in order to reduce unfavorable outcomes of ROP like retinal folds and retinal detachment. Cryotherapy however is stressful for the babies, requires general anesthesia and creates lot of periocular inflam-mation. It is therefore no more the treatment of choice.

Indirect laser photocoagulation: Laser photo-coagulation of the peripheral retina using indirect delivery system has proved to be the gold standard, time tested and successful means of treatment since many years[33,36,37]. Laser photocoagulation using infra red diode laser forms a portable mode of treatment and can be performed in the nursery by skilled professionals (Figure 3D). The biggest advantage is that it can be done under topical anesthesia. However many institutions prefer general anesthesia for patient comfort. Laser ablation covers the relatively hypoxic retina into anoxic, thereby reducing stimulus for new vessel formation and disease progression. The ETROP study from its six years analysis confirmed that eyes with type 1 ROP benefited from laser treatment at high risk pre threshold stage[38]. This failure rate of 9.6%, was better than the results shown by the CRYO-ROP study.

Cryotherapy or laser photocoagulation, ablation has its own demerits and causes destruction of the retina

amounting to significant visual field loss. Pharmacologic therapy is thus ushering a new era of ROP management.

Anti-vascular endothelial growth factors drugs: Anti-vascular endothelial growth factor (VEGF) drugs directly block the effects of VEGF, and a single intravitreal injection is less time consuming and less expensive as compared to lasers. Exceptionally successful results with anti-VEGF drugs in adult retinal vascular diseases led to its trial in paediatric retinopathy as a monotherapy as well as in combination with lasers. Intravitreal bevacizumab as an initial mono therapy was reported to cause regression of type 1 ROP in 88% cases with 9% requiring additional laser treatment and 1% requiring additional injection[39]. The BEAT-ROP (Bevacizumab Eliminates the Angiogenic Threat of ROP) study[40] is the only randomised trial done comparing anti-VEGF *vs* conventional laser. It suggested superiority of anti-VEGF treatment over conventional laser therapy for stage 3+ ROP in zone Ⅰ. Superiority in severe ROP in zone Ⅱ could not be established due to inadequate sample size. Safety is a major concern with use of anti-VEGF drugs in paediatric age group and this study could not prove it because of a short follow up. Recent studies also shown that that systemic VEGF levels remain suppressed for 8 wk after intravitreous bevacizumab injection[41].

Regarding the best approach, laser treatment is still the gold standard and anti-VEGF therapy should be tried only in selected cases.

Surgical management is reserved for advanced stages of ROP (stages 4 and 5). The stage of ROP and features specific to each eyes guide the choice of surgical technique. It is shown that best anatomical and visual outcome can be attained if surgical intervention is done at 4A ROP as it halts progression to worse stages[42]. The surgical options available for stage 4 ROP are lens sparing vitrectomy or scleral buckling. For stage 5, vitrectomy with lensectomy or open sky vitrectomy can be performed. Visual outcome for stages 4B and 5 is very poor and can lead to permanent visual impairment[42].

Periodic follow up and the burden of visual morbidity then become the prime concerns after the retinopathy is adequately treated. Visual rehabilitation can be achieved only through an integrated coordination between the pediatricians, ophthalmologists, paramedicals and parents. With the advances in screening tools, it may be hoped that occurrence of severe retinopathy or severe visual morbidity from ROP may be reduced in future.

FUTURE

Future trends in screening of ROP

Newer predictor of ROP: Timely screening of ROP is crucial for early management and improved outcomes. Current screening guidelines use only two most important risk factors gestational age and birth weight, and not the post-natal factors. However only approximately 10% of the premature babies screened need treatment[43]. Various neonatal scoring systems such as clinical risk index for babies, scores for neonatal acute physiology (SNAP), and SNAP-perinatal extension-Ⅱ have also been attempted to predict ROP, but none showed sufficient power to predict severe ROP[44]. Thus there is a need for improvement of the current screening protocols by developing new better predictors to reduce the number of ROP screening examinations[44].

Low weight gain proportion: Currently, low weight gain by six weeks of life after premature birth is being accepted as a risk factor for causing ROP. Proportion of the weight gain is defined as the weight at 6 wk of life minus the birth weight divided by the birth weight. Low weight gain proportion, *i.e.*, weight gain less than 50% of the birth weight in the first 6 wk of life is being considered superior to birth weight and gestational age alone as predictors for severe ROP[45,46]. In order to develop an efficient clinical prediction model, Binenbaum *et al*[47] found that a birth weight-gestational age-weight gain model could reduce the need for examinations by 30% in a high-risk cohort, while still identifying all infants requiring laser therapy.

WINROP algorithm: A surveillance algorithm WINROP was developed by Löfqvist *et al*[33] to detect infants at risk for developing severe ROP. WINROP is based on the weekly measurement of body weight and serum IGF-1 level from birth until postconceptional age of 36 wk. In their first prospective study, which included 50 preterm infants, the WINROP algorithm could identify all preterm babies diagnosed with severe ROP later. Since then WINROP algorithm has been validated in different cohorts of many countries with sensitivity ranging from 85% to 100%[48-51]. These studies have validated WINROP algorithm as a useful ROP screening tool that can be used to focus care on those at high risk for ROP. Currently, WINROP is being tested in a large multi-center multinational trial to validate it as universal screening tool.

ROPScore: ROPScore is based on birth weight, gestational age, weight gain and blood transfusions from birth to 6th week of life and use of oxygen. Eckert *et al*[44] initially analyzed 16 variables and established this score after linear regression. The study with 474 patients, and the area under the receiver operating characteristic curve for the score were 0.77 and 0.88 to predict any stage and severe ROP respectively. They concluded ROPScore as a promising tool which maybe more predictable than birth weight and gestational age in predicting the occurrence of ROP in very low birth weight preterm infants. Also, the score is easy enough to be routinely used by ophthalmologists or the nursing staff during screening for ROP.

IGF-1: Apart from use of IGF-1 in WINROP algorithm, the usefulness of IGF-1 level was evaluated in a prospective study by Pérez-Muñuzuri *et al*[52] They studied 74 premature newborn babies and concluded that determination of IGF-1 serum levels in the 3rd week post-partum, is a good prognostic tool to identify babies that are at a high risk of

developing ROP.

Plasma soluble E-selectin: Elevated plasma soluble E-selectin (sE-selectin) levels have been found to have an association with ROP and have been reported as independent risk predictor for ROP by Pieh *et al*[53]. They concluded that a score based on the gestational age of the preterm child and sE-selectin plasma levels would improve prediction of ROP. Increase of 10 ng/mL increases the ROP risk by 1.6 fold. For this purpose, plasma concentrations should be assessed 2 to 3 wk after birth, in premature infants.

Thus in the future, new screening tools would be developed with a hope to reduce the burden of ROP screening on the ophthalmologist and also reduce these stressful examinations on the preterm babies. Further studies are needed to validate the usefulness of these predictors. Once validated, these post-natal variables can be used successfully for early prediction of severe ROP.

Telescreening: Timely referral by pediatricians and meticulous examination by an experienced ophthalmologist is the gold standard for ROP screening. Digital retinal imaging is emerging as an important tool for ROP screening. Non-ophthalmologists like the neonatal nurses and technicians are being trained to use these digital imaging devices effectively. Pediatric ophthalmologists' services can be extended to the remote areas by electronic transfer of the images captured by these paramedical staffs[54]. Daniel *et al*[55] validated that remote evaluation of the digital retinal images by trained technicians taken by them can reduce referral warranted ROP. The result of these studies suggest that telescreening provides future strategies for outreach ROP screening and will allow access of diagnostic expertise to underserved areas in developing as well as developed countries[56].

Optical coherence tomography: It is an imaging tool which gives cross sectional images of the retina and has been extensively used in adults. Although it is not widely used in ROP, this technology is already providing new insights at a cellular and subcellular level into normal retinal development, the acute ROP process, and its long-term sequelae[57].

Newer therapeutic modalities for ROP
Anti-VEGF, systemic propranolol, IGF-1 replacement, granulocyte colony stimulating factor, Jun kinase inhibitor and omega-3 polyunsaturated fatty acid supplementation are the newer preventive strategies being evaluated through insights into the molecular pathogenesis of ROP in animal studies[58]. Newer emerging therapeutic options have the potential to complement current therapies and improve treatment outcomes. However, any new therapeutic option must be thoroughly evaluated before existing treatment paradigms can be modified, as these newer agents are mostly systemically administered and may have unknown widespread side effects.

Anti-VEGF: Recently various anti-VEGF agents are being evaluated as promising treatment modality for various stages of ROP. Bevacizumab is the most widely used anti-VEGF for treatment of acute ROP since 2007, and evidences from case reports and small studies suggest that intravitreal bevacizumab monotherapy may be a viable first-line treatment for select cases of ROP[58]. Other anti-VEGF agents are also being evaluated as adjunctive or alternate therapy. A recent study showed that administration of intravitreal pegaptanib along with laser is useful therapy with stable regression of ROP in 90% of eyes compared to 61% of only laser treated eyes[59]. Recently one prospective nonrandomized interventional case series study has evaluated 1-year outcomes of intravitreal aflibercept injection in 26 eyes with type 1 prethreshold ROP and found favorable anatomical and visual outcome in 96% and 80% eyes[60]. The efficacy of ranibizumab and bevacizumab for the regression of ROP have been compared and found similar in retrospective studies. However, high myopia was more prevalent in the bevacizumab-treated eyes, while reactivation rate was significantly higher following treatment with ranibizumab, probably due to shorter half-life[61,62]. Lutty *et al*[63] studied the effect of VEGF trap on normal retinal vascular development and oxygen-induced retinopathy in dog and concluded that it inhibits the retinal neovascularisation, however dose selection is an important variable as higher doses also inhibit vasculogenesis or retinal revascularization.

In future, intravitreal anti-VEGF injection may become the first choice treatment replacing laser therapy for zone I stage 3 ROP or cases with media opacity, if efficacy and safety are validated. Also anti-VEGF can be considered as an adjunctive therapy in patients treated with laser photocoagulation or vitrectomy.

Propranolol: Reports of use of systemic propranolol for an effective treatment of infantile hemangioma resulted in exploration of anti-angiogenic role of propranolol in ROP. A study on oxygen-induced retinopathy in a mouse model showed that propranolol decreases VEGF overproduction in the hypoxic retina. In addition, beta-AR blockade has no effect on VEGF levels in the heart, brain or lungs, as VEGF expression is these organs is independent of hypoxia[64]. Based on these findings, the safety and efficacy of propranolol in newborns with ROP (PROP-ROP) study[65], was conducted. It evaluated safety and efficacy of oral propranolol given to preterm newborns infants having early stages of ROP. In this study, 26 preterm babies with stage 2 ROP treated with oral propranolol (0.25 or 0.5 mg/kg per 6 h) showed less progression to stage 3 or stage 3 plus and a 100% relative reduction of risk for progression to stage 4. However serious adverse effects like bradycardia and hypotension were observed in about 20% of infants treated with propranolol, and the study was halted due to increased mortality in the treatment arm[65]. Recently, an experimental study reported failure of propranolol treatment to suppress retinopathy development in mice[66]. Thus, the safety of oral

propranolol to the vulnerable premature infants is uncertain, and further studies including animal as well as prospective clinical trial are needed.

IGF: IGF plays an important role in fetal development during pregnancy. The levels IGF-1 rise significantly during the 3rd trimester of pregnancy, and it controls VEGF-mediated vascular growth in the retina[67]. However, IGF-1 levels fall rapidly after preterm birth, and prolonged period of low IGF-1 in preterm children have been reported to be associated with development of ROP. Conversely, normal vessel development occurs, if the IGF-1 levels are sufficient after birth[68]. Recent studies have shown that intravenous administration of recombinant IGF-1 (rhIGF-1) with its binding protein 3 (rhIGFBP-3) to premature infants increases the serum concentrations of IGF-1 and IGFBP-3, and is found to be safe[69,70]. Can *et al*[71] studied the effect of early aggressive parenteral nutrition (APN) *vs* conservative nutrition and found that IGF-1 levels were higher in the APN group.

Granulocyte colony stimulating factor: The potential role of granulocyte colony stimulating factor (G-CSF), a biologic cytokine commonly used to increase leukocyte count in neutropenic patients, is currently being evaluated to prevent ROP[72]. In a retrospective review of 213 neonates who received G-CSF for non-ophthalmic indications, Bhola *et al*[73] studied 50 infants with birth weight < 1500 g and gestational age < 32 wk. Only 10% of the infants who received G-CSF required laser compared to 18.6% in the control group. However the observed difference was not statistically significant. Another retrospective study determined the vitreous level of 27 types of cytokines in eyes with ROP, and levels of 6 cytokines including G-CSF were found significantly higher ($P < 0.05$) in eyes with ROP compared to the control group[74]. Recently, in an animal study of oxygen-induced retinopathy, G-CSF significantly reduced the vascular obliteration ($P < 0.01$) and neovascular tissue formation ($P < 0.01$) mainly by increasing levels of IGF-1[75]. The results of these studies suggest a potential role of G-CSF in ROP prevention, however further studies are needed to establish the same, and to determine the dose required, side effects and safety.

Omega-3 polyunsaturated fatty acids: Like IGF-1, omega-3 and 6 polyunsaturated fatty acids (PUFAs) are non-oxygen-regulated angiogenic factors, which are transferred from mother to the fetus in the third trimester of pregnancy. Consequently, premature newborns lack the maternal supply of PUFAs[72]. The mouse model studies of ROP have shown that omega-3 PUFA supplementation as well as an increased retinal omega-3 and omega-6 PUFA ratio result in a protective effect against pathologic retinal neovascularisation[76,77]. The protective action of omega-3 PUFA is considered to be mediated through the suppression of tumor necrosis factor-alpha[78]. These lipids supplementation can be provided through total parenteral nutrition in premature infants, and this may be

an interesting therapeutic approach for ROP prevention. However, larger studies are required to establish the safety and efficacy of omega-3 PUFA supplementation therapy in premature babies.

Gene therapy: Association of mutations and poly-morphism of various genes (*e.g.*, *Norrin*, *Frizzled 4*, *Lrp5*) with severity of ROP or failure of treatment has been investigated in a number of small studies[79]. A recent study performed about the genetic and environmental influences on ROP in 257 infants including 38 monozygotic twins, 66 dizygotic twins, and 153 simple births found the heritability of ROP to be 0.73[80]. Interestingly, Good *et al*[81] demonstrated in a rat model of ROP that local gene transfer into retinal blood vessels was possible using recombinant viruses carrying genes of interest. They also found that adenovirus vector was specific to the inner retinal blood vessels and does not appear in deeper neural retina, when compared to other vectors like retroviruses and herpes virus[81]. Though no significant association between genetic abnormality and ROP has been reported till now, targeting the expression and regulation of various cytokines and growth factors involved in the pathogenesis of ROP by gene therapy appears as a promising future treatment method to restore an anti-angiogenic state[72].

Medico legal implications
Screening for ROP needs to be initiated timely after birth to prevent blindness. It is the responsibility of the caring pediatrician to initiate screening by referring to an ophthalmologist and it is the responsibility of the ophthalmologist to do correct screening and treatment. This has immense medico legal implications because if a child goes blind due to missed or late screening then the pediatrician and the ophthalmologist are at a very high risk of getting into a law suit[82,83].

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Sublingual immunotherapy for pediatric allergic rhinitis: The clinical evidence

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Abstract

Allergic rhinitis is estimated to affect 10%-20% of pediatric population and it is caused by the IgE-sensitization to environmental allergens, most importantly grass pollens and house dust mites. Allergic rhinitis can influence patient's daily activity severely and may precede the development of asthma, especially if it is not diagnosed and treated correctly. In addition to subcutaneous immunotherapy, sublingual immunotherapy (SLIT) represents the only treatment being potentially able to cure allergic respiratory diseases, by modulating the immune system activity. This review clearly summarizes and analyzes the available randomized, double-blinded, placebo-controlled trials, which aimed at evaluating the effectiveness and the safety of grass pollen and house dust mite SLIT for the specific treatment of pediatric allergic rhinitis. Our analysis demonstrates the good evidence supporting the efficacy of SLIT for allergic rhinitis to grass pollens in children, whereas trials regarding pediatric allergic rhinitis to house dust mites present lower quality, although several studies supported its usefulness.

Key words: Allergic rhinitis; Grass pollen allergy; House dust mite allergy; Sublingual immunotherapy

Core tip: This manuscript aims at describing objectively the current evidences of sublingual immunotherapy (SLIT) for the treatment of pollen and house dust mite allergic rhinitis in children, based upon the available randomized, double-blinded, placebo-controlled trials. All these studies have been directly analyzed by the authors and have been summarized in this manuscript, in order to be readily available to the reader. We concluded that there is a good evidence of efficacy for grass pollen SLIT, while the benefit seems to be weaker for house dust mite SLIT, in the specific setting of pediatric allergic rhinitis.

BACKGROUND

Rhinitis is the term indicating the inflammatory disease of nasal mucosa and, clinically, is defined by the onset of two or more of the following symptoms: Nasal discharge, sneezing, nasal itching and congestion.

If these symptoms last longer than 10 d, the rhinitis is defined as chronic. Chronic rhinitis can persist weeks and even months or can have a recurrent trend. While acute rhinitis are usually caused by transient viral illnesses, infectious agents are not the main etiology of chronic rhinitis and, when it is so, these are due to an overlapping bacterial infection, leading to rhino-sinusitis, characterized by purulent nasal discharge, persistent fever, headache, facial pain and cough.

Actually, chronic rhinitis can recognize several etiologies (vasomotor, occupational, hormonal, atrophic, iatrogenic, idiopathic), but the most consistent group is represented by allergic rhinitis, which is estimated to affect 10%-20% of pediatric population worldwide.

Allergic rhinitis is caused by an IgE-mediated sensitization to environmental allergens, such as dust, pollens, domestic animals and moulds. Depending upon the specific pattern of sensitization, allergic rhinitis can be intermittent or persistent and seasonal or perennial, although the distinction is not always obvious, as some people can be sensitized to several allergens. Therefore, the diagnosis of allergic rhinitis is correctly made whenever the nasal symptoms are associated to a profile of allergic sensitization (which must be documented by skin prick tests and/or the dosage of serum of allergen specific IgE), which is consistent with the clinical picture and its temporal pattern[1,2].

Once the diagnosis of allergic rhinitis is established, the general clinical management is constituted by the avoidance of allergen exposure, whether it is practicable, and by the control and/or the prevention of nasal symptoms by nasal or systemic anti-histamine drugs, intranasal steroids, leukotriene-receptor antagonists and, in a lesser extent, cromolyn sodium. Among those drugs, intranasal steroid have been demonstrated to be able to produce the greatest relief, being able to improve significantly the symptoms related to the nasal obstruction. Unfortunately, all these drugs control the symptoms, but cannot cure the allergic disease[3].

Allergic rhinitis has been considered for long time as being just a nuisance disorder. However, nasal symptoms can interfere with daily activities importantly and can disrupt or alter the sleep pattern, leading to negative consequences on patient's social life and intellectual performance. Moreover, according to the "allergic/atopic march" hypothesis and to the "united airways disease" concept, allergic rhinitis can be associated to lung function test abnormalities and/or anticipate the onset of asthma. Thus, the appropriate therapy of allergic rhinitis could help to prevent the progression to more serious respiratory diseases, in addition to ameliorating patient's life quality. Such a goal may be reached through the inclusion of specific immunotherapy (SIT) in the early treatment of allergic rhinitis, before it evolves to asthma: Indeed, SIT - unlike symptomatic drugs - aims at modulating the immune mechanisms underlying the allergic disease and, currently, it is the only available treatment which modifies the disease process[4].

SUBLINGUAL IMMUNOTHERAPY FOR ALLERGIC RHINITIS

Basically, SIT consists in the administration of increasing doses of specific allergens up to a maintenance dose, which can be repeated according to different schedules depending on the allergic disease and its pattern of sensitization.

SIT can be mainly administered by two ways: Subcutaneously (SCIT) or sublingually (SLIT). Although in many European countries SCIT is still the most common way to administer allergy immunotherapy, actually sublingual immunotherapy (SLIT) is getting growing success, especially in the pediatric population: It is usually preferred because it is easier to be administrated and it hasn't been associated to systemic and life-threatening adverse reactions[5,6].

SLIT should result in the progressive acquisition of the immune tolerance against a specific allergen. Several cellular and humoral immune mechanisms have been proposed. The main immunological modifications related to the SLIT desensitization process can be summarized briefly, as it follows: Emergence of regulatory T cells (Treg), shifting of T helper polarization toward Th1 cells, increased production of interleukin (IL)-10 and transforming growth factor-β, immunoglobulin class-switching from specific IgE to IgA and IgG4 isotypes (which would compete with IgE reducing allergen-mediated release of inflammatory molecules)[7].

In 2013, the European Academy of Allergy and Clinical Immunology (EAACI) edited an important position paper on pediatric rhinitis, providing several evidence-based insights on diagnostic and therapeutic aspects. In this document, SLIT is confirmed as an effective treatment for grass pollens and house dust mite allergic rhinitis and this concept is labeled through a force of this statement of grade A, according to the system for grading clinical recommendation in evidence-based guidelines[1,8].

Such a recommendation was reached through the evaluation of available studies, considering the results of several reviews and meta-analysis. However, most clinical trials regarded mainly the adult population and specific pediatric studies are much fewer. For instance, the important systematic review of the literature made by Radulovic *et al*[9] concluded that SLIT is an effective and safe therapy for allergic rhinitis. Although this analysis considered randomized, double-blind, placebo-controlled (RDBPC) clinical trials, actually it included

patients of any age, both children and adults, affected with allergic rhinitis (with or without allergic asthma)[9].

Similarly, the World Allergy Organization (WAO) Position Paper also stated that the indication to SLIT in the treatment of IgE-mediated allergic respiratory diseases is well established in children, provided the diagnostic work-up has been appropriate[10]. Moreover, several systematic reviews supported the specific use of SLIT in the treatment of allergic rhinitis in children. In 2006, Penagos *et al*[11] made a pivotal meta-analysis of randomized controlled trials (RCTs) of SLIT in the treatment of allergic rhinitis in pediatric patients, concluding that SLIT with standardized extract is an effective therapy in this field. More recently, Kim *et al*[5] and Larenas Linnemann *et al*[12], in their reviews, reinforced the evidence supporting the efficacy and the safety of SLIT for the treatment of allergic respiratory diseases.

However, in the medical literature, the RCTs concerning specifically the role of SLIT in pediatric allergic rhinitis are, actually, fewer than expected. We performed a specific search through PUBMED (search terms: Allergic rhinitis, children, SLIT) and it returned 201 references: Almost all (195) were published after the year 2000. Among those, we found 56 reviews and/or meta-analysis; in the remaining part, considering only english-written papers, we found 35 RCTs and 11 retrospective and/or observational studies regarding pediatric allergic rhinitis (associated to grass pollen and house dust mite sensitization). Moreover, many of these RCTs were small trials, including less than 100 patients.

In this practical review, we attempt to highlight and comment the major evidences on the use of grass pollen and dust mite SLIT against allergic rhinitis in children, deriving from available RCTs being strictly oriented to allergic rhinitis and limited to pediatric population.

SLIT FOR GRASS POLLEN ALLERGIC RHINITIS IN CHILDREN: RCTS

In our search, the first randomized, double-blind, placebo-controlled (RDBPC) study on SLIT in children ($n = 22$) affected with seasonal allergic rhinitis was written by Wüthrich *et al*[13] in 2003: After 2 years of treatment, authors detected a statistically significant reduction ($P < 0.05$) in the drug consumption in the SLIT group and such an effect resulted to be more relevant in the second year of therapy.

In 2004, Bufe *et al*[14] published a multicenter RDBPC study, including 161 children with seasonal rhino-conjunctivitis: The authors were able to find a significant ($P = 0.046$) benefit of SLIT after 3 years of treatment, but such a positive result was limited to the group of children with severe symptoms. Similarly, Rolinck-Werninghaus *et al*[15] enrolled 97 children (3-14 years) with allergic rhinoconjunctivitis to grass pollen: They treated the active group by a 5-grass mixture SLIT (3 times/wk), documenting a positive effect in term of

reduction of both multiple symptoms-medication score ($P < 0.05$) and medication score ($P = 0.0025$) rather than isolated symptom score.

Again in 2004, the multicenter study by Novembre *et al*[16], including 113 children (5-14 years) supported the beneficial effect ($P < 0.05$) of 3 years' coseasonal SLIT based upon the medication score. Another important value of this work was the demonstration that SLIT could reduce the incidence of asthma in children with grass pollen rhino-conjunctivitis. Indeed, they calculated a 3.8 relative risk of development of asthma in the control group, which was not related to differences in sex, presence of household pets, family allergic background or exposure to passive smoking[16].

A large perspective study was performed by Röder *et al*[17] in a primary care setting: 204 patients (aged 6-18 years), coming from general practitioners' office (not from allergy referral centers), were randomized to receive SLIT (five grasses pollen extract) or placebo, based on a diagnosis of allergic rhino-conjunctivitis. However, in this clinical setting, SLIT did not result to be effective in ameliorating the allergic symptoms nor in reducing the need of medications[17].

In 2009, Wahn *et al*[18] published the largest multinational RDBPC study ($n = 278$ children, aged 5-17 years), by using five-grass pollen tablet (300 IR, Index of reactivity) according to a pre-co-seasonal scheme. SLIT treated patients showed a highly significant improvement compared to placebo, both in term of symptoms score ($P = 0.001$) and in term of use of medications ($P = 0.0064$). The clinical benefit was evident also considering each symptom individually, including nasal congestion and conjunctivitis. Interestingly, this study covered a period as long as 8 mo, which means that the clinical improvement became evident in a relatively short period of time, compared to previous studies encompassing 2-3 years' follow-up. Moreover, this study included also poly-sensitized children, showing a comparable improvement as well as mono-sensitized patients: that reinforced the concept that the multi-sensitization is not an absolute limit to the access to SLIT, provided that the allergy evaluation is appropriate[18].

In the same year, Bufe *et al*[19] carried out a similar research by the use of a different SLIT product, namely 75.000 Units SQ-standardized grass allergen tablets, which contain approximately 15 μg Phl p5 (Phleum pratense major allergen 5), whereas a dose of 300 IR five grasses extract corresponds to 20 μg Phl p5. A total of 253 children (aged 5-16 years) were randomized and treated according to a pre-co-seasonal scheme. SLIT group showed a significant 24% reduction of symptom score ($P = 0.0195$) and 34% reduction of medication score ($P = 0.0156$) compared to placebo group, considering the entire grass pollen season[19].

In 2010, Halken *et al*[20] published another multinational RDBPC study, which included 267 pediatric patients (aged 5-17 years). Patients were treated according to a pre-co-seasonal schedule for one year and the SLIT group received five-grass pollen 300 IR tablet daily. As previously

reported, the benefit of SLIT was confirmed even in the first pollen season: The relative mean improvement of symptom score was around 28% compared to placebo group (*P* < 0.001) and the relative mean improvement of medication score was almost 50% (*P* = 0.01)[20].

In 2011, the first RDBPC trial performed in North America has been published. Blaiss *et al*[21] randomized 345 pediatric subjects to receive 75000 SQ tablets or placebo. Study population was made by children and adolescents affected with grass pollen induced rhino-conjunctivitis and some presented asthmatic co-morbidity too; moreover, 85% patients were multi-sensitized. Patients were treated according to pre-co-seasonal scheme before and during the 2009 grass pollen season and all efficacy parameters (symptom score, medication score, total combined score) improved significantly in the treated group compared to controls (in the extent of 25%, 81% and 26%, respectively)[21].

Recently, in 2012, a small RDBPC trial by Ahmadiafshar *et al*[22] published the only english-written study performed outside western countries. It included 24 children (5-18 years) and patients were treated for 6 mo with five-grass pollen 300 IR extract: As well as previous trials, the benefit of SLIT was confirmed and was reported as being evident after 4 mo of therapy[22]. In the same year, Wahn *et al*[23] published another multicentric RDBPC trial including 207 children affected with rhinitis/rhinoconjunctivitis with/without asthma, where the outcome was evaluated by the comparison of the area under the curve (AUC) of the symptom-medication score before and after the treatment. Patients experienced a clear improvement thank to the SLIT and the efficacy resulted to be statistically significant, even considering separately the symptoms score and the medication score[23].

SLIT FOR POLLEN-INDUCED PEDIATRIC ALLERGIC RHINITIS: GLOBAL CLINICAL EVIDENCE

A summary of pediatric RDBPC trials reported in the previous paragraph is made in Table 1. It is evident that almost all the aforementioned studies supported the efficacy of grass pollen SLIT in order to improve the burden of symptoms and medications of allergic rhinitis in children. Among those, many are well-conducted trials and some are multicentric studies being large enough to draw consistent conclusions on the efficacy of SLIT in this setting as well as its good safety.

The only trial where a clear benefit of SLIT was not evident is the one performed by Röder *et al*[17], but it seems important to underline actually the fact that the study population was not made of patients evaluated at allergy referral centers[19]. This aspect could have affected the results for several reasons. Patients managed in the primary care setting could show greater variability in the severity of allergic rhinitis; moreover, many patients included in this study resulted to be sensitized to several environmental allergens and a good correlation between

symptoms and sensitization, which can require some specific allergy expertise, has been stressed as being a main aspect for the correct indication and efficacy of SLIT. Indeed, the large multicentric trials performed in North America, which included mostly multisensitized patients (85% of study population), provided results consistent with a clear benefit of SLIT in grass-pollen induced rhino-conjuctivitis[21].

Therefore, in our opinion, these experiences together demonstrate how the multi-sensitization is not a limit for the use of SLIT in children affected with grass-pollen allergic rhinitis, provided that an accurate selection of allergic patients, that SLIT is offered to, can be made. According to the EAACI position paper, a clear relationship between the occurrence of nasal symptoms and the exposure to grass pollen, especially in multi-sensitized patients, should be ascertained before prescribing SLIT, in order to obtain a good efficacy from this expensive treatment[1].

Moreover, also the severity of allergic rhinitis can impact on the evidence of a clinical improvement after the treatment with SLIT. Indeed, in order to get a study population as more homogeneous as possible, the inclusion criteria of RCTs performed at allergy referral centers are often more restrictive than those used in the daily allergy practice; however, recent observational and multi-centric studies supported the effectiveness of SLIT in real life practice[24,25].

Finally, in the measurement of the effectiveness of therapies against grass pollen allergy, allergen exposure must be considered too, as it is different over several seasons and regions. Such an aspect is thought to have even a greater impact on the analysis of grass pollen SLIT than on the evaluation of other drugs used to treat allergy symptoms acutely. Indeed, in seasonal allergy trials with grass pollen SLIT, the treatment effect resulted to be greater in presence of higher pollen exposure[26]. This aspect must be considered in the individual clinical trials and this effect can be overcome through multi-centric studies and through meta-analysis pooling data from several RCTs. Recently, several post-hoc analysis have been published by using pooled data from some randomized, placebo-controlled and double-blind North American trials on timothy grass SLIT against allergic rhinitis and/or rhinoconjunctivitis in children and adolescents. This research confirmed that grass pollen SLIT administered daily, pre-seasonally and during the grass pollen season, is clinically effective and safe in children older than 5 years[27].

Another aspect to be discussed is that physicians could have the impression that SLIT is less effective than it is actually or compared to the other classes of drugs for allergic rhinitis, despite all the evidences we reported. Although that is not specific for pediatric age, a huge meta-analysis by Devillier *et al*[28] deserves to be reported, as it provided an indirect comparison between SLIT and pharmacotherapy: The administration of pollen SLIT tablets resulted in a relative clinical impact (RCI) *vs* placebo greater than that observed with second-

Table 1 Randomized double-blinded placebo controlled trials on grass pollen sublingual Immunotherapy for the treatment of allergic rhinits in children

Ref.	Year	Age	No. of patients	Product	Efficacy parameters	Duration	Statistical significance	Other observation
Wüthrich *et al*[13]	2003	4-11	22	ALK-Abello'	Medication score	2 yr	$P = 0.05$	A difference in drug consumption has been shown only in the second year
Bufe *et al*[14]	2004	6-12	161	Sublivac BEST, HAL-allergy	Clinical Index (combining symptom and medication score)	3 yr	$P = 0.046$	A significant difference was shown in patients with severe symptoms
Rolinck-Werninghaus[15]	2004	3-14	97	Pangramin-SLIT ALK-SCHERAX	Multiple symptom – medication score	32 mo	$P = 0.498$	Symptom score did not reveal significant difference; medication score improve significantly ($P = 0.0025$)
Novembre *et al*[16]	2004	5-14	113	ALK-Abello'	Medication score	3 yr	$P < 0.05$	Significant improvement was shown after the second year; symptom score did not improve significantly
Röder *et al*[17]	2007	6-18	204	Oralgen Grass Pollen, Artu Biologicals	Medication and symptom score	2 yr	NS	Study population was enrolled from general practices
Wahn *et al*[18]	2009	5-17	278	5-grass tablets 300IR,	Rhinoconjunctivitis total symptom score	6 mo	$P = 0.001$	SLIT was started 4 mo before before the pollen season; both symptom score and medication score improved singularly too
Bufe *et al*[19]	2009	5-16	253	Stallergenes SQ-standardized grass allergen tablet (Grazax)	Medication and symptom score	4-6 mo	$P < 0.02$	SLIT was started 8 to 23 wk before the estimated pollen season in 2007
Halken *et al*[20]	2010	5-17	267	5-grass tablets 300IR,	Medication and symptom score	6 mo	$P < 0.01$	SLIT was started 4 mo before the estimated pollen season
Stelmach *et al*[29]	2011	6-18	60	Stallergenes Staloral 300IR, Stallergenes	Combined symptom and medication score	2 yr	$P < 0.01$	Both pre-coseasonal and continuous regimen were efficacious in the same extent
Blaiss *et al*[21]	2011	5-17	345	SQ-standardized grass allergen tablet (Grazax)	Medication and symptom score	6 mo	$P < 0.01$	SLIT started 8 wk before the pollen 2009 season; 89% patients were multi-sensitized
Wahn *et al*[23]	2012	4-12	207	6-grass pollen aqueous extract (AllerSlit, Allergopharma)	Area under the curve of symptom-medication score	6-8 mo	$P = 0.004$	Patients were treated with a pre-coseasonal regimen; after this first phase, unblinding was made and all patients were treated
Ahmadiafshar *et al*[22]	2012	5-18	24	Staloral 300IR, Stallergenes	Medication and symptom score	6 mo	$P < 0.05$	SLIT was started 8-10 wk before pollen season

SLIT: Sublingual Immunotherapy; NS: Not significant.

generation H1-antihystamines and montelukast, and it was comparable to nasal corticosteroids[28]. Most recent RCTs demonstrated that SLIT is beneficial even since the first year of treatment, provided that an appropriated scheme of treatment is instituted before the pollen season. Previously, in the study by Stelmach *et al*[29], where the pre-co-seasonal and the continuous schedule were compared after a 2-years perspective RDBPC trial, both protocols resulted to be associated to a significant improvement in the total symptom and medication scores and there was no significant difference between them. Actually, the pre-co-seasonal group showed a lower improvement for nasal symptoms than the continuous schedule[29]. Similarly, the results emerging from an open randomized controlled study by Pajno *et al*[30] observed that the continuous protocol performed in a better way than the pre-co-seasonal schedule in the first pollen season, whereas in the following years both were rather equivalent.

Therefore, based upon most recent studies, a good

efficacy of a pre-co-seasonal treatment beginning around 4 mo before the pollen season has been showed. Differences in both the efficacy endpoint - in the research setting - and the clinical results - in the daily allergy practice - could be due not only to the variable scheme of vaccine administration, but also to different allergen formulation and product standardization, whose discussion overcomes the purpose of the present analysis.

Finally, it must be underlined the optimal profile of safety of grass pollen SLIT, which is confirmed by all RCTs and systematic revisions regarding children and adolescents affected with allergic rhinitis. No death or life-threatening events resulted to be associated to the treatment. Treatment related adverse events have been limited to mild to moderate local symptoms, such as oral pruritus, ear pruritus and throat irritation, reported in 15%-30% of subjects.

In conclusion, available pediatric RDBPC trials as well as reviews/meta-analysis clearly demonstrated the effectiveness and the safety of five-grass pollen

SLIT administered with the appropriate scheme and formulation (*e.g.*, 300 IR drops, 300 IR tablets, 75000 SQ-standardized tablets). Particularly, the pre-co-seasonal schedule is the most used and it is beneficial even in the first year of treatment, if it is started appropriately (3-4 mo before the supposed beginning of the pollen season).

Of course, despite these good evidences supporting grass pollen SLIT in the treatment of allergic rhinitis, some issues need consideration and further research, such as the use of different vaccines, the variable follow-up in the aforementioned studies and the lack of SLIT *vs* SLIT and SLIT *vs* SCIT trials. However, current evidences can be considered strong enough to support prescription of grass pollen SLIT to all pediatric patients suffering from grass pollen allergic rhinitis, after an appropriate diagnostic assessment by an allergy specialist, who will plan a correct schedule for SLIT administration and will provide an adequate follow-up.

SLIT FOR HOUSE DUST-MITE ALLERGIC RHINITIS IN CHILDREN: RCTS

Available RCTs concerning the efficacy of dust mite SLIT on pediatric allergic rhinitis are relatively poor and most have been made in the last few years. Indeed, the first multicenter RDBPC trial was produced by Tseng *et al*[31] in 2008. This study included 59 children (aged 6-18 years) from Taiwan and the treatment group received a standardized extract of Dermatophagoides pteronyssinus (D.p.) and Dermatophagoides farinae (D.f) up to 20 drops of a 300 IR/mL formulation, as a 5 mo' maintenance dose, which was reached in a period of 3-4 wk. Here, the authors were not able to demonstrate a significant benefit in either symptoms or medication score after 6 mo of SLIT. However, they described a significant serological response in patients treated with SLIT, in term of increase of specific IgG4 to D.f-D.p. ($P < 0.001$) and specific IgG4/IgE ratio ($P = 0.01$), which is reputed to be one mechanism of the potential efficacy of SLIT in allergic diseases[31].

Previously, we were able to find one retrospective analysis by Nuhoglu *et al*[32] in 2007, regarding 39 children affected with dust mite allergic rhinitis, which reported a positive impact of SLIT on nasal symptoms, in addition to a significant decrease of asthma attacks[32]. Moreover, in 2003 Marcucci *et al*[33] performed a 3 years' partially double-blind case-control clinical study including 24 children (aged 4-15 years) complaining dust mite allergic rhinitis for at least 2 years. In the first year of follow-up, patients were randomized to receive dust mite SLIT or placebo; subsequently, also children in the placebo group were switched to the SLIT treatment until the end of the study. The first double-blind placebo-controlled phase was not able to demonstrate a significant amelioration of symptoms and drug scores for rhinitis; however, intra-group comparison of the effect of SLIT in term of cumulative yearly nasal symptoms score revealed a significant reduction in the second ($P = 0.01$)

and, even more, in the third year ($P < 0.001$) of SLIT treatment compared to first year[33,34].

All these studies suggested the potential role of SLIT on dust mite pediatric allergic rhinitis, but none satisfied the standard quality parameters needed to draft strong evidence-based conclusions.

The first small RDBPC trial supporting the safety and the effectiveness of SLIT in house dust mite allergic rhinitis in children (aged 7-15 years) was published in 2010 by Yonekura *et al*[35]. They randomized 31 subjects and used a dust mite extract (containing 5 μg/mL of Der f 1 allergen) for 40 wk. The authors were able to find a significant reduction of symptom scores between the active group and the placebo group after 32 wk of treatment ($P < 0.05$); furthermore, whereas the placebo group reported no significant benefit at the 40th week (compared to the beginning of the study), in term of symptom scores, the active group showed a significant intra-group amelioration after SLIT treatment ($P = 0.03$). Indeed, at the end of the trial, 33% patients reported a clear improvement of symptoms, whereas placebo patients showed no more than a slight amelioration; moreover, the authors reported that half children, showing an important reduction of nasal symptom scores at the end of the treatment, had a beneficial effect persisting up to one year later. However, this study was not able to document a parallel improvement on medication score and the response to SLIT was quite variable among all the patients[35].

The paper written by de Bot *et al*[36] in 2012 investigated the results of SLIT for house dust mite allergic rhinitis in a population of children recruited in primary care settings rather than in referral centers for allergy. They included 251 patients, aged from 6 to 18 years, and performed a 2 years' RDBPC trial, being the greatest RCTs so far. Unfortunately, this study found no significant improvement in allergic children treated with dust mite SLIT compared to placebo. However, the authors themselves hypothesized some probable limitations of the present study, such as the relative low cumulative dose of allergen they used to treat the patients or a lower clinical severity of symptoms presented by patients followed in a primary care setting, compared to a referral center[36].

In the same year, we can find two more studies on dust mite pediatric allergic rhinitis, which showed some points of interest, in our opinion, despite their numerical and/or design limitations. Han *et al*[37] treated with SLIT 54 youngsters (aged 6-18 years) in parallel to 22 adults, showing a similar tendency to the amelioration of symptom and medication scores in both age groups after one year of treatment[37]. Barberi *et al*[38] performed a 2 years' small case-control study, treating 30 children with dust mite respiratory allergy with symptomatic drugs alone or with SLIT and drugs on demand. They observed a significant amelioration of symptoms and of drug utilization in patients treated with SLIT, in addition to the evidence of the induction of a condition of allergenic hypo-reactivity through the measurement of serum IL-10

Table 2 Randomized double-blinded placebo controlled trials on house dust mite sublingual Immunotherapy for the treatment of allergic rhinitis in children

Ref.	Year	Mean age (yr)	No. of patients	Product	Efficacy parameters	Duration	Statistical significance	Other observation
Marcucci *et al*[33,34]	2005	4-15	24	Aqueous solution (ALK-Abello')	Symptoms score	1 yr	NS	A significant difference was recorded in the last trimester of the year; the study was carried on after the first year in open way
Tseng *et al*[31]	2008	6-18	59	Staloral (Stallergenes)	Symptoms score	6 mo	NS	In treated group a slight improvement was recorded. Specific IgG4 and IgG4/IgE significantly increased in SLIT group
Yonekura *et al*[35]	2010	7-15	31	Extract of house dust mite (Torii Pharmaceutical)	Symptom score	40 wk	$P < 0.05$	The improvement in SLIT group increased progressively according to the duration of the therapy
de Bot *et al*[36]	2012	6-18	251	Oralgen House Dust Mite (Oralgen Mijten)	Symptom score	2 yr	NS	Study population was recruited in primary care setting
Aydogan *et al*[41]	2013	5-10	22	Staloral (Stallergenes)	Medication and symptom score	12 mo	NS	-

SLIT: Sublingual Immunotherapy; NS: Not significant.

and Th2-dependent cytokines[38].

In 2013, Wang *et al*[39] obtained a significant result supporting the efficacy of SLIT in dust mite allergic rhinitis in a multicenter RDBPC trial, including 120 patients (aged 4-60 years). A similar output was previously described also by another RDBPC study (by Lee *et al*[40]), which enrolled 134 patients (aged 4-53 years). They showed that both mono-sensitized and poly-allergic patients, recruited in allergy referral centers, had achieved a comparable and significant improvement of nasal symptom and medication scores, after at least 1 year of treatment with house dust mite SLIT[40]. Unfortunately, both trials included a small proportion of children and an age-specific analysis was not made.

In the same year, Aydogan *et al*[41] published a small RDBPC trial with 22 children (aged 5-10 years), but they were not able to demonstrate the superiority of SLIT to placebo after 12 mo of treatment.

However, very recently, Shao *et al*[42] published the results of a large (*n* = 264) randomized and placebo-controlled, but open-label trial, including children (aged 3-13 years) affected with dust mite allergic rhinitis. They were able to demonstrate a significant (*P* < 0.01) reduction of nasal symptoms and medication scores, starting from 6-7 mo of treatment. Moreover, as the study included even 133 children aged 3-5 years, they reported also that, in the SLIT group, the therapeutic response was comparable in children older and younger than 5 years[42].

SLIT FOR HOUSE DUST-MITE PEDIATRIC ALLERGIC RHINITIS: GLOBAL CLINICAL EVIDENCE

Our brief analysis showed that the clinical research, addressed to evaluate specifically the effectiveness of SLIT against house dust mite allergic rhinitis in children, is quite poor (Table 2). We were not able to find randomized double-blind placebo controlled trial showing clearly and conclusively the improvement of children affected with house dust mite allergic rhinitis through SLIT. Thus, evidences supporting the effectiveness of SLIT on dust mite rhinitis in children are largely derived from studies on adults and from trials where actually patients were affected by asthma and the nose disease represented more a co-morbidity than a primary end-point of the research[6]. Actually, the only multicenter RDBPC trial assessing the specific effect of SLIT on pediatric rhinitis was not able to show a significant clinical improvement: Maybe a six month's period of study was too short in order to achieve a positive conclusion, as actually several immunological changes, proposed as inducing tolerance in the setting of allergy, have been described in patients receiving dust mite SLIT[30].

Environmental pollutants might affect the outcome of SLIT, worsening the nasal inflammation due to house dust mite allergy. However, as regards pediatric allergic rhinitis, we found very few studies addressing this topic. Interestingly, Marogna *et al*[43] conducted a prospective study showing that the exposure to passive smoke significantly reduced the clinical response to SLIT in children affected with allergic rhinitis due to house dust mite.

However, as a final remark, it deserves to be told that the usefulness of HDM-SLIT must be sought in some indirect beneficial effects, as the prevention of asthma development, through the potential modification of the natural history of the respiratory allergic disease, and the reduction of respiratory infections too. As regards the latter aspect, allergic children are known to have more frequent and more severe respiratory infection than non-allergic controls. Indeed, the persistent mucosal inflammation in the nose of house dust mite allergic people compromises the mechanical barrier against

external infectious agents and can constitute a favorable environment for microbial proliferation; moreover, the defective production of anti-viral cytokines and the over-expression of some epithelial adhesion molecules in patients with allergic rhinitis increase the susceptibility to viral infections. Recent evidences supported that HDM-SLIT can reduce the burden of recurrent respiratory infections in allergic children and some observational studies suggested that SLIT-treated children significantly developed fewer respiratory infections compared to controls and also the use of antibiotics was reduced[44,45].

CONCLUSION

As well as the EAACI position paper on pediatric rhinitis, several reviews and meta-analysis concluded for a general efficacy and safety of AIT for pediatric rhinitis and rhino-conjunctivitis. Recently, Kim *et al*[5], trough their systematic review, inferred a moderate-strength and general evidence that SLIT improves pediatric allergic rhinitis and conjunctivitis through a reduction of symptoms and/or a decrease of drug consumption. Similarly, Pleskovic *et al*[46] concluded that SLIT is a good option for the treatment of children with grass pollen and dust-mite allergic rhino-conjunctivitis.

Similarly, our brief and practical review supports the global effectiveness of SLIT intended to treat grass pollen and house dust mite allergic rhinitis in children, but some differences must be made, in our opinion, based on current clinical evidences.

As concerns grass pollen SLIT, several RDBPC trials of good standard quality are available and almost all produced clinical data showing a positive effect of SLIT in the control of allergic symptoms and/or drug request and also in the prevention of the development of asthma.

However, the evidence of the clinical efficacy of house dust mite SLIT on pediatric allergic rhinitis is milder. Indeed, RCTs and good standard quality studies exploring this aspect are less abundant and smaller; therefore, in our opinion, more trials are needed to consolidate the recommendation for dust mite SLIT in pediatric allergic rhinitis.

These conclusions are comparable to the evidences emerging from the analysis performed recently by Larenas Linnermann *et al*[12] These authors concluded that the evidence is strong for grass pollen SLIT efficacy in the treatment of pediatric allergic rhinitis, whereas the evidence for house dust mite SLIT effectiveness is still considered "of moderate-low quality".

Finally, we think that it should be stressed the concept that SLIT for pediatric allergic rhinitis seems to be more efficacious if the prescription of SLIT derives from an experienced diagnostic pathway and if an appropriated follow-up is planned.

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MINIREVIEWS

Clinical spectrum of primary ciliary dyskinesia in childhood

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Abstract

Although the triad of bronchiectasis, sinusitis and situs inversus was first described by Kartagener in 1933, the clinical spectrum of primary ciliary dyskinesia is still under investigation. Heterotaxy defects as well as upper and lower respiratory tract symptoms are the main manifestations in childhood. It is now recognized that situs inversus is encountered in only half of patients. The first lower respiratory symptoms may be present from infancy as neonatal respiratory distress. The most common lower airway manifestations are chronic wet cough, recurrent pneumonia and therapy resistant wheezing. Patients are at risk of developing bronchiectasis which may even be the presenting finding due to delayed diagnosis. Upper respiratory tract infections such as nasal congestion, nasal drainage and recurrent sinusitis as well as otologic manifestations such as otitis media or otorrhea with conductive hearing loss are also often encountered. It seems that the type of ciliary ultrastructure defects and the involved mutated genes are associated to some extent to the clinical profile. The disease, even in nowadays, is not recognized at an early age and the primary care clinician should have knowledge of its clinical spectrum in order to select appropriately the children who need further investigation for the diagnosis of this disorder.

Key words: Primary ciliary dyskinesia; Kartagener's syndrome; Immotile cilia; Heterotaxy; Respiratory tract

Core tip: The clinical spectrum of primary ciliary dyskinesia (PCD) has been recently better understood through the evolution of electron microscopy techniques, molecular genetics and imaging of the respiratory tract. Herein, we highlight the clinical profile of the disease from infancy to adolescence, focusing on clinical studies of children with a laboratory confirmed diagnosis of PCD. Additionally, the currently recognized associations of the type of ciliary ultrastructure defects and involved mutated genes with the clinical spectrum of the disease are presented. This information is of interest for the paediatrician in order to conduct a timely investigation of children with symptoms suggestive of PCD.

INTRODUCTION AND HISTORICAL REVIEW

Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder characterized by cilia dysfunction. It is a rather rare disorder with a prevalence which ranged from 1.3 to 111 diagnosed cases per million inhabitants aged 5-14 years in different European countries[1].

A case of PCD was first reported in 1904 as an association of bronchiectasis and situs inversus by Siewert[2]. Later in 1933, Kartagener described the classic triad of bronchiectasis, sinusitis and situs inversus in a group of patients[3]. Today it is well known that situs inversus is present in only half of PCD cases and therefore it is not a necessary prerequisite for the characterization of the disease. In 1976, Afzelius[4] studied mucocilia motion in subjects with PCD who produced immotile sperm while electron microscopy indicated absence of dynein arms of cilia from the relevant cells.

The aim of this review was to describe the clinical spectrum of PCD in childhood an entity which is recently better recognized thanks to the availability of modern screening techniques and the genetic identification of this disorder. Heterotaxy defects, upper and lower respiratory tract features are the main manifestations of PCD in childhood which are herein presented. Infertility which may affect males and females with PCD in adulthood is beyond the scope of the current review.

CILIA STRUCTURE AND FUNCTION

A basic knowledge of cilia structure and related function is a prerequisite for the understanding of the clinical features of PCD. Cilia are long protuberances extending from the cell body of certain eukaryotic cells. They are categorized as motile and non-motile (sensory) cilia. Herein the structure and function of motile cilia are described as only this type of cilia is involved in the pathogenesis of PCD.

The axoneme is the core of a motile cilium. The axonemal cytoskeleton consists of a "9 + 2" arrangement of microtubules. Nine peripheral microtubules surround a central inner pair[5]. Nexin links connect the nine peripheral doublets which are connected to the central pair by radial spokes. Outer and inner dynein arms are motor proteins that are attached to the outer microtubules providing energy for ciliary movement. Each cilium is anchored to the cell by the basal body. Each ciliated epithelium cell has approximately 200 cilia which beat in a coordinated fashion and contribute through this function to the respiratory epithelial defence mechanisms.

There is also a specific type of cilia with a "9 + 0" arrangement which has nine peripheral doublets and dynein arms but lacks the central pair of microtubules[6]. Cells of the ventral node have a single motile cilium of this type per cell. The rotary pattern of the beating of the nodal cilia may confer to organ laterality during embryogenesis[7].

HETEROTAXY DEFECTS

Situs inversus totalis is encountered in approximately half of patients with PCD as it is corroborated by many studies[8-10]. Heterotaxy defects are attributed to the loss of function of nodal cilia during embryogenesis[7]. There is a case report[11] of monozygotic female twins with PCD, one with situs solitus and the other with situs inversus totalis. This observation suggests that situs inversus is a random event in the foetal development of patients with PCD. However, with the advances in genetics it has been shown that subjects with central apparatus defects and RSPH1 mutated genes do not have laterality defects which is in line with the recognition that the "9 + 0" embryologic nodal cilium does not contain radial spokes and is unaffected by central apparatus defects[12]. Boon *et al*[13] also found that situs inversus was significantly less frequent in subjects with PCD and normal ultrastructure and in those with central pair abnormalities, compared to those with dynein deficiency.

Patients with normal ultrastructure, as the researchers suggested, may represent a combined group of patients with authentic normal ultrastructure and patients with unrecognizable subtle central pair abnormalities. The latter group was not expected to have situs inversus and therefore the proportion of situs inversus occurrence in the whole group of patients with presumably normal ultrastructure would have been expected to be lower.

In addition to situs inversus, other heterotaxy defects have been encountered in patients with PCD. Situs solitus and situs inversus totalis were identified in 46% and 47.7% of patients respectively in a retrospective review of 337 patients with PCD[10], whereas 6.3% of patients had other heterotaxy defects (situs ambiguous). It is of interest that approximately half of patients with heterotaxy defects also had cardiac and/or vascular malformations, a prevalence much higher than in the general population. In another series of patients with PCD[14], situs ambiguous prevalence was nearly twice (12.1%) than that reported by Kennedy *et al*[10]. However, in accordance with Kennedy's study results[10] cardiovascular malformations were also identified in nearly half of patients with PCD and situs ambiguous.

It should be mentioned that the presence of situs inversus contributes to the earlier diagnosis of PCD as median age at diagnosis of PCD in Europe was 3.5 years for subjects with situs inversus compared with 5.8 years for those with situs totalis[1].

LOWER RESPIRATORY TRACT MANIFESTATIONS

Features in neonatal period

The first lower respiratory symptoms of PCD may be present from infancy as neonatal respiratory distress.

However, even in the presence of early symptoms the diagnosis may be delayed until late childhood[15]. Transient tachypnoea of the newborn characterized by tachypnoea starting soon after birth and resolving by the 5th day of life is a well known cause of respiratory distress in term and near term neonates[16]. It is speculated that it is associated with the delayed absorption of foetal lung fluid. It seems that ciliary motility contributes to the foetal lung fluid clearance and as a consequence fluid is not cleared rapidly in neonates with PCD and respiratory symptoms present often shortly after birth. In different series of patients with PCD, neonatal respiratory distress frequency range from 43% to 74% and this percentage may be rather an underestimation due to recall bias, which is usually anticipated in retrospective studies[8,9,17-19]. In a case control study[15], the most common diagnosis in the PCD cases with neonatal respiratory distress was neonatal pneumonia, whereas the most common diagnosis in the control subjects of that study was transient tachypnoea. Furthermore the administration of oxygen treatment was more frequent in the PCD cases as well as the length of treatment in days. The combination of situs inversus, oxygen therapy for longer than 2 d and/or lobar collapse on chest X-ray (CXR) has a sensitivity of 87% for detecting PCD, whereas the combination of oxygen therapy for longer than two days and/or lobar collapse on CXR has a sensitivity of 83% for predicting PCD. Approximately half of the PCD cases (48%) had situs inversus. The median age of diagnosis in this subgroup was 0.83 years compared with a median age of 5 years at diagnosis for the subgroup without situs inversus.

Features from infancy to adulthood

The most common clinical manifestations of lower airways involvement are chronic wet cough, recurrent pneumonia and therapy-resistant wheezing. Recurrent cough and lower respiratory tract infections were among the presenting clinical history features in more than 60% of children in a series of children with PCD from Australia[9], in 83% in another series from the United Kingdom[17], whereas all children with PCD had a clinical history of a productive cough in another study from the United States[18]. Patients with PCD are at risk of developing bronchiectasis which may even be the presenting finding due to delayed diagnosis. In the series of patients from Australia[9] bronchiectasis was the presenting history in 32% of children. A diagnosis of PCD was established as the underlying cause of bronchiectasis in 1%-17% of children with imaging evidence of non-cystic fibrosis (CF) bronchiectasis who attended paediatric chest clinics[20-23]. Although development of bronchiectasis increases with age[18], it has even been described in toddlers with PCD[24].

As is now well recognized, high resolution computed tomography (HRCT) is a highly sensitive imaging modality for diagnosing bronchiectasis[22,25]. Using this non invasive technique in children with PCD and indications of severe lung involvement, based on clinical and/or radiological parameters, bronchiectasis was found in 73% of them in a series of 26 children with PCD who also had available a HRCT[8]. Bronchiectasis were detected in all of the adults and in 56% of children with PCD in another series of patients with PCD reported by Kennedy *et al*[26]. Other findings of HRCT imaging were mucus plugging, peribronchial thickening, consolidation, ground glass opacification, air trapping, atelectasis[8,26]. The most frequently affected lobes were the right middle lobe and lingula followed by the lower lobes[8,26-28] in contrast to what is observed in CF patients[28]. Using a modified Brody composite HRCT scan scoring system, Santamaria *et al*[28] showed that the total HRCT score was significantly higher in CF patients compared to PCD patients. In that study the total HRCT scan score as well as the bronchiectasis subscore was significantly negatively related to FEV1 and FVC indices. Nevertheless spirometry was less accurate than HRCT for the evaluation of the progression of lung disease in PCD patients, as it was shown by Maglione *et al*[27]. It was of note that in that retrospective study[27], patients underwent lung function tests and HRCT at least twice and no relationship was found between the change of a total HRCT scan score and FEV1. In contrast it was observed that lung function remained stable or even improved despite the deterioration of total HRCT scan score at the second evaluation. It is also of interest that, although at both evaluations the total HRCT scan score as well as bronchiectasis subscore were significantly negatively related to FEV1 and FVC, there were patients with normal spirometry and substantial lung imaging abnormalities at the baseline HRCT. The abovementioned data suggest that spirometric indices are not accurate enough to detect patients with structural lung changes. Most recently it was shown by Boon *et al*[29] that lung clearance index (LCI) was a more sensitive functional marker of lung structural abnormalities compared with FEV1 in patients with PCD.

The microbiology of lower airways infections in PCD patients was investigated in a cohort of children and adults with PCD[18] revealing that the most common culprit was *Haemophilus influenzae* followed by *Staphylococcus aureus, non-mucoid* and *mucoid Pseudomonas aeruginosa,* and nontuberculous mycobacteria. However, in this cohort *mucoid Pseudomonas aeruginosa* was isolated mainly in subjects older than 30 years of age. In another cohort[30] of children and adolescents with PCD, *Streptococcus pneumonia* and *Moraxella catarrhalis* were also isolated, whereas *mucoid Pseudomonas aeruginosa* was recovered in 5% of children.

UPPER RESPIRATORY TRACT MANIFESTATIONS

Nasal congestion and nasal drainage typically present from the neonatal period are among the characteristic symptoms that occur in 76%-100% of children in different cohorts of patients with PCD[17,18]. Acute and/or

chronic rhinosinusitis are also common in older children and it was seen among the symptoms that were present at the time of diagnosis in 11% of children in the series described by Coren *et al*[17] and in 71% of children in the series presented by Hosie *et al*[9]. Sommer *et al*[31] found that 59% of children who attended a PCD clinic documented recurrent problems of rhinosinusitis with 32% of them having needed antibiotics more than 30 times. Frontal and/or sphenoidal aplasia or hypoplasia also seem to be common in children with PCD as Pifferi *et al*[32] disclosed these findings in 73% of children with PCD (aged 8-17 years) who underwent a computed tomography (CT) scan of paranasal sinuses. Nasal polyps were identified in about one-third of patients in the cohort of Boon *et al*[13] during the follow up when the median age of the total group was 17.7 years. In contrast Rollin *et al*[33] did not find any polyps in a group of 30 children with PCD. However, the patient age of the latter group ranged from 1-14 years, with a mean age for the Kartagener's syndrome patients being 6 years and the mean age of the PCD patients being 9 years. The differences therefore between the findings from these two studies may simply imply that nasal polyposis tends to occur in patients with PCD at an older age.

Otologic manifestations, such as chronic otitis media with effusion, recurrent acute otitis media and chronic or recurrent otorrhea with conductive hearing loss[2] are also encountered very often among children with PCD. Serous otitis media was present at the time of diagnosis in 28/55 patients in the series presented by Coren *et al*[17] while hearing loss was found in 14/55 patients in this study. Similarly recurrent otitis media was among the presenting symptoms in 49% of children with PCD in another cohort[9]. It is of note that 38% of patients with PCD were diagnosed by their ENT doctor as found by Sommer *et al*[31] but at an older age compared to patients diagnosed by other specialists.

The prevalence of otologic manifestations is even higher than the abovementioned proportions during the course of the disease. In the survey by Sommer *et al*[31] it was shown that 81% of children with PCD had a history of recurrent otitis media and as much as 38% of the patients needed more than 30 antibiotic treatments in their life. However, it seems that recurrent acute otitis media decreases with age and is not present in patients older than 18 years of age[34] while otitis media with effusion is still frequent even in subjects over 18. In the same study[34], it was shown that the occurrence of chronic otitis media increased until the age of 18 and the majority of these patients experienced otorrhea. Retraction pocket, cholesteatoma and tympanic perforation were among the otologic complications observed in this group of patients.

It should also be mentioned that the majority of patients with recurring otitis media received ventilation tubes and about one-third of them needed more than three tympanostomies according to the findings of Sommer *et al*[31] The role of tube placement in children with PCD and recurrent ear problems is controversial but it is beyond the aim of this review to present and evaluate the management options for recurrent ear problems in this population. Although data from different studies differ regarding the frequency of auditory impairment[31,34,35], it seems that its prevalence progressively decrease with age.

UPPER AND LOWER RESPIRATORY TRACT MORBIDITY AND ULTRASTRUCTURE

Recent advances in PCD genetics have allowed the investigation of the relationship between specific ciliary ultrastructure defects and disease associated mutated genes with the clinical profile and progression of PCD. It was found that children with outer dynein defects (ODA) and ODA plus inner dynein defects (IDA) do not differ significantly to children with IDA and central apparatus defect with microtubular disorganization (IDA/CA/MTD) with respect to clinical respiratory features and respiratory pathogens[30]. In another genetic study, Knowles *et al*[12] showed that subjects with PCD and biallelic mutations in RSPH1 differed from those with classic PCD and dynein arm defects, showing a lower prevalence of neonatal respiratory distress and later onset of recurrent wet cough. In accordance to this observation, FEV1 was higher in RSPH1 cases compared to those with classic PCD.

Boon *et al*[13] evaluated PCD patients with normal and abnormal ultrastructure and they did not find any difference regarding lung function parameters, imaging findings and lower respiratory tract features. The type of ciliary ultrastructure did not seem to have any association with the prevalence of upper respiratory tract manifestations in children with PCD[13,30,36].

RARE FEATURES

Hydrocephalus has been described in association with PCD in a small number of patients[37] and is attributed to the impaired beating of cilia which is necessary for cerebrospinal fluid circulation.

Recently it was recognized that a number of clinical phenotypes exist that are associated with the dysfunction of non motile cilia[6]. These diseases are known as ciliopathies. However, it is beyond the scope of this review to present all human ciliopathies.

PSYCHOSOCIAL IMPACT OF THE DISEASE

As is evident by the abovementioned features, PCD would be expected to have a significant impact on health which could possibly affect the quality of life of affected children and their families. It was found that quality of life was worse in patients who had severe disease which required more aggressive treatment[38].

It was also recently shown[39] that children with PCD had higher scores compared to the control group

regarding internalizing problems such as withdrawn, somatic complaints and anxiety/depression. Therefore the psychosocial impact of the disease should also be taken into consideration by the physicians who take care of children with PCD. However, it is beyond the scope of this review to present the treatment options[40] for children with PCD, such as regular respiratory monitoring, physiotherapy, antibiotic treatment and maybe psychological support

CONCLUSION

The clinical features of PCD are nowadays well recognized but clinical suspicion of the disease in absence of heterotaxy remains rather low, although first manifestations may be present from infancy. The primary care clinician should have knowledge of the clinical spectrum of this condition in order to select appropriately the children who need further investigation for the diagnosis of PCD.

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MINIREVIEWS

Cutting-edge technologies for diagnosis and monitoring of snoring in children

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Abstract

Snoring is a very common problem in children and may be an indication of obstructive sleep apnea (OSA). Appropriate diagnosis is of importance due to detrimental effects of OSA. Polysomnography is considered the gold standard for the diagnosis of OSA. However, it is impractical for several reasons and this is why other tests have been developed as alternatives to formal polysomnography (PSG) for the assessment of children with snoring. In this mini-review basic features of PSG as well as alternative tests are presented and future perspectives are provided in addition to current guideline for the diagnosis and monitoring of childhood snoring. The aim of this review is to highlight briefly currently developed technologies that seem promising for the evaluation of snoring.

Key words: Snoring; Sleep apnea; Polysomnography; Molecular markers; Microelectronics

Core tip: There are several methods allowing for the screening of obstructive sleep apnea (OSA) patients in a large scale, either in the field of molecular diagnosis or in the field of microelectronics Miniaturization technology as well as advances in wireless devices connectivity and data processing allows for more affordable, convenient and reliable recording of parameters such as oxygen saturation, actigraphy and others. In addition, advances in molecular biology allows for the detection of genetic and non-genetic biomarkers of sleep apnea. However the aforementioned markers and their combinations remain to be validated. Until then polysomnography is considered the gold standard for the diagnosis of OSA.

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INTRODUCTION

Snoring is the most commonly presented symptom

of obstructive sleep apnea (OSA) in children. The estimated prevalence for snoring is 10% to 12%, whereas the estimated prevalence of OSA is only 1% to 3%[1-3]. However, due to the detrimental effects of OSA, children who snore need medical advice and possibly polysomnography (PSG), the sleep test that is considered the gold standard for the diagnosis of OSA[4].

The American Academy of Pediatrics (AAP) clinical practice guideline on diagnosis and management of childhood obstructive sleep apnea syndrome recommends overnight PSG for the confirmation of OSA[5]. In addition to identifying the presence of OSA, PSG also helps define its severity, which can aid in perioperative planning. However, despite the AAP recommendations and documented utility of PSG, only about 10% of pediatric otolaryngologists in United States[6] and probably much less in the rest of the world, obtain a preoperative PSG before tonsillectomy for sleep disordered breathing.

There are several reasons that can explain the variability in obtaining PSG prior to tonsillectomy or for the evaluation of snoring in general. Lack of access, cost, time expended, and concern over the child's emotional distress are the main reasons that explain why other tests have been developed as alternatives to formal PSG for the assessment of children with snoring. However, their role is still controversial.

In this mini-review basic features of PSG as well as alternative tests are presented and future perspectives are provided in addition to current guideline for the diagnosis and monitoring of childhood snoring. The aim of this review is to highlight briefly currently developed technologies that seem promising for the evaluation of snoring even though they have not been proven and qualified in real field.

Polysomnography

Formal PSG requires hospitalization or one night stay in a sleep laboratory. Several parameters are recorded simultaneously (Table 1) that allow for the estimation of specific indexes, with apnea-plus-hypopnea index (AHI) being the most utilized for diagnosis and staging of obstructive sleep apnea. Nevertheless, even the use of AHI is problematic in children since the clinically valid cut-off for normal AHI is unclear in this age group and no consensus has been achieved as to whether children with AHI values between the normal cut-off [< 1/h of total sleep time (TST)] and 5/h TST should undergo adenotonsillectomy[7,8].

Portable monitoring devices

Portable monitoring devices, also referred to as out-of-center sleep testing (OCST), have evolved as an alternative to overnight, attended, in-laboratory PSG in selected patients. Advantages of portable monitoring devices (PM) include its convenience (it can be performed in the patient's home) and its lower costs. However, the major disadvantage is that for most of these devices, fewer physiologic variables are measured than with PSG,

which can lead to misinterpretation of the results.

The United States Centers for Medicare and Medicaid Services have released guidelines that state that results from OCST can be used to support a prescription for positive airway pressure therapy in adults[9]. The American Academy of Sleep Medicine has also released clinical practice guidelines to guide clinicians in the use of OCST[10,11]. There are three types of PM: Type II device, which has a minimum of 7 channels (*e.g.,* EEG, EOG, EMG, ECG-heart rate, airflow, respiratory effort, oxygen saturation). This type of device monitors sleep staging so the AHI can be calculated; Type III device which has a minimum of 4 channels and Type IV device which usually measure only 1-2 parameters (*e.g.,* oxygen saturation); Type I refers to polysomnography which is not actually a portable monitoring device.

At minimum, PM must record airflow, respiratory effort, and blood oxygenation[10,12], thus type IV devices are not supported officially. For example, oximetry has a high positive predictive value (97%) for diagnosis of obstructive sleep apnea, but because not all apneas result in a drop in saturations the negative predictive value is low (53%)[13]. This is because simple oximetry cannot detect: (1) events that result in arousal without desaturation; (2) how long the patient slept; (3) carbon dioxide elevation; (4) prolonged flow limitation without discrete desaturation; or (5) whether they achieved rapid eye movement sleep (the period when respiratory events are most common)[4,14].

In general, false negative rates may be as high as 17% in unattended PM studies and the role of PM, as an alternative to formal PSG, in assessing children with sleep disordered breathing is controversial[10]. Unattended PM for the diagnosis of OSA should be performed only in conjunction with a comprehensive sleep evaluation. Moreover, in-laboratory polysomnography should be performed in case the PM test is technically inadequate or does not provide the expected result[10].

Miniaturization technology as well as advances in wireless devices connectivity and data processing allows for more affordable, convenient and reliable recording of parameters such as oxygen saturation, actigraphy and others. Thus the future of portable monitoring, especially in conjugation with other tests, seems promising.

Additional tests

An extended review of PSG and related monitoring is beyond the scope of this article. Furthermore, measures derived from PSG are poor predictors of OSA-associated morbidities[15]. The aforementioned tests can be combined with a validated questionnaire (*e.g.,* health status or quality of life questionnaire). Such questionnaires have been shown recently to be of benefit for childhood obstructive sleep apnea course prognosis[16] and can be utilized in the decision making process, *e.g.,* prior to a tonsillectomy.

Other modalities that are being investigated for the diagnosis and management of sleep apnea are genetic

Table 1 Parameters most commonly recorded in a polysomnography study
Pulse oximetry
Airflow from nasal canula thermistor and/or X-flow (AASM recommends RIP technology)
Snoring
End-tidal CO_2
Esophageal pressure and other methods for monitoring respiratory effort
ECG/heart rate or heart rate variability
Arterial tonometry
Electroencephalography
EOG
Actigraphy
Body position
Chin EMG
Limb EMG
Additional channels, *e.g.*, for CPAP/BiPap levels, pH, *etc.*

AASM: American academy of sleep medicine; RIP: Routing information protocol; ECG: Electrocardiograph; EOG: Electro-oculogram; EMG: Electromyography; CPAP: Continuous positive airway pressure.

Table 2 Potential biomarkers of obstructive sleep apnea and/or its comorbidities in children	
Non-genetic	**Genetic**
8-isoprostane	CRP 1444C/T
Adiponectin	CRP 1919A/T
APOEe4	IL-6-174C/IL-6 597A
Catecholamines	NOS1 and NOS3 16SNPs
Catestatin	EDN2 and EDN3 5 SNPs
CRP	MIF gene SNP rs10433310
IL-6	NADPH oxidase (NOX) rs6520785 and rs4673
HOMA	ApoE rs405509
MRP8/14	
TNF-α	
Urinary neurotransmitters	

CRP: C-reactive protein; NADPH: Nicotinamide adenine dinucleotide phosphate; IL: Interleukin; TNF: Tumor necrosis factor.

and non-genetic biomarkers (Table 2). As mentioned earlier, PSG results are poor predictors of OSA-related morbidities and there is the need of tests that can identify the most "vulnerable" patients, who would more likely benefit for specific therapeutic interventions[8]. Of the potentially promising morbidity biomarkers, plasma IL-6 and high sensitivity C-reactive protein appear to exhibit a favorable profile, and may discriminate OSA patients with and without morbidities in both adults and children. MRP 8/14 have been utilized as a cardiovascular morbidity-associated biomarker in children. In addition, urinary neurotransmitters may also provide a good tool for screening OSA cognitive morbidity in children[8].

The above mentioned biomarkers are non-genetic, their concentrations can be measured with various methods, *e.g.*, ELISA, chromatography and others, and depend on the course of the disease, thus they have the potential to provide information related to prognosis and response to treatment. On the contrary, gene polymorphisms (single nucleotide polymorphisms, insertion/deletion and copy number variants) show the genetic predisposition for OSA and are independent of the course of the disease. Because of the lack of genome-wide studies on the field, especially in children, there are only very few SNPs (Single Nucleotide Polymorphisms) that have been associated with obstructive sleep apnea and its comorbidities[17-19] (Table 2). Since simple SNPs or sequences cannot be patented, what is expected in the near future is that panels (or combination) of them that can be IP protected, to arise and to be validated.

Later developments in the field of chromatography and molecular biology techniques, such as multiplex PCR and sequencing, allow for the detection of various markers not only in serum but also in other samples such as saliva, urine and exhaled breath condensate. This is of special important for children, because nconvenient and painful blood tests can be avoided. Moreover, the cost of these methods has already been reduced and is almost certain that we will witness further costs reductions in the near future. Thus, methods allowing for the screening of OSA patients in a large scale already exist, either in the field of molecular diagnosis or in the field of microelectronics. These methods have the potential to provide us with affluent data. The field of sleep disorders will be revolutionized in case accurate verification of this data, probably in the form of validated and patented algorithms, is accomplished.

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MINIREVIEWS

Short and long term prognosis in perinatal asphyxia: An update

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Abstract

Interruption of blood flow and gas exchange to the fetus in the perinatal period, known as perinatal asphyxia, can, if significant, trigger a cascade of neuronal injury, leading on to neonatal encephalopathy (NE) and resultant long-term damage. While the majority of infants who are exposed to perinatal hypoxia-ischaemia will recover quickly and go on to have a completely normal survival, a proportion will suffer from an evolving clinical encephalopathy termed hypoxic-ischaemic encephalopathy (HIE) or NE if the diagnosis is unclear. Resultant complications of HIE/NE are wide-ranging and may affect the motor, sensory, cognitive and behavioural outcome of the child. The advent of therapeutic hypothermia as a neuroprotective treatment for those with moderate and severe encephalopathy has improved prognosis. Outcome prediction in these infants has changed, but is more important than ever, as hypothermia is a time sensitive intervention, with a very narrow therapeutic window. To identify those who will benefit from current and emerging neuroprotective therapies we must be able to establish the severity of their injury soon after birth. Currently available indicators such as blood biochemistry, clinical examination and electrophysiology are limited. Emerging biological and physiological markers have the potential to improve our ability to select those infants who will benefit most from intervention. Biomarkers identified from work in proteomics, metabolomics and transcriptomics as well as physiological markers such as heart rate variability, EEG analysis and radiological imaging when combined with neuroprotective measures have the potential to improve outcome in HIE/NE. The aim of this review is to give an overview of the literature in regards to short and long-term outcome following perinatal asphyxia, and to discuss the prediction of this outcome in the early hours after birth when intervention is most crucial; looking at both currently available tools and introducing novel markers.

Key words: Perinatal asphyxia; Neurological outcome; Hypoxic ischaemic encephalopathy; Cerebral palsy; Cognitive

outcome

Core tip: Perinatal asphyxia is a significant cause of acquired brain injury occurring in the neonatal period. A reliable early marker for predicting injury severity and sequelae remains elusive. The advent of therapeutic hypothermia as an effective neuroprotective intervention has changed the prognosis for affected infants. In this review we summarise what is known about the short and long term outcome for infants with perinatal asphyxia in the pre- and post-cooling era. We also describe currently available early indicators of outcome and introduce the exciting field of emerging novel biomarkers, both chemical and physiological.

BACKGROUND

Perinatal asphyxia describes the interruption of blood flow or gas exchange to and from the fetus in the perinatal period[1]. This may be prolonged partial asphyxia, sudden sub-total asphyxia due to a sentinel event or a combination of both[2]. Hypoxic-ischaemic injury to the brain and vital organs may result if the perinatal asphyxia is of a sufficient degree or prolonged beyond the ability of the fetus to compensate[3-5]. Approximately 20 per 1000 deliveries will require significant resuscitation, with biochemical and clinical evidence of perinatal asphyxia[6]. Of these only 1.6 per 1000 will go on to develop signs of evolving encephalopathy consistent with hypoxic-ischaemic encephalopathy (HIE)[7]. HIE must be differentiated from other causes of neonatal encephalopathy (NE), such as sepsis, meningitis or a metabolic disorder[1,8,9]. There may be a high suspicion of hypoxic-ischaemic injury following a known perinatal insult such as placental abruption or cord accident or if typical clinical signs, biochemical evidence of metabolic acidosis or depressed Apgar scores are present. However it can be very difficult to make this differentiation quickly after birth[10]. Approximately 50%-80% of NE can be attributed to hypoxia-ischaemia and given the potential benefit of early treatment, the need to identify infants with hypoxic-ischaemic induced encephalopathy is becoming increasingly important[1,11-13].

The aim of this review is to provide an overview of the literature on short and long-term outcome following perinatal asphyxia in the pre- and post-cooling era. We also aim to discuss the ability of currently available tools and novel markers to predict outcome in the early hours after birth when intervention is most crucial.

CURRENT MARKERS FOR PREDICTION OF OUTCOME

The advent of therapeutic hypothermia as a neuro-protective treatment for those with moderate and severe encephalopathy has improved prognosis[14,15]. Hypothermia is, however, a time sensitive intervention, with a very narrow therapeutic window and must be instigated within 6 h or ideally sooner following delivery to be effective[16]. So the challenge has become the prompt identification of those infants with signs of perinatal asphyxia who are most at risk of developing moderate or severe HIE. Prompt identification of those who will benefit from current and emerging neuroprotective therapies will help guide appropriate application of resources and permit prognostication. Currently available indicators such as blood biochemistry, clinical examination and electrophysiology all have limitations and their predictive power has been affected by the interceding intervention of therapeutic hypothermia; yet they still remain at the core of our predictive armamentarium in the critical first postnatal hours (Table 1).

Acid-base balance
A disturbance in acid-base balance is one of the earliest and most sensitive signs of fetal distress. The degree of acidosis is measured by scalp or cord pH, with acidosis being used to determine the need for intervention. A pH of < 7.00 gives a 50% chance of abnormal outcome, however the positive predictive value for significant encephalopathy is low[17]. This prediction might be improved by focusing on metabolic acidosis, and in particular lactate level. However several large trials have shown that lactate monitoring during labour does not improve our ability to detect or prevent adverse labour outcomes compared to pH monitoring alone and may in fact increase rates of instrumental deliveries unnecessarily[18,19].

Apgar score
Almost all infants are scored at birth through the eyes of Virginia Apgar, with midwives worldwide using her score for describing the condition of the infant at birth. However, Apgar scores suffer from poor sensitivity and specificity, as 80% of those with an Apgar score of ⩽ 7 at 5 min will have a normal outcome[17]. Often felt to be useful at extremes, 1 in 5 babies with an Apgar score of 0 at 10 min will survive to school age without moderate or severe disability[20]. A further difficulty is the subjective nature of the Apgar score, which leads to high levels of inter-observer variability. Subjective real time clinical scores have been shown to overestimate Apgar scores by a median value of 2.4 compared to later video enhanced estimation[21]. Attempts have been made to improve on the conventional Apgar score with Expanded and Combined versions that take aspects of neonatal resuscitation into account[22,23]. In particular, the Combined Apgar score at 5 min after birth has shown some promise in the prediction of perinatal acidosis

Table 1 Predictors of outcome

Predictors of outcome	Pros	Cons
Standard		
Acid-base balance	Widely available test, can be measured early by scalp and cord sampling	Cannot differentiate degree of severity of injury, invasive testing
pH	Responds early to HI	Low PPV for abnormal outcome
Lactate	Better reflects metabolic mechanism	No advantage over pH
Apgar score	Quick assessment of neonatal condition at birth, non-invasive	High inter-observer variability, poor predictor of long-term outcome
Clinical examination	Non-invasive, good to track changes in clinical state as injury evolves, predictive at discharge	Requires clinical experience, affected by intubation and medications and hypothermia, poor predictor of long-term outcome
EEG/aEEG	"Gold standard", early predictive value if normal, value of subclinical seizure detection, non-invasive	Requires resources, equipment to apply, clinical expertise to interpret
Novel		
HRV	Differentiates severity of HIE, non-invasive	Requires specialist equipment
MRI/MRS	Specific patterns of injury aid prognosis, early changes apparent	Requires specialist equipment, requires transfer of sick infant to MRI machine/department, requires infant to remain still for prolonged periods
Biomarkers	Very promising in pilot studies	None validated for clinical use
Serum	Reflects systemic biochemical state	Mixed markers from cerebral and other organ dysfunction, only small volumes available, invasive testing
Cord blood	Large volumes possible, available early	Mixture of fetal and placental blood
CSF	Reflects cerebral markers	Very difficult to sample
Urine	Relatively easy to sample	Affected if significant renal disease
Proteomics	Relatively stable and easy to test	Requires specialist equipment, response to injury may be delayed
Metabolomics	Rapidly responsive to changes in biochemical state	Requires specialist equipment, highly sensitive to environmental factors
Transcriptomics	Involved in critical processes of cell cycle and cell death, very stable	Requires specialist equipment, most markers are completely novel and difficult to identify, they may also regulate multiple pathways

The current standard tools and the novel emerging techniques to predict outcome in perinatal asphyxia are outlined with their respective advantages and disadvantages. HI: Hypoxia-ischaemia; EEG: Electroencephalograph; aEEG: Amplitude-integrated electroencephalograph; HRV: Heart rate variability; MRI: Magnetic resonance imaging; MRS: Magnetic resonance spectroscopy; HIE: Hypoxic-ischaemic encephalopathy; CSF: Cerebrospinal fluid.

(sensitivity 97% and specificity 99%) and HIE ($P = 0.01$ if score is < 10), though it cannot distinguish severity of HIE, and long term outcome data is unavailable[24].

Clinical examination

The neurological examination of a neonate is a clinical skill learnt through experience and exposure. Standardised scores have been developed, and widely used in an attempt to improve interobserver reliability[25-27]. However, the examination of a sick neonate is hampered by the need for sedative medications, anti-convulsants and intubation. We have shown that in full term infants, the best prediction of outcome is achieved by the examination at discharge. Examination on the first day after birth, even using a standardised method is not good for the prediction of outcome at two years[28]. More recent studies have shown that therapeutic hypothermia reduces our ability to accurately estimate the neurological state of the infant[29].

Electrophysiological monitoring

Electroencephalography (EEG) and amplitude integrated EEG (aEEG) have both been shown to offer excellent predictive ability as early as 3-6 h following delivery[30]. Outcome is strongly linked with the severity of EEG abnormalities seen. EEG and aEEG abnormalities evolve over the first 72 h, and so the timing of the recording is crucial to interpretation[31]. Early return of sleep wake cycling and normalization of background EEG abnormalities are good prognostic indicators[32,33]. The link between prognostic ability and timing of this evolution is altered by therapeutic hypothermia, so that delayed recovery may still be associated with a normal outcome[34]. A normal EEG recorded soon after birth is highly associated with a normal outcome at 2 years[35]. However, an abnormal EEG soon after birth may recover over subsequent days but if it remains abnormal at 48 h a poor prognosis is highly likely[35]. The PPV of a severely abnormal aEEG for death or disability at 6 h is 0.63 when assessed by the voltage grading scheme[34] and 0.59 when the aEEG is assessed by the pattern grading scheme[30,36,37]. These values drop slightly but not significantly in cooled infants[34]. In experienced hands EEG or aEEG provide an accurate assessment of the grade of encephalopathy and are excellent adjuncts to clinical decision making.

Multichannel EEG monitoring is essential for the detection of neonatal seizures, which occur frequently in infants with moderate and severe HIE. These seizures are difficult to predict or detect clinically and are associated with poor prognosis[38-41]. aEEG alone will miss focal or low amplitude seizures and requires expert interpretation[42,43]. We hope that in the future cot-side automated seizure

detection tools will be available to improve detection, and thereby the treatment of seizures in HIE[44].

NOVEL MARKERS FOR THE PREDICTION OF OUTCOME

There is increasing interest in the possibility of developing more accurate, early and reliable markers for predicting long term outcome in HIE. These bio- and physiomarkers may take the form of physiological monitoring [EEG and heart rate variability (HRV)], neuroradiological, or biochemical. In fact the ideal marker may be a combination of many of these (Table 1).

Reduced HRV has shown potential for the assessment of HIE severity and the prediction of long term outcomes[45,46].

Radiologically improvements in magnetic resonance imaging has improved our ability to delineate patterns of injury and thereby, aid in prognosis[47]. Piglet models of phosphorous-MRS profiles within the first 2 h post-injury can predict the evolution of injury severity[48].

Blood biomarkers have also shown promise in predicting injury severity and outcome. Although no definitive blood biomarker has entered into routine clinical use, there are a number which have shown promise based on pilot work in small cohorts. Protein markers, such as UCH-L1, IL-6 and IL-16 and Activin A are altered significantly in cord bloods taken at birth from infants with HIE[49-51]. In addition GFAP and S100B have shown elevations slightly later, reaching a peak at 24 h[49,52]. Animal and, more recently, human studies have shown significant alterations in the metabolomic profile of infants with HIE[53-55]. Transcriptomics has also shown promise in differentiating infants with perinatal asphyxia and HIE[56] Some evidence is also available showing that circulating microRNAs in maternal blood may be useful for the detection of hypoxia in the intrapartum period[57]. Other bodily fluids such as urine and CSF have also been the subject of biomarker discovery work[58]. A previous meta-analysis by Ramaswamy *et al*[59] in this area reported cerebrospinal fluid neuron-specific enolase and IL-1β to be a potential markers of abnormal outcome in survivors.

This list of novel biomarkers is by no means exhaustive but gives an indication of the proactive research ongoing in this rapidly emerging field. In the future one or a combination of these markers may help to offer early, rapid and reliable identification of infants suitable for neuroprotective intervention and may also provide further insight into the complex biochemical responses of the body to hypoxic-ischaemic injury.

TREATMENT OF HYPOXIC-ICHAEMIC ENCEPHALOPATHY

Based on substantial evidence from multiple randomized controlled trials, therapeutic hypothermia is now standard of care for infants with moderate and severe HIE in the majority of neonatal units where the necessary resources

are available[14,15,60-62]. Indications for treatment vary somewhat between centres but usually involve some combination of biochemical and clinical evidence of perinatal asphyxia with overt clinical manifestations of encephalopathy often based on the recruitment criteria of the larger trials of therapeutic hypothermia[60,61].

Unfortunately, using these current standard clinical markers, it is estimated that approximately 15%-20% of infants are mis-classified as having either a mild or no encephalopathy and are therefore not offered therapeutic hypothermia, worsening their long term prognosis[63].

OUTCOME IN PERINATAL ASPHYXIA

The majority of infants who require significant resuscitation at birth recover quickly and have no signs of encephalopathy. These children, in general have a normal outcome and function in line with their peers academically[64]. For this reason, at present, neuroprotective intervention has been reserved for infants with moderate or severe HIE as outlined above. However, several large population based studies now suggest that outcome in children with perinatal asphyxia without clinical encephalopathy is not completely normal. Odd *et al*[65] demonstrated an increased risk of low IQ at 8 years in this group compared to a control group. This is concerning due to the potential risk of a huge burden of more subtle disability (Table 2).

SHORT TERM OUTCOME IN HIE

For those infants who develop HIE, the most commonly used grading system remains the Sarnat score, with infants graded as mild, moderate or severe depending on their clinical signs[66]. The approximate breakdown tends to be mild (39%), moderate (39%) and severe (22%)[7]. The management and outcome varies significantly with grade of HIE.

Of those with moderate HIE, approximately one third will develop clinical and electrographic seizures in the neonatal period[39]. These seizures will usually commence between 18 and 20 h following delivery and will last for minutes to hours[67]. Following the cessation of seizures the encephalopathy may gradually improve to the point where oral feeding can recommence and care normalised. Both seizure burden and the time to achieve full oral feeding are useful in predicting the long term outcome of the infant[39,41]. The overall death rate in NE of all grades is 9.9% in developed countries but this rises acutely to 30% among those who qualify for cooling and precipitously to 76.8% when we consider severe encephalopathy alone[7,14].

LONG TERM OUTCOME IN HIE

Prior to the cooling era approximately 26.4% of infants with NE survived with moderate to severe neurodevelopmental impairment and a further 14% survived with mild impairment. Reported rates of cerebral palsy following NE vary but are generally around 10%-13% among survivors

Table 2 Outcomes in perinatal asphyxia

Short-term		
	Death	
	HIE	
	Seizures	
Long-term		
	Motor	Cerebral palsy
	Sensory	Hearing loss
		Visual impairment
	Cognitive	Episodic and working memory
		Attention
	Educational	Increased support requirements
		Lower test scores
	Behavioural	Attention
		Explosiveness
		Irritability
	Neuropsychiatric	Psychotic symptoms
	Neurodevelopmental	Autistic spectrum

A summary table of reported outcomes in perinatal asphyxia and hypoxic-ischaemic encephalopathy; HIE: Hypoxic-ischaemic encephalopathy.

of moderate to severe encephalopathy[68,69]. The risk is increased threefold where there is a history of neonatal seizures[68]. Dyskinetic CP and spastic quadriplegia are the most common subtypes with 80% of dyskinetic CP attributable to perinatal hypoxia-ischaemia at term[69]. Sensory disruption is also increased following hypoxic-ischaemic injuries. Rates of hearing loss are reported to be as high as 17.1% in those with other persistent neurological deficits[70]. Up to 41% of infants with a diagnosis of NE have an abnormality in some element of visual function in the first year of life, and where associated with moderate to severe basal ganglia changes and severe white matter changes on MRI this rises to 100%[71].

Therapeutic hypothermia has improved the outlook for infants with moderate to severe HIE, with increased likelihood of survival with normal IQ (RR = 1.31) and improved survival without neurological abnormalities (RR = 1.6) following therapeutic hypothermia at follow-up at 6-7 years of life[14].

It is important to note that learning deficits may be present with or without motor or sensory dysfunction. Impairments in episodic memory associated with reduced hippocampal volume has been found in children following perinatal hypoxic-ischaemic injury but without associated neurological deficits[72]. Robertson and Finer showed a reduction in school readiness scores as well as attention scores and increases in symptoms of explosiveness and irritability at 5.5 years in survivors of moderate encephalopathy without other disability[73]. Marlow *et al*[74] also demonstrated memory and attention/executive function impairments in the severe encephalopathy group and increased special educational needs and lower achievement on national curriculum attainment scores in both moderate and severe groups at 7 years[74]. Odd *et al*[64] have shown that infants with encephalopathy had lower working memory, reading accuracy and comprehension scores and increased requirement for educational support (OR = 6.24) between 8 and 11

years. A Swedish population based study examining the long term outcome following moderate encephalopathy has shown that in late adolescence the rates of disability are even higher, with 30% having CP, and 70% of those without CP having cognitive disability which interfered with their daily life[75].

NE has also been associated with increased behavioural difficulties. Those children with a history of moderate and severe encephalopathy have a significant increase in parent and teacher reported hyperactivity[74]. There is also a reported increase in autistic spectrum disorders in these children by 5 years (RR = 5.9)[76]. Adverse perinatal events are also associated with an increased risk of psychotic symptoms including schizophrenia[77,78].

The longer we follow these children the more evident it becomes that perinatal asphyxia and HIE have significant long term non-motor effects.

CONCLUSION

It is important to end with the note that the statistics quoted thus far have predominantly focused on high income countries, where research is most active. However, the greatest burden of disease is in low and middle income countries. Worldwide 10 million infants a year will suffer perinatal respiratory depression, of which 1.15 million will develop clinical encephalopathy. In countries with low neonatal mortality rates (NMR < 5) the incidence of NE is 1.6 per 1000 births rising to 12.1 per 1000 deliveries in countries with high NMR (> 15)[7]. It is estimated that 23% of neonatal deaths worldwide can be attributed to asphyxia which equates to nearly 1 million neonatal deaths per year; and in a countries with high neonatal mortality rates the death rate is 8 times that of countries with low NMRs[79]. Lack of modern obstetric care, inadequate neonatal resuscitation and lack of therapeutic hypothermia will cause this gap to widen. We need to strive for effective, reliable and inexpensive measures to enable early identification of infants at risk of long term injury, where low cost interventions, such as cooling, are potentially feasible and can produce significant and lifelong improvements on quality of life for these children, their parents and their communities[80].

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MINIREVIEWS

Oral medications regarding their safety and efficacy in the management of patent ductus arteriosus

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Abstract

Patent ductus arteriosus (PDA) is a common clinical condition in preterm infants which is inversely related to birth weight and gestational age. Cyclooxygenase inhibitors such as indomethacin and ibuprofen which block the prostaglandin conversion from arachidonic acid are the most commonly used drugs for ductal closure. This review focuses on the safety and efficacy oral medications in the management of PDA in preterm infants. Ibuprofen seems to be the first choice due to its higher safety profile, as it is associated with fewer gastrointestinal and renal side effects when compared to indomethacin. PDA closure rates are better with oral than with intravenous ibuprofen probably due to the pharmacokinetic of the drug. However, these medications were reported to be associated with several adverse including transient renal failure, gastrointestinal bleeding and perforation, hyperbilirubinemia and platelet dysfunction. Paracetamol seems be an alternative to PDA therapy with lower adverse events and side effects.

Key words: Efficacy; Ibuprofen; Oral; Paracetamol; Patent ductus arteriosus; Preterm infant; Safety

Core tip: Regarding to the management of patent ductus arteriosus (PDA) in preterm infants, neonatologists and cardiologists have not reached a consensus on which PDAs to treat, when to treat, and how to treat. Currently, ibuprofen seems to be the first choice due to its higher safety profile, as it is associated with fewer gastrointestinal and renal side effects when compared to indomethacin. PDA closure rates are better with oral than with intravenous ibuprofen. Recent studies suggest that paracetamol can be a medical alternative in the management of PDA with similar efficacy but lower side events than nonsteroidal anti-inflammatory drugs.

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INTRODUCTION

Patent ductus arteriosus (PDA), of which incidence is inversely related to gestational age (GA), is the most common cardiac condition among preterm infants. It is estimated to be 55% in preterm infants born before 28 wk' GA and weighing < 1000 g[1,2]. Several comorbidities are associated with a PDA, but whether PDA is responsible for their development or not is still unclear[3,4].

The treatment options for PDA closure are pharmacological and surgical. Prostaglandin-H₂ synthetase (PGHS) enzyme system, which has two active sites as cyclo-oxygenase (COX) and peroxidase (POX), produces circulating prostaglandins that regulate ductal patency[5,6]. Nonsteroidal anti-inflammatory drugs (NSAIDs), especially indomethacin and ibuprofen, are widely used for management of hemodynamically significant (hs)-PDA[7,8]. Ductal closure rate of PDA's pharmacological treatment is among 70%-85%[9-11]. COX-1 is constitutively expressed in different tissues, all NSAIDs can determine many side effects, mainly in cerebral, gastrointestinal and renal districts. In addition, there are some known contraindications such as renal failure, thrombocytopenia, intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC) and severe hyperbilirubinemia for ibuprofen or indomethacin administration. Therefore, there is a burden for alternative therapies which may result in at least equal closure rates but with fewer side effects[12-14].

Paracetamol (acetaminophen), unlike ibuprofen, directly inhibits the activity of prostaglandin synthase by acting at the POX region of the enzyme. Paracetamol inhibition is facilitated by a decreased local concentration of hydroperoxides[15,16]. The role of paracetamol as an alternative therapy for hs-PDA closure has gained attention in recent years because of the potential adverse effects of COX inhibitors[17-19]. In our previously reported case series, we showed that intravenous (IV) paracetamol could be an alternative treatment in patients in whom feeding was contraindicated or who had feeding intolerance[19]. Our recent studies show that paracetamol in oral form can be used successfully as the primary choice in PDA closure[20,21].

The main point of this review is the safety and efficacy oral medications in management of PDA in preterm infants. One commonly used therapeutic (oral ibuprofen) and a new alternative medication as oral paracetamol are discussed.

ORAL IBUPROFEN

Ibuprofen currently appears to be the drug of choice depending on its similar efficacy but lower side effect profile in comparison to indomethacin in closing PDA.

It is effective in closing PDA without reducing renal, cerebral or intestinal blood flow[22-24]. The rate of PDA closure in preterm infants varies both due to the dose regimens and the repeated courses of ibuprofen. The recommended dose regimen is 10 mg/kg loading dose followed by 5 mg/kg per day every 24 h for next two days[25,26]. A higher dose regimen as 20-10-10 mg/kg can result in a higher closure rate especially in lack of response to ordinary regimen, but must be balanced with side effect profile of the drug[26]. Individualizing the treatment by dosing COX inhibitors based on plasma concentrations has been discussed in the literature. The serum concentration after the first dose of ibuprofen was reported as the most important factor for a successful ductal closure[27].

Ibuprofen prophylaxis is also reported in various studies. Recently, Cochrane update[28] evaluated seven studies comparing prophylactic ibuprofen with placebo/no intervention. According to their results ibuprofen decreases the incidence of PDA on day three, the need for PDA treatment with NSAIDs and surgical ligation. In aspect of side effects, ibuprofen prophylaxis negatively affects renal function, increases risk of gastrointestinal bleeding but has no significant differences in mortality, IVH and bronchopulmonary dysplasia (BPD). In conclusion, this meta-analysis included two prophylaxis studies with oral ibuprofen and concludes that until long-term follow up results of these studies are published, no further trials of prophylactic ibuprofen are recommended. Similarly, we had terminated our recent study with oral ibuprofen prophylaxis according to observed serious side effects such as gastrointestinal bleeding, isolated intestinal perforation and renal failure in first days of life[29].

Aly *et al*[30] compared intravenous indomethacin and oral ibuprofen and the results of the study showed that oral ibuprofen is as effective as intravenous indomethacin. Because of preference of ibuprofen in contrast to indomethacin, new researches have focused on oral ibuprofen. A prospective- randomized study by Cherif *et al*[31] showed that ductal closure rate with oral ibuprofen was at least as effective as the intravenous route (84.3% *vs* 62.5%, *P* = 0.04) and oral ibuprofen was associated with fewer side effects. Our randomized controlled trial (RCT) demonstrated that oral ibuprofen was more successful than IV ibuprofen (84.6% *vs* 62%) in the management of PDA in very low birth weight (VLBW) infants. A higher increase in cystatin-C level, a marker of impaired renal function, with oral ibuprofen than intravenous form indicated that infants with borderline renal function should be carefully monitored[10]. In a similar designed study now in extremely low birth weight preterm infants, we demonstrated a similar initial closure rate but a higher reopening rate in infants who received ibuprofen when compared to our previous study in VLBW preterm infants[10,11]. A meta-analysis including three trials comparing oral with IV ibuprofen (*n* = 236) showed a significantly lower failure rate of PDA closure in favor of oral ibuprofen (RR = 0.42; 95%CI: 0.26-0.67); (RR =

-0.22; 95%CI: -0.35 to -0.11); NNT 5 (95%CI: 3-9) with similar rates of side effects[32]. Recent Cochrane review in 2015 concluded that oral ibuprofen was associated with a decreased risk of failure to close a PDA in comparison to IV ibuprofen[33].

Oral ibuprofen is associated with GI bleeding, NEC and spontaneous intestinal perforation (SIP)[34-36]. However, SIP was reported in both arms of intravenous and oral administration of the drug in RCTs comparing oral and intravenous ibuprofen or intravenous ibuprofen and indomethacin for PDA treatment. Additionally, the meta-analysis by Ohlsson failed to detect a statistically significant difference between oral ibuprofen and intravenous ibuprofen for all the gastrointestinal complications associated with administration of NSAIDs (GI bleeding, NEC and SIP)[37]. We suggest to use oral ibuprofen after oro-gastric feeding and flush it with 1-2 mL of distilled water to decrease its osmolarity related side effects on GIS.

Ibuprofen can compete with bilirubin for albumin binding sides and may induce bilirubin encephalopathy[38,39]. There is no definitive opinion on the effect of ibuprofen on bilirubin. *In vitro* studies suggest that ibuprofen may displace bilirubin from albumin binding sites, since it is 99% protein bound, increasing the risk of kernicterus[38]. Zecca *et al*[40] discussed the role of competition between ibuprofen and bilirubin and showed that ibuprofen use was associated with increase in total serum bilirubin levels and longer phototherapy duration.

Persistent pulmonary hypertension of the newborn (PPHN) was also observed, soon after the administration of IV ibuprofen, in the context of a randomized prophylactic trial, which was prematurely discontinued before full enrolment due to the development of this adverse effect[41]. After administration of oral ibuprofen, PPHN has not been observed in any study. Gournay *et al*[41] alerted physicians about the possibility of PPHN after the loading dose of ibuprofen, and hypothesized that PPHN could be related either to the early drug administration or to a drug-induced pulmonary microembolism.

In conclusion, ibuprofen is contraindicated in treatment of PDA in preterm infants with PDA-dependent congenital heart disease, renal failure, severe hyperbilirubinaemia, sepsis, NEC, gastrointestinal perforation, active bleeding from any site, severe thrombocytopenia and hypersensitivity to ibuprofen[42].

Oral paracetamol: A new approach to PDA treatment
Paracetamol is emerging as a possible alternative to indomethacin and ibuprofen following a chance observation made by Hammerman *et al*[17] in a baby with PDA who was given paracetamol for pain relief. The effect of paracetamol is at the peroxidase segment of prostaglandin synthase[43].

There has been increasing interest on the use of paracetamol for the treatment of PDA in the last few years. In the first case series by Hammerman *et al*[17] oral paracetamol (15 mg/kg per dose/6 h for 3 d)

was effective in five patients who did not respond to ibuprofen. In our previous case series with a median GA of 28.5 wk and a median birthweight of 995 g, paracetamol was administered after a median of 9.5 d (5-27) from birth in 8 preterm infants who did not respond to 2 sequential courses of ibuprofen and/or for whom treatment with ibuprofen was contraindicated[18]. The hs-PDA closed in 7 of the infants[18]. In our other case series, we used intravenous paracetamol in 10 preterm infants with hs-PDA in whom feeding was either not tolerated or contraindicated, and the PDA closure was successful in all patients[19]. In a case series by Yurttutan *et al*[20] that was conducted to investigate the efficacy of paracetamol as the first choice for the treatment of PDA in 6 preterm infants, 5 infants were successfully treated.

Our recent prospective RCT demonstrated that the PDA closure rate was similar for ibuprofen (77.5%) and paracetamol (72.5%) after the first course of the treatment[21]. In addition, both oral medications were well-tolerated and deemed safe in terms of renal and liver variables, as well as a lack of statistically significant difference in major complications (renal tolerance, hypertransaminasemia, hyperbilirubinemia, gastrointestinal bleeding, NEC, IVH, BPD, and ROP). Similarly, Dang *et al*[44] randomized 160 babies born before 34 wk to oral ibuprofen *vs* oral paracetamol in a non-blinded trial. Overall closure rates were similar at 79% *vs* 81% respectively with less gastrointestinal bleeding and less jaundice in the paracetamol group. There was not any significant differences in other side effects.

In particular, a reduced efficacy of paracetamol was observed in uncontrolled studies for extremely preterm neonates (GA < 28 wk)[45]. This phenomenon is not surprising because in more immature neonates, the expression of prostaglandin receptors is greater in the wall of the ductus, and extremely preterm neonates have a thin-walled ductus arteriosus that fails to develop extensive neointimal mounds. Due to these structural limitations in these subjects, functional closure induced by PGHS inhibitors is less frequently followed by the structural closure of the ductus[5,46].

IV paracetamol may transiently increase liver enzymes concentration in patients[47]. Alan *et al*[48] reported 3 patients with transient increased transaminases, which they observed with IV paracetamol use. Moreover, serious acute liver toxicity events have been reported in neonates when using intravenous formulation of paracetamol[49-52]. The slow oxidative metabolism of neonates, production of toxic metabolites in their livers, and increased rate of glutathione synthesis are mechanisms that may confer protection in the context of an overdose[52-54]. N-acetylcysteine, which detoxifies the toxic metabolite N-acetyl-p-benzoquinone imine, appears to be safe in the neonate but there is no data on its use in patients treated for PDA[52,55].

Measurement of serum paracetamol concentration was performed in only two studies with PDA management.

Table 1 Summary of the studies comparing oral ibuprofen and others (IV ibuprofen/indomethacin, oral paracetamol)

Ref.	Comparison (*n*)	Gestational age (wk)	Method	Ductal closure rates		Comparison of adverse effects	Conclusion
Aly *et al*[30] (LOE 1A)	IV INDO (9) Oral IBU (12)	IV INDO: 32.9 ± 1.6 Oral IBU: 31.2 ± 2.5	Prospective, randomized, single mask	Oral IBU 83%	IV INDO 78%	Oral IBU = IV INDO	Oral IBU could be an easy to administer and efficacious alternative treatment.
Cherif *et al*[31] (LOE 1A)	IV IBU (32) Oral IBU (32)	IV IBU: 28.3 ± 1.1 Oral IBU: 29.3 ± 1.2	Prospective, randomized, single mask	Oral IBU 70.30%	IV IBU 70%	Oral IBU < IV IBU	Early ductal closure with oral IBU is as good as IV route
Gokmen *et al*[10] (LOE 1A)	IV IBU (50) Oral IBU (52)	IV IBU: 28.7 ± 2.1 Oral IBU: 28.5 ± 1.9	Prospective, randomized	Oral IBU 84.6%[1]	IV IBU 62%	IV IBU = Oral IBU	Oral IBU is more effective than IV IBU for ductal closure in VLBW infants
Erdeve *et al*[11] (LOE 1A)	IV IBU (34) Oral IBU (36)	IV IBU: 26.3 ± 1.3 Oral IBU: 26.4 ± 1.1	Prospective, randomized	Oral IBU 83.3%[1]	IV IBU 61.70%	BPD is lower with oral IBU	Oral IBU is as effective as IV IBU for PDA closure even in < 1000 g preterm infants.
Keady *et al*[42] (LOE 1B)	Oral PARA (80) Oral IBU (80)	Oral PARA: 31.2 ± 1.8 Oral IBU: 30.9 ± 2.2	Prospective, randomized	Oral PARA 81.20%	Oral IBU 78.80%	Oral PARA < Oral IBU	Oral PARA was comparable to IBU in terms of the rate of ductal closure and even showed a decreased risk of hyperbilirubinemia or gastrointestinal bleeding.
Oncel *et al*[21] (LOE 1B)	Oral PARA (40) Oral IBU (40)	Oral PARA: 27.3 ± 1.7 Oral IBU: 27.3 ± 2.1	Prospective, randomized	Oral PARA 72.50%	Oral IBU 77.50%	Oral PARA = Oral IBU	Oral PARA is as effective as oral IBU for PDA closure.

[1]Differences were statistically significant ($P < 0.05$). LOE: Levels of evidence; IBU: Ibuprofen; INDO: Indomethacin; IV: Intravenous; PARA: Paracetamol; PDA: Patent ductus arteriosus; BPD: Bronchopulmonary dysplasia; VLBW: Very low birth weight.

Table 2 Comparison of adverse effects of oral ibuprofen and paracetamol[3]

	Oral ibuprofen	Oral paracetamol
Renal side effects	+/-	-
NEC	+	-
Spontaneous intestinal perforation	+	-
Gastrointestinal system bleeding	++	-
IVH	+	-
BPD	+/-	-
Alteration of platelet function	+	-
Decrease in cerebral blood flow	-	-
Hyperbilirubinemia	+/-	-
Hypertransaminasemia	-	+

NEC: Necrotizing enterocolitis; IVH: Intraventricular hemorrhage; BPD: Bronchopulmonary dysplasia.

In particular, Oncel *et al*[19] measured serum levels of paracetamol on days 1 (7.3 mcg/mL), 2 (15.5 mcg/mL) and 3 (14.7 mcg/mL) of treatment; while in the study by Yurttutan *et al*[20] evaluated serum paracetamol only after 24 h from the first dose and values ranged from 5 to 18 mcg/mL. All these values were within the therapeutic range for children (10-30 mcg/mL)[56].

Terrin *et al*[45] evaluated 2 RCTs and 14 uncontrolled studies on paracetamol use for the management of PDA. This meta-analysis of RCTs does not demonstrate any difference in the risk of ductal closure. Proportion meta-analysis of uncontrolled studies demonstrates a pooled ductal closure rate of 49% (95%CI: 29%-69%)

and 76% (95%CI: 61%-88%) after 3 and 6 d of treatment with paracetamol, respectively. Safety profiles of paracetamol and ibuprofen are similar. Meta-analysis demonstrated an efficacy of paracetamol comparable with that reported for ibuprofen. Efficacy of paracetamol seems to depend on GA and postnatal age of neonate and on modalities of drug administration[45].

The Cochrane review[57] which compared the effectiveness and safety of paracetamol *vs* ibuprofen combined two studies with 250 preterm infants in total. The success rate for paracetamol in ductal closure was similar to that of ibuprofen in addition to similar adverse events. However, infants who were treated with paracetamol had a lower risk of hyperbilirubinaemia than those treated with ibuprofen. Data on adverse effects on the developing brain from paracetamol in an experimental study and an association between prenatal paracetamol and the development of autism or autism spectrum disorder in childhood limits its wide use. Since no long term follow-up data are available for paracetamol use, it can not be recommended as the first line treatment choice.

CONCLUSION

Regarding to the management of PDA in preterm neonates, neonatologists and cardiologists have not reached a consensus on which PDAs to treat, when to treat, and how to treat. Currently, more neonates are managed conservatively, and the number of infants receiving surgical ligation is declining; however, there is a need for RCTs concerning the effect of this approach on long-term cardiovascular, pulmonary, and neurodevelopmental health.

Ibuprofen seems to be the first choice due to its

higher safety profile, as it is associated with fewer gastrointestinal and renal side effects when compared to indomethacin. PDA closure rates are better with oral than with intravenous ibuprofen. Indomethacin and ibuprofen remain the mainstays of medical management, whereas acetaminophen use is emerging as a less toxic option. Recent studies suggest that paracetamol can be an alternative in the management of PDA with similar efficacy but lower side events than NSAIDs.

The summaries of the some of the studies about oral ibuprofen and paracetamol are shown in Table 1. Considering the potential adverse effects of drugs, a careful monitoring including feeding intolerance, abdominal distension, oliguria and hypertension, and laboratory evaluation for renal and hepatic side effects in case of any need during and following day after the treatment is highly recommended. However, safety evaluation should also always consider long-term consequences of clinical and subclinical side effects. Comparison of adverse effects of oral ibuprofen and paracetamol are summarized in Table 2. Safety should be investigated especially in extreme preterm infants before routine use of paracetamol for PDA closure. We suggest that further prospective, randomized controlled trials are needed to evaluate the efficacy of oral *vs* intravenous paracetamol or intravenous paracetamol *vs* intravenous ibuprofen/indomethacin for the closure of PDA.

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MINIREVIEWS

Current views of the relationship between *Helicobacter pylori* and Henoch-Schonlein purpura in children

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Abstract

Helicobacter pylori (*H. pylori*) is one of the factors involved in the pathogenesis of various gastrointestinal diseases and may play a potential role in certain extra-intestinal diseases. *H. pylori* infection are mainly acquired during childhood, and it has been reported that in endemic areas of China the infection rates are extraordinarily higher in HSP children, particular those with abdominal manifestations. Furthermore, eradication therapy may ameliorate Henoch-Schonlein purpura (HSP) manifestations and decrease the recurrence of HSP. Therefore, results suggested that detection of *H. pylori* infection by appropriate method ought to be applied in HSP children. Current evidences indicate that local injury of gastric mucosa and immunological events induced by *H. pylori* infection are involved in the development of HSP. Increased serum IgA, cryoglobulins, C3 levels, autoimmunity, proinflammatory substances and molecular mimicry inducing immune complex and cross-reactive antibodies caused by *H. pylori* infection might play their roles in the course of HSP. However, there are no investigations confirming the causality between *H. pylori* infection and HSP, and the pathogenesis mechanism is still unclear. More bench and clinical studies need to be executed to elaborate the complex association between *H. pylori* and HSP.

Key words: *Helicobacter pylori*; Henoch-Schonlein purpura; Children

Core tip: This is the first comprehensive review to report current clinical and bench studies focusing on the potential role of Helicobacter pylori infection in Henoch-Schonlein purpura children. We also presented the possible mechanism underlying their association and the questions need to be addressed in the future studies.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative spiral, flagellated and microaerophilic bacterium colonizing human gastric mucosa as a significant factor involved in the pathogenesis of various gastrointestinal diseases. Evidences indicate that this bacteria may participate in certain extra-intestinal disease progression *via* various comprehensive mechanisms[1-3]. Recently, *H. pylori* infection or *H. pylori* induced gastritis was observed to be related to iron-deficiency anemia (IDA)[4-8], and the eradication therapy was reported to be effective in improving ferrintin level or even curing IDA[4-12]. Similar relationship between idiopathic thrombocytopenic purpura (ITP), a common hematologic disease in children, and *H. pylori* infection was also revealed by amount of physician-conducted clinical studies[13-18]. In the Asia-Pacific Consensus Guidelines and European Helicobacter and Microbiota Study Group Consensus 2012, it was recommended that eradication of *H. pylori* infection was necessary in certain patients with chronic ITP[19,20]. Furthermore, IDA and ITP were considered to be the extra-intestinal diseases related to *H. pylori* infection confirmed by many clinical trials[4,6-8,21-24]. Several relatively weaker evidences indicated that *H. pylori* infection or its related immune response possibly had interferences with some other extra-intestinal diseases, like cardiovascular, neurological and endocrine disorders[25-30].

H. PYLORI INFECTION AND HENOCH-SCHONLEIN PURPURA

Henoch-Schonlein purpura (HSP) is a common disease in children. It is characterized by IgA-deposits in vessel walls and renal mesangium and defined as acute leukocytoclastic vasculitis of small vessels. Although it is known that the cause of HSP is various, infectious agents are considered as the most important etiological factors[31,32]. Besides the purpura, gastrointestinal manifestations, usually noted as abdominal colic and intra-gastrointestinal hemorrhage, are concomitant during the course of disease and associated with therapeutic strategy and prognosis.

On the other hand, the prevalence of *H. pylori* ranges from 20% to 80%, which makes its infection popular worldwide[33]. Patients get infected predominantly in childhood and persists germ-carrying status mostly through their lifetime[34]. Few infected-individuals will develop upper gastrointestinal disorder, majority of them keep asymptomatic lifelong. Thus many of these children get diagnosed only after other diseases causing intestinal

manifestations.

The possible relation between *H. pylori* infection and HSP was firstly enlightened by several cases reports in adults suffering HSP and gastritis simultaneously[35-37]. The *H. pylori* infection could be detected by both ^{13}C-urea breath test. The golden standard of diagnostic relied on gastric mucosal biopsy. Reinauer *et al*[35] reported that in a HSP and chronic gastritis case with diagnosed *H. pylori* infection, the purpura, intestinal symptoms and albuminuria disappeared after eradication treatment. However, the patient was detected to be infected again while the purpura recurred 10 mo later, while the symptoms improved after elimination of *H. pylori*. Mozrzymas *et al*[38] and Mytinger *et al*[39] respectively presented the cases of children with this issue. It was also reported that in children with HSP and duodenal ulcer, purpura manifestations were ameliorated after *H. pylori* eradication was utilized.

Quantity of articles in China reported their investigations on the potential association between *H. pylori* infection and HSP[40-50]. Different from the literatures of cases from Western country, quite a lot of Chinese studies had enough sample sizes to perform cohort study, thus the results might be more reliable. Yuan *et al*[46] reported a retrospective study with the largest sample size, which included 186 HSP patients and 150 control cases. Anti-Hp IgG test was utilized as diagnostic method to detect *H. pylori* infection. Forty point nine percent (76/186) of patients in HSP group and 15.3% (23/150) of controls were confirmed to be infected. Based on the outcomes, the authors concluded that the infection was related to HSP occurrence[46]. In another research with the largest follow-up sample size, Li *et al*[43] claimed that OCA (O: Omeprazole; C: Clarithromycin; A: Amoxicillin) eradication treatment was effective in preventing HSP recurrence in those cases infected by *H. pylori* simultaneously, which were confirmed by rapid urease test (RUT).

A meta-analysis had been made to get the pooled *H. pylori* infection rate and identify the relativity between the two diseases[51]. One thousand three hundred and nine cases, including 749 HSP children and 560 healthy controls were enrolled in the pool analysis. The infection rates among the HSP children showed a wide range, which was from 22% to 75%, while those were only 3% to 44% in the healthy controls. Utilizing the data from 10 studies, the meta-analysis got a conclusion that this bacterial infection was statistically significantly associated to the increased occurrence of HSP with nearly 4 folds of risks in Chinese children (OR = 3.80, 95%CI: 2.54-5.68, $P < 0.001$). This study also claimed that the eradication therapy might play a protective role in the HSP recurrence, based on the data of 4 available studies (RR = 0.38, 95%CI: 0.25-0.58, $P < 0.001$) (Table 1).

It was interesting to see that the infection rate in HSP children reported in China varied in regions, which might be the outcome of combination of the different *H. pylori* infection prevalence and HSP incidence rate. In addition, researcher agreed that there was a geographic

Table 1 Brief view of current clinical control studies that focusing on the relationship between Henoch-Schonlein purpura and *Helicobacter pylori* infection in Chinese children

Ref.	Year	Total	Healthy control	HSP	Gastrointestinal HSP	HP in HSP children (%)	HP in control (%)	Eradication therapy
Wang *et al*[40]	2004	65	30	35	30	22.86	3.33	Yes
Zhang *et al*[41]	2004	120	60	60	-	38.33	23.33	No
Lv *et al*[42]	2005	62	28	34	11	23.53	3.57	No
Li *et al*[43]	2006	270	120	150	90	60.00	44.17	Yes
Chen *et al*[44]	2006	62	28	34	11	23.53	3.57	No
Wang *et al*[45]	2007	98	30	68	36	44.12	6.67	Yes
Yuan *et al*[46]	2007	336	150	186	118	40.86	15.34	Yes
Li *et al*[47]	2008	69	30	39	-	74.36	43.33	Yes
Li *et al*[48]	2008	102	42	60	40	58.33	28.57	Yes
Xia *et al*[49]	2008	67	30	37	37	54.05	23.33	No
Gao *et al*[50]	2009	120	40	80	51	62.50	12.50	No

HSP: Henoch-Schonlein purpura.

variation of *H. pylori* strains varies around the world[52]. Cytotoxin-associated gene A (Cag-A) positive strains was dominant among the isolated East-Asia *H. pylori*, whereas cag-A negative could be identified in up to 20% to 40% strains from Europe or Africa. Cag-A positive *H. pylori* strains from above continent showed differences in the repeating sequences numbers from each other, which resulted in variant abilities to infect the host and cause manifestations[53,54]. Similar status was observed when taking *vacA*, another important virulence of *H. pylori*, into consideration[55,56]. This might be the background of why HSP cases with concurrent *H. pylori* infection were rare in western countries (especially those developed one) but commonly encountered in East-Asia area. Thus, the current foundation of assuming the potential relation between *H. pylori* infection and HSP in Western population might not be that solid as in Eastern population.

The diagnosing method was another part that might influence the practice of exploring such possible relations. All the case reports confirmed bacterial infection with biopsy, which was the gold standard of *H. pylori* infection[35,38,39]. However, only 3 of these cohort studies above applied RUT, which was based on the tissues biopsied using endoscopy[43,45,47]. Five studies diagnosed the infection with urease breath test, the others only adopted serum anti-Hp immunoglobin (IgG mainly) test to identify the diagnosis[40,42,46,48,50]. It was known to all that IgG could be detected in the humoral immune system long after *H. pylori* infection or eradication, thus there might be several false positive cases confounded in the samples.

Another concern was the gastro-duodenoscopic manifestations of HSP. In these patients with obvious abdominal symptoms, endoscopy was considered to be a useful tool to confirm the diagnosis and exclude other surgical emergencies. It was reported that duodenum was the most common site of lesions, other sites like gastric antrum, body and angle, but never cardia or esophagus[57]. Endoscopic findings include Erythema, edema, petechiae, ulcers and other intraluminal lesions consisted of common endoscopic findings of HSP[58].

These manifestations overlapped with those of *H. pylori* infections more or less, thus bacteria detection was crucial in the treatment of certain patients.

It was unable to distinguish the *H. pylori* infection timing during the progression of HSP by using current study evidences. Intestinal manifestations may occur at any period of HSP courses, which would be the clue to detecting the *H. pylori* infection. No evidences were able to clarify whether the patients got infected before or after HSP symptoms appeared. It was reported that anti-*H. pylori* IgG level was relatively higher in HSP patients serum, comparing to that in healthy controls, while it was far from revealing the influence of bacterial infection on this autoimmune disorder[59]. Even clinic studies suggested there was strong relationship between *H. pylori* infection and HSP; researchers could not confirm that bacterial infection triggering the development of HSP. In addition, although HSP could be triggered by other infectious conditions, particular some respiratory infections, the limitation of their retrospective background made it impossible to exclude all the infectious or allergic diseases.

Moreover, there were no uniform criteria or parameters to evaluate the effectiveness of *H. pylori* eradication therapy in treating those HSP children suffering the infection. Because of their retrospective basics, the studies which indicated the effectiveness of eradication therapy in control HSP recurrence were not high quality evidences. Prospective well-designed clinical trials might eliminate the skepticism. Therefore, it was urgent to find out the appropriate diagnosing methods and indicators for detecting *H. pylori* infection. It was also necessary to establish the standard to assess the effectiveness of eradication therapy in HSP children.

BIOLOGIC MECHANISM BENEATH HSP AND *H. PYLORI* INFECTION

It is known to all that pathogenesis of HSP remained unclear. The clinical characteristics of HSP were the consequences of systemic leukocytoclastic vasculitis

with polymeric immunoglobulin A (pIgA), activated complements (C3 or C5) and certain fibrinogen/fibrin deposited in vessel walls, without IgG or IgM deposition. The immune complex between these elements in skin, gut, kidney and other organs resulted in the purpura, intestinal manifestation, nephritis and other relatively rare symptoms. Most investigators agreed that IgA1 was crucial in the progression of HSP[60-62]. Thus it could be speculated that any pathogens that were capable of initiation type Ⅲ allergic reaction with elevating serum IgA1 antibody levels and conducted systematic vasculitis might be indispensable in HSP progression.

H. pylori infection could also be diagnosed in IgA nephropathy patients, which shared several similarities with HSP. High level of serum anti-Hp IgA and disposition of pIgA in glomerular were two significant ones among the characteristics[63]. *H. pylori* infection can cause the incline of the serum levels of IgA, C3 and cryoglobulins, which is deduced to promote the immune complexes formation and increase the risk of HSP occurrence[64]. A study in adult patients revealed that, when compared to healthy controls, anti-Hp IgG levels in the acute phase of HSP and anti-Hp IgA/IgG ratios in the remitting phase were significantly higher[65]. However, there was no solid evidence of bench studies clarifying that whether the immune responses or abnormalities induced by *H. pylori* infection was associated with HSP or responsible for triggering the pathological process of the disease.

H. pylori infection resulted in bacterial invasion into gastric mucosa, which led to the direct damage to the physical barrier. Strong systemic humoral and cellular immune responses might be induced. It was assumed that such immune response might be able to coordinate the cross-talk between the infection of *H. pylori* and certain extra-gastrointestinal diseases, embracing autoimmunity, pro-inflammatory substances and molecular mimicry inducing immune complex and cross-reactive antibodies[66-69]. During the course, Ig A was secreted by the mucosa. Although this antibody was capable of inhibiting the adoption of bacterial antigen, preventing the adhesion and movement of *H. pylori*, and neutralization of toxin[63], the secretion was commonly over-activated.

H. pylori infection prognosis relied on the interaction among variant factors, such as virulence of dominant bacteria strain, host characteristics, and environmental influences. The product of vacuolating toxin gene A (*vacA*) and *cagA* were the main virulence factors of *H. pylori*. The *vacA* and *cagA* alleles, encoding the most important *H. pylori* virulence proteins *VacA* and *CagA*, contribute to the isolation of China and Western countries bacterial strains for the functional polymorphism. Based on the high toxigenicity of Chinese *H. pylori* strains and relatively low toxigenicity of strains in western countries, we hypothesize that *vacA* or *cagA* might participate in the progression of HSP through a complicate and unknown mechanism. Experimental research focusing relationship of *H. pylori* and atherosclerosis indicated that *cagA* antigen mimicry

the peptides of vascular wall, which also suggested that *cagA* antibody would damage the endothelium[70]. Another study suggested that *cagA* increased the secretion of IgA1 a dose-/time-dependent manner. Furthermore, it also indicated that *cagA* could promote the underglycosylation of IgA1 in B cells[71].

H. pylori infection also conducted the massive secretion of inflammatory mediators, like interleukin (IL)-6, IL-12, IFN-γ, TNF-α, *etc*. By their complicated interaction network, these cytokines participated in the inflammatory response directly or indirectly. Cellular immune response triggered by the infection was another mechanism that might influence the course of HSP. It was reported that CD4+/Treg cells proliferation was incited by *H. pylori* infected dendritic cells with the mediation of IL-1β, the secretion of which was stimulated by *vacA* and γ-glutamyl transpeptidase[72-74]. However, no significant difference in Treg cell level was identified between HSP patients and healthy controls[75,76]. In contrast, Th17 cells activation was also reported to be a functional part of *H. pylori* induced inflammation, and its concentration was demonstrated to be higher in HSP cases[77]. These results suggested that more details of cellular immune reaction beneath the fact of *H. pylori* infection needed further studies to explore.

Molecular mimicry was another approach of *H. pylori* in inducing autoimmune diseases. For example, human Lewis determinants [Le(x) and/or Le(y)] and H determinants expression could be detected in a majority of isolated *H. pylori* strains. While in some other strains, the detected components changed to Le(a), Le(b) and sialyl-Le(x)[78,79]. All the determinants were located in the O-chain of the surface lipopolysaccharide. In the preliminary researches, it was indicated *H. pylori* could evade host responses and evoke autoantibody responses to Le antigens with the help of certain molecular mimicry. Moreover, one study hypothesize that anti-Le autoreactive antibodies induced by *H. pylori* infection were involved in the progression of autoimmune disorders[80]. However, there is lack of clinical evidences could support this issue till now. The role of molecular mimicry in immune disorders, like HSP, requires further comprehensive analysis of T cells and autoantibodies functions. More functional research and clinical studies may focusing on Le antigens and other components in surface lipopolysaccharide of *H. pylori*.

CONCLUSION

Extra-gastric disorders were important aspects of *H. pylori* infection and diseases-correlated to the progression, which was proved by more and more clinical researches in children. Current studies suggested the latent relationship between the infection and HSP in children. Therefore, detecting *H. pylori* carrying status in HSP children, particular those with abdominal manifestations is indispensable in endemic areas. Diagnostic methods which are able to confirm the current infection situation are recommended to detect existence of *H. pylori* in HSP patients. However, it remained unclear how the

bacterial infection got involved in the progression of HSP. Considering the evaluation of the eradication therapy effectiveness in HSP children with *H. pylori* infection is not available, more robust evidences, such as randomized, placebo-controlled, double-blind large sized studies with appropriate diagnostic methods, would be conducted to reveal the potential association between *H. pylori* and HSP and to judge whether eradication therapy should be applied in those children.

Yet, despite some investigation suggested correlations of *H. pylori* infection with HSP in children, there remained many unanswered questions need to be addressed, which may lead to a further comprehension of *H. pylori*'s role in HSP, and to improve therapeutic and preventative strategies: (1) Latest clinic reports had significant drawbacks of sample size and study method. Large sample sized prospective clinic studies or nation-wide epidemiological studies need to be conducted to confirm the correlation or causality between *H. pylori* infection and HSP; (2) Most of current clinical studies were from Asia, particularly from China. Researchers might need to consider whether *H. pylori* strains with high toxicity differed from those with low toxicity in inducing or exacerbating HSP; (3) Further researches are also required to explore whether *H. pylori* infection is the cause of HSP or just only concurrent infection; (4) It is also necessary to know whether the existence of *H. pylori* induces those abdominal manifestations in HSP progression; (5) Long-term intensive follow-up of HSP recurrence post radical therapy is needed to identify the possible relationship between HSP infection and *H. pylori*, and to see the effect of such treatment on controlling recurrence; (6) Future studies answer that whether endoscopy can be a supplementary diagnostic tool when suspected HSP patient with significant digestive symptoms but no typical purpura; and (7) Basic mechanism of crosslink between the two diseases requests more bench studies to illuminate.

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ORIGINAL ARTICLE

Retrospective Study

Validation of a pediatric bedside tool to predict time to death after withdrawal of life support

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Abstract

AIM: To evaluate the accuracy of a tool developed to predict timing of death following withdrawal of life support in children.

METHODS: Pertinent variables for all pediatric deaths (age ≤ 21 years) from 1/2009 to 6/2014 in our pediatric intensive care unit (PICU) were extracted through a detailed review of the medical records. As originally described, a recently developed tool that predicts timing of death in children following withdrawal of life support (dallas predictor tool [DPT]) was used to calculate individual scores for each patient. Individual scores were calculated for prediction of death within 30 min (DPT30) and within 60 min (DPT60). For various resulting DPT30 and DPT60 scores, sensitivity, specificity and area under the receiver operating characteristic curve were calculated.

RESULTS: There were 8829 PICU admissions resulting in 132 (1.5%) deaths. Death followed withdrawal of life support in 70 patients (53%). After excluding subjects with insufficient data to calculate DPT scores, 62 subjects were analyzed. Average age of patients was 5.3 years (SD: 6.9), median time to death after withdrawal of

life support was 25 min (range; 7 min to 16 h 54 min). Respiratory failure, shock and sepsis were the most common diagnoses. Thirty-seven patients (59.6%) died within 30 min of withdrawal of life support and 52 (83.8%) died within 60 min. DPT30 scores ranged from -17 to 16. A DPT30 score $\geqslant$ -3 was most predictive of death within that time period, with sensitivity = 0.76, specificity = 0.52, AUC = 0.69 and an overall classification accuracy = 66.1%. DPT60 scores ranged from -21 to 28. A DPT60 score $\geqslant$ -9 was most predictive of death within that time period, with sensitivity = 0.75, specificity = 0.80, AUC = 0.85 and an overall classification accuracy = 75.8%.

CONCLUSION: In this external cohort, the DPT is clinically relevant in predicting time from withdrawal of life support to death. In our patients, the DPT is more useful in predicting death within 60 min of withdrawal of life support than within 30 min. Furthermore, our analysis suggests optimal cut-off scores. Additional calibration and modifications of this important tool could help guide the intensive care team and families considering DCD.

Key words: Death; Organ donation; Children; Donation after circulatory death

Core tip: Donation after circulatory death (DCD) has gained acceptance as a way of increasing the number of organs available for transplantation. In order for DCD to occur, organs must be harvested within 30 or 60 min of withdrawal of support. A tool that predicts time of death after withdrawal of support in children has been created but not validated by an external source. In this study, we apply the newly created Dallas Predictor Tool to an external pediatric sample and show it to be an accurate predictor of death within 60 min of withdrawal of support. The tool would require additional calibration to be a good predictor of death within 30 min.

INTRODUCTION

In the United States, the number of patients awaiting organ transplantation far exceeds the number of organs available from living or brain dead donors[1,2]. In August 2015, there were 133661 patients waiting for organ transplantation in the United States, yet there were only 29532 transplants performed during the previous year with organs from 14413 donors, highlighting the marked disparity between need and supply[3]. That disparity also exists in the pediatric age range, considering that there are currently 2036 patients younger than 18 years of age awaiting transplants, despite 1795 transplants performed in 2014[3].

In donation after circulatory death (DCD), organs are recovered from a donor that dies during controlled withdrawal of life support[4-6]. Typically, in the United States, a potential DCD donor is a terminally ill patient with a clear advance directive or a surrogate decision maker who, in conjunction with the medical team, believes that the best course of action is withdrawal of life support. Should there be agreement on the opportunity for DCD and proper consent, life support is withdrawn under controlled conditions and organs (usually kidneys and liver) can be harvested upon declaration of death by cardiopulmonary criteria following a pre-determined time interval. Although DCD has become more frequent in the past decade, still the majority of transplanted organs are recovered after donation following neurological death (DND)[3]. Various studies have shown that outcomes following DCD transplants are similar to those following DND transplants[6-8]. Therefore, increasing utilization of DCD is one mechanism to increase the availability of organs for patients on the transplant wait list and decrease waiting time[9].

Organ viability from DCD donors is predicated on a minimal interval between withdrawal of support and organ removal. If excessive time elapses between withdrawal of support and circulatory death, the donor will become ineligible. Although no evidence-based consensus on what constitutes "excessive time" exists, an organ typically is no longer considered transplantable if time from withdrawal to death is greater than 30 min for a liver and 60 min for kidneys[10]. The uncertainty of suitability of organs relative to the time of death in addition to usual end-of-life considerations may lead to undue stress on the donor's family, potential transplant recipients and medical teams. Therefore, improved ability to predict the amount of time from withdrawal of support to circulatory death could enhance the DCD process and facilitate increased donation rates.

A tool that predicts the likelihood of death within the organ recovery window has been developed and used in adult patients for several years[11]. The Wisconsin DCD Evaluation Tool predicted suitability for DCD in adults 83.7% of the time within 60 min after withdrawal of support[11]. More recently, a pediatric tool was developed through analysis of 518 deaths at Children's Medical center dallas, referred here henceforth as the dallas predictor tool (DPT)[1]. The DPT was created using data from a single institution and external validation has not been reported. Validation of this tool could help physicians determine *a priori* whether a pediatric patient might be eligible for organ donation following withdrawal of life support and help inform families considering this type of organ donation.

The objective of this study is to characterize the process of death following withdrawal of support in the pediatric intensive care unit (PICU) of an academic

Table 1 Dallas predictor tool score calculation[1]		
	30 min predictor (DPT30)	60 min predictor (DPT60)
Model parameter	Point score	Point score
Age 1 mo or younger	-9	-9
Norepi, epi or phenyl > 0.2 mcg/kg per minute	11	10
ECMO	11	17
PEEP > 10 cmH2O	5	11
Spontaneous ventilation	-8	-12
Lowest possible score	-17	-21
Highest possible score	27	38

DPT: Dallas predictor tool; Norepi: Norepinehrine; Epi: Epinephrine; Phenyl: Phenylephrine; ECMO: Extracorporeal membrane oxygenation; PEEP: Positive end-expiratory pressure.

children's hospital and evaluate the performance of the DPT in predicting time to death after withdrawal of support in this remote pediatric sample.

MATERIALS AND METHODS

After obtaining Institutional Review Board approval, we performed a detailed retrospective chart analysis of all deaths that occurred after withdrawal of support in the PICU at UH Rainbow Babies and Children's Hospital from January 1st 2009 to June 30th 2014. The inclusion criteria for this study were all patients 21 years of age or younger admitted to PICU who died after withdrawal of life support. Patients were excluded if they were older than 21 years of age, died during active resuscitation or were declared brain dead.

Patients were identified using our own PICU database. Medical records for these patients were reviewed to evaluate whether they met the inclusion criteria. Data from patients meeting the inclusion criteria were abstracted into a protected spreadsheet for subsequent analysis. Extracted data included demographic information, time of admission, time of withdrawal of support, time of death, diagnoses, co-morbidities, vital signs, support modalities (*e.g.,* mechanical ventilation, renal replacement therapies, extracorporeal life support), vasoactive and inotropic support, results from laboratory testing related to renal and hepatic function, infectious status and mechanism of death. The dataset was then used to externally validate the accuracy of the existing pediatric DCD tool (DPT)[1] for this remote sample.

We utilized the DPT to calculate scores for likelihood of death within 30 min (DPT30) and 60 min (DPT60) for each patient with clinical data obtained just prior to withdrawal of life support using the criteria specified in Table 1. The score was then used to predict the likelihood of a patient dying within 30 min or 60 min from withdrawal of life support. The predictive accuracy of the DPT tool was calculated by correlating the scores and the actual time interval between withdrawal of life support and death.

Data were treated with descriptive statistics using dedicated software (SigmaPlot, Systat Software Inc,

San Jose, CA). Receiver operating characteristic (ROC) curves were created using a dedicated web-based ROC analysis calculator[11]. ROC data were used to determine the optimal cut point in the range of DPT scores for best sensitivity and specificity. This optimal cut point was then used to determine the overall classification accuracy, which we defined as the added percentage of patients correctly predicted to be dead or alive at 30 min and 60 min by using the optimal score cut point.

RESULTS

During the 66-mo study period, there were 8829 admissions to the PICU resulting in 132 deaths and a mortality rate of 1.5% (Figure 1). Death followed withdrawal of life support in 70 patients (53%). Of those, 8 patients were excluded from the data analysis for not having sufficient data for retrospective calculation of the DPT scores. Therefore, 62 patients who died following withdrawal of life support were included in the data analysis. Among the remaining 62 patients for whom support was not withdrawn, 37 deaths (28%) occurred during attempts to resuscitate (failed CPR), 16 (12%) patients met brain death criteria, and 9 (7%) deaths occurred in patients with a "do not attempt resuscitation" (DNAR) order but without active withdrawal of life support.

The mean age of patients analyzed in our sample was 5.3 years (SD: 6.9 years). The median time to death after withdrawal of life support was 25 min (range: 7 min to 16 h 54 min). Thirty-seven patients (59.6%) died within 30 min of withdrawal of life support and 52 (83.8%) died within 60 min. Common diagnoses included respiratory failure (32.2%), hypoxic-ischemic encephalopathy (19.3%), cardiorespiratory arrest (16.1%), congenital heart disease (16.1%) and shock (14.5%).

Death within 30 min after withdrawal of life support (DPT30) scores ranged from -17 to 16 (Table 2). A DPT30 score $\geq$ -3 was most predictive of death, with sensitivity of 0.76, specificity of 0.52, area under curve (AUC) of 0.69 and an overall classification accuracy of 66.1% (Table 2 and Figure 2). Death within 60 min after withdrawal of life support (DPT60) scores ranged from -21 to 28 (Table 3). A DPT60 score $\geq$ -9 was most predictive of death, with sensitivity of 0.75, specificity of 0.8, AUC of 0.847 and an overall classification accuracy of 75.8% (Table 3 and Figure 3). Organs were actually donated after circulatory arrest following withdrawal of life support (DCD) from 2 patients in our sample. The interval time to death after withdrawal of life support in those patients was 35 min and 38 min, with liver and kidneys harvested in those procedures. There was also 1 case of attempted but unsuccessful DCD, where parents consented to donation but the child died after the 60-min time limit following withdrawal of life support.

DISCUSSION

Data from the Organ Procurement and Transplant
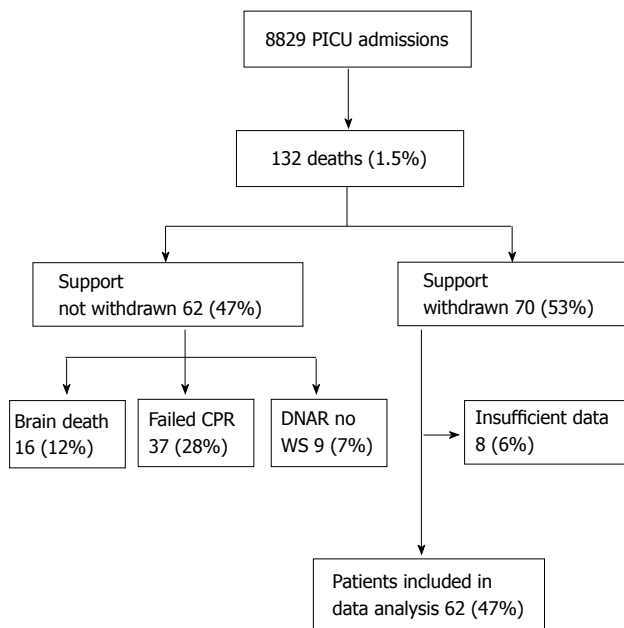
Figure 1 Pediatric intensive care unit admissions and deaths. PICU: Pediatric intensive care unit; CPR: Cardiopulmonary resuscitation; DNAR: Do not attempt resuscitation; WS: Withdrawal of support.

Table 2 Scores for dallas predictor tool 30 min

Cutpoint	Dead (1)	Alive (0)	Total	Sensitivity	Specificity
-17	1	1	2	1.0000	0.00
-9	1	0	1	0.9730	0.04
-8	7	12	19	0.9459	0.04
-3	1	0	1	0.7568	0.52
0	10	7	17	0.7297	0.52
2	2	1	3	0.4595	0.8
3	1	0	1	0.4054	0.84
5	0	2	2	0.3784	0.84
7	1	0	1	0.3784	0.92
8	0	1	1	0.3514	0.92
11	7	1	8	0.3514	0.96
16	6	0	6	0.1622	1.00
Total	37	25	62		

Table 3 Scores for dallas predictor tool 60 min

Cutpoint	Dead (1)	Alive (0)	Total	Sensitivity	Specificity
-21	1	1	2	1.0000	0.0
-12	12	7	19	0.9808	0.1
-9	1	0	1	0.7500	0.8
-1	1	0	1	0.7308	0.8
0	15	2	17	0.7115	0.8
1	1	0	1	0.4231	1.0
5	1	0	1	0.4038	1.0
8	2	0	2	0.3046	1.0
10	7	0	7	0.3462	1.0
11	2	0	2	0.2115	1.0
16	1	0	1	0.1731	1.0
17	1	0	1	0.1538	1.0
19	1	0	1	0.1346	1.0
21	3	0	3	0.1154	1.0
28	3	0	3	0.0577	1.0
Total	52	10	62		

Network show that the number of patients awaiting an organ transplants continues to grow every year[2]. A national survey of donor hospitals identified 1330 eligible pediatric organ donors with consent rates of nearly 69%[12]. However, within that group there were only 37 pediatric DCD donors yielding 103 transplanted organs[12]. Another study evaluating the potential for DCD at a children's hospital showed that 5.5% of all patients who died in a PICU would have been potential candidates for organ donation through DCD[13]. However, that figure is higher (58%) when considering only those patients not receiving CPR or without a contraindication for donation, such as target organ dysfunction[13]. A more recent study involving children in the neonatal, cardiovascular and pediatric intensive care units found that the number of pediatric potential candidates for DCD was significantly larger than the number of potential candidates for donation after neurologic determination of death, but that the actual donation rate was significantly lower[9]. With external validation of the DPT and its increased use, it might be possible for pediatric intensivists to identify with a greater degree of certainty which patients might be eligible for DCD following withdrawal of life support and counsel the family accordingly. This tool may also help minimize the stress, frustration, and inefficient use of resources associated with donation failure by enrolling patients found to be highly likely to die within 60 min following withdrawal of life support.

The criteria used to calculate the DPT score are simple and intuitive. It is reasonable to expect that critically-ill patients who are incapable of producing spontaneous respirations, those who require significant mechanical ventilator support, high doses of vasoactive or inotropic drugs, or extracorporeal life support would have a shorter interval between withdrawal of life support and circulatory death. The original development and application of the DPT has an overall classification accuracy of 74.5% and 87.3% for death within 30 and 60 min after withdrawal of life support[1]. However, those figures were obtained by applying the DPT to the very sample used to develop it. While the DPT score has shown promise in that initial publication, it had not been validated through a remote sample until the current study.

Our data suggest that the DPT is clinically relevant in predicting time from withdrawal of life support to death. We note that the DPT60 score has higher classification accuracy than the DPT30 score and a more robust AUC. In general, the classification accuracy in our data was lower than that noted in the original Dallas study[1]. The overall classification accuracy for DPT30 at our institution was 66.1% while the accuracy in the Dallas study was 74.5%[1]. Similarly, the accuracy for DPT60 at our institution was 75.8% compared to the accuracy of 87.3% noted in the Dallas study[1]. Despite these differences, we believe that the DPT60 score can be used as an accurate predictor of death within 60 min following withdrawal of life support. Our analysis also suggests that optimal
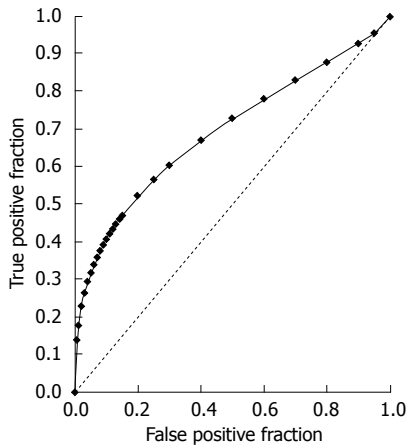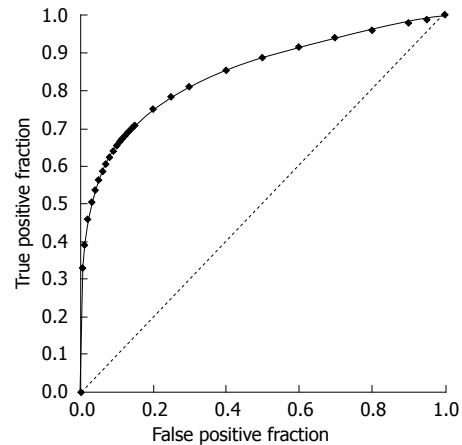
Figure 2 Receiver operating characteristic for prediction of death within 30 min of withdrawal of life support (DPT30). Area under the curve = 0.69.

Figure 3 Receiver operating characteristic for prediction of death within 60 min of withdrawal of life support (DPT60). Area under the curve = 0.87.

cutoff scores for best accuracy vary between samples. The optimal cutoff scores of -3 and -9 for DPT30 and DPT60 for our study, respectively, differed considerably from the original Dallas study[1], underscoring the need for additional calibration and modifications of the tool so as to arrive at more widely applicable cutoff points.

In our study, there were only 3 attempts at DCD and only 2 successful donations, highlighting the fact that this form of organ donation is still the exception among pediatric patients. However, should each one of the 62 eligible patients have consented for DCD prior to withdrawal of support, 37 patients would have been eligible to donate a liver (death within 30 min) and 52 patients would have been eligible to donate kidneys (death within 60 min).

Our study is limited by factors inherent to its retrospective nature, specifically the accuracy of documentation of end-of-life events for these patients. However, clinical data and times of withdrawal of life support and death are extensively and redundantly documented at our institution, so the likelihood of this type of error is minimal. Nevertheless, a prospective study would be required to completely validate these data and test the real time prospective applicability of this tool. The sample size in our study was considerably smaller than in the original Dallas study (62 patients *vs* 518 patients, respectively). This relatively small sample could lead to sampling error and potentially impact the accuracy of the DPT score in an external cohort. However, if the DPT score is accurate it should be predictive in any cohort irrespective of the diagnostic profile and associated comorbidities of the external cohort.

In conclusion, A simple, convenient and accurate tool that predicts time to death after withdrawal of life support in children, such as the DPT would be an important adjunct to the decision-making process regarding DCD. In this external cohort, the DPT is clinically relevant in predicting time from withdrawal of life support to death. Our data show that the DPT is more useful in predicting death within 60 min than within 30 min of withdrawal

of life support. The predictive accuracy of the DPT30 score is not as high and may require recalibration or incorporation of additional variables to become more clinically useful.

COMMENTS

Background
The number of patients awaiting organ transplantation far exceeds the number of organs available from living or brain dead donors in the United States. Donation after circulatory death (DCD), a form of donation where organs are recovered from a donor that dies following controlled withdrawal of life support, has been seen as an alternative to increase availability of organs and decrease waiting time. The ability to accurately predict the time interval between withdrawal of support and death is important in the DCD process because it can help inform the medical decision-makers (patient and family) and the medical team on the likelihood of death within the acceptable donation time window. This can help decrease the emotional stress associated with unsuccessful DCD on the donor family, potential organ recipient and medical teams. A pediatric tool that predicts the likelihood of death within the DCD time window has recently been develop but not yet validated externally. In this study, the authors apply this newly developed tool to a remote pediatric sample to evaluate its sensitivity, specificity and overall classification performance.

Research frontiers
Accurately predicting the time elapsed between withdrawal of support and death could have major implications in the process of DCD. This study is the first attempt to externally validate the dallas predictor tool (DPT); its results will help guide the application of this tool to remote samples.

Innovations and breakthroughs
In this study, the DPT accurately predicted death within 30 min of withdrawal of support in 66.1% of subjects, and death within 60 min of withdrawal of support in 75.8% of subjects. The authors have shown that the DPT accuracy is lower when applied to an external sample. The DPT may require recalibration or incorporation of additional variables to become more clinically useful, particularly for the 30 min time window.

Applications
This study suggests that the DPT can predict death within 60 min in over 75% of patients and can be used to inform the suitability of a potential pediatric donor being considered for DCD.

Terminology
DCD: Organ donation after circulatory death is a process by which organs are

recovered from a donor that dies during controlled withdrawal of life support. DPT: The dallas predictor tool is method that predicts the likelihood of death within 30 or 60 min of withdrawal of life support in children.

Peer-review

The manuscript is well written and covers a gap in knowledge on this topic. In this manuscript, DPT is clinically relevant in predicting time from withdrawal of life support to death. Precisely, DPT is more useful in predicting death within 60 min of withdrawal of life support than within 30 min. Additional calibration and modifications of this important tool could help guide the intensive care team and families considering DCD.

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ORIGINAL ARTICLE

Retrospective Study

Analysis of the therapeutic evolution in the management of airway infantile hemangioma

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Abstract

AIM: To analyze the evolution in the management of airway infantile hemangioma (AIH) and to report the results from 3 pediatric tertiary care institutions.

METHODS: A retrospective study of patients with diagnosis of AIH and treated in 3 pediatric tertiary care institutions from 1996 to 2014 was performed.

RESULTS: Twenty-three patients with diagnosis of AIH were identified. Mean age at diagnosis was 6 mo (range, 1-27). Single therapy was indicated in 16 patients and 7 patients received combined therapy. Two therapeutic groups were identified: Group A included 14 patients who were treated with steroids, interferon, laser therapy and/or surgery; group B included 9 patients treated with oral propranolol. In group A, oral corticosteroids were used in 9 patients with a good response in 3 cases (no requiring other therapeutic option), the other patients required additional treatment options. Cushing syndrome was observed in 3 patients. One patient died of a fulminant sepsis. Open surgical excision and endoscopic therapy were performed in 11 patients (in 5 of them as a single treatment) with a response rate

of 54.5%. Stridor persisted in 2 cases, and one patient died during the clinical course of bronchial aspiration. In group B, oral propranolol was used in 9 patients (in 8 of them as a single treatment) with a response rate of 100%, with an mean treatment duration of 7 mo (range, 5-10); complications were not observed.

CONCLUSION: Our experience and the medical literature support the use of propranolol as a first line of treatment in AIH.

Key words: Infantile hemangioma; Propranolol; Surgery; Airway; Fibrobronchoscopy

Core tip: Through this study we want to highlight the importance of early use of propranolol in the treatment of airway infantile hemangioma. We also want to show our experience with other treatment options including corticosteroids, interferon and surgical and endoscopic treatments used before the propranolol era.

INTRODUCTION

Even though infantile hemangiomas (IH) are the most common head and neck tumors during childhood, the airway is uncommon location, accounting for only 1.5% of all pediatric laryngeal lesions[1]. Symptoms at presentation of infantile hemangioma (AIH) are related to the grade of airway obstruction, which becomes more evident during periods of agitation, crying, or respiratory infections. Stridor, usually biphasic but more prominent during inspiration, is the most common presentation symptom. Diagnosis is performed by bronchoscopy image which typically reveals a unilateral, soft, submucous and reddish mass[2]. IHs are usually not present at birth; they proliferate during the first year of life, and then they involute. For AIH, the treatment goal is to provide an airway adapted for the development of these children. Multiple modalities, both medical and surgical, have been used for its treatment including external irradiation[3], tracheostomy[4], surgical resection[5], systemic or intralesional corticosteroids[6,7], laser vaporization[8] and interferon[9], but many have significant risks and complications. Until recently, the most common medical therapy for AIH was high-dose systemic corticosteroids, but this often results in significant well-known adverse effects including hypertension, irritability, and cushingoid

appearance[10]. The introduction of propranolol by Leaute-Labreze in 2008 for the treatment of IH has revolutionized its management. Potential modes of actions for propranolol include vasoconstriction, a down-regulation of angiogenetic factors like vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and an up-regulation of apoptosis of capillary endothelial cells[11]. Our aim was to analyze the evolution in the management of AIH and to report the results from 3 pediatric tertiary care centers.

MATERIALS AND METHODS

A retrospective study of all patients with diagnosis of AIH treated in 3 pediatric tertiary care institutions during a period of 18 years (1996-2014) was performed. Variables analyzed included gender, age at diagnosis, symptoms at presentation, lesion location, grade of airway obstruction (according to Cotton classification), treatment, complications and survival. In all the patients, diagnosis was obtained by endoscopic direct visualization of the lesion using a flexible fibrobronchoscope. Assessment of airway compromise was performed according to Cotton classification which divides airway obstruction into four categories[2] (grade I: Lesions have less than 50% obstruction, grade II: Lesions have 51% to 70% obstruction, grade III: Lesions have 71% to 99% obstruction and grade IV: Lesions have no detectable lumen or complete stenosis). Treatment option depended on symptoms at presentation, grade of respiratory impairment and time at diagnosis. Retrospective analysis divided patients into 2 groups according to the treatment received. Group A: Patients treated with oral corticosteroids (methylprednisolone at 2 mg/kg per day or dexamethasone at 0.5 mg/kg per day), interferon (1-3 $\times 10^6$ U/m^2, 3 times a week), endoscopic laser therapy (Diode laser) and/or surgery (open surgical excision through an anterior midline cricothyroidotomy, resection of the hemangioma and laryngotracheoplasty with costal cartilage graft if required). Group B: patients treated with oral propranolol, at a maximum dose was 2 mg/kg per day, divided in 2 doses. Prior to propranolol treatment initiation, an electrocardiogram, blood pressure and heart rate monitoring and serum glucose level were performed in all the patients. The response to each treatment was evaluated in all patients according to the clinical course and fibrobronchoscopic exam.

RESULTS

From 1996 to 2014, twenty-three patients with diagnosis of AIH were treated in 3 pediatric tertiary care hospitals (Table 1). There were 16 females and 7 males with a mean age at diagnosis of 6 mo (range, 1-27). All the patients were referred to our hospitals due to stridor and respiratory distress. Of the 23 patients, 14 were between the age range of 1-3 mo with impossibility for extubation. The remaining older patients, had sequelae from AIH as stridor and dyspnea by airway obstruction.

Table 1 Patient characteristics

	Age (mo)	Gender	Symptoms at diagnosis	Lesion location	Degree of SGE (Cotton scale)	Treatment	Outcomes
1	2	F	Stridor	Subglottic	Ⅱ	Corticosteroids	Asymptomatic
2	3	F	Stridor	Glottis and subglottic		Interferon, corticosteroids	Asymptomatic
3	1	M	Stridor	Subglottic	Ⅱ	Corticosteroids	Asymptomatic
4	1	F	Stridor	Subglottis and trachea	I	Resection and cricothyroidotomy	Asymptomatic
5	1	M	Stridor, dyspnea	Supraglottis and subglottis	Ⅲ	Laryngotracheoplasty	Dysphonia
6	14	M	Stridor, dyspnea	Subglottic	Ⅲ	Corticosteroids, laser, resection and cricothyroidotomy	Asymptomatic
7	27	F	Stridor	Subglottic	Ⅱ	Laser, laryngotracheoplasty	Exitus
8	2	F	Stridor, dyspnea	Subglottic	Ⅲ	Laryngotracheoplasty	Dysphonia
9	4	F	stridor, dyspnea	Subglottic	Ⅲ	Corticosteroids, laryngotracheoplasty	Dysphonia
10	3	F	Stridor, dyspnea	Subglottic	Ⅲ	Laryngotracheoplasty	Transitory stridor
11	5	F	Stridor, dyspnea	Subglottic	Ⅲ	Laryngotracheoplasty	Asymptomatic
12	16	M	Stridor, dyspnea	Glottis and subglottic	I	Corticosteroids	Asymptomatic
13	5	F	Stridor, dyspnea	Subglottic	Ⅲ	Corticosteroids, resection and cricothyroidotomy	Transitory stridor
14	3	F	Stridor	Subglottis and trachea	I	Corticosteroids, laryngotracheoplasty	Asymptomatic
14	19	M	Stridor	Subglottic	I	Propranolol	Exitus
16	5	F	Stridor	Supraglottis	-	Propranolol	Asymptomatic
17	26	M	Stridor	Subglottic	I	Propranolol	Asymptomatic
18	5	F	Stridor, dyspnea	Subglottic	Ⅲ	Corticosteroids, propranolol	Asymptomatic
19	3	F	Stridor	Subglottic	Ⅱ	Propranolol	Asymptomatic
20	3	M	Stridor	Subglottic	I	Propranolol	Asymptomatic
21	2	F	Stridor	Subglottic	I	Propranolol	Asymptomatic
22	2	F	Stridor	Subglottic	I	Propranolol	Asymptomatic
23	3	F	Stridor	Subglottic	I	Propranolol	Asymptomatic

F: Female; M: Male; SGE: Subglottic stenosis.
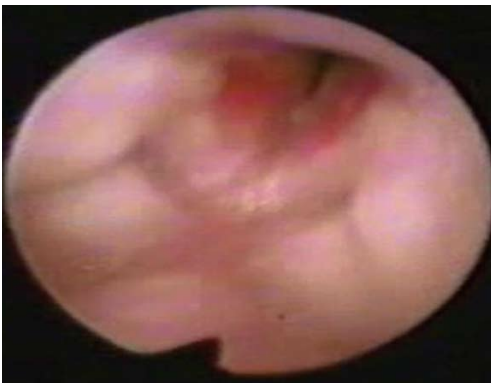
Figure 1 Fibrobronchoscopic view of a supraglottic infantile hemangioma.

Flexible fibrobronchoscope showed airway diameters ranging from 2.8 to 3.6 mm. Lesions showed a unique location in 18 patients (17 in subglottis and 1 in supraglottis). Five patients presented at joint location (1 in supraglottis and subglottis, 2 in glottis and subglottis and 2 in subglottis and trachea). Reduction of the cross-sectional area of the airway at the subglottic region prior to treatment initiation was observed in 22 patients: Grade I of Cotton classification in 10 patients, grade Ⅱ in 4 patients and grade Ⅲ in 8 patients. The patient with the supraglottis lesion did not have reduction of the cross-sectional area of the airway (Figure 1). Six patients had an associated facial hemangioma and 2 of them were diagnosed with PHACES syndrome (Figure 2).

Single therapy was indicated in 16 patients, whereas 7 non-responder patients received combined therapy. In group A (n = 14), 4 patients had subglottic stenosis grade Ⅰ, 3 grade Ⅱ and 7 grade Ⅲ. In 8 patients single therapy was indicated, 3 of them received exclusive oral corticosteroids (methylprednisolone at 2 mg/kg per day or dexamethasone at 0.5 mg/kg per day) for a mean time of 8 wk (range, 4-24) with complete response and without complications. Five patients underwent surgery exclusively. Four patients were treated with laryngotracheoplasty with graft and one with resection and cricothyroidotomy. Mean time of intubation after surgery was 7 d (range, 4-11 d) with need for reintubation in 1 case secondary to an increase of respiratory distress.

Complete response was observed in 40% of the patients. Stridor persisted in 1 patient and dysphonia was observed in 2 cases. Complications included one pneumothorax after reintubation and 1 infection of surgical wound. The other 6 patients in this group A, received combined therapy with corticosteroids, interferon (in one patient at a dose of 1×10^6 U/m^2, gradually increased to 3×10^6 U/m^2, 3 times a week) and open surgery/laser due to a poor response to single therapy (three patients were treated with laryngotracheoplasty and graft, three patients with resection and cricothyroidotomy and 2 patients initially were treated with endoscopic therapy by Diodo laser, that were subsequently treated with surgical resection). Complications in this group included Cushing

Table 2 Patient evolutions after treatment *n* (%)

Evolution after treatment	Group A *n* = 14					Group B Oral propranolol *n* = 9	
	Oral corticosteroids		INF	Open surgical resection and endoscopic therapy			
	Single treatment *n* = 3	Combined treatment *n* = 6	Combined treatment *n* = 1[1]	Single treatment *n* = 5	Combined treatment *n* = 6	Single treatment *n* = 8	Combined treatment *n* = 1
Asymptomatic	3 (100)	0 (0)	0 (0)	2 (40)	4 (66.6)	8 (100)	1 (100)
Symptomatic	0 (0)	6 (100)	1(100)	3 (60)	2 (33.3)	0 (0)	0 (0)
Dysphonia	0 (0)	0 (0)	0 (0)	2 (40)	1 (16.6)	0 (0)	0 (0)
Stridor	0 (0)	5 (83.3)	1 (100)	1 (20)	1 (16.6)	0 (0)	0 (0)
Complications	0 (0)	3 (50)	0 (0)	1 (20)	1 (16.6)	0 (0)	0 (0)
Exitus	0 (0)	1 (16.6)	0 (0)	0 (0)	1 (16.6)	1 (12.5)[2]	0 (0)

[1]Only one patient was treated with interferon, but the same patient was also treated with corticosteroids and surgery, which is included in the 6 patients with combined treatment; [2]Not related to propranolol therapy. INF: Interferon.

Figure 2 PHACES syndrome in a patient with airway infantile hemangioma.

syndrome in 3 cases that required other therapeutic alternatives for poor response to steroids. Dysphonia was observed in 2 cases after surgical treatment, and in 2 cases reintubation was necessary secondary to increased respiratory distress.

Response rate after surgery in patients with combined therapy was 66.6%. One patient died of a fulminant sepsis and other patient died during the clinical course of bronchial aspiration (Table 2).

In group B (*n* = 9) patients were treated with propranolol at a maximum dose of 2 mg/kg per day, divided in 2 doses. Six patients presented with subglottic stenosis grade Ⅰ, 1 grade Ⅱ and 1 grade Ⅲ; the lesion was localized in the supraglottis in 1 patient. In 8 patients, propranolol was used as monotherapy. One patient had previously received corticosteroids and endoscopic therapy with Diodo laser, without response. Response rate after propranolol therapy was 100%. Mean duration of treatment was 7 mo (range, 5-10), and complications were not observed. One patient died secondary to a congenital hypertrophic cardiomyopathy, not related to treatment with propranolol (Table 2).

DISCUSSION

AIH is a challenging entity that usually presents with inspiratory and expiratory stridor at 3 or 4 wk after birth which becomes more evident during periods of agitation, crying, or respiratory infections. This delayed presentation after birth is secondary to the natural course of IH with a progressive growth during the early proliferative phase[12-14]. Most authors agree that IH shows a rapid growth until 6-12 mo of age followed by involution after 18 or more months[15]. Up to 50% of these patients have cutaneous IH[16], with a typical beard-area distribution and whose presence may guide to the clinical diagnosis of AIH in patients with respiratory symptoms (Figure 3). Some authors have described association of AIH with PHACES syndrome[17]. In our series, 6 (26%) of the patients had an associated facial hemangioma and 2 of them had a PHACES syndrome.

Management and treatment guidelines for the treatment of AIH are not well established and different treatment options have been reported[12,15,17]. There seems to be a consensus regarding tracheostomy as a therapeutic approach that currently seems to be abandoned by virtually all authors[18]. Systemic corticosteroids can be effective in halting further growth of AIH during the proliferative phase, with success rates ranging from 60% to 90%[19,20]. However, efficacy rates may be lower in large, function-threatening AIH, and adverse effects may be intolerable (Cushing syndrome, growth retardation, hypertension, and immunodeficiency), reported in 12.9% of the cases and verified in our experience[21-23]. Interferon was widely heralded for treatment in refractory AIH, but it has a significant risk of neurotoxic effects (spastic diplegia), especially in very young infants under 6 mo of age[24]. In our series, interferon was only used in a patient, without complications, but with poor response.

Open surgical resection, first described by Sharp in 1945[25], showed a success rate of 98%. Bitar *et al*[23] operated on 50 patients with AIH who required a mean intubation or stenting period of 9 d, and carried a 10% complication rate, including subglottic stenosis, bleeding, and wound infections. Although in our experience we observed a global response rate in surgical patients of 54.5% (patients treated with single and combined therapy), we believe this therapeutic option is too invasive in the propranolol era. However, a role for

Figure 3 Infantile hemangioma in a beard-area distribution in a patient with airway infantile hemangioma.

surgical resection in combination with propranolol may exist for early emergency cases in which waiting for medical treatment response is not an option. Complications associated with AIH surgery include dysphonia (observed in 3 of our patients who underwent laryngotracheoplasty with graft). Open surgical resection should be considered only for selected cases, after failure of other treatments.

The CO_2 laser was considered as the initial treatment of choice although it is not very specific for the treatment of these lesions and it has a limited effectiveness in coagulation of the hemangioma. Published series observed up to 20% of residual subglottic stenosis in patients treated with this technique. The neodymium laser (Laser Nd:YAG) is considered an useful laser coagulator although large lesions may cause damage to the surrounding tissues and probably increase the risk of subglottic stenosis[17,24]. Other authors prefer the potassium-titanyl-phosphate laser (KTP), arguing that it is absorbed mainly by hemoglobin, making it ideal for treating vascular lesions[26]. Saetti *et al*[20] carried out a retrospective medical records review of all patients treated for congenital subglottic hemangiomas, and they observed a success rate of 95% in patients treated with diode laser as primary treatment, with a complication rate of 9%. In our experience, the 2 patients treated with laser therapy required additional surgery for persistent symptoms.

Peridis *et al*[27] performed a meta-analysis, on the effectiveness of propranolol for the treatment of AIH in 36 patients. In a retrospective manner, they analyzed the effectiveness of propranolol *vs* steroids, CO_2 laser or vincristine in predominantly case reports with relatively small sample sizes in each treatment group. It could be demonstrated that propranolol is the most effective treatment as compared to former treatments. In our series, we observed a response rate of 100% for patients treated with oral propranolol. No adverse reactions were documented. Lou *et al*[28], carried out a meta-analysis including 35 studies to identify studies which estimated the efficacy of propranolol therapy in infants with hemangiomas of all sites of the body. They evaluated the efficacy of propranolol *vs* other treatments. Sixteen studies

with 45 IH cases and 45 controls compared the efficacy of propranolol with other treatment modalities in treating AIH. Heterogeneity was absent (Q = 5.00, I^2 = 0.0%, P = 0.986). They observed that propranolol therapy is more effective in treating AIH (OR = 20.91, 95%CI: 7.81-55.96, P < 0.001). Potential risks associated with propranolol include bradycardia, hypotension, and hypoglycemia[29,30]. To reduce the rate of adverse reactions, Bajaj *et al*[17], gave some recommendations for the use of propranolol in infantile isolated subglottic hemangioma prior to treatment that included a detailed history and clinical examination, inspection of the whole body for hemangiomas, as well as cardiovascular and respiratory assessment. Pre-treatment tests should include electrocardiogram, heart rate and blood pressure monitoring.

Not only the effectiveness of propranolol in the management of AIH is important, but also its efficiency in terms of cost when it is compared to other therapeutic options which involve more use of hospital resources in these complex cases. Further cost-effectiveness studies are required to better define the exact cost of treating patients with AIH. The optimal duration of propranolol treatment is unknown, but it is currently accepted that the patients should remain on propranolol until the hemangioma enters the phase of involution, which usually occurs after the first year of life. Vlastarakos *et al*[31] performed a meta-analysis that included 17 studies with 61 patients treated with propranolol, observing a rate of relapse of 11.5% after withdrawal of propranolol. This was not observed in our patients cohort.

Though our retrospective study has several limitations that should be considered in the interpretation of our findings, including the small number of patients in the study cohort and the referral bias inherent in our status as referral centers for pediatric airway disorders, we consider propranolol is currently the first line treatment for symptomatic AIH, considering its efficacy and relatively mild side effects. The two cohorts, pre-propranolol group and propranolol group, were not randomized and other reasons beside propranolol might have contributed to the better outcome of the propranolol group. Pre-propranolol group contained more patients with advanced disease compared to propranolol group (Cotton grade Ⅱ and Ⅲ, 71% *vs* 22%). This could be explained by the fact that patients with more advanced disease may have a delayed referral to institutions with pediatric airway expertise. Early initiation of oral propranolol might avoid this advanced stage.

In conclusion, the management of AIH has evolved from surgical resection and systemic steroids to oral propranolol in the last 7 years. Bronchoscopy plays an important role in the diagnosis and evaluation of treatment response. Our experience as referral centers for pediatric airway disorders and the medical literature support the early use of propranolol as a first line of treatment in AIH due to its benefits in terms of effectiveness and efficiency. Surgical and/or endoscopic approach represent a second line therapeutic option for

non-responder patients to propranolol. Management of children with AIH should be performed in pediatric institutions with expertise in both, vascular anomalies and airway disorders.

COMMENTS

Background
The management of airway infantile hemangioma has evolved from surgical resection and systemic steroids to oral propranolol in the last 7 years. The authors present an experience as referral centers for pediatric airway disorders, which is in accordance with the most recent published medical literature regarding the use of propranolol as a first line of treatment in airway infantile hemangioma.

Research frontiers
Further research studies should be performed in order to investigate the role of other betablocker agents in the treatment of airway infantile hemangioma.

Innovations and breakthroughs
The authors' status as referral centers for pediatric airway disorders and vascular anomalies has permitted us to obtain a greater experience in the management of these challenging patients.

Applications
Since the introduction of propranolol in 2008 for the treatment of infantile hemangioma, this agent has become first line treatment for lesions located in the airway. Historically, this has been a challenging site for the occurrence of infantile hemangioma, but propranolol treatment has dramatically changed the prognosis of these young patients. The study analyzes the evolution in the management of airway infantile hemangioma.

Terminology
Infantile hemangioma is the most common vascular tumor in children that has a rapid growth phase (1-3 mo of age), followed by a slow growth phase (3-12 mo of age) and involution (1-10 years of age).

Peer-review
In the paper, the authors present a nice and conclusive overview on the clinical presentation and the treatment of airway infant hemangiomas, highlighting the revolutionary advance achieved by the introduction of propranolol in hemangioma treatment. The paper is well written, with good language, its content is conclusive and it is very important to distribute the beneficial experiences with propranolol.

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ORIGINAL ARTICLE

Retrospective Study

Expression of pain and distress in children during dental extractions through drawings as a projective measure: A clinical study

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Abstract

AIM: To evaluate the efficacy of drawings as a projective measure of pain and distress in children undergoing dental extractions.

METHODS: Children in the age range of 4-13 years with existence of untreatable caries or over-retained primary teeth, indicated for extractions were included. Pain was assessed using one behavioral [faces, legs, activity, cry and consolability (FLACC)] scale; and a self report measure; faces pain scale-revised (FPS-R), at two points of time, after completion of local anesthetic administration and after extraction. The general behavior of children was assessed with Wright's modification of Frankl rating scale. At the end of the session, children were instructed to represent, themselves along with the dentist, and their experiences of the dental treatment through drawing. The drawings were scored utilizing Child drawing: Hospital scale (CD: H) manual and correlated with FLACC, FPS-R and Frankl using Pearson correlation test.

RESULTS: A positive correlation, though statistically not significant, was observed between CD: H scores and all other considered parameters (Frankl, FPS-R and FLACC) in the present study.

CONCLUSION: Drawings could not act as surrogate measure of child's pain; however, they acted as a narrative of his/her experiences and reflection of inner

emotions. Hence, drawings can be used as an additional dental armamentarium.

Key words: Anxiety; Child; Distress; Drawings; Pain

Core tip: Assessing the effect of an invasive dental treatment, such as, extractions, on children is very important. To achieve this, drawings can be addressed as a method for working with children. They act as narrative of children's painful experience and emotions.

INTRODUCTION

Pain is an unpleasant combination of sensations and emotions, which is difficult to describe. As the threshold for the pain varies from person to person, only the person experiencing the pain can explain its intensity and nature. Child's pain is complex and varies with his/her cognitive, emotional and social experience[1,2], necessitating an accurate assessment. Dentistry involves numerous procedures, which may be perceived as painful by a child; local anesthetic administrations and extractions being the most painful of all, which can cause psychological distress[3,4]. Hence, a multilevel approach of assessing the procedural pain in dentistry is essential, as neglecting their experience can lead to development of anxiety in the child, which becomes a major barrier in accepting dental treatment during their future visits[5]. Thus, correct appraisal of pain helps in understanding their interest in seeking treatment in the future, assessing their behavior in succeeding visits as well as customizing the guidance for the child.

Procedural pain can be assessed using behavioral [faces, legs, activity, cry, consolability (FLACC) scale/sound, eye, motor scale][6,7], self report measures [facial pain scale-revised (FPS-R)/pain thermometer/visual analogue scale/colour analogue scale/finger span test][8-12] and/or a combination of these approaches. However, depending on the child's age and development, the ability of these measures to quantify and qualify the pain experience of a child varies[13]. Thus, communication with the child, in verbal/non-verbal/compounded means, plays a vital role in evaluating their pain. However, children may or may not have the ability and/or vocabulary to express their feelings, fears and concerns verbally[14]. Most of the children disguise the inner fears of their painful experience[15], which becomes another drawback of verbal communication. Thus, there is a need to use some non-verbal technique that explores their inner emotional status and enhances the verbal communication. One such technique is the use of drawings, a pleasant exercise, which tends to project the things felt as important by a child[16,17]. Children's drawing is thought to reflect his/her inner world; depicting various feelings and relating information concerning intelligence, psychological status and interpersonal style[18-24]. Thus, drawings ameliorate the communication capacity of the child and help in verbalizing their distress. Free drawings (child is free to draw anything without directions or instructions), bridge drawings (child is asked to draw about future expectations and relative threat), volcano drawings (child is asked to draw his/her means to manage anxiety), person picking an apple from a tree (to know the child's coping ability and resourcefulness), kinetic family drawings (child asked about family dynamics), human figure drawings (asked to draw a picture of a person) are the various means employed in studies on children drawings, of which, human figure drawings are popular clinically[25].

Scoring systems for drawings were also developed, of which Good enough-Harris test, Koppitz developmental scoring system, Draw-a-person quantitative scoring system are renowned[26,27]. To assess the emotional status of hospitalized school age children, Child drawing: Hospital (CD: H) manual was specially developed[28,29]. This manual was applied in pediatric dental settings to assess the effect of pulp therapy and/or restorative treatments for carious primary molars[30]. The present study was performed to determine the efficacy of drawings using CD: H manual in depicting the experiences of children undergoing local anesthetic (LA) administrations and extractions of primary teeth.

MATERIALS AND METHODS

The study was performed in Narayana Dental College and Hospital, Nellore, India during the period July 2012 to June 2014.

Sample

After obtaining institutional ethical clearance (as per Code of Ethics of the World Medical Association and Declaration of Helsinki, 1964, as revised in 2004), children who met all inclusion criteria were selected: (1) age range of 4 to 13 years (irrespective of gender and ethnic characteristics); (2) existence of untreatable carious or over-retained primary teeth, indicated for extraction; (3) complete physical and mental health without any confounding medical history; (4) interested in drawings; and (5) whose parents gave their consent to participate in the study.

Children indicated for extraction of teeth as a part of emergency/immediate phase treatment, those with very negative behavior[31] during initial examination and who were reluctant to draw picture were excluded.

About CD: H scale

CD: H scale was employed in the present study, as it

is a proven instrument with good internal validity[28] developed as a means of measuring the emotional status of hospitalized school aged children based on the theoretical foundation of drawings as a projective measure of children's state of anxiety. This manual consists of three parts, A, B and C. Part A focuses on the facets such as position, action, length, width, size of the child, his/her eyes and facial expression, colour predominance, number of colors used, use and placement on paper, stroke quality, inclusion and size of dental equipment and developmental level of the child as projected from their drawings. Part B focuses on omission, exaggeration, de-emphasis and distortion of body parts along with transparency and shading, whereas, part C represents general gestalt of the picture. The levels of anxiety, based on the scores obtained from CD: H scores are, $\leqslant$ 43: Very low stress, 44-83: Low stress, 84-129: Average stress, 130-167: Above average; and 168 and over: Very high stress; the detailed description of which can be read from CD: H manual[28].

CD: H scores obtained in the present study were correlated with FLACC, FPS-R scores and behavior of the children as assessed with Frankl's behavior rating scale. FLACC scale was considered due to its simplicity of application in clinical settings, that consists of five behavioral categories, facial expression, leg movement, bodily activity, cry or verbalization, and consolability[32]; each rated on a scale of 0 to 2 to provide a maximum overall pain score of 10, an acceptable ordinal convention point. Its validity was also proved in children, adults with cognitive impairment, and critically ill adults[33-35]. To achieve, self report of pain possible on the widely accepted 0 to 10 metric, FPS-R, adapted from the Faces pain scale was employed[8,36]. This was considered in the study due to the ease of administration and absence of smiles and tears in the faces, which is an added advantage[36].

Interventions

LA was administered for all the recruited children and extraction of the intended tooth performed following a standard protocol with routine behavior guidance techniques consistently by all the operators (two male and two female pediatric dentists) which was videotaped. The behavior of the child during oral examination, intraoral radiography, topical anaesthetic application, LA administration, extraction and departure from dental chair was rated using Wright's modification of Frankl rating scale. The overall score was obtained by summing the ratings on all the above mentioned occasions; if the child was positive on at least half of the situations, he/she was designated as positive (+) and if otherwise as negative (-). If there was no negative score in any of the occasion, the child was designated as definitely positive (++)[31]. As a behavioral measure of pain and distress, FLACC scale[37] was used to score the LA administration and extraction procedures separately. FPS-R[8], a self-report measure, was also recorded at two points of

time, after completion of LA administration and after extraction. All the above scorings were recorded by two investigators who were not involved in the treatment procedure (RK and SP).

At the end of the therapeutic session, one of the investigators (SP) seated each child in a position where they can observe the complete clinical area. The A4 sheet paper and crayons box (exposing all the colours) were placed on the table in front of children. They were instructed to represent, by drawing, themselves along with the dentist and their experience of the dental treatment; while drawing neither parent/s nor dentist guided the children. If the children were not eager to draw at that point of time, they were excluded from the study. No time limit was given and children were informed that they can stop drawing whenever they want to. If the children were very distracted, the above directions were repeated. After completion of drawing, the details of the children (including outpatient number, date of birth and gender) were noted on the back of the drawing paper, whereas, explanations for the drawing were noted on separate paper. The drawings were analyzed by one Pediatric dentist (SN) and a clinical psychologist (who was blinded to the behavior of children in clinic) separately based on the manual. Any disagreements between the two were discussed and crosschecked with explanations given by the children; and after getting common consensus, final scores for drawings were given.

Sample size determination

Based on the findings of a previous study conducted with sample size of 54, which compared drawing scores, applying CD: H manual, with behavioral measure of pain; and considering the findings of our pilot study on 10 children with behavioral measure as the primary outcome, and self report measure and behavioral ratings as secondary outcomes, with the level of significance set at 0.05, power of 80%, a minimal sample size of 100 was determined.

Statistical analysis

Cohen's kappa was employed to measure the reliability of the obtained data (both inter-rater and intra-rater). Inter-rater reliability between two investigators (SP and RK) for FLACC and Frankl scores were 0.91 and 0.89 respectively. Intra-rater reliability for FLACC and Frankl (scored after two weeks on the basis of videotaped treatment procedure) were 0.96 and 0.90. The drawings of these children were scored once by both the Pediatric dentist (SN) and a clinical psychologist and reproducibility of CD: H scores were found to be $r = 0.85$, $r = 0.88$.

The data was assessed for the difference in distribution of participants based on age, gender and influence of accompanying person using χ^2 test; the differences between/among the variables in various groups was evaluated using one way ANOVA followed by post hoc comparisons. The correlation between the variables (bivariate correlation) was assessed with Pearson
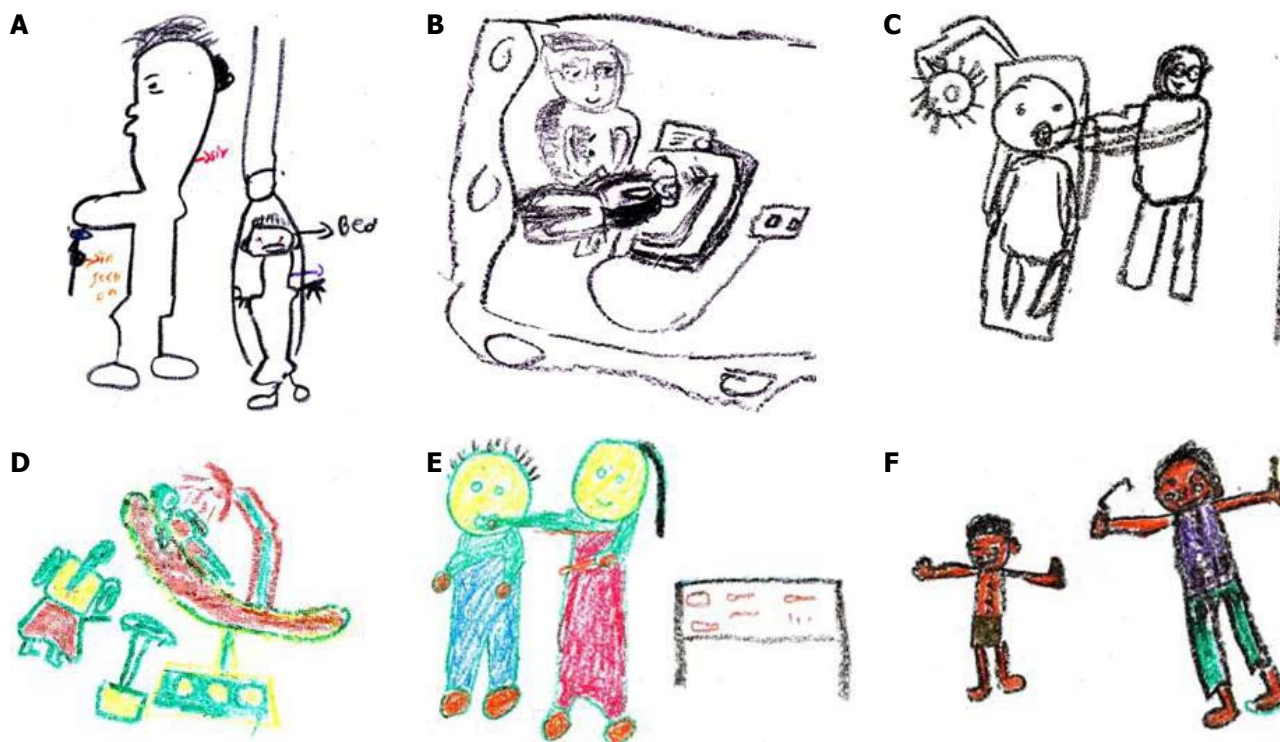
Figure 1 Samples of children's drawings. A: Ages 11 years; gender: boy; Franki: 4. FLACC score (LA): 2 (mild discornfort), FLACC score (Ext): 2 (mild discornfort); FPS-R score (LA): 6, FPS-R score (Ext): 2. Child drawing: Hospital score: 94 (average stress). The predominant colour in the drawing was black. The child included dental equipment in the drawing (syninge and dental chair) represented himself crying in the dental chair. B: Ages 12 years; gender: boy; Franki: 4. FLACC score (LA): 4 (moderate pain), FLACC score (Ext): 6 (moderate pain); FPS-R score (LA): 8, FPS-R score (Ext): 8. Child drawing: Hospital score: 67 (low stress). The child used only black colour and included dental equipment in the drawing including cabin partitions. C: Ages 13 years; gender: boy; Franki: 4. FLACC score (LA): 2 (mild discornfort), FLACC score (Ext): 1 (mild discornfort); FPS-R score (LA): 8, FPS-R score (Ext): 0. Child drawing: Hospital score: 92 (low stress). The child used only black colour and included dental equipment. It was an action picture showing extraction of his tooth by the doctor. Child omitted noses, ears and hair for himself. D: Ages 11 years; gender: boy; Franki: 3. FLACC score (LA): 3 (mild discornfort), FLACC score (Ext): 0 (mild discornfort); FPS-R score (LA): 2, FPS-R score (Ext): 0. Child drawing: Hospital score: 68 (low stress). The child used only small part of paper for his drawing and included dental equipment (syninge and dental chair). However, the predominant colour used green. E: Ages 12 years; gender: boy; Franki: 4. FLACC score (LA): 2 (mild discornfort), FLACC score (Ext): 0 (mild discornfort); FPS-R score (LA): 2, FPS-R score (Ext): 6. Child drawing: Hospital score: 49 (low stress). Child drew an action picture if he is undergoing extraction and also included dental equipment (Try with instruments arranged on it). Note that the noses and ears are missing in his human figures. F: Ages 10 years; gender: boy; Franki: 4. FLACC score (LA): 5 (mild discornfort), FLACC score (Ext): 0 (mild discornfort); FPS-R score (LA): 2, FPS-R score (Ext): 0. Child drawing: Hospital score: 64 (low stress). Child drew himself in a very happy mood, although he included dental equipment (extraction instrument and LA spray). FLACC: Faces, legs, activity, cry, consolability scale; FPS-R: Facial pain scale-revised; LA: Local anaesthetic administration; Ext: Extractions.

correlation test.

RESULTS

A total of 107 children (58 boys and 49 girls) completed the study, out of the 110 participants. Three children willingly participated at the beginning of the study, but, after extraction dissented to draw. The mean age of the children who completed the study was 10.1 years (range: 4-13). The mode for the Frankl score of participants was 4 (range: 2-4). The mean FLACC score during LA administration was 2.8 ± 1.7 (range: 0-10) and during extractions it was 2.24 ± 2.04 (range: 0-10). The mode for FPS-R after LA administration was 2 (range: 0-10) and after extraction it was 0 (range 0-10). The mean CD: H score of participants was 74.1 ± 16.2 (range of 36-112). Some samples of children's drawings are presented in Figure 1.

Differences in distribution of participants
The sample was grouped based on the age, gender and

accompanying person. Thus, 7 participants (6.5%) were 4-6 years old, 33 (30.8%) were > 6-13 years and 67 (62.6%) were > 9-13 years old; 58 participants (54.2%) were boys and 49 (45.8%) were girls; 55 (51.4%) children were accompanied by mother, 31 (29%) and 21 (19.6%) by father and guardian respectively. The distribution of participants in various scoring categories of Frankl, FLACC, FPS-R and CD: H scores based on age, gender and accompanying person are presented in Tables 1 and 2. Significant differences were not observed with the distribution of participants in various categories of CD: H, FPS-R and Frankl. However, there was a statistically significant difference in the distribution of participants based on FLACC scores among various age groups ($P < 0.01$ during LA administration and extractions) (Table 2).

Differences based on age, gender and influence of accompanying person
The differences between/among scores recorded in groups divided based on age, gender and accompanying

Table 1 Distribution of Child drawing: Hospital scale and facial pain scale-revised scores based on age, gender and accompanying person

Variables		Mean ± SD	CD: H Classification					FPS-R (local anaesthetic administration)					FPS-R (extractions)				
			≤ 43	44-83	84-129	130-167	> 168	Mode	0	2-4	6-8	10	Mode	0	2-4	6-8	10
Age	4-6	76.43 ± 16.08	0	4 / 3.73%	3 / 2.80%	-	-	8	1 / 0.93%	2 / 1.86%	2 / 1.86%	2 / 1.86%	0	2 / 1.86%	0	3 / 2.8%	2 / 1.86%
	> 6-9	76.06 ± 15.36	0	21 / 19.6%	12 / 11.20%	-	-	10	5 / 4.67%	11 / 10.28%	7 / 6.54%	10 / 9.34%	0	8 / 7.47%	8 / 7.47%	10 / 9.34%	7 / 6.54%
	> 9-13	72.87 ± 16.75	3 / 2.80%	43 / 40.18%	21 / 19.60%	-	-	2	6 / 5.60%	38 / 35.51%	17 / 15.88%	6 / 5.60%	0	17 / 15.88%	24 / 22.42%	18 / 16.82%	8 / 7.47%
	Significance		0.70[NS]					0.10[NS]					0.44[NS]				
Gender	Boys	78.03 ± 15.29	0	35 / 32.71%	23 / 21.49%	-	-	2	7 / 6.54%	22 / 20.56%	17 / 15.88%	12 / 11.2%	0	14 / 13.08%	17 / 15.88%	17 / 15.88%	10 / 9.34%
	Girls	69.41 ± 16.18	3 / 2.80%	33 / 30.8%	13 / 12.14%	-	-	2	5 / 4.67%	29 / 27.10%	9 / 8.41%	6 / 5.60%	0	13 / 12.14%	15 / 14.01%	14 / 13.08%	7 / 6.54%
	Significance		0.08[NS]					0.17[NS]					0.97[NS]				
Accompanying person	Mother	72.55 ± 15.61	1 / 0.93%	39 / 36.4%	15 / 14.01%	-	-	2	5 / 4.67%	27 / 25.23%	14 / 13.08%	9 / 8.41%	0	13 / 12.14%	14 / 13.08%	15 / 14.01%	13 / 12.14%
	Father	77.61 ± 17.15	1 / 0.93%	16 / 14.95%	14 / 13.08%	-	-	2	5 / 4.67%	14 / 13.08%	8 / 7.47%	4 / 3.73%	2	10 / 9.34%	15 / 14.01%	6 / 5.60%	3 / 2.80%
	Guardian	72.90 ± 16.36	1 / 0.93%	13 / 12.14%	7 / 6.54%	-	-	2	2 / 1.86%	10 / 9.34%	4 / 3.73%	5 / 4.67%	8	4 / 3.73%	13 / 12.14%	10 / 9.34%	1 / 0.93%
	Significance		0.47[NS]					0.90[NS]					0.12[NS]				

CD: H: Children drawing: Hospital scale; FPS-R: Facial pain scale-revised; NS: Not significant, SD: Standard deviation.

Table 2 Distribution of faces, leg, activity, cry, consolability scale and frankl scores based on age, gender and accompanying person

Variables		FLACC (local anaesthetic administration)					FLACC (extractions)					Frankl (total score)			
		Mode	0	1-3	4-6	7-10	Mode	0	1-3	4-6	7-10	Mode	2	3	4
Age	4-6	4	0	2 / 1.86%	5 / 4.67%	0	3	1 / 0.93%	3 / 2.80%	1 / 0.93%	2 / 1.86%	3	0	4 / 3.73%	3 / 2.80%
	> 6-9	4	0	16 / 14.95%	14 / 13.08%	3 / 2.80%	4	4 / 3.73%	14 / 13.08%	12 / 11.20%	3 / 2.80%	4	4 / 3.73%	5 / 4.67%	24 / 22.42%
	> 9-13	2	4 / 3.73%	52 / 48.59%	11 / 10.28%	0	2	19 / 17.75%	41 / 38.31%	7 / 6.54%	0	4	7 / 6.54%	9 / 8.41%	51 / 47.66%
	Significance	< 0.01[1]					< 0.01[1]					0.06[NS]			
Gender	Boys	2	2 / 1.86%	37 / 34.57%	17 / 15.88%	2 / 1.86%	2	14 / 13.08%	29 / 27.10%	12 / 11.20%	3 / 2.80%	4	3 / 2.80%	11 / 10.28%	44 / 41.12%
	Girls	2	2 / 1.86%	33 / 30.80%	13 / 12.14%	1 / 0.93%	2	10 / 9.34%	29 / 27.10%	8 / 7.47%	2 / 1.86%	4	8 / 7.47%	7 / 6.54%	34 / 31.77%
	Significance	0.95[NS]					0.82[NS]					0.16[NS]			
Accompanying person	Mother	2	1 / 0.93%	34 / 31.77%	19 / 17.75%	1 / 0.93%	2	9 / 8.41%	32 / 29.99%	11 / 10.28%	3 / 2.80%	4	7 / 6.54%	9 / 8.41%	39 / 36.44%
	Father	2	3 / 2.80%	21 / 19.6%	6 / 5.60%	1 / 0.93%	0	13 / 12.14%	14 / 13.08%	4 / 3.73%	0	4	2 / 1.86%	2 / 1.86%	27 / 25.23%
	Guardian	2	0	15 / 14.01%	5 / 4.67%	1 / 0.93%	2	2 / 1.86%	12 / 11.20%	5 / 4.67%	2 / 1.86%	4	2 / 1.86%	7 / 6.54%	12 / 11.20%
	Significance	0.34[NS]					0.07[NS]					0.10[NS]			

[1]Significant at 0.01 level. FLACC: Faces, leg, activity, cry, consolability scale; NS: Not significant.

person, were analyzed, and significant differences were not observed based on age and accompanying person in CD: H, FPS-R or Frankl scores. However, there was a statistically significant difference in CD: H scores between boys and girls (Table 3) and in the FLACC scores recorded among the age groups, during LA administration (post hoc showing difference between > 6-9 and > 9-13 age groups) as well as extractions (post hoc showing difference between > 6-9 and > 9-13 and 4-6 and > 9-13 age groups). Significant difference was also observed in FLACC values recorded during extractions among the groups divided based on the accompanying person (post hoc showing difference between the group of children, accompanied by mother and those by father as well as between the groups accompanied by father and guardian) (Table 4).

Correlations

There was a positive correlation between CD: H scores and all the other considered parameters (Frankl, FPS-R

Table 3 Differences between/among scores (Child drawing: Hospital scale and facial pain scale-revised) in groups divided based on age, gender and accompanying person

Variables	Groups	Mean ± SD	One way ANOVA *P* valve	Post hoc comparisons	Post hoc *P* valve
CD: H	Age groups (4-6, > 6-9, > 9-13)	4-6: 76.4 ± 16.1	0.61[NS]	4-6 *vs* > 6-9	1.00[NS]
		> 6-9: 76.1 ± 15.4		> 6-9 *vs* > 9-13	0.66[NS]
		> 9-13: 72.9 ± 16.7		4-6 *vs* > 9-13	0.86[NS]
	Gender (boys and girls)	Boys: 78.0 ± 15.3	0.01[1]	--	--
		Girls: 69.4 ± 16.2			
	Accompanying person (mother, father, guardian)	Mother: 72.6 ± 15.6	0.36[NS]	Mother *vs* father	0.38[NS]
		Father: 77.6 ± 17		Mother *vs* guardian	1.00[NS]
		Guardian: 72.9 ± 16.4		Father *vs* guardian	0.59[NS]
FPS-R (LA)	Age groups (4-6, > 6-9, > 9-13)	4-6: 6.0 ± 4.0	0.07[NS]	4-6 *vs* > 6-9	0.92[NS]
		> 6-9: 5.5 ± 3.8		> 6-9 *vs* > 9-13	0.14[NS]
		> 9-13: 4.1 ± 2.9		4-6 *vs* > 9-13	0.33[NS]
	Gender (boys and girls)	Boys: 5.0 ± 3.5	0.24[NS]	--	--
		Girls: 4.2 ± 3.1			
	Accompanying person (mother, father, guardian)	Mother: 4.8 ± 3.9	0.63[NS]	Mother *vs* father	0.64[NS]
		Father: 4.1 ± 3.3		Mother *vs* guardian	1.00[NS]
		Guardian: 4.8 ± 3.6		Father *vs* guardian	0.80[NS]
FPS-R (Ext)	Age groups (4-6, > 6-9, > 9-13)	4-6: 6.0 ± 4.3	0.28[NS]	4-6 *vs* > 6-9	0.78[NS]
		> 6-9: 4.9 ± 3.9		> 6-9 *vs* > 9-13	0.54[NS]
		> 9-13: 4.0 ± 3.5		4-6 *vs* > 9-13	0.41[NS]
	Gender (boys and girls)	Boys: 4.7 ± 3.8	0.50[NS]	--	--
		Girls: 4.2 ± 3.6			
	Accompanying person (mother, father, guardian)	Mother: 5.0 ± 3.9	0.06[NS]	Mother *vs* father	0.08[NS]
		Father: 3.1 ± 3.3		Mother *vs* guardian	1.00[NS]
		Guardian: 5.0 ± 3.4		Father *vs* guardian	0.70[NS]

[1]Significant at 0.01 level. CD: H: Children drawing: Hospital scale; FPS-R: Facial pain scale-revised; L.A: Local anaesthetic administration; Ext: Extractions; NS: Not significant.

and FLACC) which was not statistically significant. However, there were some statistically significant positive correlations, as well as some non-significant negative correlations between CD: H and other parameters based on age, gender and accompanying person which are represented in Table 5. In children belonging to 4-6 year age group, FPS-R and FLACC during LA administration were significant and correlating positively with CD: H scores, whereas others were not. In > 6-9 and > 9-13 year age groups, there were non-significant associations between CD: H scores and all other considered parameters. In the data segregated based on the gender, there were no statistically significant correlations between the CD:H scores and other parameters. The data segregated based on accompanying person also showed non-significant associations, except FLACC scores, during extraction in children accompanied by mother and FPS-R during LA administration in children accompanied by guardian, showing significant positive correlations.

DISCUSSION

Drawing ability in children shows predictable, observable and measurable stages that coincide with cognitive and motor development; better representational and detailed with age. By the age of 4 years, children drawings emerge to have identifiable human figures and by the end of 13 years they reach a stage where drawings tend to become more natural, with true representation of things. As CD: H is a manual based on human figure drawings (HFDS), in the present study, children in the age range of 4 to 13 years were included. The data was also segregated for analysis into 4-6, > 6-9 and > 9-13 based on the development of the quality and content of HFDS[16]. Scoring systems also exist in human drawing tests, such as Good enough-Harris, Koppitz developmental system and Draw-a-person quantitative system, however, CD: H was employed in the present study, as it is exclusively developed for assessing the emotional status of hospitalized children.

Before discussing the correlations, the distribution of participants as observed in the present study needs attention, as it revealed fluctuations on the observational scale. Significant differences were observed in FLACC scores among the three age groups considered. In > 9-13 year age group, all the children during LA administrations and majority of the children during extractions, scored 0 in FLACC. The mean scores were also less in > 9-13 year age group, for the differences among the scores recorded. These observations are in accordance with the reported drawback of FLACC, *i.e.,* older children tend to mask the expression of pain[38-42]. Another observation in the present study was; the mean FLACC scores were statistically less significant in children accompanied by father, compared to those accompanied by mother/ guardian, which can be due to the authoritative nature of father in the culture of the study population that might have influenced the externalization of pain by the children accompanied by their father.

Correlations of CD: H scores with FPS-R, FLACC and

Table 4 Differences between/among scores (faces, leg, activity, cry, consolability dcale and frankl) in groups divided based on age, gender and accompanying person

Variables	Groups	Mean ± SD	One way ANOVA *P* valve	Post hoc comparisons	Post hoc *P* valve
FLACC (LA)	Age groups (4-6, > 6-9, > 9-13)	4-6: 3.9 ± 1.9	< 0.01[2]	4-6 *vs* > 6-9	0.97[NS]
		> 6-9: 3.7 ± 2.2		> 6-9 *vs* > 9-13	< 0.01[2]
		> 9-13: 2.3 ± 1.2		4-6 *vs* > 9-13	0.06[NS]
	Gender (boys and girls)	Boys: 2.9 ± 1.8	0.61[NS]	--	--
		Girls: 2.8 ± 1.6			
	Accompanying person (mother, father, guardian)	Mother: 3.0 ± 1.6	0.60[NS]	Mother *vs* father	0.62[NS]
		Father: 2.6 ± 1.8		Mother *vs* guardian	1.00[NS]
		Guardian: 3.0 ± 1.9		Father *vs* guardian	0.75[NS]
FLACC (Ext)	Age groups (4-6, > 6-9, > 9-13)	4-6: 3.9 ± 2.8	< 0.01[2]	4-6 *vs* > 6-9	0.67[NS]
		> 6-9: 3.2 ± 2.4		> 6-9 *vs* > 9-13	< 0.01[2]
		> 9-13: 1.6 ± 1.5		4-6 *vs* > 9-13	0.01[2]
	Gender (boys and girls)	Boys: 2.3 ± 2.2	0.93[NS]	--	--
		Girls: 2.2 ± 1.9			
	Accompanying person (mother, father, guardian)	Mother: 2.5 ± 1.9	< 0.01[2]	Mother *vs* father	0.02[1]
		Father: 1.3 ± 1.5		Mother *vs* guardian	0.62[NS]
		Guardian: 3.0 ± 2.4		Father *vs* guardian	0.01[2]
Frankl (total)	Age groups (4-6, > 6-9, > 9-13)	4-6: 3.4 ± 0.5	0.68[NS]	4-6 *vs* > 6-9	0.86[NS]
		> 6-9: 3.6 ± 0.7		> 6-9 *vs* > 9-13	0.69[NS]
		> 9-13: 3.7 ± 0.7		4-6 *vs* > 9-13	0.94[NS]
	Gender (boys and girls)	Boys: 3.7 ± 0.6	0.17[NS]	--	--
		Girls: 3.5± 0.8			
	Accompanying person (mother, father, guardian)	Mother: 3.6 ± 0.7	0.17[NS]	Mother *vs* father	0.32[NS]
		Father: 3.8 ± 0.5		Mother *vs* guardian	0.82[NS]
		Guardian: 3.5 ± 0.7		Father *vs* guardian	0.22[NS]

[1]Significant at 0.05 level; [2]Significant at 0.01 level. FLACC: Faces, leg, activity, cry, consolability scale; LA: Local anaesthetic administration; Ext: Extractions; NS: Not significant.

Table 5 Correlation of Children drawing: Hospital Scale with facial pain scale-revised, faces, leg, activity, cry, consolability scale and frankl

Variables	Groups		CD:H	FPS-R (LA)	FPS-R (Ext)	FLACC (LA)	FLACC (Ext)	Frankl (Total)
Age	4-6	Correlation	1	0.87	-0.17	0.84	0.71	-0.14
		Significance	-	0.01[2]	0.72[NS]	0.02[1]	0.07[NS]	0.76[NS]
	> 6-9	Correlation	1	-0.09	-0.24	0.20	-0.12	0.27
		Significance	-	0.63[NS]	0.19[NS]	0.26[NS]	0.50[NS]	0.63[NS]
	> 9-13	Correlation	1	0.21	0.17	-0.08	0.05	0.07
		Significance	-	0.09[NS]	0.17[NS]	0.53[NS]	0.67[NS]	0.56[NS]
Gender	Boys	Correlation	1	0.11	-0.12	0.12	0.09	0.01
		Significance	-	0.40[NS]	0.37[NS]	0.38[NS]	0.52[NS]	0.94[NS]
	Girls	Correlation	1	0.18	0.18	0.11	0.07	0.15
		Significance	-	0.22[NS]	0.21[NS]	0.46[NS]	0.65[NS]	0.32[NS]
Accompanying person	Mother	Correlation	1	0.18	0.10	0.19	0.28	0.01
		Significance	-	0.20[NS]	0.47[NS]	0.17[NS]	0.04[1]	0.98[NS]
	Father	Correlation	1	-0.06	-0.01	0.02	0.08	0.1
		Significance	-	0.74[NS]	0.97[NS]	0.91[NS]	0.68[NS]	0.60[NS]
	Guardian	Correlation	1	0.55	0.11	0.20	-0.12	0.33
		Significance	-	0.01[2]	0.65[NS]	0.38[NS]	0.61[NS]	0.14[NS]
Total		Correlation	1	0.17	0.04	0.12	0.08	0.12
		Significance	-	0.09[NS]	0.72[NS]	0.21[NS]	0.44[NS]	0.24[NS]

[1]Significant at 0.05 level; [2]Significant at 0.01 level. NS: Not significant; CD: H: Children drawing: Hospital Scale; FPS-R: Facial pain scale-revised; FLACC: Faces, leg, activity, cry, consolability scale; L.A: Local anaesthetic administration; Ext: Extractions.

Frankl revealed interesting findings. Considering the total sample, CD: H was positively correlating with all the other parameters though not significant statistically. These findings are in accordance with a previous study, which proved drawings as a projective measure for children's distress in pediatric dentistry[30]. However, these correlations showed variations when the sample was segregated in the present study. In the age specific groups, we found significant positive correlation of CD: H with FPS-R and FLACC for LA administration was observed in 4-6 year group, and non-significant relations in older age groups. This can be due to curtailment of emotions on the dental chair by these older children, as well as drawing activity, considered as unrelated to

dentistry by them, might have lead to disparity in CD: H and FLACC/FPS-R scores. In the accompanying person category, a significant positive correlation of drawing scores with FPS-R for LA administration were observed in the guardian group; liberty to choose their expression of pain in the self-report scale by those children, who were not accompanied by parents can be a possible explanation for this. Significant positive correlation was also observed between CD: H and FLACC during extractions, in the children accompanied by mother. This can be due to free expression of pain physically, when companioned by mother.

Gender difference in anxiety of children was reported frequently in the existing literature; some reporting high anxiety scores in girls[40,43,44], where as others depicting no difference[42]; In the present study we attempted to assess the gender difference in expression of pain, using self report, observational measures and in the drawings. Significant differences were observed in CD: H scores, with boys reporting high mean scores, compared to girls. All the remaining parameters, like FLACC and FPS-R, the mean scores were higher in boys, compared to girls, which can be due to the tolerance capacity of girls being more, compared to boys[45].

Scoring of drawings using CD: H was practically easy, but, this manual was originally developed to determine the effect of hospitalization on children[29]. When the same instrument was employed for assessment of dental treatments, some of the items in the scoring system were not applicable to dental settings; necessitating revision and simplification of this instrument. In part A; the first item position of the child, needs modifications, most of the proposed positions were not suitable for drawings in the dental operatory. The scoring of items; action, length and width of the person, considered in the CD: H, might have been subjected to bias because, the differences noted might be due to drawing abilities of children, rather than pain and anxiety. Other controversial aspects found in the present study were the colour predominance and stroke quality. As children were provided with only crayons, almost all the children used black crayon as replacement for pencil to draw the outline of their drawings, which became the predominant color most of the times. Difficulty in scoring the quality of strokes, which were drawn with crayons, is a point to ponder. In part B, transparency, exaggeration and de-emphasis items can be eliminated as they do not adapt well to our dental scenario. Finally, the part C is prone for subjective variations, thus, omitting that part can lead to simplification of the instrument.

An attempt was made to observe drawings of children and their FLACC as well as FPS-R scores at an individual level which disclosed the utility of the present study. Some of the children with low scores on both FLACC and FPS-R drew dental equipment in their drawings and represented themselves in either helpless condition or crying in the dental chair. On the other hand, children who scored high values in FLACC and FPS-R, scored low in CD: H and presented themselves in happy mood. This

clearly projects the major difference between drawings and other parameters; as, observational and self report measures represent fleeting emotions when the child is on dental chair, whereas, drawings symbolize the lasting feelings of a dental treatment. These enduring emotions are crucial for customizing our guidance techniques in future visits and for assimilating dental interest in children. This study, thus, has been proved as a means to discern the inner emotional disturbances originated in a child due to a painful dental treatment, and the way this can be used to guide the behaviour of the child in his/her future dental treatments. Drawings in the field of pediatric dentistry can be furthered studied by testing their validity in assessing the emotional condition of the child before treatment and depicting his/her subjective fears in their first dental visit.

The LA administration and extractions in the present study were performed by more than one pediatric dentist. However, this will not bias the results of the present study, as it is a factor that has a consistent influence on all the parameters considered to measure pain of a single child. The major limitation of the present study was disregarding the effect of schooling and intelligence, which are proposed to influence the drawings of children[19,26,27,46]. However, we substantiate our study, with the studies that proved no effect of these factors on drawing talent of children[18,47-49].

In conclusion, the present study clearly demonstrated that, scoring of children's drawing using CD: H manual, though authentic, has limited validity to measure the pain experience of children undergoing local anesthetic administration and extraction of primary teeth. Drawings could not act as surrogate measure of pain; however, we should not condude an end to the use of drawings in a dental setting, as they act as narrative of children's painful experience and emotions. They are an easy, interesting exercise for children that can be employed as an additional measure of understanding the exact source of anxiety and/or to know the objective fears created due to a painful experience. Drawings address a method for working with children, and we should never underestimate the effect of our behavior and responsiveness on children. The most affirmative point in the present study was, the children after experiencing a stressful activity, got distracted due to the drawing and were leaving the dental operatory with a happy mood.

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COMMENTS

Background

During day to day pediatric dental practice, communication with children is significant, to assess the procedural pain and its impact on them. Non-verbal communication can explore the inner emotional condition, compared to verbal, as children may or may not have the ability and/or vocabulary to express their feelings, fears and concerns verbally. Drawings, being a pleasant exercise for children, have been considered as a measure to determine the pain and

distress in children undergoing dental extractions.

Innovations and breakthroughs

In pediatric settings, this is the second study in literature that determined the procedural pain experienced by children during dental treatments, first being the study done by Aminabadi *et al* in 2011. Aminabadi *et al* have tested the procedural pain during pulp therapy and/or restorative treatments for carious primary molars. However, in dentistry, out of the numerous procedures perceived as painful by a child, local anesthetic (LA) administrations and extractions are the most painful of all, which can cause psychological distress. Hence, the present study is a breakthrough to know the efficacy of drawings in depicting the experiences of children undergoing LA administration and extraction of primary teeth.

Applications

Drawings acted as a narrative of children's painful experience and emotions. They were an easy, interesting exercise for children and hence, can be employed as an additional measure of understanding the exact source of anxiety and/or to know the objective fears created due to a painful experience. Drawings addressed a method for working with children; after experiencing pain, they got distracted due to the drawing exercise and left the dental operatory with a happy mood.

Terminology

Pain drawings*:* Pain drawings are simple line drawings of the human figure on which patients can indicate their pain for both clinical information and research. Anxiety: Anxiety is a personality trait and is an apprehension, tension or uneasiness that stems from anticipation of danger, the source of which is largely unknown or unrecognized. Objective fear: It is acquired objectively or produced by direct physical stimulation of the sense organs, but not of parental origin, which are disagreeable and unpleasant in nature.

Peer-review

The work stresses on the relevance of non-pharmaceutical efforts to relieve childrens' pain in medical procedures, as well as, presents an interesting and helpful methodology that should also be made available to others.

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ORIGINAL ARTICLE

Observational Study

Dental knowledge and awareness among grandparents

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Author contributions: Oberoi J contributed to the principal investigator, data collection, classification, and compilation; Kathariya R made the contributions to drafting of article, statistical analysis, formatting, and revising it critically for important intellectual content and made the final approval of the version to be submitted; Panda A conceived, guided and design of the study; Garg I, as the secondary investigator, made the classification, and acquisition of data; Raikar S revised and read the final version.

Institutional review board statement: The study was approved by the Institutional Scientific and Ethics committee, and reviewed by the Institutional Review Board of Padmashree Dr. D.Y. Patil Dental College and Hospital, Nerul, Navi Mumbai- 400614, Maharashtra India, With the Ethics Clearance Number: 2013-2014/5/1301.

Informed consent statement: Participation in the study was voluntary. The grandparents, who volunteered to take part in the study, signed a consent form. The questionnaire was translated into a local language for ease of understanding. The questionnaire was read for the grandparents who could not read and their answers were recorded. The questionnaires were completed in the waiting area prior to the patient's appointments.

Conflict-of-interest statement: I confirm that the manuscript is original and has not been published elsewhere. Nor is it sent to any other journals for consideration. The manuscript in its submitted form has been read and approved by all authors. There is no financial relationship between any author and any commercial firm(s), which may pose a potential, perceived, or real conflict of interest.

Data sharing statement: Study participants gave informed consent for data sharing. Technical appendix, statistical code, and dataset are available with the corresponding author at rkathariya@gmail.com.

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Abstract

AIM: To investigate grandparent's knowledge and awareness about the oral health of their grandchildren.

METHODS: Grandparents accompanying patients aged 4-8 years, who were living with their grandchildren and caring for them for a major part of the day, when both their parents were at work were included in the study. A 20-item questionnaire covering socio-demographic characteristics, dietary and oral hygiene practices was distributed to them. The sample comprised of 200 grandparents (59 males, 141 females). χ^2 analysis and Gamma test of symmetrical measures were applied to assess responses across respondent gender and level of education.

RESULTS: Oral health related awareness was found to be low among grandparents. In most questions asked, grandparents with a higher level of education exhibited a better knowledge about children's oral health. Level of awareness was not related to their gender.

CONCLUSION: Oral hygiene and dietary habits are established during childhood. There is a great need for dental education of grandparents as they serve as role models for young children.

Key words: Grandparents; Awareness; Primary teeth; Children; Oral health

Core tip: An observational study evaluating the attitude and knowledge of grandparents as they serve an important role as caregivers in their grandchildren's lives. There is a great need for dental education of grandparents as they serve as role models for the young.

INTRODUCTION

With changing family patterns, increased life expectancy, growing numbers of dual-worker households and higher rates of family breakdown, grandparents are now playing an increasing role in their grandchildren's lives[1-3]. Grandparents are an important resource for both parents and children. They routinely provide child care, financial assistance and emotional support. Occasionally they are called upon to provide much more, including temporary or full time care and responsibility for their grandchildren. Child and adolescent psychiatrists recognize the important role many grandparents play in raising their grandchildren[1].

The changing role of grandparents in society has led to a greater responsibility on them as nurturers, mentors, and teachers and they also play a crucial part in modelling a good lifestyle for the child. Moreover, in everyday life, grandparents function as role models for their grandchildren, and therefore, grandparents' knowledge, awareness and dental hygiene practises may play an important role in influencing children's behaviour and attitude towards good oral health care. Hence, in order to improve children's oral health status, grandparents should be considered as one of the key people involved in and influencing children's lives and the health care that they receive. A considerable amount of research dealing with the role that parents[4,5] (and

mothers in particular)[6,7] play in influencing the oral health status of children has been done, but there is dearth of literature investigating the role grandparents play in influencing children's oral health status. A review of literature revealed only one study[8], where knowledge of caregivers, which included grandparents (107 grandparents out of 615 caregivers, *i.e.*, 17.4%) was studied. Thus, this study was carried out to investigate the knowledge, awareness, beliefs and practises of grandparents related to their grandchildren's oral health.

MATERIALS AND METHODS

Before the start of the study, the study protocol was approved by the Ethics Committee of the Dr. D.Y. Patil Dental College and Hospital, Navi Mumbai. A questionnaire was prepared to assess the knowledge and awareness of grandparents regarding the oral health of their grandchildren. Grandparents accompanying patients aged 4-8 years attending the outpatient department of Pediatric and Preventive Dentistry of the Dr. D.Y. Patil Dental College and Hospital, Navi Mumbai, who were living with their grandchildren and caring for them for a major part of the day, when both their parents were at work, were explained the purpose of the study. Participation in the study was voluntary. The grandparents who volunteered to take part in the study and signed a consent form, were distributed the questionnaires in the waiting area of the outpatient department. The questionnaire was translated into a local language for ease of understanding. The questionnaire was read for the grandparents who could not read and their answers were recorded. The questionnaires were completed in the waiting area prior to the patient's appointments. The questionnaire comprised of 20 questions and the participants were asked to select one appropriate option for each question. The demographic information collected from the questionnaire included age of the grandchild, and gender and educational qualification of the grandparent. Literature was consulted regarding the knowledge of mothers/parents in regard to their children's dental health and the existing questions were adapted for this survey[4-8]. A pilot study was conducted by asking 20 grandparents and members of staff to complete the questionnaire. The feedback was positive but indicated that use of the terms "pedodontists" and "malocclusion" was causing some confusion. As a result, these were replaced with "pediatric dentists" and "crooked teeth" respectively, to improve understanding. Completed questionnaires were collected and passed blind to an independent statistician where they were analysed for response frequency and the results tabulated. The statistical analysis was carried out using Statistical Package for Social Sciences (SPSS Inc., version 15.0 for Windows). Descriptive statistics and χ^2 analysis for non-parametric data with the appropriate degrees of freedom were performed on the data to assess responses to the questionnaire items across respondent
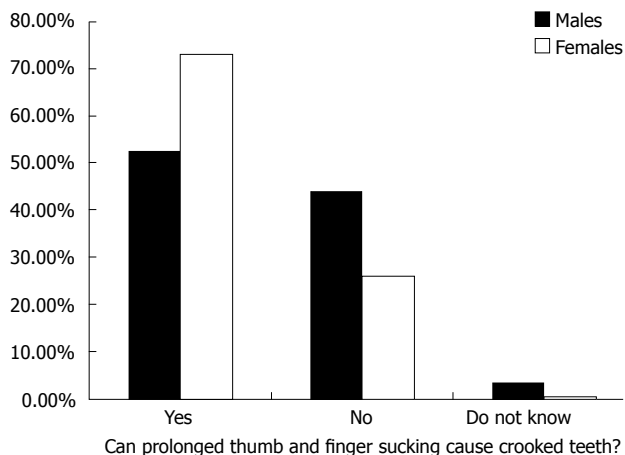
Figure 1 Gender based analysis.

gender and level of education. In addition, Gamma test of Symmetrical Measures was also applied for analysis across education levels. Statistical significance was determined at $P \leqslant 0.05$.

RESULTS

A total of 200 grandparents participated in the study. Out of these 141 (70.5%) were females and 59 (29.5%) were males. Out of 200 grandparents, 40 (20%) were illiterate and 114 (57%) had up to higher secondary education. The number of grandparents having graduate and post graduate qualifications, was 38 (19.0%) and 8 (4%) respectively. The results were analysed on the basis of gender and level of education.

Statistically significant difference ($P \leqslant 0.05$) between genders was seen only in one question, that is, female grandparents (73%) were more aware than their male counterparts (52.5%) that prolonged sucking habits could result in malocclusion (Figure 1). In all other questions, the difference seen between the responses of both the genders was statistically insignificant.

When response of the grandparents was compared to the level of education, a higher level of awareness was positively correlated with a higher level of education but not in all questions and not consistent with the increase in the level of education. Statistically significant difference ($P \leqslant 0.05$) between different levels of education was seen in five questions (Figure 2). When asked whether they were aware of paediatric dentists, 30%, 39.5%, 57.9% and 62.5% of illiterates (henceforth referred to as category Ⅰ), higher secondary educated (henceforth referred to as category Ⅱ), graduates (henceforth referred to as category Ⅲ) and post graduates (henceforth referred to as category Ⅳ) respectively (total 42%), replied in the affirmative (statistically significant) (Figure 2). A total of 51% grandparents (47.5%, 48.2%, 60.5% and 62.5% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively), thought frequent visits to the dentist were important. When asked whether they had ever tried to treat the child for any dental related problem at home, 35%, 30.7%, 23.7%

and 50% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively, replied in the affirmative (total 31%). Majority (67%) of the grandparents (65%, 64.9% 68.4% and 100% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) said that prolonged thumb and finger sucking can cause crooked teeth. A vast majority (88.5%) of the grandparents (92.5%, 84.2%, 94.7% and 100% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) knew that brushing teeth could prevent dental problems. Regarding the availability of specialized toothbrushes and pastes only for children, 37.5%, 57%, 65.8% and 75% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively, were aware of the same (statistically significant) (Figure 2), whereas 17.5%, 13.1%, 5.3% and 12.5% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively, were aware only of specialized brushes for children and not of pastes. A majority (78.5%) of grandparents (65%, 81.6%, 78.9% and 100% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) said that they gave as much importance to the care of their teeth as to other parts of their body. When asked whether maternal dental health could affect child's future dental health and general health, only 30%, 40.4%, 50% and 75% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively, replied in the affirmative (statistically significant) (Figure 2). Many of the grandparents were also unaware of the age at which the first dental check up of the child should be done. Only 17.5%, 11.4%, 7.9% and 37.5% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively, said that the first dental checkup should be at or before 1 year of age (total 13%), whereas the rest of the grandparents gave varied answers like 2 years (20.5%), 3 years (13%), after 4 years (20.5%) and when required (33%). The high level of unawareness was also evident by the responses to the question about when one should start brushing the child's teeth. A total of 27.5% grandparents (22.5%, 23.7%, 42.1%, and 37.5% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) said that brushing should be started at or before 1 year of age, whereas others gave varied answers like 2 years (31%), 3 years (25.5%), after 4 years (14.5%) and do not know (1.5%). Most of the grandparents were well aware of the total number of permanent teeth with 87.5%, 79.8%, 84.2% and 100% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively giving the answer as 32, but most were not aware of the number of milk teeth with only 7.5%, 15.8%, 13.1% and 50% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively (total 15%), giving the answer as 20. A total of 60.5% grandparents (42.5%, 59.6%, 76.3% and 87.5% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) said that milk teeth were important (statistically significant) (Figure 2), whereas a total of 47.5% grandparents (45%, 38.6%, 68.4% and 87.5% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) said that problems of milk teeth could affect permanent teeth. Only 36% of grandparents (37.5%, 28.9%, 50% and 62.5% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) knew that dental decay could be transmitted by sharing of spoons and cups. When asked whether prolonged bottle or breast feeding could affect dental health, 49.5% grandparents (47.5%,
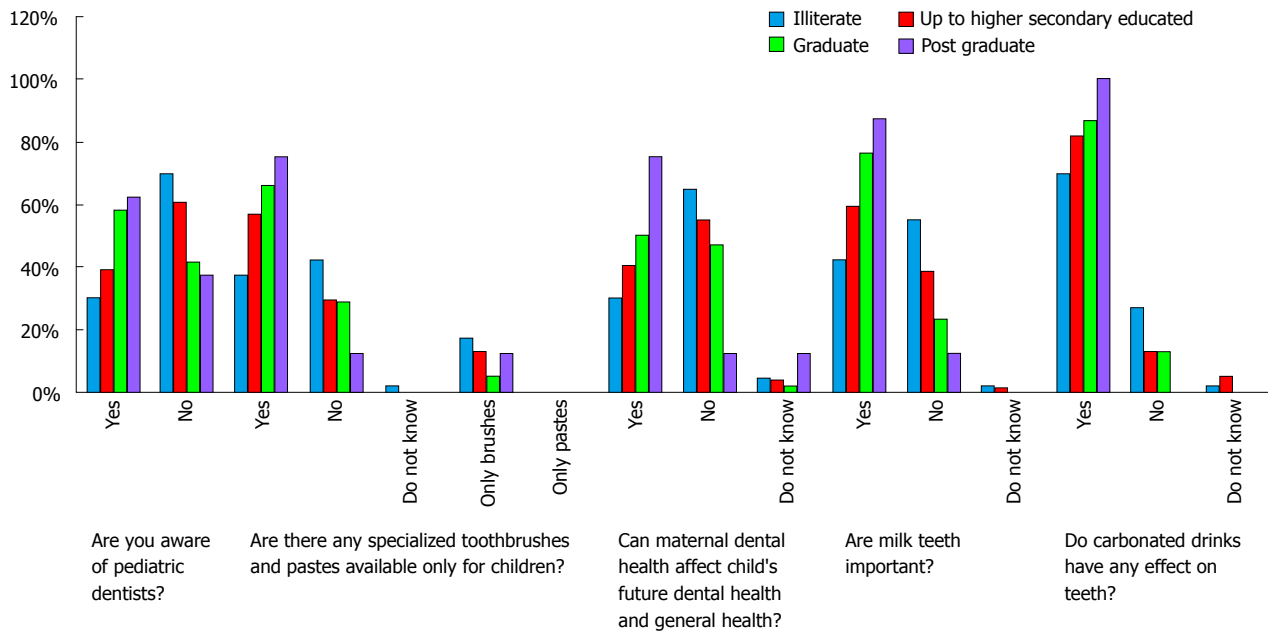
Figure 2 Analysis based on level of education of the grandparents.

45.6%, 60.5% and 62.5% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) replied in the affirmative. A total of 32.5% grandparents (40%, 33.3%, 28.9% and 0% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) said that it was alright to put children to bed with a bottle. A total of 36.5% of grandparents (37.5%, 28.9%, 50% and 75% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) said that use of fluorides could strengthen teeth. A vast majority (81%) of grandparents (70%, 81.6%, 86.8% and 100% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) knew that carbonated drinks had ill effects on teeth (statistically significant) (Figure 2). A vast majority (95%) of grandparents (100%, 93.8%, 92.1% and 100% of categories Ⅰ, Ⅱ, Ⅲ, and Ⅳ respectively) also knew that frequent snacking of sweet and sticky foods had ill effects on the teeth.

DISCUSSION

In the present study, the sample selected was dominated by female grandparents (70.5%) and a majority of the grandparents fell in the up to higher secondary educated group (57%). To the best of our knowledge no other study has been done to evaluate the level of dental awareness among grandparents, so a direct comparison of the above findings was not possible. In this study it was seen that the level of awareness about oral health was low among grandparents. A majority of them were not aware about paediatric dentists or specialized dentists for children. Awareness about the same was higher in the more educated groups but even among the post graduates, many were not aware of the same. There was no significant difference found between the genders. The grandparents were also not aware about the importance of frequent visits to the dentist so that any decay or condition can be recognized and rectified at

an early stage. This indicates that there is an immediate and great need for education of caregivers like grandparents. The importance of such facts needs to be emphasized by paediatricians who see children at regular intervals or for vaccinations. Alternatively nurses can also be trained to impart this information in paediatric and vaccination wards of hospitals so that it can have an impact on maximum people.

When asked whether they had tried to treat their grandchildren at home with home remedies, the results obtained were surprising. It was found that people who were illiterates were in the habit of taking the children to a dentist whereas 50% of the postgraduates had tried their hand at treating the child themselves. People need to be educated that they should not try to treat children themselves, especially when the child cannot be relied upon to explain the symptoms correctly due to young age and lack of previous experience of dental pain. Children's understanding of pain and their ability to describe it changes in a predictable developmental sequence[9]. As the experience of pain is inherently personal and subjective, it is not directly accessible to others and requires considerable judgment and skill on the part of observers in the use of cues that are available, if inferences are to be accurate[10]. It should be stressed to the caregivers that they should always consult a specialist and not try to treat the children themselves as this may lead to wastage of precious time and the child may have to bear consequences that are permanent and irrevocable.

The grandparents showed good knowledge about the harmful effects of prolonged sucking habits. The female grandparents were much more aware about the same than their male counterparts, the difference being statistically significant (*P* < 0.05) (Figure 1). The reason for this can be that as females are more aesthetically

oriented[11] and spend more time looking after and caring for their grandchildren, they may notice little changes in the facial structure that occur during the growing up stages of the child.

When the responses of the same question were compared with the level of education, the surprising result was that the level of awareness was the same in the illiterates, up to higher secondary educated and the graduates (65%) but jumped to 100% in the postgraduates. The reason for this could be that the postgraduates, because of higher level of knowledge and understanding, probably come in contact with information from different sources such as media and newspapers, books and internet and hence, are more aware. The awareness about the deleterious effects of prolonged sucking habits need to be imparted to everybody so that such habits can be stopped before they have any effect on the oral structures.

The knowledge about the advantages of regular brushing was very good across genders and at all levels of education, probably because of the role of media in promoting tooth brushes. The awareness about specialized toothbrushes and pastes for children need to be improved, especially in caregivers of young children as children below 2 years of age should not be given fluoridated toothpastes, unless considered at moderate or high caries risk[12] and such small children won't be able to manoeuvre an adult brush in their small mouths. This can be achieved through the use of broadcast media, such as televisions. Another important source of information can be pharmacists who can help promote these by giving information to customers, especially as old people are likely to visit them more.

A majority of grandparents did not know about the transmission of cariogenic bacteria from mothers to their children[13,14], and the fact that it could increase the risk and severity of caries in very young children[15,16]. This knowledge was higher in the groups with a higher level of education but even among the well educated grandparents, a considerable proportion was not aware of the same (Figure 2). To improve this, workshops/lectures can be started in community centres or in places like meditation centres, laughter clubs, societies and book clubs etc. which are routinely frequented by the aged. Regarding the awareness about the appropriate age for the first dental visit and the age to start brushing the child's teeth, mixed results were obtained and no particular trend was followed. This shows that even people who have the highest level of education are not aware about basic and important health recommendations while caring for a child. Again the paediatrician or the nurses at hospitals and vaccination centres can help in spreading awareness about the same. Caretakers should be educated that regular tooth cleaning needs to be started early in life, as soon as the first primary tooth erupts[12].

Most grandparents knew about the number of permanent teeth in the mouth but not about the primary teeth. This could be due to the age old myth that as milk teeth are ultimately going to fall off, they are not important. A large number of the grandparents even acknowledged that they did not consider primary teeth to be of any importance and that they did not think that problems of primary teeth could affect permanent teeth. This misconception leads to neglect of the child's oral health by caregivers and because of bad habits instilled early in life, such children grow up to be adults who do not take good care of their teeth. The importance of primary teeth needs to be emphasized by paediatricians and general dentists so that this mindset can be changed. It has been reported in previous studies[17,18] that the low value attributed to primary teeth is an obstacle to developing effective prevention programs.

The practice of sharing foods and utensils by adults has been associated with early infection with *Streptococcus mutans* in infants[19,20]. Grandparents are often in the habit of checking the temperature of the food before giving it to the child by tasting it. This can be harmful as it can introduce caries causing microorganisms into the child's mouth if the same spoon or cup is used. Grandparents need to be educated about the harmful effects of such practices which they consider normal and routine.

Majority of the grandparents did not know about the harmful effects of prolonged bottle or breast feeding or the advantages of fluorides. Awareness of the same was more in the higher educated but not consistently or significantly. Knowledge about the deleterious effects of putting a child to bed with a bottle and use of carbonated drinks by older children was more in the higher educated grandparents (Figure 2).

There is a great need for education of grandparents regarding their grandchildren's oral health. This can be done by holding demonstrations and lectures/workshops in community centres. Also small demonstrations can be prepared and delivered in waiting areas of hospitals, nursing homes and dental clinics by volunteers or small documentaries can be made for televisions which are repeatedly played in the waiting area. Also nurses and other health personnel can be trained to impart information in vaccination centres. Paediatricians can also help in imparting information and guidance when the child is brought for any treatment. Posters regarding oral health related facts about children can be put up in paediatric wards and dental clinics/vaccination centres. All these measures can improve the preventive dental care children receive at home and their use of professional dental services, ultimately, bringing us closer to our international oral health goals for children.

COMMENTS

Background

In today's changing society where both parents are working, grandparents assume an important role as caregivers in their grandchildren's lives. There is a great need for dental education of grandparents as they serve as role models for the young. The attitude and knowledge of grandparents was evaluated in this study so as to implement changes to improve the oral hygiene standard of children.

Research frontiers

To the best of our knowledge, no such study has been done where only grandparents were evaluated for the role that they play in influencing children's oral health status. A review of literature revealed only one study where knowledge of caregivers, which included grandparents (107 grandparents out of 615 caregivers, *i.e.*, 17.4%) was studied. It has been seen that improving the knowledge of mothers has a positive influence on the children's oral health status.

Innovations and breakthroughs

The major conclusion from the article is that there is a great need for education of grandparents regarding their grandchildren's oral health. Since a lot of grandparents are playing a major role in caring for the children while both their parents are working, steps must be taken to ensure that they know how to take care of the oral hygiene of the children. Till now, major stress was given in imparting this knowledge to only parents, especially mothers.

Applications

To eliminate any disease, the first step is to gain knowledge of how that disease is contracted. Since dental caries cannot be reversed, to control it, we need to prevent its onset. This can only be done by maintaining proper oral hygiene. If children are educated about the value of maintaining proper oral hygiene, good habits can be instilled in them from a young age. Since grandparents play a big role in taking care of the children and serve as role models for them, they need to be educated about how to take care of their grandchild's oral health.

Terminology

Streptococcus mutans is facultative anaerobic, Gram-positive coccus-shaped bacterium commonly found in the human oral cavity and is a significant contributor to tooth decay.

Peer-review

An interesting article that provides a different perspective to the prevention of dental caries. It shows how caretakers need to be educated if we want the next generation to be free of oral diseases.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Effects of carob-bean gum thickened formulas on infants' reflux and tolerance indices

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Abstract

AIM: To examine the effect of carob-bean gum (CBG) thickened-formulas on reflux and tolerance indices in infants with gastro-esophageal reflux (GER).

METHODS: Fifty-six eligible infants (1-6 mo old) were randomly allocated to receive for two weeks a formula with either 0.33 g/100 mL (Formula A) or 0.45 g/100 mL (Formula B) of cold soluble CBG galactomannans respectively, or a formula with 0.45 g/100 mL of hot soluble CBG galactomannans (Formula C). No control group receiving standard formula was included in the study. Data on the following indices were obtained both at baseline and follow-up from all study participants: 24 h esophageal pH monitoring indices, anthropometrical indices (*i.e.*, body weight and length) and tolerance indices (*i.e.*, frequency of colics; type and frequency of

defecations). From the eligible infants, forty seven were included in an intention-to-treat analysis to examine the effects of the two-week trial on esophageal 24 h pH monitoring, growth and tolerance indices. Repeated Measures ANOVA was used to examine the research hypothesis.

RESULTS: Regarding changes in 24 h pH monitoring indices, significant decreases from baseline to follow-up were observed in the "Boix Ochoa Score" (*i.e.*, an index of esophageal acid exposure), in the total number of visible refluxes and in all symptoms related indices due to acid reflux only for infants provided with Formula A, while no significant changes were observed for infants provided with Formulas B and C. In addition, the significant decreases observed in two symptoms related pH monitoring indices (*i.e.*, "Symptom index for reflux" and "Percentage of all reflux") for infants provided with Formula A were also found to differentiate significantly compared to the changes observed in the other two groups ($P = 0.048$ and $P = 0.014$ respectively). Concerning changes in anthropometric indices, body weight significantly increased among infants provided with Formulas A and C, but not for infants provided with Formula B. As far as tolerance indices were concerned, the numbers of total and diarrheic defecations increased significantly only in infants provided with Formula B and these changes were significantly higher compared to the decreases observed in infants fed with Formulas A and C ($P = 0.003$ and $P = 0.015$ respectively). Lastly the number of colics significantly decreased in all infants, irrespective of the tested formula.

CONCLUSION: Formula A (*i.e.*, 0.33 g/100 mL of cold galactomannans) was effective in reducing certain pH-monitoring indices of uncomplicated GER, increased body weight and was well-tolerated by infants.

Key words: Reflux; Carob bean gum; Galactomannans; Infants; Formula

Core tip: The present study showed that Formula A was more effective in decreasing esophageal acid exposure, the total daily number of visible and measurable refluxes, as well as acid reflux related symptoms, while such changes were not observed for the infants fed with Formulas B and C. Furthermore, a significant increase of body weight was observed for infants fed with Formulas A and C while that was not observed for infants fed with Formula B, probably due to the increased number of diarrheic and total defecations recorded in this group. These findings indicate that Formula A, containing 0.33 g/100 mL of cold soluble galactomannans, seems to be more effective in reducing certain pH-monitoring indices of uncomplicated gastro-esophageal reflux, increasing body weight and being well-tolerated by infants.

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INTRODUCTION

Gastro-esophageal reflux (GER) is defined as the involuntary passage of gastric contents (*e.g.*, saliva, ingested foods and drinks, gastric secretions, pancreatic or biliary secretions) into the esophagus and does not refer to any specific etiology with or without regurgitation and vomiting[1]. The term regurgitation is specifically used if the reflux dribbles effortlessly out of the mouth[2]. GER is a common and global problem affecting about 50% of all babies up to the age of two months and has a peak incidence at the age of three months. Only some infants will develop pathologic gastro-esophageal reflux disease (GERD), in which clinical problems are related to excessive passage of acid gastric contents. GERD should be suspected if the regurgitating infant shows one or more other symptoms such as crying, fussing or arching of the back, refusal to feed, failure to thrive, hematemesis, occult blood in the stool, anemia or refusal to eat[2,3]. Uncomplicated GER should be suspected in infants with uncomplicated recurrent regurgitation[1]. In some cases GER may affect thrive because of caloric insufficiency and potentially lower dietary nutrients' intake. There is often abnormal sucking and swallowing and weight gain may be poor.

Since infant regurgitation is a transient problem, treatment goals are to provide effective reassurance and symptom relief. Infants with GER may feel unhappy and parents often seek medical attention. The use of anti-reflux formulas and formulas with added thickening agents [*e.g.*, processed rice, corn or potato starch, guar gum or carob-bean gum (CBG)] results in a decrease of visible regurgitation[4]. CBG or locust bean gum is refined from the endosperm of seeds of the carob tree (Ceratonia siliqua). Eighty-five percent of the product is in the form of galacto-mannose oligo/polysaccharide having the monosaccharides mannose and galactose in a ratio of 4:1, about 5% is protein, and the final 10% is water. This galactomannan is indigestible but fermentable by colonic bacteria[5]. Because of this fermentable characteristic, some infants may react with abdominal pain, colic and diarrhea. In fact these adverse effects are normal for fiber ingestion and not specifically associated with CBG. Nevertheless, it seems that CBG is safe for its therapeutic use in term infants to treat GER from birth onwards[6]. Commercially available anti-reflux formulas currently contain CBG galactomannans at a concentration of 0.45 g/100 mL. However, Miyazawa *et al*[7,8] published studies in 2006 and 2007 reporting that lower dosages of CBG (0.35 g/100 mL) are effective too, at least with regard to visible refluxes. Although for these reasons the amount of CBG could be reduced to 0.35-0.40 g/100 mL, the

effect of these lower concentrations on measurable refluxes is not known.

The primary objective of the current study was to examine the efficacy of formulas containing cold *vs* hot soluble CBG galactomannans (at a concentration of 0.45 g per 100 mL) and the effect of feeding infants with a lower concentration of galactomannans (*i.e.*, 0.33 g per 100 mL) on visible and measurable refluxes assessed by 24 h pH impedance monitoring. Furthermore, a secondary objective was to determine whether the decrease in the concentration of galactomannans and the change from hot to cold soluble galactomannans affects weight gain and tolerance indices (*i.e.*, stool frequency and consistency, colic) in infants.

MATERIALS AND METHODS

Study design

The current study was a randomized, partly double blind clinical trial initiated on July 2013 and completed on July 2014 at the Second Pediatric Clinic in the University Hospital "St. Marine" Varna, Bulgaria. Informed consent was obtained from the parents of all infants that were found to be eligible to be included in the study. Prior to study's initiation and during the first screening phase, eligibility of infants to participate in the study was assessed according to the following inclusion criteria: Availability of parents/infants to participate in the study throughout the intervention period; less than ¼ of daily milk consumption coming from breast milk; no use of any anti-reflux formulae or medications that can affect gastrointestinal tract motility; no history, diagnosis or illness from cow's milk protein allergy (*i.e.*, positive IgE and/or positive skin prick test to cow's milk), wheezing, aspiration caused pneumonia, apnea, anemia, bleeding, laryngitis, urinary tract infection, diarrhea, neurologic deficits and any known organic or metabolic cause of reflux. Further to the initial screening phase, a total number of 56 one to six month-old infants that were born full-term, fulfilling all above inclusion criteria and diagnosed with GER (based on a score > 7 in the GER Orenstein questionnaire[9] as filled in by parents at inclusion) were considered eligible and entered the study. Eligible infants were randomized into three study groups based on the type of formula provided to them: Formula A containing 0.33 g/100 mL cold soluble galactomannans; Formula B containing 0.45 g/100 mL cold galactomannans; and Formula C containing 0.45 g/100 mL of hot soluble galactomannans. The cold soluble form of galactomannans is heated during production to be pre-gelatinised and gets gelatinised when dissolved in lukewarm water (*i.e.*, of approximately 45 ℃). The hot soluble form of CBG galactomannans is only minimally heated during production and needs to be dissolved in hot water (*i.e.*, of approximately 90 ℃) to be gelatinised. The difference in water temperature explains why this study could not be double blind for all study groups. More specifically, parents whose infants were allocated to Formulas A and B were instructed

to use lukewarm water, whereas those parents whose infants were allocated to Formula C were instructed to use hot water for the preparation of the relevant milk formulae. Further to the above although the intervention was double blind for the study groups receiving Formula A and Formula B, this was not feasible for the Formula C treatment arm.

Following the first screening and before allocation of eligible infants to the study groups all infants were fed with a standard infant formula (Frisolac Gold 1, Friesland Campina, the Netherlands) for seven consecutive days, which served as a "run-in" period before the initiation of the intervention. On day seven, baseline anthropometric and 24 h pH impedance monitoring measurements were conducted. From day eight to day 21 the infants received the intervention Formula A, B or C. Allocation of infants to each one of three treatment arms was based on a standard table developed by a statistician (StatistiCal B.V., Wassenaar, The Netherlands), randomly assigning a different numerical code to each study participant receiving one of the three test formulae. On day 22 the final anthropometric and 24 h pH impedance monitoring measurements were conducted. Formula C was the reference formula and was provided to parents in the standard Friso Comfort packaging. The other two test formulae were provided in blank sachets labeled with either "A" or "B". The product developer kept the decoding information in a sealed envelope, which was opened after completion of the intervention and evaluation of the study results.

Measurements

The measurements conducted and the data collected in the present study are summarized below.

Gastro-esophageal reflux questionnaire: The gastro-esophageal reflux questionnaire (GERQ) is an instrument developed and validated for diagnosis of GER in infants and toddlers from 1 to 14 mo old[9,10]. Based on the scoring (*i.e.*, GERQ score) derived from the answers provided by mothers, infants with a GERQ score > 7 (*i.e.*, score indicative of possible GER) were considered eligible to participate in the study. The appropriateness of using the Orenstein questionnaire to identify infants with GER in the current study was also confirmed by the pH monitoring indices values obtained at baseline. Specifically, all eligible children identified by Orenstein questionnaire were also found to have pH indices above the references values suggested by Kitz *et al*[11] at baseline.

Three-day diaries: A 3-d diary was provided to mothers both at the start and at the end of the intervention period (*i.e.*, the diaries were filled in by mothers from day four until day six and from day 18 to day 20), in order to record "tolerance" indices (*i.e.*, type and frequency of colic and defecations) and information regarding the amount of formula consumed by their infants during the day. Regarding colic, that was defined based on the classic

definition of infantile colic and specifically an approach based on the rule of threes: *i.e.*, fussy crying that lasts for 3 h per day and for 3 d per week[12]. Regarding defecations, the total number of infants' defecations was recorded by mothers in the diaries, while a visual chart, *i.e.*, the *Bristol Stool chart* (BSC), that classifies defecations based on 7-point stool hardness scale (1, hard; 7, watery) was used to define constipation; diarrhea and ideal-stool defecations. BSC is currently the most popular scale/tool used in many clinical trials also conducted on infants and children to assess stool consistency[13]. Regarding the amount of formula consumed by their infants during the day, mothers were asked to keep a record reporting the exact volume of milk formula prepared and the exact volume of milk formula left over after each feeding. This information was recorded during the total intervention period in relevant record sheets that were provided to mothers. Mothers received both written and verbal instructions for the correct completion of the diaries and the record sheets.

Anthropometrical measurements: Body weight of infants was measured, as an average of two separate measurements, on a calibrated scale (Digital baby weight scale Seca 374) to the nearest 10 g, without cloths and diapers. Recumbent length of infants was measured as an average of two separate measurements, using a length board (Seca 416 infantometer for measuring babies and toddlers) to the nearest 1 cm according to standard instructions.

Gastro-esophageal reflux monitoring: Gastro-esophageal reflux was quantitatively assessed *via* combined measurements of the intra-esophageal pH and multiple electrical impedance[14,15], using the Digitrapper pH-Z ambulatory 24 h pH and impedance recorder (Digitrapper, Sierra Scientific Instruments, Los Angeles, CA) and the relevant software (AccuView pH-Z). According to its principle of operation, this method measures the electrical impedance changes between two neighboring electrodes during the passage of a bolus inside a luminal organ (*i.e.*, retrograde bolus movement in the esophagus in the current study). An age-appropriate catheter was used in the current study depending on the infant's length and was placed trans-nasally above the upper boarder of the lower esophageal sphincter. The correct positioning of the catheter during the 24 h esophageal pH monitoring was assessed *via* X-ray at both baseline and follow-up examination. The purpose of X-ray was to ensure that the catheter was positioned above the stomach and specifically three vertebrae above the diaphragm following the guidelines from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition[1].

Esophageal pH impedance monitoring was performed continuously for 19-24 h at the two time points of measurements, *i.e.*, before the initiation and at the end of the intervention. At the end of each recording, data were analyzed using the AccuView pH-Z™ software version 5.2 (Given Imaging Ltd, Israel) and results were expressed

in 10 pH impedance monitoring indices. Among the 24 h pH monitoring indices, the recording of the "symptom-related" indices required caregiver's interference by pressing an "event button" any time the baby was crying or was showing signs of anxiety or discomfort (according to parent's/caregiver's perception). This was not required for all other pH indices (*i.e.*, "non-symptom related" ones).

Statistical analysis

The sample size estimation in the present study was based on the experience gained from a previous intervention study[16] conducted also with Bulgarian infants, examining the same outcomes (*i.e.*, the same pH monitoring indices) as the current study. Based on the observed changes in pH monitoring indices observed in this previous intervention study, a minimum sample size of 30 subjects (or 10 subjects per treatment arm) was considered adequate to provide in the present study a statistical power of 90%.

The effect of the intervention scheme on pH monitoring, growth and tolerance indices was examined using intention-to-treat (ITT) analysis. Multiple imputations were performed to estimate missing follow-up data due to drop-outs and the pooled imputed data were used in all subsequent analyses. All data were reported as mean (SD) and as mean change (95%CI) over baseline. Normality tests were used to determine normality of distribution of the examined variables. Repeated measures analysis of variance (Repeated Measures ANOVA) was used to assess the significance of the differences between groups at baseline and follow-up examination (Treatment effect), the significance of the changes observed within each group (Time effect) and the significance of the differences among groups in the changes from baseline to follow-up examination (Treatment X Time Interaction effect). The between-group factor was the study groups (*i.e.*, Formula A *vs* Formula B *vs* Formula C); the within-group factor was the time-point of measurement (*i.e.*, baseline, follow-up). In all analyses, adjustments were made for the average volume of milk consumed by infants per day during the intervention period. All *P*-values reported were two-tailed. Statistical analysis was conducted with the use of the SPSS statistical analysis software for Windows (version 21.0). The level of statistical significance was set at $P \leqslant$ 0.05. The statistical methods of this study were reviewed by Dr. Kourlaba Georgia from The Stavros Niarchos Foundation-Collaborative Center for Clinical Epidemiology and Outcomes Research.

Ethics statement

The study was approved by the Medical Ethical Committee of the "St. Marina" University Hospital of Varna (Ethical approval No. 13/03.28.2013) and was implemented in accordance to the signed protocol and the rules for good clinical practice. The study was registered in the Netherlands Trial Register: NTR4334.

RESULTS

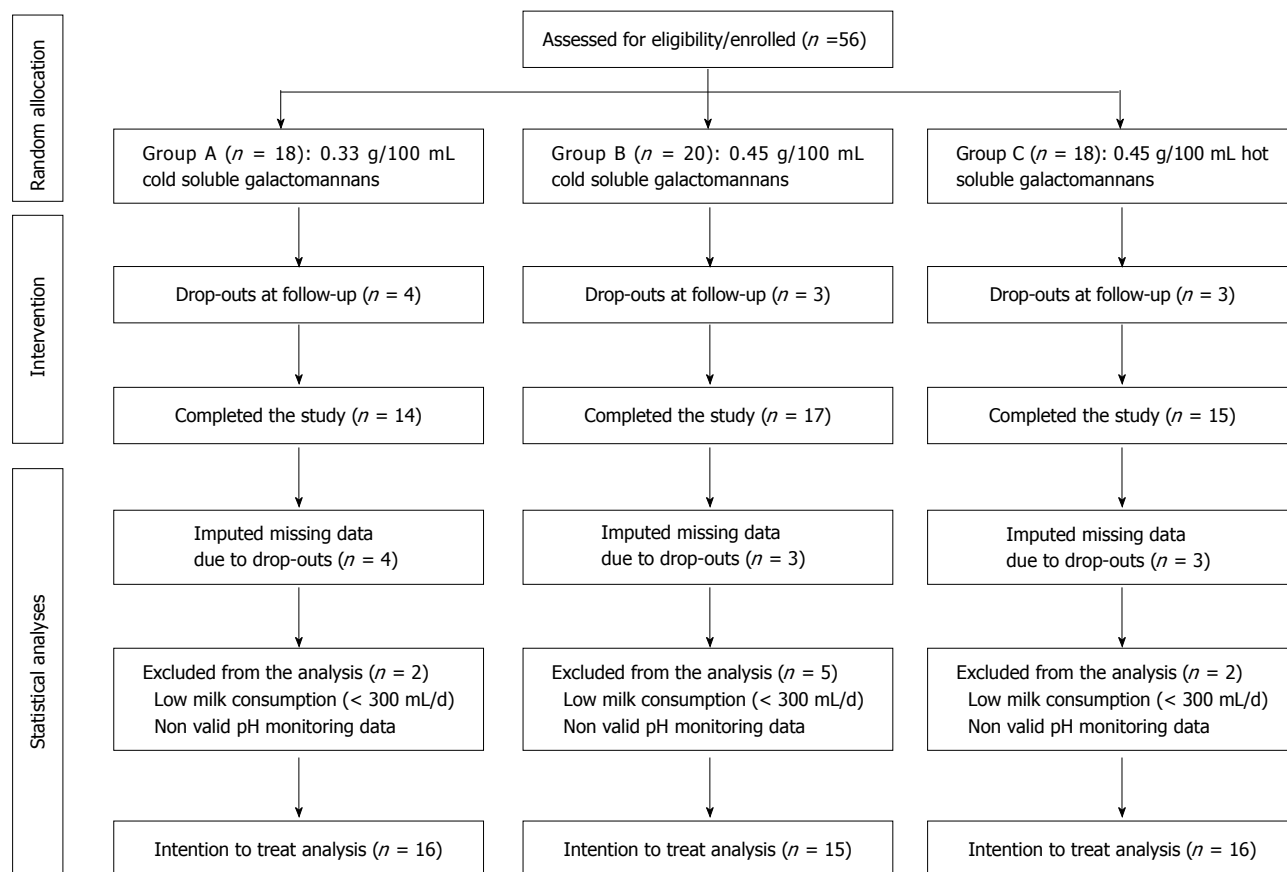Figure 1 presents a flow diagram of infants that were

Figure 1 Flow of study participants through the trial.

included in the statistical analysis. More specifically, 56 eligible infants were identified at the initial screening phase. From these 56 infants, 10 infants either dropped out at follow-up or did not perform the 2nd 24 h esophageal pH monitoring due to parental refusal to provide consent for the follow-up pH measurement. Nevertheless, as these 10 infants were actually subjected to treatment almost throughout the intervention period, multiple imputations were conducted to estimate their missing data at follow-up examination and these infants were reinstated in the study sample for which the ITT analysis was performed. Before performing the ITT analysis, a thorough examination of the collected data revealed that for five infants at least one of the two 24 h pH impedance monitoring measurements was non-valid (*i.e.*, mainly due to the incorrect positioning of the catheter), while in four infants the average daily milk consumption was quite low (*i.e.*, below 300 mL per day) throughout the intervention period. These nine infants were excluded from the ITT analysis, which was finally performed for a total sample 47 infants. The results from these analyses are presented in Tables 1-3 and in Figure 2.

Tables 1 and 2 present the changes in the "non-symptom related" and "symptom related" 24 h pH monitoring indices, respectively. According to the data presented in Table 1, significant decreases from baseline to follow-up examination were observed for the "Boix Ochoa Score" and the "Number of refluxes per day"

(by 72.0 and 231.8 respectively), only for the Formula A group (percent changes from baseline in the three study groups are also presented in Figure 2). Still, these changes did not differentiate significantly with the changes observed in the other two study groups. No other significant findings were observed for the rest of the "non-symptom related" 24 h pH monitoring indices examined in the present study. Based on the data displayed in Table 2, significant decreases were observed from baseline to follow-up examination for all "symptom related" 24 h pH monitoring indices only for infants fed with Formula A. Furthermore, in two out of the four "symptom-related" indices examined, the decreases observed for infants fed with Formula A from baseline to follow-up examination (*i.e.*, by -18.2 in SI and by -16.9% in the percentage of all refluxes) were significantly higher compared to the relevant changes in other two groups (*i.e.*, *P* < 0.05).

Table 3 summarizes the changes observed in growth and tolerance indices. As far as growth indices were concerned, body weight significantly increased for infants fed with Formula A (by 0.57 kg or 40.7 g per day) and C (by 0.79 kg or 56.4 g per day). Nevertheless these changes from baseline to follow-up examination were not found to differentiate significantly among the three study groups. Furthermore, no significant differences were observed among groups with regards to recumbent length, which increased significantly in all three study

Table 1 Changes in "non-symptom related" 24 h pH monitoring indices from baseline to follow-up examination by study group

	Baseline		Follow up		2-wk change		*P*-value
	Mean	(SD)	Mean	(SD)	Mean change	(95%CI)	(treatment x time)
Reflux Index (%)							0.484
Formula A (*n* = 16)	11.4	-10.3	8.97	-8.23	-2.52	(-9.48 to 4.45)	
Formula B (*n* = 15)	6.47	-5.67	10.1	-13.1	3.44	(-3.85 to 10.7)	
Formula C (*n* = 16)	10.3	-12.6	9.42	-10.2	-0.67	(-7.77 to 6.44)	
P value (Treatment effect)	0.247		0.901				
Longest reflux (min)							0.445
Formula A (*n* = 16)	19.3	-15.4	13.9	-8.69	-5.16	(-13.8 to 3.48)	
Formula B (*n* = 15)	15.7	-18.4	11.8	-7.27	-3.31	(-12.3 to 5.73)	
Formula C (*n* = 16)	14.4	-9.47	17.6	-16.6	2.48	(-6.32 to 11.3)	
P value (Treatment effect)	0.664	0.219					
Total time below pH 4 (min)							0.722
Formula A (*n* = 16)	12.2	-12.1	7.85	-7.64	-4.5	(-13.6 to 4.58)	
Formula B (*n* = 15)	11.5	-20.3	10.3	-13.1	-1.7	(-11.2 to 7.80)	
Formula C (*n* = 16)	9.66	-12.9	9.84	-10.3	0.72	(-8.54 to 9.98)	
P value (Treatment effect)	0.944		0.655				
Reflux below pH 4 for more than 5 min (*n*/d)							0.712
Formula A (*n* = 16)	6.42	-7.19	4.6	-5.21	-1.76	(-5.89 to 2.38)	
Formula B (*n* = 15)	4.08	-4.28	4.33	-4.83	0.48	(-3.85 to 4.80)	
Formula C (*n* = 16)	5.53	-8.34	5.96	-5.86	0.15	(-4.06 to 4.37)	
P value (Treatment effect)	0.446		0.452				
Boix Ochoa Score[1]							0.198
Formula A (*n* = 16)	107.6	-163	36.6	-29.4	-72.0[3]	(-131.6 to -12.5)[3]	
Formula B (*n* = 15)	53	-78.1	45	-41.8	-12.3	(-74.6 to 50.0)	
Formula C (*n* = 16)	57.2	-80.7	52.1	-49.8	-0.08	(-60.8 to 60.7)	
P value (Treatment effect)	0.346		0.381				
Total refluxes per day (*n*/d)[2]							0.385
Formula A (*n* = 16)	377.3	-524.5	142.9	-118	-231.8[3]	(-437.9 to -25.8)[3]	
Formula B (*n* = 15)	169.3	-217	128	-97	-30.9	(-246.6 to 184.8)	
Formula C (*n* = 16)	307.3	-475.4	220.4	-266.8	-99.2	(-309.5 to 111.1)	
P value (Treatment effect)	0.343		0.234				

[1]Global measure of esophageal acid exposure; [2]Indicative of the total number of visible and measurable refluxes during the 24 h monitoring; [3]Indicate statistical significant findings. The "non-symptom related" 24 h pH monitoring indices include those indices recorded during the 24 h pH monitoring procedure, not requiring caregiver's interference by pressing the "event button. Adjustment was made for the average volume of milk consumed by infants per day during the intervention period.
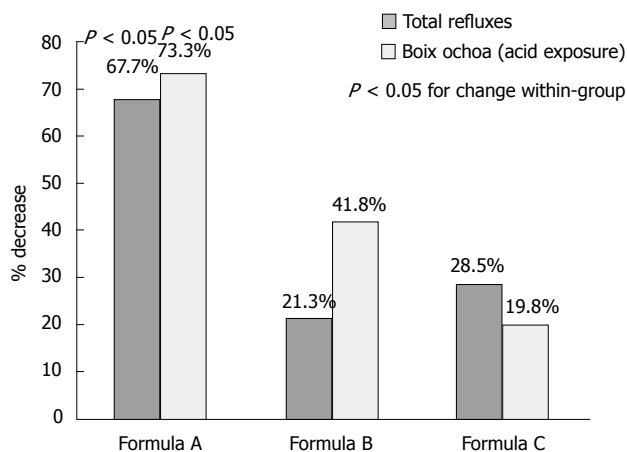
Figure 2 Percent decreases from baseline to follow-up in the "Boix Ochoa Score" index and "number of refluxes per day" in the Formula A (0.33 g/100 mL cold carob-bean gum), B (0.45 g/100 mL cold carob-bean gum) and C (0.45 g/100 mL hot carob-bean gum) groups.

groups. Regarding tolerance indices, the number of diarrheic and total defecations, increased significantly from baseline to follow-up examination by 5.47 and 6.02, respectively, only for infants fed with Formula B. On the contrary, a significant decrease by 5.72 in the number of total defecations was observed for infants fed with Formula C. Furthermore the increases observed in the number of diarrheic and total defecations for infants fed with Formula B were significantly higher compared to the changes observed in the other two groups (*P* = 0.015 and 0.003, respectively). Lastly, the total number of colics decreased significantly form baseline to follow-up examination in all three study groups, but no significant differences in these changes were observed among groups.

DISCUSSION

In the current study, among the several pH monitoring indices examined, only "Boix Ochoa score" and the "Total number of refluxes per day" showed significant decreases for infants fed with Formula A, while no significant changes were observed for infants fed with Formulas B and C. Furthermore, Formula A was found to be more effective in decreasing acid reflux-related symptom indices compared to the two other formulas (Table 2). Similarly, to the present study the majority of previous clinical trials assessing the

Table 2 Changes in "symptom related" 24 h pH monitoring indices from baseline to follow-up examination by study group

	Baseline		Follow up		2-wk change		*P*-value (treatment x time)
	Mean	(SD)	Mean	(SD)	Mean change	(95%CI)	
Symptom index for reflux (SI)							0.048[3]
Formula A (*n* = 16)	39.7	-26.2	21.6	-14.5	-18.2[3]	(-31.8 to -4.57)[3]	
Formula B (*n* = 15)	24.5	-27.2	19.7	-20.8	-5.21	(-19.5 to 9.03)	
Formula C (*n* = 16)	27.7	-20.6	33.7	-24.8	6.49	(-7.39 to 20.4)	
P value (Treatment effect)	0.213		0.119				
Symptom association probability[1]							0.096
Formula A (*n* = 16)	87.4	-25.2	49.5	-42.5	-37.9[3]	(-64.6 to -11.3)[3]	
Formula B (*n* = 15)	54.6	-47.3	58.7	-40.2	3.82	(-24.1 to 31.7)	
Formula C (*n* = 16)	82.9	-33.8	57.8	-38.3	-24.8	(-52.0 to 2.37)	
P value (Treatment effect)	0.031		0.762				
Percentage of acid refluxes[2] (%)							0.067
Formula A (*n* = 16)	39.8	-26.1	22.6	-15.0	-17.2[3]	(-30.8 to -3.66)[3]	
Formula B (*n* = 15)	24.6	-27.3	19.4	-20.8	-5.53	(-19.7 to 8.65)	
Formula C (*n* = 16)	27.7	-20.4	33.3	-25.3	5.93	(-7.89 to 19.8)	
P value (Treatment effect)	0.21		0.152				
Percentage of all reflux (%)							0.014[3]
Formula A (*n* = 16)	47.9	-22.3	31.0	-18.1	-16.9[3]	(-31.3 to -2.28)[3]	
Formula B (*n* = 15)	35.1	-23.6	36.8	-28.7	1.78	(-13.3 to 16.9)	
Formula C (*n* = 16)	33.7	-22.3	48.2	-28.8	14.4	(-0.28 to 29.2)	
P value (Treatment effect)	0.167		0.201				

[1]Probability that symptom and reflux are not associated solely by chance; [2]Percentage of acid refluxes out of the total number of refluxes occurring during the 24 h monitoring; [3]Indicate statistical significant findings. The "symptom related" 24 h pH monitoring indices include those indices recorded during the 24 h pH monitoring procedure requiring caregiver's interference by pressing the "event button". Adjustment was made for the average volume of milk consumed by infants per day during the intervention period.

effectiveness of formulas containing different types and concentrations of various thickening agents found no significant differences on the most commonly pH indices examined, *i.e.*, the Reflux Index, the number of reflux episodes lasting more than 5 min and the duration of the longest reflux episodes[4]. The only exception were two clinical trials, out of 14, that reported significant decreases in these three pH indices after providing formulas thickened with re-gelatinised corn-starch *vs* a control group receiving a standard formula, for four weeks[17,18]. However, considering the above, direct comparisons of the current study with these two studies are probably not feasible, mainly because of the shorter intervention period, the different thickening agent and the lack of a control group in the current study.

Similar favourable changes in the aforementioned three pH monitoring were also reported by Marinova and Stoimenova[16] on infants fed for two weeks with a formula containing 0.5 g/100 mL of hot-soluble CBG galactomannans. In this study infants were provided with the thickened formula after having been fed with a standard formula for two weeks. These favourable changes observed over the total intervention period of four weeks could be also partially attributed to the gastro-esophageal maturation. Although the same thickening agent was used as in the current study, these findings are not directly comparable to the current ones due to the slightly higher concentration of CBG galactomannans in the tested formula and the different equipment and analysis software used for the 24 h pH monitoring.

Reduced intake of calories and nutrients due to GER

and consequently poor growth is of concern. In line with other studies[4], the present study showed increases of body weight during the 2-wk intervention period. These increases were significant for infants fed with Formulas A and C and were 40.7 and 56.4 g per day, respectively (Table 3). The findings of the present study regarding the concentration of CBG in Formula A (*i.e.*, 0.33 g/100 mL) and the weight gain observed seem to be comparable with previous studies providing CBG in similar concentrations. More specifically, in the study of Miyazawa *et al*[19], when 0.35 g/100 mL CBG-galactomannans were provided a weight gain of 29.3 g per day was observed after one week of intervention. In the study of Vandenplas *et al*[20] when 0.33 and 0.36 g/100 mL CBG-galactomannans (*i.e.*, calculated with 13 g of infant milk powder per 100 mL and 85% galactomannans in CBG) were provided, the weight gains observed were 37 and 24 g per day, respectively, after two weeks of intervention and 27.5 and 25 g per day, respectively, after four weeks of intervention. Taken together, it seems that the increase in body weight as seen in group A is comparable to the increases reported in other studies also using CBG thickened formulas in similar concentrations as in Formula A. However, regarding the increase in body weight observed in Formula C, this was higher compared to those reported in other studies providing similar or higher concentrations. For instance in the study of Vivatvakin and Buachum[21], when a comparable product with an even higher CBG concentration (*i.e.*, 0.5 g/100 mL) than Formula C was provided, a weight gain of 24.5 g per day was observed after two weeks of intervention. The higher mean volume of milk formula consumed by infants fed with Formula C

Table 3 Changes in growth and tolerance indices from baseline to follow-up examination by study group

	Baseline		Follow up		2-wk change		*P*-value (treatment x time)
	Mean	(SD)	Mean	(SD)	Mean change	(95%CI)	
Growth indices							
Weight (kg)							0.648
Formula A (*n* = 16)	5.8	-1.34	6.37	-1.11	0.57[3]	(0.14 to 0.99)[3]	
Formula B (*n* = 15)	5.36	-1.44	5.91	-1.37	0.51	(-0.06 to 0.96)	
Formula C (*n* = 16)	5.33	-1.73	6.06	-0.97	0.79[3]	(0.35 to 1.22)[3]	
P value (Treatment effect)	0.355		0.40				
Length (cm)							0.917
Formula A (*n* = 16)	61.2	-5.57	63.2	-5.07	1.93[3]	(0.59 to 3.27)[3]	
Formula B (*n* = 15)	59.4	-6.23	61.5	-6.02	2.07[3]	(0.67 to 3.48)[3]	
Formula C (*n* = 16)	60.2	-3.76	62.5	-3.53	2.33[3]	(0.96 to 3.70)[3]	
P value (Treatment effect)	0.543		0.631				
Tolerance indices							
Total number (3 d) of hard stools[1]							0.723
Formula A (*n* = 16)	0.5	-2.00	0.48	-1.07	-0.01	(-0.81 to 0.79)	
Formula B (*n* = 15)	0.2	-0.56	0.55	-1.45	0.4	(-0.44 to 1.24)	
Formula C (*n* = 16)	0.13	-0.50	0.17	-0.47	-0.02	(-0.83 to 0.80)	
P value (Treatment effect)	0.62		0.291				
Total number (3 d) of diarrheic defecations[2]							0.015[3]
Formula A (*n* = 16)	8.38	-11.6	5.31	-5.63	-2.88	(-7.91 to 2.16)	
Formula B (*n* = 15)	5.6	-4.81	10.3	-10.1	5.47[3]	(0.0 to 10.7)[3]	
Formula C (*n* = 16)	11.1	-10.7	6.77	-6.68	-5.3	(-10.4 to -0.16)	
P value (Treatment effect)	0.163		0.176				
Total number of defecations (3 d)							0.003[3]
Formula A (*n* = 16)	10.7	-10.4	7.59	-4.23	-2.89	(-7.42 to 1.64)	
Formula B (*n* = 15)	7.33	-3.81	12.5	-9.05	6.02[3]	(1.28 to 10.8)[3]	
Formula C (*n* = 16)	12.8	-9.39	8.07	-5.70	-5.72[3]	(-10.3 to -1.10)[3]	
P value (Treatment effect)	0.153		0.040[3]				
Number of colics per day							0.569
Formula A (*n* = 16)	3.31	-2.72	1.34	-1.33	-1.99[3]	(-3.02 to -0.95)[3]	
Formula B (*n* = 15)	4.42	-2.44	1.59	-1.61	-1.87[3]	(-2.96 to -0.79)[3]	
Formula C (*n* = 16)	2.79	-1.55	1.5	-1.18	-1.24[3]	(-2.30 to -0.19)[3]	
P value (Treatment effect)	0.868		0.735				

[1]Hard stools are indicative of constipation and were corresponding to the Bristol stool chart types 1 and 2; [2]Diarrheic defecations were corresponding to the Bristol stool chart types 5, 6 and 7; [3]Indicate statistical significant findings. Adjustment was made for the average volume of milk consumed by infants per day during the intervention period.

(*i.e.*, 841.3 mL) compared to infants fed with Formulas A (*i.e.*, 756.7 mL) and B (*i.e.*, 711.9 mL) in the present study as well as to infants in the study of Vivatvakin and Buachum (*i.e.*, 589.5 mL)[21] might provide an explanation for these differences. However, as the exact volume of breast milk consumed by infants in the present study could not be recorded or estimated, the reasoning provided above might not fully explain the observed weight gain in group C.

In studies examining the effect of other thickening agents instead of CBG on weight gain some mixed results were observed. Xinias *et al*[17] reported no significant differences in weight gain between the experimental and control groups after four weeks of intervention with cornstarch-thickened formulas. Furthermore, Chao and Vandenplas[22] reported no significant differences in body weight gain between the control and intervention groups during the first two weeks, but significantly higher increases at four and eight weeks of intervention compared to the control group. Similarly, in another study by Chao and Vandenplas[23], when rice-thickened formula was provided, significantly higher weight gains were observed at four and eight weeks of intervention for

the intervention compared to the control group.

Regarding changes observed in tolerance indices, the present study showed a significant increase in the number of diarrheic and total defecations from baseline to follow-up for the infant fed with Formula B. In contrast, no such unfavorable adverse effects were observed for infants fed with Formulas A and C, potentially indicating that this might be an adverse effect only of Formula B providing 0.45 g/100 mL of cold soluble CBG-galactomannans. This observation could further provide an explanation for the non-significant increase of body weight recorded for infants fed with Formula B, while body weight significantly increased among infants in the other two study groups. Of course the subjective assessment and recording of these indices by parents/caregivers might have also produced bias that needs to be considered when interpreting these findings.

The results of the present study should be interpreted under the light of its strengths and limitations. Regarding strengths, the inclusion of a "run-in" period in the study protocol and the measurement of reflux by pH-monitoring increase methodological integrity and decrease possible bias in data collection and results.

However, the use of fairly new pH-monitoring equipment can be considered as a limitation of the current study, since direct comparisons with previous studies and results/outcomes may not be feasible or appropriate. Furthermore, the absence of a control group can be considered as another limitation of the current study, since this might have limited the ability to have a more clear view on the effectiveness and tolerance of the three anti-reflux formulas under study. Lastly, although the number of infants examined in the present study was relatively small, the imputation of missing data as part of the ITT analysis resulted to a sufficient sample size and as such to adequate statistical power for the analyses. Nevertheless, future intervention studies with larger samples sizes should be implemented in order to shed more light on this field.

In conclusion, the present study showed that Formula A was more effective in decreasing esophageal acid exposure (as indicated by the Boix Ochoa Score), the total daily number of visible and measurable refluxes, as well as acid reflux related symptoms, while such changes were not observed for the infants fed with Formulas B and C. Furthermore, a significant increase of body weight was observed for infants fed with Formulas A and C while that was not observed for infants fed with Formula B, probably due to the increased number of diarrheic and total defecations recorded in this group. These findings indicate that Formula A seems to be more effective in reducing certain pH-monitoring indices of uncomplicated GER, increasing body weight and being well-tolerated by infants.

ACKNOWLEDGMENTS

COMMENTS

Background
Gastro-esophageal reflux (GER) is a common and global problem affecting about 50% of all babies up to the age of two months and has a peak incidence at the age of three months. Only some infants will develop pathologic gastro-esophageal reflux disease, in which clinical problems are related to excessive passage of acid gastric contents. Uncomplicated GER should be suspected in infants with uncomplicated recurrent regurgitation. In some cases GER may affect thrive because of caloric insufficiency and potentially lower dietary nutrients' intake which may lead to poor weight gain. The use of anti-reflux formulas with added thickening agents, such as carob-bean gum (CBG), can decrease the frequency and intensity of GER.

Research frontiers
Commercially available anti-reflux formulas currently contain 0.45 g/100 mL hot-soluble CBG galactomannans. With the exception of one study, there are no other randomized clinical trials available in the literature examining the effectiveness of anti-reflux formulas containing less than 0.45 g/100 mL hot-soluble CBG galactomannans on reflux and tolerance indices. In addition there are no reports examining the effectiveness of cold *vs* hot-soluble CBG galactomannans on reflux and tolerance indices.

Innovations and breakthroughs
The current study is the first to examine the effectiveness of formulas containing cold or hot soluble CBG galactomannans in different concentrations (*i.e.*, 0.45 g or 0.33 g/100 mL) on reflux indices assessed by 24 h pH impedance monitoring as well as on tolerance indices (*i.e.*, defecations and colic).

Applications
The formula containing 0.33 g/100 mL of cold-soluble CBG galactomannans was effective in reducing certain pH-monitoring indices of uncomplicated GER, increased body weight and was well-tolerated by infants.

Terminology
GER is defined as the involuntary passage of gastric contents into the esophagus and does not refer to any specific etiology with or without regurgitation and vomiting. The term regurgitation is specifically used if the reflux dribbles effortlessly out of the mouth.

Peer-review
In their work, the authors present a very clear and well conducted, controlled randomized study analyzing the effects of three different anti-reflux formulas for infants with GER (excluding complicated cases). The study include not too many, but a sufficient number of patients, it was performed for a relatively short period of time, but probably just sufficient. It is well described, and the results are conclusive and helpful.

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CASE REPORT

Tourette syndrome associated with attention deficit hyperactivity disorder: The impact of tics and psychopharmacological treatment options

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Abstract

Tourette syndrome (TS) is a neurodevelopmental disorder characterized by multiple chronic motor and vocal tics beginning in childhood. Several studies describe the association between TS and attention deficit hyperactivity disorder (ADHD). Fifty percent of children diagnosed with ADHD have comorbid tic disorder. ADHD related symptoms have been reported in 35% to 90% of children with TS. Since ADHD is the most prevalent comorbid condition with TS and those with concomitant TS and ADHD present with considerable psychosocial and behavioral impairments, it is essential for clinicians to be familiar with these diagnoses and their management. This paper highlights the association between treating ADHD with stimulants and the development of tic disorders. The two cases discussed underscore the fact that children with TS may present with ADHD symptomatology prior to the appearance of any TS related symptoms. Appropriate management of TS in a patient diagnosed with ADHD can lead to quality of life improvements and a reduction in psychosocial impairments.

Key words: Tourette syndrome; Psychopharmacology; Attention deficit hyperactivity disorder; Tics

Core tip: Tics can be a symptom of Tourette syndrome

(TS). Attention deficit hyperactivity disorder (ADHD) has the highest comorbidity with TS. Psychopharmacological treatment of ADHD with stimulants may cause, or exacerbate pre-existing, tics. Because of this, providers may be reluctant to use stimulants in patients with comorbid tic disorders. However, the role of stimulants in the treatment of TS associated with ADHD, when the benefits outweigh the risks, cannot be over emphasized as a comprehensive approach, considering all treatment options for managing TS and ADHD will yield better outcomes.

INTRODUCTION

Tourette syndrome (TS) is a neurodevelopmental disorder characterized by multiple chronic motor and vocal tics developing before adulthood[1]. Tics are sudden, rapid, recurrent, non-rhythmic, stereotyped and involuntary motor movements or vocalizations. They are classified as either simple or complex, with the former affecting several muscle groups. Motor tics can affect any part of the body with varying location, frequency and complexity of movements which may change over time. Vocal tics, also known as phonic tics, are involuntary sounds produced by air motion through the nose, mouth, or throat[1,2]. See Table 1 for types and description of tics[1,3].

Although the American Psychiatric Association Diagnostic and Statistical Manual, Fifth Edition (DSM-5) diagnostic criteria for TS necessitate the presence of both multiple motor and one or more vocal tics, they need not occur concurrently. The tics may wax and wane in frequency and must have lasted for more than one year since first onset. Substance induced etiologies, for example cocaine use, and general medical conditions such as Huntington's disease or post viral encephalitis, are exclusionary criteria[1].

The Tourette Syndrome Classification Study Group defined TS as the presence of motor and vocal tics with frequent tics almost daily for at least one year, with an onset before age 21. In addition, the symptoms should be observed by an examiner[3]. See Table 2 for definitions and classification of tic disorders.

The prevalence of tic disorders in the classroom is between 5% and 20% of children, with impairments occurring in 1-10 per 1000[4-6]. Tic disorders have comorbidity with other disorders including obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD), learning difficulties and sleep abnormalities[2,5,6]. Leckman[7] (2002), reported the average age of onset

of TS symptoms as seven years with a range from three to eight years. However, the DSM-5 gives the average tic onset age as between four and six years with peak severity being between 10 and 12 years[1]. See Figure 1 for clinical course of TS.

ADHD is the most common childhood psychiatric disorder with an estimated prevalence of 2% to 15%[8]. The onset of ADHD symptoms is usually between four and five years of age, thus generally appearing before the onset of tics[9]. ADHD related symptoms have been reported in 35% to 90% of children with TS[2,4,5,10]. TS with concomitant ADHD can cause significant psychosocial and behavioral impairments[11]. We present two cases of adolescent TS associated with ADHD and hypothesize that stimulant medications did not exacerbate the former disorder in these patients.

CASE REPORT

Case 1

An 8-year-old boy was referred to the child psychiatric clinic by his school due to his use of inappropriate language. He was diagnosed with ADHD at 4 years of age. At age six he was treated with stimulants, including methylphenidate and mixed amphetamine salts. Significant improvement of his ADHD symptoms occurred with immediate release mixed amphetamine salts, but the medication was discontinued due to excessive blinking and bilateral hand tics. Of note, the child's maternal uncle had a history of TS. The stimulant was discontinued and, 4 wk later, the tics subsided. However the blinking continued intermittently. As a result, child neurology was consulted and clonidine 0.1 mg at night was prescribed to manage the ADHD and insomnia. On this regimen the boy's parents noted a lessening of his hyperactivity and impulsivity, but a continued poor attention span. The child started using profanity at school, which led to his being bullied as well as his eventual suspension from school. Both the neurologist and the child psychiatrist agreed to re-evaluate the child for possible TS. The neurological examination was essentially normal. His EKG and routine blood tests, including a CBC, CMP and a lead level, were non-significant. Based on his history and clinical examination, a diagnosis of TS was made. He was started on immediate-release dexmethylphenidate 5 mg twice daily, gradually increased after 4 wk to 10 mg twice daily for the ADHD related symptoms. His regimen was later simplified by changing to a morning dose of 25 mg of the extended-release form. The core TS symptoms were treated with risperidone 0.125 mg twice daily, increased after 6 wk to a maintenance dose of 0.5 mg twice daily. The severity of his tics was monitored using the Yale Global Tic Severity Scale (YGTSS). After 6 mo of treatment, the boy's vocal tics completely subsided. He continued to have intermittent blinking, but with a significant reduction in frequency from once every few minutes to less than 10 times per day.
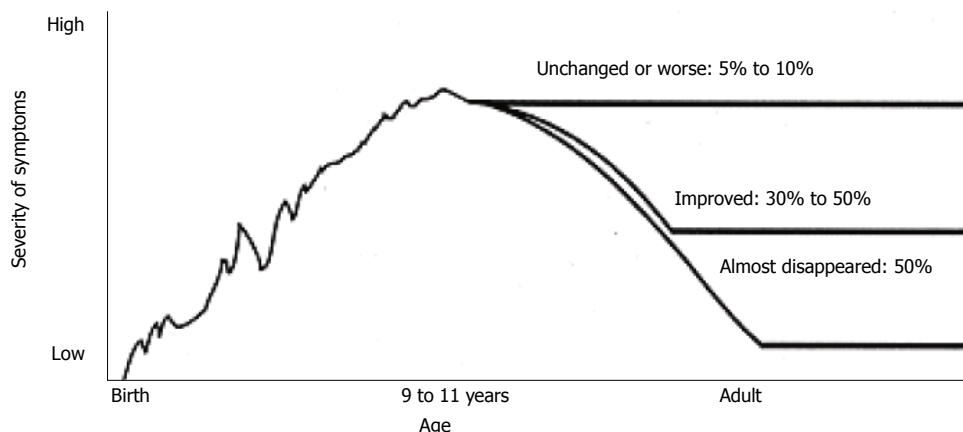
Figure 1 Onset of Tourette syndrome is before 7 years of age. It is usually recognized 2-3 years after onset. TS peak severity is at 9-11 years of age. Approximately 5%-10% have intensifying course. Approximately 85% experience a reduction of symptoms during and after adolescence[9]. TS: Tourette syndrome.

Table 1	Types and description of tics	
	Simple	**Complex**
Vocal or Phonic	Simple phonic/vocal tics: These are sudden meaningless noises or sounds Examples: Throat clearing, coughing, spitting, barking, hissing, sucking, clacking, gurgling, sniffing, grunting	Complex phonic/vocal tics: These are sudden and more meaningful words, syllables or phrases Examples: Echolalia (repeating words or phrases spoken by others), palilalia (rapid repetition of one's own words or phrases), and coprolalia (compulsive utterance of obscene words or phrases) Coprolalia is not pathognomonic of tourette syndrome. In fact less than 10% of tourette syndrome patients exhibit coprolalia. Hence, coprolalia is not required in diagnosing tourette syndrome
Motor	Simple motor tics: Rapid, meaningless contractions of one or a few muscles Examples: Eye blinking, shoulder shrugging, head jerking, hand clapping, neck stretching, mouth movements, head, arm or leg jerks, and facial grimacing	Complex motor tics: Less common, typically more purposeful movements with a slower and longer nature. The movements appear more coordinated and may involve a cluster of movements Examples: Facial gestures, dystonic postures, throwing, arm thrusting, touching objects or people, stereotyped imitation of the movements (echopraxia) and obscene gestures (copropraxia)

Table 2	Definitions and classification of tic disorders
Classification of tic syndromes/tourette's disorder. Tourette Syndrome Classification Study Group. Tourette Syndrome Criteria: (TSCSG 1993) A Both multiple motor and one or more vocal tics have been present at some time during the illness, although not necessarily concurrently B The tics occur many times a day, nearly every day, or intermittently throughout a period of more than a year C The anatomic location, number, frequency, complexity, type, severity of tics changes over time D Onset before age 21 E Involuntary movements and noises cannot be explained by other medical conditions F Motor and/or vocal tics must be witnessed by a reliable examiner directly at some point in the illness or be recorded by videotape or cinematography	

Case 2

A 10-year-old boy with a 4 year history of ADHD combined type, as well as a learning disorder, experienced an initial good response to extended release methylphenidate. However, after 8 mo of management with this formulation at 36 mg each morning, his parents discontinued the medication due to worsening bilateral eye blinking. The methylphenidate was replaced with 10 mg of atomoxetine daily, with a gradual increase over a 2 mo period to 100 mg daily. After 2 mo on this regimen, the boy's ADHD symptoms worsened, the frequency of blinking increased and he experienced vocal tics. During re-evaluation, the

child reported a history of "cursing" people for more than 2 years. He could avoid directing profanity at others by retreating to the restroom to curse. After developing a good alliance with his psychiatrist, he reported having a secret "dictionary," which was coprographic in nature, listed profane words beginning with A through Z, and included obscene drawings. The boy regularly read the dictionary to decrease the urge to directly curse at others. After a comprehensive evaluation, he was diagnosed with TS and comorbid ADHD. The ADHD symptoms were well controlled on 15 mg of immediate-release dexmethylphenidate in the morning and 10 mg in the afternoon. Clonidine

Table 3 Common differential diagnoses of tics
Stroke
Dystonia
PANDAS
Encephalitis
Head trauma
Epileptic seizures
Sydenham's chorea
Carbon monoxide poisoning
Functional movement disorders in children
Chromosomal disorders such as Down syndrome and Fragile X syndrome
Genetic conditions (such as Huntington's disease, Wilson's disease and Tuberous sclerosis)
Stereotypy (in developmental disorders such as Autism spectrum disorders and Stereotypic movement disorder)
Medication-induced tics (*i.e.*, Neuroleptics, Stimulants, Antiepileptics, Lithium)

PANDAS: Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections.

0.05 mg at night was added to treat his TS symptoms. He experienced drowsiness which subsided after 3 wk and within 2 mo clonidine was increased to 0.1 mg twice daily. The boy's ADHD and TS symptoms significantly subsided on the combination of dexmethylphenidate and clonidine.

DISCUSSION

TS affects people of all racial and ethnic groups. The Centers for Disease Control and Prevention (CDC) reports that the disease is more likely among non-Hispanic white people than among Hispanic or African American individuals[12]. There is also a predilection in males three times that of females. In the United States, an estimated 3 in 1000 children between 6 and 17 years of age have TS, with an incidence twice as high among 12-17 year olds *vs* 6-11 year olds[12]. The prevalence of the disorder varies between 0.4% and 3.8% internationally, with a lower rate in sub-Saharan Black Africans[6,13-15]. In the United Kingdom the prevalence is between 0.46% and 1.85% for those between the ages of 5 and 18, with an average prevalence of 1%[5,13].

Multifactorial pathogeneses have been attributed to TS. There is a prevalence of 5% to 15% in first-degree relatives of those with TS. A higher ratio of concordance in monozygotic twins as compared to dizygotic twin pairs exists[5,6]. The mode of inheritance is believed to operate mainly through a dominant gene. Boys with the gene(s) are three or more times likely than girls to manifest TS symptoms[12]. Longitudinal studies show some evidence that gender and stress-related hormonal factors are entwined in the disorder's pathogenesis[16]. There is also speculation about the role of gonadal androgens during the very early stages of central nervous system development *in utero*. Some clinical trials support the view that a change in the hormonal contexture during adolescence and adulthood affects tic severity[17]. In addition, monoamine neurotransmission has been

implicated in the neurobiology of TS. Positron emission tomography and single-photon emission computer tomography studies suggest an abnormal regulation of dopamine production and metabolism in TS leading to higher dopamine levels[5], and lower levels of serotonin and glutamate have been found in such individuals[5]. Brain findings are usually normal in these patients. However, in a subpopulation, brain magnetic resonance imaging (MRI) scan studies have demonstrated an increased number of subcortical hyperintensities and reduced neuronal activity in the basal ganglia. Increased brain activity in the prefrontal, parietal, temporal, and cingulate regions has also been reported[2,5,6]. Volumetric imaging studies have demonstrated smaller caudate volumes[2,5,6,9,14,15]. Furthermore, children with TS tend to have smaller corpus callosum, while adults with TS have larger corpus callosums as compared to the normal population[2,5,6,9,14,15]. The TS subgroups with ADHD comorbidity appear to have increased left amygdala volume as compared to those without comorbidity[18,19]. Other implicated etiological factors of TS include intrauterine exposure to alcohol and cigarette smoke, a complicated birth, and low birth weight[2,14,15]. Possible autoimmune causes have also been considered based on studies linking TS and exposure to group A β-hemolytic streptococcal infections (GABHS) complicated by Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections (PANDAS)[7,12].

A TS diagnosis is based on clinical history and examination. When clinically indicated, routine laboratory and radiological investigations should be considered to rule out other causes of tic disorders. See Table 3 for common differential diagnoses of TS[1,3,6,9,12,13,20]. In order to document the quality and quantity of tics, some experts recommend video-recording by parents and teachers[2]. A standardized rating scale such as the YGTSS may be useful for diagnosing and monitoring treatment response[5,21]. Although brain MRI scans will likely be normal in TS, brain imaging is indicated in those suspected of having neuroinflammatory/degenerative conditions, for example Sydenham's chorea. In addition, DNA testing should be considered in individuals with family history of Huntington's chorea (especially DNA microarray technology)[2,5,6]. Heavy metal toxicities should be considered, including lead, as well as serum copper and ceruloplasmin if Wilson's disease is suspected[5,6,14,15]. Electroencephalography (EEG) may be useful when myoclonic epilepsy is suspected[2,14,15]. A throat swab should be considered in patients who have history of pharyngitis to rule out GABHS and PANDAS[2,5,6,9,12,14,15].

The CDC has conducted a number of studies examining comorbidities in TS. Children and adolescents with TS have higher risk of comorbid learning, behavioral, and social problems. Approximately, 79% of children with TS have at least one comorbid mental health, behavioral or developmental condition[12,22]. Among children with TS, 64% have ADHD, 43% have behavioral problems such as oppositional defiant disorder or conduct disorder, 40% have anxiety, 36% suffer from depression, and 28% are
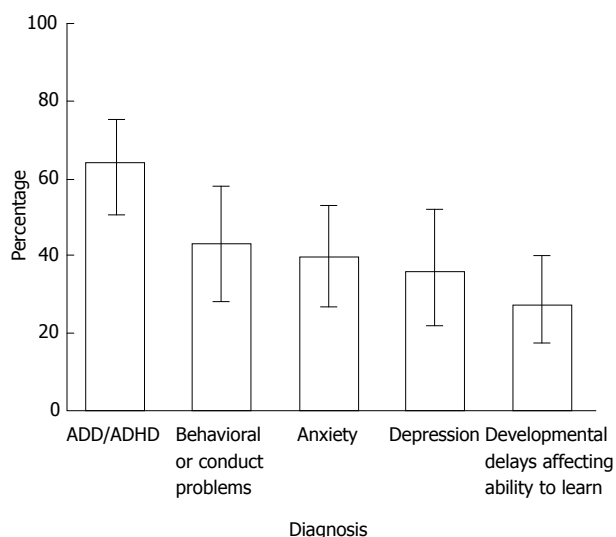
Figure 2 Adapted with permission from CDC. Prevalence of selected diagnoses among persons aged 6-17 years with tourette syndrome. ADD: Attention deficit disorder; ADHD: Attention deficit hyperactivity disorder.

developmentally delayed[22]. See Figure 2 for prevalence of comorbid diagnoses among 6 to 17 year olds with TS[22]. Other clinical symptoms associated with TS include stuttering, aggressive and antisocial behavior, impulsivity, exhibitionism, sleep disturbances and self-injurious behaviors[5]. Since ADHD is the highest comorbid condition with TS, it is essential for clinicians to be familiar with the diagnosis and management of these conditions.

In recent years, there has been considerable research on the psychopharmacology of TS associated with ADHD. The psychopharmacological management of children with TS associated with ADHD should be tailored towards the clinical presentation and severity of the illness[11,23]. Leckman *et al*[20] 2002, suggested prioritization of pharmacological interventions based on the degree of distress and impairment. Since the natural course of tics is either short-term intermittent episodes or long-term waxing and waning of symptoms, medication options should be tailored to minimize side effects while maximizing treatment benefits[2,20]. Pharmacological management of TS associated with ADHD is similar to other childhood psychiatric disorders in that few of the medications commonly utilized are FDA approved[24].

Several studies have demonstrated the effectiveness of stimulants in alleviating ADHD symptoms. Contrary to previous notions that stimulants can worsen tics in TS, some studies have shown contrary results. A critical review of the literature reported that group data analysis showed no significant increase in tics when stimulants are used in patients with tics as compared with controls[10]. This conclusion was also supported by the Tourette Syndrome Study Group in a multicenter, randomized, double-blind 16 wk clinical trial in which 136 children with ADHD and a chronic tic disorder were randomly administered clonidine, methylphenidate, combined clonidine and methylphenidate, or placebo. The group concluded that the combination of methylphenidate

and clonidine is effective for ADHD in children with comorbid tics and that prior recommendations to avoid methylphenidate because of concerns of worsening tics are unsupported by the trial[25].

Studies have shown that the motor and behavioral symptoms associated with TS respond well to most typical and atypical antipsychotic medications. The typical antipsychotic drugs, such as haloperidol, fluphenazine and pimozide, with a high tendency to block postsynaptic dopamine (D2 receptors) are the treatment of choice due to their greater effectiveness[2,22,26]. However, typical antipsychotics remain a second-line treatment option because of side effects including extrapyramidal side-effects (EPSE) and tardive dyskinesia (TD) for haloperidol, and cardiotoxicity for pimozide[22,26]. Other side-effects include sedation, orthostatic hypotension, dry eyes and mouth, urinary retention and confusion. Neuroleptic malignant syndrome (NMS) is a rare but serious adverse-event characterized by lead pipe rigidity, autonomic instability, increased heart rate, fever, and rhabdomyolysis.

The atypical neuroleptic medications such as risperidone, ziprasidone, olanzapine, aripiprazole, and quetiapine, which selectively block postsynaptic dopamine D2 receptors, have had some encouraging outcomes in the treatment of tics associated with TS[2,22,23,26,27]. Close monitoring when using these medications is essential especially in pediatric populations with higher risks of developing metabolic syndrome and EPSE. The mechanisms of action of atypical neuroleptic drugs are different from conventional medications in that atypicals have greater affinity for serotonin receptors (especially 5-HT2A) than D2 receptors, thus generally causing minimal EPSE, milder increases in prolactin and lesser tendencies to induce TD[28,29]. Risperidone is associated with orthostasis, and more EPSE than other atypical agents. It also causes significant hyperprolactinemia which in turn can cause adverse events including gynecomastia in boys. Prolactin monitoring has been recommended in those treated with risperidone. Olanzapine is associated with higher incidences of sedation and metabolic syndrome. Quetiapine can lead to sedation and anticholinergic side effects, while ziprasidone can cause dry ejaculation and a prolonged Q-T interval[2,22,23,26-29].

Clonidine and guanfacine are sympatholytic agents that lower blood pressure and heart rate. Their mechanisms of action involve alpha-2A adrenoceptor selective agonists. The use of this group of medications for TS is supported by a few controlled studies[22,26]. Clonidine in divided daily doses, ranging from 0.1 to 0.3 mg, has been associated with favorable outcomes in pediatric populations[2,22,23,26,30]. Also, guanfacine in divided daily doses of 0.5 to 3 mg has been recommended for milder TS[2,22,23,26]. Associated adverse-effects of clonidine and guanfacine include sedation, dry mouth, headache, postural hypotension and dizziness, and sudden discontinuation can induce a hypertensive crisis. It is essential to monitor blood pressure and heart rate and obtain a baseline EKG when using clonidine or guanfacine[23,31,32]. Sudden discontinuation, especially of clonidine, should be avoided due to risk of

rebound hypertension.

The data are mixed concerning the use of antiepileptic drugs such as levetiracetam and topiramate in the treatment of TS[22,33]. Other reported alternative pharmacological treatments of tics in TS include tetrabenazine, ropinirole, botulinum toxin, baclofen and clonazepam but the evidence is limited[2,22,23,26,34].

Since this paper focuses on the psychopharmacological management of TS and ADHD, psychological interventions are not addressed in detail. There are evidence-based non-pharmacological treatment options for TS associated with ADHD[35,36]. With regard to non-psychopharmacological management of tics, studies have revealed better outcomes from habit reversal therapy and, exposure and response prevention strategies[35,36]. In addition, treating OCD symptoms associated with TS may potentially reduce tics and ADHD symptoms[26,36,37]. Selective serotonin re-uptake inhibitor (SSRI) antidepressants are the preferred pharmacological treatment for OCD. Multiple studies support the role of cognitive behavioral therapy (CBT) for behavioral management of OCD in TS[26,36-38].

Recent studies have revealed promising outcomes from transcranial magnetic stimulation (TMS)[39,40] and deep brain stimulation surgery[41-43] in the treatment of medication resistant tics associated with TS.

In conclusion, ADHD has a high correlation with TS and patients with ADHD are more likely to develop tic disorders, with or without treatment with stimulants. Tics which appear during the treatment of ADHD with stimulants may be due to a naturally developing tic disorder in which the tics have the usual waxing and waning pattern of occurrence, intensity, and frequency and may have developed even without the use of stimulants. The decision to use, or to continue to use, stimulants must be made on a case-to-case basis. Overall the treatment of ADHD with appropriate psychopharmacological agents, including stimulants, is suggested if the treatment benefits outweigh the potential medication risks.

Medication management of TS associated with ADHD is quite variable. The choice of appropriate pharmacological agent(s) should depend on the severity of the impairments. Starting with a non-stimulant agent such as guanfacine or clonidine is recommended, as they are effective in alleviating both symptoms of TS and ADHD. The newer long acting preparations of guanfacine approved for ADHD may be the most reasonable agents to consider first. If the TS is not severe, but the ADHD is disabling, the option of treating the ADHD with a stimulant should be considered. The course and severity of tics should be monitored with a reliable TS rating scale, such as the YGTSS. It is also recommended that, in order to prevent any future debilitating disease process, careful history taking be done in diagnosing and managing of symptoms of TS associated with ADHD.

ACKNOWLEDGMENTS

COMMENTS

Case characteristics
Two boys, ages 8 and 10 years old, with Attention deficit hyperactivity disorder (ADHD) are treated with stimulants and experience the emergence of tics, later diagnosed as tourette syndrome (TS).

Clinical diagnosis
Case 1: Inappropriate language/profanity, vocal tics and inattention, impulsivity and hyperactivity. Case 2: Bilateral blinking, vocal tics, profanity, impulsivity and hyperactivity.

Differential diagnoses
With regard to the etiology of tics, Stroke, Dystonia, Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections, Encephalitis, Head trauma, Epileptic seizures, Sydenham's chorea, Carbon monoxide poisoning, Functional movement disorders, Chromosomal and Genetic disorders, Stereotypy and Medication-induced tics should be considered.

Laboratory diagnosis
All laboratory studies were within normal limits.

Pathological diagnoses
Both cases were examples of TS associated with ADHD.

Treatment
In both cases stimulants were used to treat ADHD, however the TS was treated with clonidine in the first case and risperidone in the second.

Related reports
Tics can present as an adverse effect of stimulants. However, given the high comorbidity of ADHD with TS, emerging tics can be symptom of TS, which typically manifests temporally later than ADHD. In the cases presented, both boys were changed to non-stimulant medications after developing tics, which led to suboptimal treatment of their ADHD symptoms. The second child actually experienced a worsening of TS symptoms on the non-stimulant medication. Despite providers' reluctance to prescribe stimulants due to fear of causing tics or worsening pre-existing tics, evidence has shown that stimulants can be beneficial in the treatment of TS associated with ADHD. In fact, using stimulants may reduce the severity of the tics and improve the quality of life of patients with these disorders.

Term explanation
TS is a neurodevelopmental disorder characterized by multiple chronic motor and vocal tics developing before adulthood. ADHD, also a neurodevelopmental disorder, presents with a persistent or on-going pattern of inattention and/or hyperactivity and impulsivity, which interferes with typical development and quality of life. ADHD is associated with difficulties in achieving and sustaining attention, and with executive function and working memory.

Experiences and lessons
There is a misperception that stimulants are contraindicated in patients with tic disorders due to the potential to cause or exacerbate tics, however, the role of stimulants in the treatment of TS, when the benefits outweigh the risks, cannot be over emphasized.

Peer-review
The manuscript is an informative review regarding the diagnosis and management of Tourette syndrome associated with ADHD. It is deemed to be of academic value and worthy of publishing.

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CASE REPORT

Acute lobar nephritis in children: Not so easy to recognize and manage

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Abstract

Acute lobar nephritis (ALN) is a localized non-liquefactive inflammatory renal bacterial infection, which typically involves one or more lobes. ALN is considered to be a midpoint in the spectrum of upper urinary tract infection, a spectrum ranging from uncomplicated pyelonephritis to intrarenal abscess. This condition may be difficult to recognize due to the lack of specific symptoms and laboratory findings. Therefore the disease is probably underdiagnosed. Computed tomography scanning represents the diagnostic gold standard for ALN, but magnetic resonance imagine could be considered in order to limit irradiation. The diagnosis is relevant since initial intravenous antibiotic therapy and overall length of treatment should not be shorter than 3 wk. We review the literature and analyze the ALN clinical presentation starting from four cases with the aim to give to the clinicians the elements to suspect and recognize the ALN in children.

Key words: Acute lobar nephritis; Children; Computed tomography; Magnetic resonance imagine; Upper urinary tract infection

Core tip: Acute lobar nephritis (ALN) is a renal bacterial infection presenting difficult diagnosis due to the lack of specific symptoms and laboratory findings. Suspecting ALN in children with septic fever with or without clinical signs should be part of the diagnostic tool of clinicians. The diagnosis is relevant both to prefer intravenous antibiotic therapy and suggest an overall length of antibiotic treatment not shorter than 3 wk. We review the literature and analyze the ALN clinical presentation, with the aim to give to the clinician the elements to suspect, diagnose and accurately treat ALN in children.

INTRODUCTION

Acute lobar nephritis (ALN), also known as acute focal bacterial nephritis, is a localized non-liquefactive inflammatory renal bacterial infection, which typically involves one or more lobes[1,2]. It presents as an inflammatory mass without frank abscess formation[3]. ALN is considered to be a midpoint in the spectrum of upper urinary tract infection (UTI), a spectrum ranging from uncomplicated pyelonephritis to intrarenal abscess[4]. According to some authors[5], this spectrum lacks a dynamic progressive nature and two patterns, based on computed tomography (CT) findings, are described: Simple ALN, which represents progression of acute pyelonephritis (APN), and complicated ALN, which may progress into renal abscess without or even with treatment[6]. The typical presentation of ALN shares some common clinical and laboratory features with both renal abscess and APN including septic fever, flank pain, sick appearance, nausea or vomiting, elevation of inflammatory markers, pyuria and bacteriuria[1,7]. According to a recent study patients with ALN are febrile for longer after hospitalization and have more nausea/vomiting symptoms than those with APN[8]. A timely diagnosis is relevant because under-diagnosis may result in late renal scarring, and/or evolution in renal abscess, which in turn may lead to hypertension or renal failure[9].

CASE REPORT

Case 1

A 4-year-old girl was admitted with a 2 d history of high fever and abdominal pain. Fever persisted despite an oral amoxicillin-clavulanate treatment prescribed by her family physician.

The patient's medical history was remarkable for an episode of APN 2 years before, successfully treated with oral antibiotics. At admission she was febrile and looked sick with an unremarkable physical examination. White blood cell count (WBC) was 24.600/mm^3, C-reactive protein (CRP) 24.52 mg/dL (normal range 0-5 mg/dL) and erythrocyte sedimentation rate (ESR) 97 mm/h (normal range 0-20 mm/h). Urinalysis revealed leukocyturia (200 WBC/mm^3 at standard optical microscopy) without bacteriuria. Urine cultures were repeatedly negative as was chest X-ray. Abdominal ultrasonography (US) showed a nonspecific diffuse increased echogenicity of the right kidney, which appeared smaller than the left; no focal masses were detected on both kidneys. A CT scan showed multiple lesions with irregular margins and variable size in the right kidney, which appeared hypodense after contrast medium administration (Figure 1). ALN was diagnosed and a three weeks intravenous antimicrobial therapy with ciprofloxacin and tobramycin was started with clinical improvement. Voiding cystourethrography (VCUG) and dimercaptosuccinic acid (DMSA) renal scintigraphy performed 6 mo later showed a reflux with associated renal scarring nephropathy.
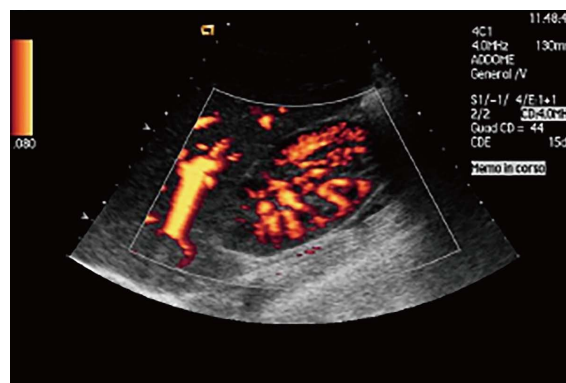
Case 2

A 13-year-old boy was admitted after 3 episodes of high fever in the last 2 mo, without an obvious focus. During the first episode, the boy was confused and agitated; CT and magnetic resonance imagine (MRI) of the brain, cerebrospinal fluid (CSF) examination, electroencephalography (EEG), chest X-rays, urinalysis with standard optical microscopy, urine culture were normal; blood test showed increased CRP (10.8 mg/dL). Empirical therapy with acyclovir and ceftriaxone was started with clinical success, maintained for 5 d and switched to oral treatment for 5 more days. The boy was discharged with a diagnosis of suspected encephalitis. After 2 wk of wellness, the patient presented fever, vomiting and drowsiness. MRI of the brain was normal. Chest X-ray and echocardiogram were also normal. CRP was 13.9 mg/dL. Abdominal US was negative. Empirical treatment with ceftriaxone was started with rapid improvement. The boy was discharged after 5 d of intravenous therapy; at home he continued with 3 d oral amoxicillin-clavulanate. Four days after the end of treatment, he was admitted for the third time with fever and vomiting. On examination, he was in good clinical condition. The abdomen was soft, slightly painful in the right flank. CRP was 10.4 mg/dL. Urinalysis with optical microscopy, chest X-rays and abdominal US were negative. An abdominal CT scan was performed. The scan (Figure 2) revealed multiple poorly defined hypodense areas after contrast medium administration in the right kidney, compatible with ALN. One of these areas presented a 5 mm colliquative part in the middle, attributable to an abscess. An abdominal US was repeated (Figure 3) with power-doppler analysis showing at least two hypoperfused areas at the right kidney. The patient's medical history was unremarkable; no previous UTIs were reported. Intravenous antimicrobial therapy with tobramycin and teicoplanin was started and maintained for 3 wk, followed by oral amoxicillin-clavulanate for other 3 wk.
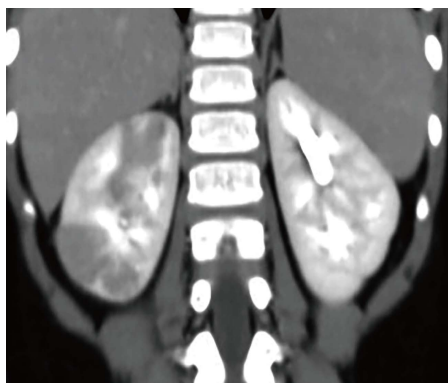
Figure 1 Case 1: Abdominal computed tomography. Computed tomography scan of the abdomen after intravenous contrast injection reveals multiple wedge-shaped hypo dense non enhanced lesions in the right kidney, more visible in the upper and lower pole.
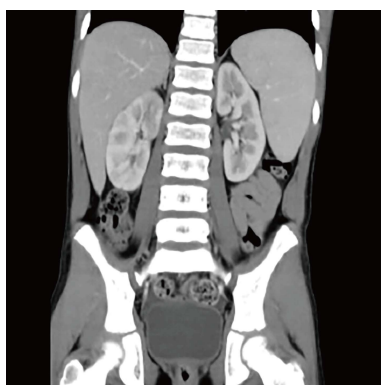
Figure 2 Case 2: Abdominal computed tomography. Abdominal computed tomography after contrast medium administration, showing a notable hypodense area in the upper pole of right kidney.

Figure 3 Case 2: Abdominal ultrasound. Abdominal ultrasonography with power-doppler analysis shows two hypoperfused areas at the upper pole of right kidney.
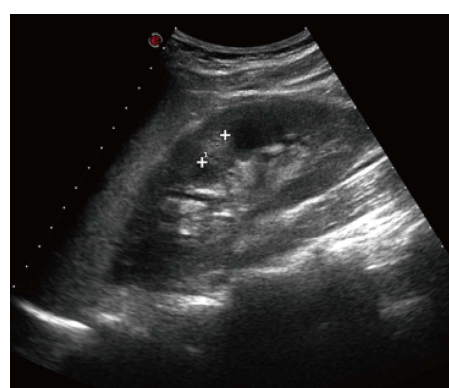
Figure 4 Case 3: Abdominal ultrasonography. Abdominal ultrasonography shows an increased volume of the left kidney with a small hyperechoic mass (1 cm diameter) in the middle zone of the kidney.

Case 3

A 17-year-old girl was admitted with a 2 d history of high fever, chills and pain on the left flank. On physical examination, she looked sick; the abdomen was soft and painful in the left flank. WBC count was normal (8340/mm^3), while there was a marked increase of both CRP (25.33 mg/dL) and ESR (62 mm/h). Urinalysis revealed leukocyturia (50 WBC/mm^3 at standard optical microscopy) but no bacteriuria. Urine and blood cultures were repeatedly negative. Abdominal US (Figure 4) showed an increased volume of the left kidney with a small hyperechoic mass (1 cm diameter), consistent with ALN. Intravenous therapy with ceftriaxone and tobramycin was started; after 48 h the patient was still febrile and sick, therefore treatment was switched to intravenous ciprofloxacin, teicoplanin and cefotaxime. There was a slow and gradual clinical improvement and after 72 h, the fever disappeared. Intravenous antimicrobial therapy was maintained for three weeks.

Case 4

A 6-year-old girl presented with a 24 h history of high fever, vomiting and abdominal pain on the left iliac fossa without diarrhea. Physical examination was unremarkable

except for an abdominal evoked pain in the left iliac fossa. WBC was 21.190/mm^3, CRP 34 mg/dL. Urinalysis with standard optical microscopy was negative and therefore urine cultures were not performed. Chest X-ray was normal. Abdominal US showed an increased volume of both kidneys; CT scan was then performed revealing multiple wedge-shaped cortical hypodense lesions in both kidneys, more represented in the left one (Figure 5). Intravenous therapy with ceftriaxone was started; after 48 h, the patient was still febrile and sick, so intravenous netilmicin was added with clinical improvement. The therapy was continued for 10 d, then switched to oral ciprofloxacin, for additional 2 wk.

DISCUSSION

When should ALN be suspected?

ALN may be difficult to recognize due to the frequent absence of specific signs and symptoms and the wide differential diagnosis (Table 1). Specific symptoms as flank pain or laboratory findings (positive urinalysis and bacteriuria) may be absent. Therefore the disease is probably underdiagnosed[10]. Nevertheless, fever with septic features (sick appearance, malaise, chills, and nausea or vomiting), increased inflammatory indexes

Table 1 Clinical and laboratoristic acute lobar nephritis differential diagnosis	
	Clinical and laboratoristic ALN differential diagnosis
APN	Leukocyturia and bacteriuria
Appendicitis[26]	Mc Burney, Blumberg and Rovsing's sign, right iliac fossa pain, typical age
Gastroenteritis[27]	Diarrhea, dehydration's signs
Infected urachal cyst[28]	Belly button discharge
Nephrolithiasis[29]	Colic pain, familiarity, previous episode, micro/macrohematuria, Giordano's sign
Pancreatitis[30]	Typical pain, serum amylase and lipase elevated
Pelvic inflammatory disease[31]	Sexually active female, irregular periods, vaginal discharge, dyspareunia, lower abdomen pain
Pneumonia[32]	Dyspnea, cough, typical auscultation, Sat O_2 < 96%
Sickle cell disease[33]	Anemia, decreased haptoglobin, sickle cell, ethnicity

Differential diagnosis with other conditions such as abdominal abscess, infected intestinal duplication and nephrolithiasis need radiological evaluation. ALN: Acute lobar nephritis; APN: Acute pyelonephritis.

Figure 5   Case 4: Abdominal computed tomography. Computed tomography scan of the abdomen after intravenous contrast injection reveals multiple wedge-shaped cortical hypodense lesions in the both kidneys, more represented in the left one.

and/or abdominal pain, should suggest a deep bacterial infection. The diagnosis of this condition is relevant due to the need of a specific therapeutic approach.

How to confirm the ALN diagnostic suspect?

Sonographically, ALN presents as focal hypoperfused lesions with poorly defined irregular margins disrupting cortico-medullary differentiation. Masses can be respectively hyper-, iso-, or hypo-echogenic depending on the temporal sequence of the lesions and resolution of the disease[11,12]. Renal pole swelling has also been reported[11,13,14]. Although renal US is an effective diagnostic method, there may be false positive and false negative results[13,15], and often a false negative US is frequently reported. A study[16] demonstrated that isolated severe nephromegaly (defined as renal length of greater than mean +3 SD for age) has a diagnostic sensitivity of 90%; the finding of a focal renal mass increases the sensitivity to 95% (compared with the gold standard CT), with a specificity of 86.4%. The diagnostic gold standard for ALN is CT scanning[11,13,15,17]. CT images of the involved areas are usually normal in non-enhanced scans but appear as wedge-shaped, poorly defined regions of decreased nephrogenic density after contrast medium administration[11,13]. With the aim to avoid radiation exposure, MRI should be strongly considered[18];

in fact CT scan results in a small but not negligible increased lifetime risk for cancer[19,20]. Enhanced ultrasound in the near future could replace CT scan because of comparable sensitivity and specificity. Unfortunately second-generation contrast agents are off-label in children, even if there are no adverse events documented in literature[21]. Static scintigraphy with DMSA, the gold standard to identify renal involvement in UTI, has no application in the differential diagnosis of this condition showing only focal uptake defects with the means of a hypoactive area in the renal parenchyma. In the literature, positive urine culture rate has a wide variability; the bias probably depends on the ability of different centers to diagnose ALN, particularly when urinalysis and urine are negative. When positive, results are not different from other forms of UTI: The most represented urinary pathogen is *Escherichia coli* in 40%-75% of the cases, while other gram negative germs are less frequent; gram positive germs infection is unusual[8,10,22].

Treatment

Treatment is based on antibiotic therapy. Empiric approach, before antibiogram response when available, should be targeted at gram negative germs. Intravenous administration is recommended at least until 2 to 3 d after defervescence with a possible switch to oral treatment. A study suggested that a 3 wk antimicrobial therapy protocol should constitute the treatment of choice for all radiographically documented patients with ALN[22]. Surgical intervention is needed in the 25% of the cases where the lesion turns to renal abscess[23].

Integrating literature evidence and daily clinical practice: The diagnostic clues

These 4 cases presented with high fever, toxic appearance and abdominal pain. Only 1 case presented with flank pain, a specific UTI symptom not reliable before age of 6-8 years. Furthermore, as a confounding factor, urinalysis showed leukocyturia only in 2 cases, both with no bacteriuria at microscopy and negative urine cultures (one of these children started antibiotic treatment prior to admission). The other 2 patients presented negative microscopic and dip stix urinalysis. In case 4, urine cul-
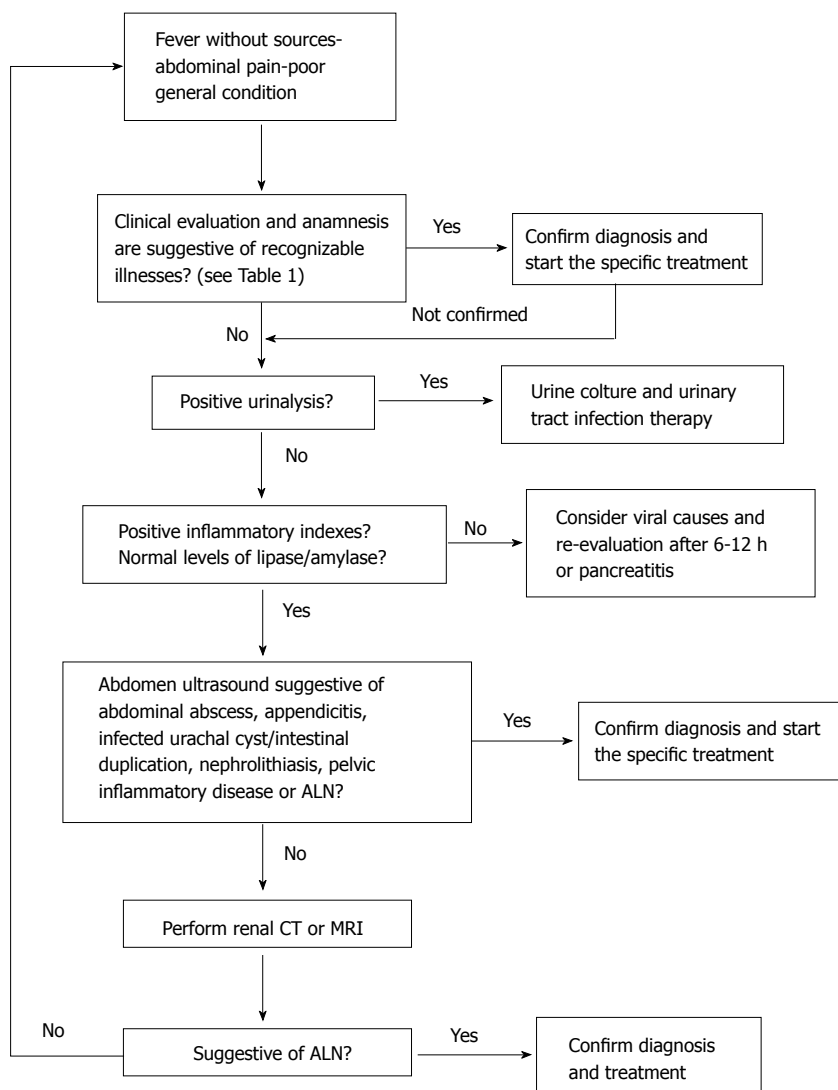
Figure 6 Diagnostic algorithm to suspect and then recognize acute lobar nephritis in children. ALN: Acute lobar nephritis; CT: Computed tomography; MRI: Magnetic resonance imagine.

ture was reported not performed because of the negative urinalysis. Our findings are in line with previous published reports. Evaluating data of 25 children diagnosed with ALN, some authors[10] showed that the main clinical presentation was represented by septic temperatures and rapid deterioration of clinical condition, characteristics that were present in all the children. Only the older children (10 out of 25), with a mean age of 8 years, suffered from specific symptoms such as flank pain, whereas younger children presented with unspecific symptoms such as vomiting, lethargy, abdominal pain or poor feeding. Laboratory findings showed always an elevation of CRP, ESR and WBC. Leukocyturia and bacteriuria were not found in all of the children but in 18/25 and 20/25 respectively. Positive urine cultures were found in 20/25 children; however 4 of 5 children with negative urine cultures had been pretreated with oral antibiotics prior to admission. In our experience, CT scan was performed in 3 cases. In case 3, US images were so typical (enlargement of the kidney and presence of a mass) that we decided not to obtain CT scans, in order

to avoid irradiation of the patient. Case 2 is noteworthy because CT revealed multiple lesions, one of these with a 5 mm colliquation area, probably an ALN evolution in abscess. US power Doppler, performed the day after, identified upper UTI compatible lesions. According to some authors, ALN should be considered as complicated UTI. Further radiological examination as VCUG is thus considered mandatory for the detection of urinary tract anomalies, known as the main underlying predisposing factors of ALN in children[24,25]. This series highlights the lack of specific clinical presentation of ALN. This condition should be suspected in front of a child with high fever, poor general condition and elevation of inflammatory markers, without an apparent infective focus even in presence of negative abdominal ultrasound, urinalysis and microscopy. Alternatively ALN should be suspected in patients with APN not responsive to antibiotic treatment. We suggest avoiding CT scan when sonographic criteria of severe nephromegaly and focal mass are present and to perform MRI instead of CT in the other cases, in order to minimize radiation exposure. We present a diagnostic algorithm

that could be used to suspect and then recognize ALN in children (Figure 6).

COMMENTS

Background
Clinicians often take on challenge with septic fever without clear clinical signs; the authors report four cases presenting with acute lobar nephritis (ALN), an underestimated condition, with the aim to give to the clinicians the instruments to suspect and diagnose ALN. A timely diagnosis is relevant because under-diagnosis may result in late renal scarring, and/or evolution in renal abscess, which in turn may lead to hypertension or renal failure.

Research frontiers
Important areas of research could be evaluate the sensitivity and specificity of both magnetic resonance and intravenously enhanced ultrasound in diagnosing ALN with the aim to avoid computed tomography and then to spare radiations.

Innovations and breakthroughs
In literature there is lack of data about ALN in childhood. The authors report of four cases of pediatric ALN, rising diagnostic and therapeutic issues. Furthermore the authors describe their experience about MR or enhanced ultrasound study of kidney in diagnosing ALN. These techniques are not mentioned elsewhere in the ALN diagnosis.

Applications
All children with septic fever with or without abdominal pain should raise in the clinicians the suspect of deep bacterial infection. For a correct diagnosis it is essential both performing urinalysis before antibiotic therapy and abdominal imaging.

Terminology
Enhanced ultrasound is a free radiation imaging technique consisting in instillation of microbubble in bladder or vein emphasizing structures. Unfortunately, its use in children is still off-label.

Peer-review
This is a series of 4 cases with acute lobar nephritis in children discussing the clinical presentation, radiological features, treatment and management issues.

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