SCIENTIFIC BULLETIN

Vol. XV

Editor:

Dr. Namganglung Golmei

Dr. Amarjit Moirangthem

Pediatric Association of Manipur (PAM)
Imphal

Scientific Bulletin, Vol.XV

December, 2023

Imphal, Manipur.

Editors:
Dr. Namganglung Golmei
Dr. Amarjit Moirangthem

Published by:
Ngangbam Sonamani
Secretary, PAM

For Pediatric Association of Manipur (PAM)
PAM Regd. No. 597/M/SR/2013
Office: IMA Complex, Lamphel, Imphal West
Manipur, India. 795004.

Designed & Printed at : Thoujalheiba Press, Uripok Sinam Leikai

Editorial

*Pediatric Association of Manipur has been publishing scientific bulletins almost every year till 2019 and due to some technical problems and miscommunications it discontinued. But it is with utmost pleasure to acknowledge the republication of our annual bulletin publication starting from this year again. This publication, Vol. XV of the scientific bulletin, will be in commemoration to the XXVII MANIPEDICON to be held on 17th December, 2023 under the theme "**Nurturing Psychosocial Health in Children: A Way Forward**" at the IMA conference hall, Lamphel, Imphal. "The contents of this scientific bulletin have been enriched by various imminent Pediatricians of our Association.*

We hope that this scientific bulletin will help everyone attending the conference in their day to day office practice and also elevate the knowledge of our post graduate trainees too. I on behalf of the editorial team and PAM would like to thank all the authors of this scientific bulletin for their valuable contributions in making this publication a success.

We would also like to thank the medical representatives of various pharmaceutical companies and state working committee of PAM for their support in releasing this bulletin in time.

We also would like to thank all the staffs of Thoujal Press, Uripok, RIMS Road, Imphal for their ever ready attitude in the working up of this scientific bulletin and completing it well within the time frame.

Dr. Namganglung Golmei

Dr. Amarjit Moirangthem

INDIAN ACADEMY OF PEDIATRICS (IAP)
MANIPUR STATE BRANCH

Year	President	Secretary	Treasurer
1989 - 1992	Dr. L. Ibemtombi Devi	Dr. L. Immo Singh	Dr. L. Ranbir Singh
1992-1996	Dr. L. Ibemtombi Devi	Dr. L. Ranbir Singh	Dr. Shyamkumar Laishram
1996-2001	Dr. Ksh. Chourajit Singh	Dr. Th. Nabachandra	Dr. Shyamkumar Laishram
2001-2003	Dr. H. Kumar Singh	Dr. Th. Nabachandra	Dr. Shyamkumar Laishram
2003-2004	Dr. L. Braja Mohan Singh	Dr. L. Ranbir Singh	Dr. A. Naranbabu Singh
2004 - 2007	Dr. Th. Nabachandra Singh	Dr. Shyamkumar Laishram	Dr. A. Naranbabu Singh
2007 - 2009	Dr. H. Ibemhal Devi	Dr. N. Kameshore Singh	Dr. H. Jasobanta Singh
2009-2012	Dr. A. Naranbabu Singh	Dr. L. Manglem Singh	Dr. Ch. Shyamsunder Singh
2012-2013	Dr. L. Ranbir Singh	Dr. H. Jasobanta Singh	Dr. Ch. Shyamsunder Singh
PEDIATRIC ASSOCIATION OF MANIPUR (PAM)			
2013-2015	Dr. Shyamkumar Laishram	Dr. N. Kameshore Singh	Dr. Ch. Shyamsunder Singh
2015-2016	Dr. Kh. Ibochouba Singh	Dr. Ch. Shyamsunder Singh	Dr. Y. Rameshwar
2016-2017	Dr. Kh. Ibochouba Singh	Dr. Ch. Shyamsunder Singh	Dr. R.K. Rupabati Devi
2017-2018	Dr. Kh. Ratankumar Singh	Dr. R.K. Rupabati Devi	Dr. N. Golmei
2018-2019	Dr. Kh. Ratankumar Singh	Dr. R.K. Rupabati Devi	Dr. N. Golmei
2019-2020	Dr. H. Jasobanta Singh	Dr. Y. Rameshwor Singh	Dr. Ng. Sonamani
2020-2021	Dr. H. Jasobanta Singh	Dr. Y. Rameshwor Singh	Dr. Ng. Sonamani
2021-2022	Dr. N. Kameshore Singh	Dr. Ngangbam Sonamani	Dr. Khumanthem John
2022-2023	Dr. N. Kameshore Singh	Dr. Ngangbam Sonamani	Dr. Khumanthem John

Contents

Role of Micronutrients on Psychological Health in Children

Dr Rajkumari Rupabati Devi
Associate Professor, Department of Pediatrics, JNIMS
Nodal officer, Nutrition Rehabilitation Centre, JNIMS

The key factors influencing the early phase of life are nutrition and nurture. The burden of mental health problems in children is increasing worldwide. The prevalence has been reported to be 10-20% worldwide. Anxiety disorders are the most common psychiatric disorders of childhood, occurring in 5-18% of all children and adolescents Similar to any non communicable disease a child may develop depression and anxiety at an early age. Studies show that nutritional inadequacy plays an important role in mental health and may contribute to pathogenesis of depression in school children aged 6-9 years.

In a child's life , the period from conception until 2 years old is crucial for growth and development . In addition to the development of vital organs and regulatory system, this phase also determine a child's personality, mental health and socio-emotional growth. Micronutrients comprising of vitamins and minerals play an essential role in child's growth and development. Many children have suboptimal intake of iron, zinc, potassium, calcium, vitamin D, and vitamin K, and excess intakes of sodium. Micronutrient deficiencies limit the body's ability to deactivate free radicals , which causes cell damage. Deficiencies of these elements are often related to the cognitive potential and physical lifelong consequences. Children who eat more micronutrient diets with more fruits and vegetables have both mental health and wellbeing. Intelligent scores can be improved by micronutrient supplementation in children and adolescent with very poor dietary status. There is significantly greater improvement in aggression and emotion regulation for those children who took micronutrients.

A study by Priyom Bose revealed that a child's early diet at 6months, 18 months, 3 and 7 years was associated with the incidence of symptoms of anxiety and depression at 8 years. The physical consequences of malnutrition , such as stunted growth, emaciation, and skeletal abnormalities, can contribute to feelings of inadequacy and a negative self perception.They may face stigmatization and discrimination due to their physical appearance or cognitive limitations. Obesity is a nutritional imbalance that negatively alters the micronutrient status of individuals, increasingly associated with an inadequate intake of minerals such as iron, calcium, magnesium, zinc and copper.

Iron and mental health in children :

Iron deficiency anemia can cause developmental delays and behavior disturbances such as decrease motor activity, decrease social interaction and attention to tasks. Several studies show an increase likelihood of mild or moderate mental retardation associated with iron deficiency, even if it has not progressed to the point of anemia. In clinical practice, it is recommended that individuals with serum ferritin levels less than 40 ng/ml are considered iron deficient. Reduce serum ferritin is known to be associated with sleep disorders due to restless leg syndrome , attention deficit/hyperactivity disorder .Fatigability and insomnia cause by chronic iron deficiency may have induced a state that is predisposed to anxiety, low concentration, and/or depression. Studies shows that iron administration for at least 12 weeks to children without anemia and with reduced serum ferritin improves symptoms such as anxiety, low concentration, depression, fatigability, and/or insomnia.

Iodine and mental health in children :

Iodine deficiency had substantial impact on mental development, 6.9 to 10.2 IQ point lower in Iodine deficient children compared with Iodine replete children. Neuro-intellectual outcomes in children appear to be more dependent on their mothers nutritional Iodine status than on maternal thyroid function. Prenatal , mild to moderate Iodine deficiency adversely affects cognitive developments later in life, with a seemingly greater impact on verbal abilities. Iodine deficiency is the main cause for potentially preventable mental retardation.

Iodine intake in the desired quantities becomes very important for neurodevelopment, including for school-aged children.

Zinc and mental health in children :

Zinc is essential for the formation and migration of neurons, along with the formation of neuronal interconnections called synapses. Evidence on the impacts of zinc deficiency has shown to be the most prominent during the stage of infancy. Its deficiencies could interfere with the formation of neuronal pathways and neurotransmission, thus affecting behavior and development. Mechanism have also been suggested for the relation between zinc and ADHD symptoms, specify through modifications in the neurotransmitter dopamine and serotonin. Zinc deficiency associated with anemia is prevalent in infant and toddlers because of their vulnerable position to micronutrient deficiencies while their growth rapidly increases. Zinc supplementation through nutritional support programs and government aid programs has demonstrated a positive effect on zinc levels through slight improvements in behavior such as increase alertness and activity levels.

Magnesium and mental health in children:

Magnesium for children is essential to support their health and development. It is crucial for cellular activity, second most abundant intra-cellular cation in the body. Magnesium is also involved in neurological diseases such as ADHD and autism due to involvement of transient receptor potential melastatin channels in the brain. An effective intervention for treatment of hyperactive children is use of vitamin B6 and magnesium besides other specific medical therapy.. Magnesium helps to turn nutrients found in the food into serotonin which regulates mental health by affecting mood and sleep schedules. Magnesium also helps neural connection to brain, achieve synaptic plasticity and help to learn concentration and retain information.

Vitamin D and Calcium on mental health in children :

Vitamin D is increasingly recognized as important for brain health, apart from its role in endocrine and bone health. Vitamin D affects early brain development by acting as a potent differentiating factor for brain cells by increasing nitrite outgrowth, regulation of neurotropic factors and reactive oxygen species

and down regulating calcium channels. Vitamin D increases calcium uptake. Low vitamin D level adversely affects the brain development, plays a role in the development of psychiatric illness. Studies in India reported that 21% of children with psychiatric illnesses had vitamin D deficiency. Lower vitamin D level was found in neurodevelopmental disorder including intellectual disability, pervasive development disorder, and disturbance of activity and attention. Calcium has a role in the electrical signaling transmission of impulses between nerve cells is of importance in the brain. Calcium deficiency also causes depression, increase irritability, anxiety and alter mood.

Vitamin B12 and mental health in children :

The most two physiologically important derivatives of vitamin B12 are cobalamine and methylcobalamine. Vitamin B12 deficiency may lead to accumulated homocycteine which is neurotoxic. Vitamin B12 also correlated to multiple behavioral problem, fatigue, socio-emotional development and obsessive compulsive disorder. Evaluation of vitamin B12 may be prudent in children and adolescents with affective complaints of abrupt onset with accompanying psychotic/motor features. The common symptoms include difficulty thinking,/ concentrating , agitation/irritability/negativism, fatigue and weakness in addition to anemia and balance problem. Vitamin B12 less than 300pg/ml in children may predispose to forgetfulness, anxiety and unhappiness. The early treatment of B12 deficiency may prevent the development of depression and anxiety in adolescent.

Folic acid and mental health in children:

Folic acid is the synthetic form of vitamin B9, found in supplements and fortified foods, while folate occurs naturally in foods. Folic acid is crucial for proper brain function and play an important role in mental and emotional health. Folic acid deficiency will have a wide range of effect on toddler. According to British Psychological society , children's emotional intelligence improved if mother take folic acid supplement throughout pregnancy. Folate supplementation in early pregnancy is important in neurogenesis , cell growth and proliferation, and myelination. Children with a fall in their IQ, neurotic disturbance and depression had significantly lower serum folic acid levels than normal children

Periconceptual use of folic acid (before conception and into the first trimester) has been associated with a decrease risk of autism spectrum disorder. In a small study finds folinic acid improves communication and ease symptoms in language among autistic children.

Vitamin A and mental health in children:

Vitamin A is a fat soluble nutrient essential for healthy vision, immune system, fertility and skin. Vitamin A , through its main metabolite retinoic acid, continues to exert important actions on brain physiology and behavior in post-embryonic and adult life. Vitamin A is essential to boost physical energy, blood Oxygen, and mental health. Its deficiency leads to mental health issues such as poor learning capacity and hampered memory.

Vitamin E and mental health in children :

Vitamin E protects cells from oxidative damage specially protection for brain and it also decreases the risk of depression and dementia. Recently , a possible association between oxidation stress and several mental disorders including depression, anxiety and bipolar disorders are observed. Studies are there that suggest for the first time that an inadequate supply of vitamin E during maternal vitamin E deficiency , is associated with reduce brain vitamin E levels at birth and increase anxiety at adult. There is an inverse relationship between vitamin E intake and the risk of stress.

Vitamin C and mental health in children :

The association between vitamin C deficiency and adverse psychiatric effects (depression and cognitive impairment) has been known for centuries. The function of vitamin C in the brain include acting as a co-factor for dopamine beta-hydroxylase in the conversion of dopamine to noradrenaline, involvement in modulating both dopaminergic and glutamatergic neurotransmission, and regulation of catecholamine and acetylcholine release from synaptic vesicles.. Vitamin C plays a role in the differentiation and maturation of neurons and in the formation of the myelin sheath that protect them and speeds impulse transmission, making the vitamin crucial to cognitive performance. Vitamin C supports immune system and it can help boost mood too. A 2015 study reported

a reduction in anxiety was observed in high school students given 500mg/day of vitamin C.

Take home message:

Emerging dietary habits and poor levels of micronutrients in our food supply are considered major factors in micronutrient deficiency. Although it is generally advise that micronutrients should be obtained from food, many children do not reach daily intake recommendations for select micronutrients, including vitamin A, C. D, E and some minerals such as calcium and magnesium. The RDA in a specific life stage and gender group should be used in the planning of diets for children to meet the increase growth and metabolism of the body.

Right to food has been one of the most contentious and highly debated issues in relation to the right to development of children. A healthy dietary pattern can affect mental health and well being through anti-inflammatory, antioxidant, neurogenesis, microbiome and immune modifying mechanism, as well as through epigenetic modification.. Early identification of micronutrient deficiency and supplementation is important in preventing psychological illnesses in children.

References:

1. Lam LF, Lawlis TR . The effect of micronutrient intervention diet in cognitive performance among school-aged children A systematic review of randomize control trial.Clin. Nutr 2017 Aug;36 (4):1007-14
2. Singh S, Awasth S, Kumar D, Samab SR, Pandey AK et all Micronutrients and cognitive functions aqmong urban school-going children and adolescent: Acrossectional multicentric study from India. PlosS One 2023 Feb 2:8(2);e0281247 PMID 36730336
3. Rubio-Lopez N, Morales-Suarez-Varela M et all. Nutrient intake and Depression symptom in Spanish Children: The ANIVA study. Int. J. Environ. Res Public Health. 2016; 13:352
4. K Mikami, H Okazawa, K Kimbo et all . Effect of oral Iron Administration on Mental State in Children with Low Serum Ferritin Concentration. Glob Pediatr Health 2019, 6: 2333794X19884816
5. N Chawhan, SK Padhy, R Shah, S Malhotra. Vitamin D deficiency in children with Psychiatric illness in a Tertiary Care Hospital in North India, 2019 J Neurosci Rural Pract Jan-Mar;10(1):16-20
6. K Bougma, FE Aboud, KB Harding, GS Marquis. Iodine and Mental Development of Children 5 years old and under: A systematic review and meta-analysis Nutrients 2013 Apr 22; 5(4):1384-416
7. Gogia S, Sachdev HS. "Zinc supplementation for mental and motor development in children. Dec. 2022. Cochrane study.
8. Ali FT, Rabic B, Genco V, Ayten F. Mood disorder with mixed, psychotic features due to vitamin B12 deficiency in an adolescent: Case Report. Jume 2012 Child Adoles psychiatry Ment health 6;25(2012)
9. Christopher R Olson and Chaudo V Mello. Significance of Vitamin A to Brain function, behavior and learning. Mol Nutr Fool Res 2010 Apr ; 54(4)(:489-95
10. David P, Cheme G. The neuropsychiatric effects of vitamin C deficiency: A systemic review. BMC Psychiatry.2020;20:35

DEVELOPMENTAL DELAY IN CHILDREN

Dr. A. Kiran Devi
Specialist Pediatrics, DH, Senapati

The most crucial period for development of cognitive, language, social, behavioural, emotional and physical skiills in a human life is the period from birth to five years (1).

What is developmental delay?

Developmental delay is said to occur when a child fails to attain age appropriate milestones or skills in one or more domains of development (i.e gross motor, fine motor, speech/language, cognitive, social/personal or activities of daily living).A discrepancy of 25 percent or more from the expected rate, or a discrepancy of 2 standard deviations from the norm is considered a significant delay .Global developmental delay is defined as a delay in two or more developmental domains.(2,3).

Common etiologies of developmental delay (4)**:**

Prenatal

- Genetic disorders: Down syndrome, fragile X syndrome, chromosomal microdeletion or duplication
- Cerebral dysgenesis: microcephaly, absent corpus callosum, hydrocephalus, neuronal migration disorder
- Vascular: occlusion, haemorrhage
- Drugs: cytotoxic, anti epileptic
- Toxins: alcohol, smoking
- Early maternal infections: rubella, cytomegalovirus, toxoplasmosis
- Late maternal infection: varicella, malaria, HIV

Perinatal :

- Metabolic: symptomatic hypoglycaemia, bilirubin induced neurological dysfunction

Postnatal :

- Infections: meningitis, encephalitis
- Metabolic: hypernatraemia, hyponatraemia, hypoglycaemia, dehydration
- Anoxia: suffocation, near drowning, seizure
- Trauma: head injury, either accidental or non accidental
- Vascular: stroke

Others

- Social: severe understimulation, maltreatment, malnutrition (deficiency of iron, folate and vitamin D)
- Maternal mental health disorders

The socioeconomic risk factors were identified as important as the biological risk factors in the age group 3 months to 5 years (1). Hence , early identification and intervention is of prime importance and can positively alter the child's long term trajectory in terms of preventing future developmental disability and secondary problems like family dysfunction, school failure and peer difficulties. However, in countries like India , there is minimal awareness of developmental delay among the parents and caretakers where speech delay in most cases is the only delay concerned to them (5,6)

Barriers to early identification:

There are multiple challenges or barriers to screening for developmental delay in clinical practice. Lack of time, training and resources are some of the common barriers to early identification. Other notable barriers include scarcity of available subspecialists for referral, inadequacy of staffs, poor consensus on the best screening tool etc. . A study shows that nearly 82% of primary care physicians cited time constraints as the most prominent barrier (4,7). Apart

from these factors, the primary care physician's referral to a specialist may not be activated by the parents/caretakers. Reasons could be due to parents not willing for referral,poor understanding of the importance of early intervention or family's unfavourable social circumstances like single parenthood and poor financial resources(8).

Identifying developmental delay at primary care level:

For early identification of developmental delay and treatment, it is imperative that the pediatricians be skilled with the proper usage of screening tests and be aware of the strengths and limitations of developmental screening (9). As stimulation begins at birth itself, it is vital that at every given opportunity the child's developmental status be evaluated. It is very important that the parents be encouraged to share their concerns regarding their child's development or behavior, conduct a developmental surveillance at every given opportunity. Based on the consultation, decision can be made either to review again, refer or discharge. In the absence of any red flag sign and no abnormality on clinical examination (i.e mild developmental delay) parents/caretakers can be adviced about appropriate stimulation activities with a review conducted at 3 month's time. However, if there is any risk factor associated or there is significant delay , a prompt referral to a developmental pediatrician is advisable(4,8).

Developmental surveillance questions (10)

1. Do you have any concerns about your child's development, behaviour or learning?
2. What concerns do you have about your child?
3. Age and domain specific queries, e.g. to evaluate language in an 18 month old child, you could ask, 'How does your child communicate with you?'
4. How has your child's development improved since the last visit?

Approach to a case of developmental delay requires a thorough history taking and a good physical examination:

Key points in history taking (11,12)

- **Family history**

Three generations, maternal and paternal ,consanguinity , previous pregnancy outcomes: miscarriages, stillbirths, neonatal or childhood deaths, infertility,family history of birth defects, childhood deaths, mental retardation, speech delay, learning disabilities, autism and known genetic conditions – note level of education in parents and siblings, ethnic backgroun

- **Antenatal history**

Maternal health: underlying medical condition, use of medications, drugs, smoking, alcohol consumption, presence of any infections in pregnancy

- **Birth and neonatal history**

Gestational age at birth, birth weight, Apgar scores, perinatal events, cord blood thyroid stimulating hormone level, universal newborn hearing screening results

- **Developmental milestones**

Current functioning of the child across the various developmental domains (gross motor, fine motor and vision, language and hearing, personal social skills including activities of daily living), play (solitary vs. parallel vs. interactive play, choice of toys), atypical development, e.g. perseverance/obsessions/ compulsions, rigidity and poor task transitioning, motor mannerisms, sensory issues, atypical language (echolalia, odd prosody), behaviour in different settings, e.g. home vs. school ,school performance, e.g. academics, behaviour, socialisation skills with peers, reports from teachers, reports from professionals working with the child, e.g. external therapists, early temperament in infancy, e.g. social responsiveness, feeding, sleeping, crying, history of any developmental regression, dietary history, sleep habits

- **Social history**

Main caregiver(s) spoken language at home, medical illnesses (especially mental wellness) in the family, recent stressors, domestic violence, financial difficulty, any past or current concerns of child abuse/neglect

Key points in physical examination (11,12)

Growth parameters (weight, height, occipitofrontal circumference)

Dysmorphism, Neurocutaneous stigmata, systemic examination, full neurological examination (tone, reflexes, gait, cranial nerves, cerebellar), spine, hips , behavioural observations during consult.

Hearing assessment if there are concerns regarding hearing like poor response to name when called and language delay

Vision assessment if the child(>= 6 weeks) is does not show fixing and following or has history of frequent bumping into objects for a mobile child or have delayed acquisition of fine motor skills

Baseline investigations:

Full blood count (possible iron deficiency), bone mineral profile and vitamin D levels (if rickets are suggested), thyroid function tests (especially for children with GDD and growth problems), urea levels and electrolyte levels(4).

Further tests may be required as per the different etiologies.

Screening tests for developmental delay(9)

Name	Age range
Denver Development Screening Test (DDST)	0-6 yrs
Denver II Developmental profile (DP II)	0-6 yrs 0-9 yrs
Cognitive Adaptive Test Clinical linguistic Auditory Milestone Scale (CAT/CALMS	0-3 yrs
Early Language Milestone Scale (ELM)	0-3 yrs
Vineland Social Maturity Scale	0-15 yrs
Tests for Indian Children	
Trivandrum Development Screening Chart (TDSC)	0-2 yrs
Haroda Development Screening Test for Infants	0-2 yrs

DDST is by by far the most commonly used screening test.

TAKE HOME MESSAGES

1. Developmental delays are common and can involve either a single domain or multiple domains of the child's functioning.
2. Early identification of developmental delays and appropriate management can positively alter the child's developmental trajectory.
3. Primary care physicians play a pivotal role in early identification of developmental delays through developmental screening and surveillance.
4. For children presenting with mild developmental delays and in the absence of any red flags, appropriate stimulation activities can be suggested, with close monitoring of the child.
5. There should be a low threshold for specialist referral for children at high risk for developmental problems.

References:

1. Ozkan M, Senel S, Arslan EA, Karacan CD. The socioeconomic and biological risk factors for developmental delay in early childhood. Eur J Pediatr. 2012 Dec;171(12):1815–21.
2. Gupta S, Shrivastava P, Samsuzzaman M, Banerjee N, Das DK. Developmental delay among children under two years of age in slums of Burdwan Municipality: A cross-sectional study. J Fam Med Prim Care. 2021 May;10(5):1945–9.
3. Poon JK, Larosa AC, Shashidhar Pai G. Developmental delay: Timely identification and assessment. Indian Pediatr. 2010 May;47(5):415–22.
4. Choo Y, Yeleswarapu S, How C, Agarwal P. Developmental assessment: practice tips for primary care physicians. Singapore Med J. 2019 Feb;60(2):57–62.
5. Kaur P, Chavan BS, Lata S, Kaur A, Tinku S, Arora Y, et al. Early intervention in developmental delay. Indian J Pediatr. 2006 May;73(5):405–8.
6. Bennett FC, Guralnick MJ. Effectiveness of Developmental Intervention in the First Five Years of Life. Pediatr Clin North Am. 1991 Dec;38(6):1513–28.
7. Morelli DL, Pati S, Butler A, Blum NJ, Gerdes M, Pinto-Martin J, et al. Challenges to implementation of developmental screening in urban primary care: a mixed methods study. BMC Pediatr. 2014 Dec;14(1):16.
8. Choo Y, Agarwal P, How C, Yeleswarapu S. Developmental delay: identification and management at primary care level. Singapore Med J. 2019 Mar;60(3):119–23.
9. Malhi P and Singh P. Indian Pediatrics. In: Screening young children for delayed development. 1990th ed. 1990. p. 569–77.
10. Committee on Children With Disabilities. Developmental Surveillance and Screening of Infants and Young Children. Pediatrics. 2001 Jul 1;108(1):192–5.
11. Vasudevan P, Suri M. A clinical approach to developmental delay and intellectual disability. Clin Med. 2017 Dec;17(6):558–61.
12. Choo Y, Agarwal P, How C, Yeleswarapu S. Developmental delay: identification and management at primary care level. Singapore Med J. 2019 Mar;60(3):119–23.

ADHD
(Attention Deficit Hyperkinetic Disorder)

Dr. Nelson Loitongbam
Assistant Professor, Dept. of Psychiatry
JNIMS

ADHD is one of the most common neuro developmental disorders of childhood. It is usually first diagnosed in childhood and often lasts into adulthood. Children with ADHD may have trouble paying attention, controlling impulsive behaviors (may act without thinking about what the result will be), or be overly active.Globally, it has been estimated that approximately 5% of children and adolescents are affected by ADHD. In India, an estimated 5 to 8% of schoolchildren are affected by ADHD. ADHD significantly impairs multiple aspects of life, leading to educational underachievement, unemployment, unsuccessful marriage and criminality, etc. Moreover, ADHD shows significant correlations with a wide range of comorbid psychiatric disorders, including affective disorders, defiant, antisocial personality disorder, self-harm, substance misuse, placing a considerable burden on society and family.

Signs and Symptoms

It is normal for children to have trouble focusing and behaving at one time or another. However, children with ADHD do not just grow out of these behaviors. The symptoms continue, can be severe, and can cause difficulty at school, at home, or with friends.

A child with ADHD might:

- daydream a lot
- forget or lose things a lot
- squirm or fidget
- talk too much

- make careless mistakes or take unnecessary risks
- have a hard time resisting temptation
- have trouble taking turns
- have difficulty getting along with others

Diagnosis

Deciding if a child has ADHD is a process with several steps. There is no single test to diagnose ADHD, and many other problems, like anxiety, depression, sleep problems, and certain types of learning disabilities, can have similar symptoms. One step of the process involves having a medical exam, including hearing and vision tests, to rule out other problems with symptoms like ADHD. Diagnosing ADHD usually includes a checklist for rating ADHD symptoms and taking a history of the child from parents, teachers, and sometimes, the child.

The American Academy of Pediatrics (AAP) recommends that healthcare providers ask parents, teachers, and other adults who care for the child about the child's behavior in different settings, like at home, school, or with peers.

Healthcare providers use the guidelines in the American Psychiatric Association's Diagnostic and Statistical Manual, Fifth edition (DSM-5), to help diagnose ADHD. This diagnostic standard helps ensure that people are appropriately diagnosed and treated for ADHD. Using the same standard across communities can also help determine how many children have ADHD, and how public health is impacted by this condition.

DSM-5 Criteria for ADHD

People with ADHD show a persistent pattern of inattention and/or hyperactivity–impulsivity that interferes with functioning or development:

1. Inattention: Six or more symptoms of inattention for children up to age 16 years, or five or more for adolescents age 17 years and older and adults; symptoms of inattention have been present for at least 6 months, and they are inappropriate for developmental level:

- Often fails to give close attention to details or makes careless mistakes in schoolwork, at work, or with other activities.
- Often has trouble holding attention on tasks or play activities.
- Often does not seem to listen when spoken to directly.
- Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (e.g., loses focus, side-tracked).
- Often has trouble organizing tasks and activities.
- Often avoids, dislikes, or is reluctant to do tasks that require mental effort over a long period of time (such as schoolwork or homework).
- Often loses things necessary for tasks and activities (e.g. school materials, pencils, books, tools, wallets, keys, paperwork, eyeglasses, mobile telephones).
- Is often easily distracted
- Is often forgetful in daily activities.

2. Hyperactivity and Impulsivity:

Six or more symptoms of hyperactivity-impulsivity for children up to age 16 years, or five or more for adolescents age 17 years and older and adults; symptoms of hyperactivity-impulsivity have been present for at least 6 months to an extent that is disruptive and inappropriate for the person's developmental level:

- Often fidgets with or taps hands or feet, or squirms in seat.
- Often leaves seat in situations when remaining seated is expected.
- Often runs about or climbs in situations where it is not appropriate (adolescents or adults may be limited to feeling restless).
- Often unable to play or take part in leisure activities quietly.
- Is often "on the go" acting as if "driven by a motor".
- Often talks excessively.
- Often blurts out an answer before a question has been completed.
- Often has trouble waiting their turn.

- Often interrupts or intrudes on others (e.g., butts into conversations or games)

In addition, the following conditions must be met:

- Several inattentive or hyperactive-impulsive symptoms were present before age 12 years.
- Several symptoms are present in two or more settings, (such as at home, school or work; with friends or relatives; in other activities).
- There is clear evidence that the symptoms interfere with, or reduce the quality of, social, school, or work functioning.
- The symptoms are not better explained by another mental disorder (such as a mood disorder, anxiety disorder, dissociative disorder, or a personality disorder). The symptoms do not happen only during the course of schizophrenia or another psychotic disorder.

Based on the types of symptoms, three kinds (presentations) of ADHD can occur:

- ***Combined Presentation*:** if enough symptoms of both criteria inattention and hyperactivity-impulsivity were present for the past 6 months
- ***Predominantly Inattentive Presentation*:** if enough symptoms of inattention, but not hyperactivity-impulsivity, were present for the past six months
- ***Predominantly Hyperactive-Impulsive Presentation*:** if enough symptoms of hyperactivity-impulsivity, but not inattention, were present for the past six months.

Because symptoms can change over time, the presentation may change over time as well.

Causes of ADHD

Scientists are studying cause(s) and risk factors in an effort to find better ways to manage and reduce the chances of a person having ADHD. The cause(s) and risk factors for ADHD are unknown, but current research shows that genetics plays an important role. Recent studies link genetic factors with ADHD.

In addition to genetics, scientists are studying other possible causes and risk factors including:

- Brain injury
- Alcohol and tobacco use during pregnancy
- Premature delivery
- Low birth weight
- Blood relatives, such as a parent or sibling, with ADHD or another mental health disorder
- Exposure to environmental toxins — such as lead, found mainly in paint and pipes in older buildings

Treatment of ADHD

When a child is diagnosed with attention-deficit/hyperactivity disorder (ADHD), parents often have concerns about which treatment is right for their child. ADHD can be managed with the right treatment. There are many treatment options, and what works best can depend on the individual child and family. To find the best options, it is recommended that parents work closely with others involved in their child's life—healthcare providers, therapists, teachers, coaches, and other family members.

Types of treatment for ADHD include

- Behavior therapy, including training for parents; and
- Medications.

Treatment recommendations for ADHD

For children with ADHD younger than 6 years of age, the American Academy of Pediatrics (AAP) recommends parent training in behavior management as the first line of treatment, before medication is tried. For children 6 years of age and older, the recommendations include medication and behavior therapy together — parent training in behavior management for children up to age 12 and other types of behavior therapy and training for adolescents. Schools

can be part of the treatment as well. AAP recommendations also include adding behavioral classroom intervention and school supports. Good treatment plans will include close monitoring of whether and how much the treatment helps the child's behavior, as well as making changes as needed along the way.

Behavior Therapy, Including Training for Parents

ADHD affects not only a child's ability to pay attention or sit still at school, it also affects relationships with family and other children. Children with ADHD often show behaviors that can be very disruptive to others. Behavior therapy is a treatment option that can help reduce these behaviors; it is often helpful to start behavior therapy as soon as a diagnosis is made.

The goals of **behavior therapy** are to learn or strengthen positive behaviors and eliminate unwanted or problem behaviors. Behavior therapy for ADHD can include

- Parent training in behavior management;
- Behavior therapy with children; and
- Behavioral interventions in the classroom.

These approaches can also be used together. For children who attend early childhood programs, it is usually most effective if parents and educators work together to help the child.

Children younger than 6 years of age

For young children with ADHD, behavior therapy is an important first step before trying medication because:

- Parent training in behavior management gives parents the skills and strategies to help their child.
- Parent training in behavior management has been shown to work as well as medication for ADHD in young children.
- Young children have more side effects from ADHD medications than older children.

- The long-term effects of ADHD medications on young children have not been well-studied.

Overview for parents

School-age children and adolescents

For children aged 6 years and older, AAP recommends combining medication treatment with behavior therapy. Several types of behavior therapies are effective, including:

- Parent training in behavior management;
- Behavioral interventions in the classroom;
- Peer interventions that focus on behavior; and
- Organizational skills training.

These approaches are often most effective if they are used together, depending on the needs of the individual child and the family.

Medications

Medication can help children manage their ADHD symptoms in their everyday life and can help them control the behaviors that cause difficulties with family, friends, and at school.

Several different types of medications are FDA-approved to treat ADHD in children as young as 6 years of age:

- **Stimulants** are the best-known and most widely used ADHD medications. Between 70-80% of children with ADHD have fewer ADHD symptoms when taking these fast-acting medications.
- **Non stimulants** were approved for the treatment of ADHD in 2003. They do not work as quickly as stimulants, but their effect can last up to 24 hours.

Medications can affect children differently and can have side effects such as decreased appetite or sleep problems. One child may respond well to one medication, but not to another.

Healthcare providers who prescribe medication may need to try different medications and doses. The AAP recommends that healthcare providers observe and adjust the dose of medication to find the right balance between benefits and side effects. It is important for parents to work with their child's healthcare providers to find the medication that works best for their child.

Parent Education and Support

CDC funds the National Resource Center on ADHD (NRC), a program of Children and Adults with Attention-Deficit/Hyperactivity Disorder (CHADD). The NRC provides resources, information, and advice for parents on how to help their child.

Tips for Parents

The following are suggestions that may help with your child's behavior:

- **Create a routine.** Try to follow the same schedule every day, from wake-up time to bedtime.
- **Get organized.** Encourage your child to put schoolbags, clothing, and toys in the same place every day so that they will be less likely to lose them.
- **Manage distractions.** Turn off the TV, limit noise, and provide a clean workspace when your child is doing homework. Some children with ADHD learn well if they are moving or listening to background music. Watch your child and see what works.
- **Limit choices.** To help your child not feel overwhelmed or over stimulated, offer choices with only a few options. For example, have them choose between this outfit or that one, this meal or that one, or this toy or that one.
- **Be clear and specific when you talk with your child.** Let your child know you are listening by describing what you heard them say. Use clear, brief directions when they need to do something.

- **Help your child plan.** Break down complicated tasks into simpler, shorter steps. For long tasks, starting early and taking breaks may help limit stress.
- **Use goals and praise or other rewards.** Use a chart to list goals and track positive behaviors, then let your child know they have done well by telling them or by rewarding their efforts in other ways. Be sure the goals are realistic—small steps are important!
- **Discipline effectively.** Instead of scolding, yelling, or spanking, use effective directions, time-outs or removal of privileges as consequences for inappropriate behavior.
- **Create positive opportunities.** Children with ADHD may find certain situations stressful. Finding out and encouraging what your child does well—whether it's school, sports, art, music, or play—can help create positive experiences.

Play and Play Based Interventions for Autism: Benefits and Implications

Priyanka Konsam
Child Psychologist, JNIMS and RCI Licensed Rehabilitation Counsellor)

Autism Spectrum Disorder is a childhood development disability that significantly affects the individuals' ability to communicate, build and maintain social relationships and interact meaningfully with the world around them. Autism is a neurodevelopmental disability caused by differences in the way the brain develops, sometimes having underlying genetic causes but most often the direct cause is not known. Research is still underway to reveal the mystery behind this disability. As per the DSM 5, Autism Spectrum disorder is characterized by the presence of deficits in the areas of social interaction, social communication across multiple contexts along with restricted and repeated patterns of behaviors, interests or activities. Further, as per the diagnostic criteria Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities or may be masked by learned strategies in later life) and the deficits are not better explained by intellectual disability or global developmental delay.

In recent times the number of children diagnosed with ASD has been on the rise in India and according to a report by ET Health World, about 18 million people in India are diagnosed with autism. About 1 to 1.5 per cent of children aged two to nine years are diagnosed with ASD. However further research on the prevalence in India is required.

Once the diagnosis has been made, the requirement is of focused rehabilitative services. The usual range of rehabilitative intervention for ASD include, behavior therapy, social skills training, occupational therapy, speech therapy, group based therapy. Over and above these, as per the requirement of the child, play therapy, arts based therapy, Cognitive behavior therapy etc., are also used. Such services are best implemented as early as possible. According to the

early intervention model, a child's brain is rapidly developing till about the age of 6 years and during this period the new connections are being formed hence, the earlier support and interventions are implemented, the better the outcomes for a child who is at risk of developmental difficulties. One of the techniques used is play based interventions. Play-based interventions are **practices designed to improve socio-emotional, physical, language, and cognitive development through guided interactive play.** Play based interventions may be defined as either the socio-cognitive techniques that are specifically built on the elements of play or the implementations that are delivered during the playtime or within the play settings (Ingersoll & Walton, 2013).

The aim of this article is to highlight the importance and the use of play and play based interventions for ASD.

Play and its benefits

Play is the most natural way of being for a child. Play is a child's work. There is no doubt about the many benefits of play in the life of a child. When a child is immersed in play they are carefree and without any stress. Different types of play contribute to the development of many skills in different developmental domains i.e. physical, motor, cognitive, socio-emotional etc.

While there are many definitions of play, for the purpose of this article, play will be defined as a physical or mental leisure activity that is undertaken purely for enjoyment or amusement and has no other objective

Play can be classified into three categories: functional, symbolic, or socio-dramatic play (Jung & Sainato, 2018). Functional play refers to the appropriate and functional uses of an object and cause-and-effect actions (e.g., a pop-up toy). Symbolic play is defined as a child's ability to act on an object as if it were something else. This type of play involves three forms: object substitution (e.g., using a block as a car), the attribution of false properties (e.g., pretending the dolls are eating), and the attribution of presence to imaginary objects (e.g., sailing a boat over an invisible lake). Socio-dramatic play is an advanced form of symbolic play that involves engagement in role-playing (Cardinal, 2021)

Autism and Play

One of the features of ASD is stereotypical play, limited pretend, imaginative and social play. Children with ASD do not follow the typical pattern of play development and may show delays in developing play skills. The core characteristics of ASD, deficits in social interaction and communication, and restricted repetitive patterns of behaviors and interest contribute to the differences in the way play develops or how children with ASD play.

Play for autistic children may be characterized by functional play skills that are repetitive (e.g. lining objects spinning, mouthing). Children with ASD have difficulties with social skills like initiating and sustaining interactions with their peers, further they lack social behaviors like, eye contact, sharing, turn taking, reciprocal interaction which inhibit their ability to engage in play with their peers. It is common to find children with ASD being described as 'being lost in their own world', as they process the environment in a different way than neuro typical children. With respect to social play, children across the autism spectrum share some common characteristics. Studies have shown that compared to typically developing children and children with developmental delays, children with autism direct fewer overt social initiations to peers (Hauck et al. 1995; Sigman and Ruskin 1999)

They may have restricted interests which limit their scope for variety in play, or playing with new themes. Difficulties with transitions, change, emotional reciprocity etc., are all related features of ASD which also affect their play.

Developmental delays and differences in underlying abilities for joint attention, imitation, and social reciprocity all contribute to the limited play capacities. In sharp contrast to the diverse social and imaginary play of typically developing children, the play of children with autism is typified by restricted, repetitive, and stereotyped patterns of behavior, interests, and activities, which they often pursue in isolation (American Psychiatric Association 2012)

Play and Play based interventions and Autism

Play in itself has multiple benefits not only for the neurotypicaly developing child but for neurodivergent children as well because of its inherent characteristics of being stress relieving, fun, non judgmental etc.

Since children with Autism engage in atypical play, and may be delayed in developing play skills, one may assume that they may not engage or benefit from play based interventions, however on the contrary, play interventions have shown to have benefits for children with Autism. Though it may be stated here that research is limited in this field about the efficacy of play, it has shown to have positive effects on various skills of autistic children as well as on 'positive mental health and parent child interaction and relationship.

Play-based interventions appear to have a beneficial effect on positive, but not negative, mental health in children with ASD (Francis et al, 2022)

In a literature review by Neijis et. al. (2023), it was concluded with that the affectivity of play-based interventions differed across randomized Control Trials, most reported improvements are found in ASD symptoms, everyday functioning, and parental attunement.

In a randomized intervention study conducted by Schottelkorb et al. based on self-directed play sessions, showed that children aged 4–10 years with ASD who received play based intervention experienced a reduction in ASD behavioral symptoms such as attention problems and aggression compared to children in the waiting list group.

There is also research that shows that Parent mediated play based interventions led to improvements in children with ASD. In one study by Barret et al, 2020 it was found that parent-led play-based intervention for children with ASD improves language and interaction at 1–4 years of age.

With respect to ASD, play has the following benefits:

Develop language and communication

By integrating toys, games, and interactive activities, these interventions become a conduit for the development of both verbal and nonverbal communication

skills. The use of language during play by the caregiver and providing appropriate reinforcement s, language and communication skills are demonstrated

Relationship building

During play interactions, the caregiver/therapist builds a close bond with the child which results in the child gaining skills in recognizing faces, sounds, speech and certain emotions. These skills in turn develop vital cognitive and social skills that help. With improving communication and also understanding relationships.

Awareness of the world around

A child's engagement and understanding of their environment are hindered by sensory processing issues and hence the way they interact with their environment is atypical or functional. When engaged in therapeutic play, there is scope to work through these issues and help the child attain improved understanding of the environment

Reciprocal interaction

Play activities and games can be used to teach turn taking, and two way communications. A child's understanding of cause of cause and effect, is enhanced which help in the building of these cognitive and social skills.

Nonverbal Communication

During play the use of facial expressions, verbal and nonverbal expressions, body language and gestures are ways in which the child will learn to figure out the meaning of other people's nonverbal communication.

Emotional Expression

Play activities focusing on emotional ideas and emotions can be used to help the child with ASD explore their emotional world and interpret facial expressions and hence emotions of others. Further pretend play is lacking in children with autism and play can help develop this area as well.

Implications

One of the findings pertaining to the benefit in the presence of parents and the improvement in parent child interaction as an outcome of play and play

based interventions, has important implications as this can be used as a strategy to empower parents in joining the intervention for children with ASD.

Even if parents are not trained formally in play based interventions, parents can be equipped with skills to join in the play of their child, skills to reinforce positive responses from the child and thus improving parent child bonding.

Further, allied professionals who are involved in the rehabilitation of children with Autism can also use play or play based intervention strategies in their practice.

There is also scope for research on how play can be incorporated into a home based intervention program for parents and caregivers.

In conclusion, Play and Play based interventions have positive outcomes in children with ASD and can be incorporated into practice as well as by parents and caregivers. However, there is limited evidence about its efficacy in every case. Hence, there is further scope for research to evaluate how play or play based interventions is beneficial to children with ASD especially in the Indian context.

References:

- Cardinal, B. Teaching Play Skills to Children with Autism: A Review of the Literature, Journal of Graduate Studies in Education, Volume 13, Issue 1, 2021
- Dijkstra-de Neijs L, Tisseur C, Kluwen LA, van Berckelaer-Onnes IA, Swaab H, Ester WA. Effectivity of Play-Based Interventions in Children with Autism Spectrum Disorder and Their Parents: A Systematic Review. J Autism Dev Disord. 2023 Apr;53(4):1588-1617. doi: 10.1007/s10803-021-05357-2. Epub 2021 Dec 2. PMID: 34853960.
- Francis, G., Deniz, E., Torgerson, C., & Toseeb, U. (2022). Play-based interventions for mental health: A systematic review and meta-analysis focused on children and adolescents with autism spectrum disorder and developmental language disorder. Autism & Developmental Language Impairments, 7. https://doi.org/10.1177/23969415211073118
- Ingersoll B., Walton K. (2013). Play intervention. In Volkmar F. R. (Ed.), *Encyclopedia of Autism Spectrum disorders* (pp. 2287–2291). Springer New York.
- Jung, S., & Sainato, D. M. Teaching play skills to young children with autism. Journal of Intellectual & Developmental Disability,2013, 38(1), 74-90. https://doi.org/10.3109/13668250.2012.732220
- Schottelkorb, A.A.; Swan, K.L.; Ogawa, Y. Intensive Child-Centered Play Therapy for Children on the Autism Spectrum: A Pilot Study. *J. Couns. Dev.* **2020**, *98*, 63–73. [**Google Scholar**] [**CrossRef**]
- Lantz, J. Play Time: An Examination of Play Intervention Strategies for Children with Autism Spectrum Disorders. Play Time: An Examination Of Play Intervention Strategies for Children with Autism Spectrum Disorders | Request PDF (researchgate.net)

Antimicrobial Resistance: Major Challenge in Pediatric Therapeutics

Dr. Kh. Ratankumar Singh
Consultant, Mother's Care Children Hospital and Research Centre, Imphal

Antibiotics remain the major boon to the humanity in respect of the healthcare interventions. Antimicrobials have made it possible for people to live longer with extended life expectancy across the globe. It has also made major contribution towards development in cancer therapy, organ transplant in addition to infectious diseases treatment. However, anytime a new antibiotic is introduced, drug resistance follows inevitably. In fact, resistance occurred within a decade after introduction of Penicillin during the clinical trials with more than 50% resistance among Staph. aureus by the end of 1940. Alexander Fleming in 1945 warned about injudicious use of antimicrobials to the medical fraternity. But the rampant abuse of antibiotics still continues in agriculture, veterinary as well as in medical practice.

We have so far escaped from the devastating impact of Multi Drug Resistant Organisms (MDRO) because of new antibiotics being introduced regularly. But these newly inducted antimicrobials are bound to become ineffective if they are overused injudiciously. Vancomycin is one single antibiotic which remained without inducing resistance for 16 long years while Daptomycin, a newly introduced drug showed resistance in 1 yr of induction for clinical use. Hence new antibiotics are needed to be brought in short period. Unfortunately, in the last 20 yrs the number of pharma companies investing in making new antibiotic has dwindled from 18 to just 4. Moreover, the development of resistance, helped by our indiscipline in antimicrobial prescription, far outpace the development of new antibiotics which is a long drawn process.

Hence if prompt decisive action is not taken now, we are headed towards era of pre-antibiotics where simple infections will become fatal. WHO projects,

if the trend is not reversed, drug resistant disease could cost 10 million lives by 2050 with cost of healthcare and gross domestic product loss of 300 billion US Dollars. (Antimicrob. Resist. Infect.Control.2018, 7, 58)

Every year,infection with drug resistant organism accounts for estimated 7,00,000 deaths across all ages. Out of this, overwhelming 2,00,000 deaths are contributed by newborns with MDR infection. (4rth Global Conference of Women Deliver,WHO: Copenhagen) In Europe, MDR contributes to 30% of all infections in children while 90% of all newborns admitted in NICU are having MDRO infection in some regions in Middle East. In some areas of SE Asia, 83% of children have E coli resistant to first line antibiotics (Br. J. Clin. Pharmacol, 2015,79:446-455) In subSaharan Africa, 66% of neonatal sepsis and meningitis are due to MDRO. In USA, 20.5% of pediatric patients are found to be receiving colistin, because of MDR gram negative organisms.

Among the infections in children lower respiratory tract infection remains most frequently treated with antibiotics. More than 50% of all the antibiotics prescribed are beta lactams. The ARPEC (Antibiotic Resistance and Prescription in European Children) study involving 226 hospitals in 41 countries in 2012, found that 36.7% percent of children receive at least one antibiotic at a given time. Ceftriaxone was prescribed in 31.3% while Meropenem contributed to 13.15 percent of antibiotic usage. In different studies , antibiotic usage rates have been found to be between 33% to 78% . (Am.J. Infect. Control 40,491-496.) Of this huge volume of antibiotic usage, 50 % were found to be inappropriate. (Arch.Intern.Med.,163: 972-978.)

Antibiotic abuse is not restricted to countries and regions just as antimicrobial resistance has no national or international boundaries. And it is not restricted to professions and sectors. Antimicrobial abuse is rampant in agricultural sector where it is used as crop enhancers. In animal husbandry, it is use as growth promoters by putting in small quantities in animal feeds. This prophylaxis is becoming an alternative to cleanliness in farm.

But the most impactful abuse, by far, is from the medical practitioners. 80-90% of antibiotic prescriptions come from general practitioners. Over 30% of all antibiotic prescriptions are unnecessary.(US Centers for Disease Control

and Prevention. CDC: 1in 3 antibiotic prescriptions unnecessary. May, 2016.) Of these, viral respiratory infections, viral diarrhea, allergic wheezing and viral croup are common diseases. Antibiotic may be indicated and necessary but it can still be given inappropriately which can be to the tune of 50% of all antibiotic prescriptions.

For an antibiotic to be offered optimally, the site of infection, suspected organism, age of patient, spectrum of the antibiotic and PK-PD are the important considerations. Failure to de escalate , unwarranted overlap of spectrum, wrong dosing and wrong duration of treatment are the major points in inappropriate use.

Most common abuse is in cases viral infections. It is true that some viral respiratory tract infections are very difficult to differentiate from acute bacterial pneumonia. Hence many physicians specially in ICUs end up giving antibiotics specially when there is lack of facilities for rapid diagnostic tests (RDT). Subsequently also, the de-escalation after getting reports negating bacterial infection also is also delayed many a time .

The inevitable result of the indiscipline is evolution of multi drug resistant organisms (MDRO). MDRO are very frightening to come across specially in neonatal population. Disease is much more protracted and severe, obviously with longer hospitalization. In many instances, Tigecycline which has no sufficient safety studies below 18 yrs, is called up in cases of MDR Klebsiella pnemoniae. MDR E. coli is another organism finally needing Colistin which has its inherent dosing problems and very narrow therapeutic window. The MDR phenomenon is also fast extending towards fungal organisms too.

With this grave scenario of MDR to overcome, it is very pertinent for us to look at the roots of this abuse of antimicrobials. The most important factor is the poor perception of organisms expected at that particular age during the infection at a particular site. Community acquired pneumonia (CAP) in children above 1 yr, Strept pneumonia, H. influenza, Staph aureus , Klebsiella pneumoniae need to be considered at that order (CAPES 2015,APC, Chandigarh) . Pneumonia in children above 5 yrs, Clamydophila pnenumoniae also needs to be considered

in addition to viral causes. In all the cases the status of vaccination needs to be considered before deciding about the antibiotic.

For effective killing of bacteria, the availability of the drug in the infection site in sufficient concentration for optimum time is required. Consideration of PK-PD is another important requirement so as to ensure optimum concentration of the antimicrobial in the site of infection for optimum period of time which is required to achieve the clinical cure thereby decreasing chances of developing resistance. Lastly, the diagnosis of bacterial infection needs to be established to justify the antibacterial administration using modern culture techniques. Role of rapid diagnostics and cultures will be eminent for this goal.

The quality of antibiotic prescription in a hospital or a country may be generally indicated by certain factors. The prevalence of broad spectrum antibiotic prescription is the most important indicator of quality in prescription. The rate of de-escalation in antibiotic usage indicates the state of diagnostics and awareness. Rate of antibiotic usage by individual, hospitals and country is indicative of antibiotic abuse. De-escalation will also include switching from parenteral to oral route. The documentation of reasons for antibiotic prescription or change need to be recorded in charts for review. Good amount of antibiotic is used as surgical prophylaxis for multiple conditions. Reduction of duration in peri operative antibiotic is also an important intervention.

The first step towards correct antimicrobial usage will be to understand the drugs we use and the patient characteristics. Pharmacokinetics of a drug is the concentration of the drug in plasma and different body fluids and sites at a period of time. Pharmacokinetics will be different in different patient populations which are exceedingly diverse in pediatric age group. Glomerular filtration rate GFR) in newborn increases quickly in the first two weeks of life then slows down to reach adult rate at 8-12 months. Tubular secretion is also immature at birth and reaches adult function during the first year. The implication will be in drug clearance from the body, hence the dosing frequency. Organ dysfunction is another important consideration during drug administration as it changes pharmacokinetics in a major way. The description of this variability of antimicrobial concentrations across the population is known as Population Pharmacokinetics.

Pharmacodynamics is the relationship of concentration of a antimicrobial in a infection site in enough concentration for enough time so as to attain clinical cure. This defined exposures, indexed to MIC, of the antibiotic to the pathogen have been used to evaluate the PK-PD measure that describe the antimicrobial activity of the antimicrobial /pathogen pair. Three most common PK-PD measures associated with microbial killing are: i.Duration of time during which the antimicrobial concentration is above the MIC . Amoxycillin is a good example which demonstrate time dependent bacterial killing. It is enough to have concentration above MIC for 40% of dosing interval for the drug to kill bacteria. Increasing drug concentration further or increasing doses frequency will not increase the efficacy. ii. 2nd measure is ratio of maximal drug concentration to MIC. In this measure more concentration of the drug in the site, greater will be killing of bacteria. Aminoglycosides have this character which is why giving maximal dose at one time is preferred to divided smaller doses. iii. Fluoroquinolones also are concentration dependent but in a little different way. They are dependent on concentration above the MIC in the area under the curve, which is the third measure.

Clinical breakpoint is the most important consideration for successful killing of bacteria. Required drug concentration over a period of time for effective killing is indexed to MIC. The probability of cure using a certain drug dose given to all children population, considering the MIC value and antibiotic exposure in population can be calculated using Monte Carlo simulation. This will show the clinical breakpoint for the antibiotic in the population. Mention may be made that trials of drug in pediatric population is not easy. It ethically is difficult to subject children to prospective studies where antibiotics are progressively increased experimentally to reach the clinical breakpoint. It is unethical to administer a drug in a lower dose knowing that it will not produce cure. (Pedtr. Infect. Dis. J. 2010; 29:143-1046)

In addition to PK-PD target, certain other factors need to be considered during treatment of a child with antimicrobial. Site of infection is important consideration which will determine the antibiotic class. Similarly, patient specific

GFR, tubular secretion and renal function will modify the drug dosing. Urgency with which the cure is sought is also a factor that will modify our antibiotic prescription. ELBW or immune compromised child will demand immediate killing of bacteria.

After all these considerations of lack of discipline in antibiotic prescription, inappropriate use and abuse ,it is obvious that there is urgent need for action against the real and present danger of MDRO and impending catastrophe. Antibiotic stewardship (AS) is the concept formed over the decades. It is a set of coordinated interventions, philosophy and ethics. Term was first coined in 1996 by John Mc Gowan, an internist in John Emery University School of Medicine and Dale Gerding, a specialist in Clostridium difficile pathogen. They proposed large scale, well controlled studies in use of antibiotics through sophisticated epidemiological methods, molecular typing of organisms and precise analysis of mechanisms of resistance. In 2001, Gerding et al considered AS as optimal selection, dosing of antibiotics for best clinical outcome with minimal toxicity , resistance and prevention of infection . In 2017, it was refined as set of coherent action promoting responsible use of antibiotics applicable at individual, national and global levels with beneficial effect in humans, animal and environmental health.

For the execution of AS , a multidisciplinary team is required. The team is ideally comprised of infectious disease specialist, clinical pharmacologist, medical microbiologist, nurses and pediatrician with supporting administrator. The job will be providing local guidelines, advise on antimicrobials use and implement educational interventions. They will also review and audit local antimicrobial prescriptions.

The foremost intervention in this stewardship is the study of the data on antibiotic usage in the population . ARPEC and its global version GARPEC(Gloal Antibiotic Resistance and Efficacy among Children) have series of Point Prevalence Surveys(PPS) and have provided various data. Repeated PPSs are needed to safeguard against inappropriate antibiotic usage and assess efficacy of AS programme.

In March 2017, WHO essential medicine list created three sub categories of antibiotics. The first group ACCESS which is indicated by green include antibiotics with broad spectrum with lower resistance potential. These antibiotics are used as first line in common infections. Second group, WATCH , (Yellow) include antimicrobials with broader spectrum and higher potential for resistance. They are used as second line in selective organisms. They are subject to local or national monitoring and review programmes. The third is RESERVE(Red) category. They are reserved for confirmed and suspected cases of multidrug resistant organisms when alternative approaches fail. This measure is thought to control abuse and inappropriate use of antibiotics.

The need for controlling prescription of antimicrobial is of enormous importance. Pre Prescription Authorisation (PPA) is one way to control the prescription of antimicrobial. This effectively will reduce abuse and inappropriate prescriptions. Disadvantages are some clinicians may feel that autonomy is threatened. Moreover, the authorization may be influenced by the variability of the subject. Finally there can be increased unauthorized use of antimicrobials is the fear. Post Prescription Review Feedback (PPRF) is probably a better option as team member have direct access to index case and evaluate, recommend guideline based on clinical and laboratory data . Presently ,data shows that PPRF approach is associated with substantial reduction of inappropriate antimicrobial prescription.

Another important factor that will help in avoiding antimicrobial as well as in de escalation is Rapid Diagnostic Tests.(RDT). Ideal RDT should be cheap, easy to perform, stable at extremes of temperature. One major shortcoming of RDTs is most of the time it is qualitative, hence the severity of the disease cannot be highlighted by RDT.

Antibiotic cycling was appealing as it was thought that withdrawing an antibiotic for a certain period of time and reintroducing is likely to decrease chances of developing resistance. The finding in different studies remain discordant. Latest study by Van Dujin found little usefulness in reducing resistence. (Lancet Infect. Dis. 18, 401-409)

Diagnostic algorithm is an effective tool leading to decrease in inappropriate antibiotic use. Example is Procalcitonin in community acquired pneumonia where antibiotics are avoided in many instances.

A look at the future threats gives us major incidence of methicillin resistant Staph aureus(MRSA) in 1960s in adults, 30yrs later, in pediatric population. Vancomycin previously thought to be ultimate solution for MRSA had a major jolt when vancomycin intermediate sensitive Staph aureus was isolated from the wound of a Japanese child . Daptomycin and Linezolid are the alternative drugs. Daptomycin will not be of much use in pneumonia as the drug binds to surfactant thereby rendering itself ineffective. Ceftaroline, a 5th generation cephalosporin , has shown good activity against MRSA.

ESBL producing Enterobacteriacaea is another looming threat particularly in low birth weight babies, neonates with prolonged ventilation, prolonged antibiotic treatment and chronic diseases like cystic fibrosis. Therapeutic alternatives are piperacillin- tazabactam, ceftazidime- avibactam, cefepime, fluoroquinolones, aminoglycosides and carbapenems. Resistance to carbapenems has been increasingly reported in pediatric population. Enterobacter resistant to carbapenem has increased from 0% in 2000 to 4.5% in 2012. (Emerg. Infect. Dis. 2015,21:919-925). ESBL Enterobateriacae and carbapenemase producing bacteria are often carriers of plasmid transferred genes that can confer resistance to aminoglycosides and fluoroquinolones.

To conclude, antibiotic resistance is real and present danger raising menace of post antibiotic era. Specific pediatric problems of reckless prescription with wrong diagnosis , lack of pediatric trials and the evolving nature of the diverse population renders it more complex. Creation of team for AS program, use of software to consider multiple variables for specific drug dosage, implementation quick antibiotic susceptibility test to direct definitive therapy and finally encouraging new antibiotic research are few of the practicable interventions towards prevention MDRO development.

Back To Basics: Recognition Of Pediatric Sepsis

Dr. Khangembam Sachikumar
Assistant Prof., SAHS

Sepsis, a syndrome of physiologic, pathologic, and biochemical abnormalities induced by infection, is a major public health concern. Sepsis is the primary cause of death from infection, especially if not recognized and treated promptly. Sepsis is a syndrome shaped by pathogen factors and host factors with characteristics that evolve over time. What differentiates sepsis from infection is an aberrant or dysregulated host response and the presence of organ dysfunction. Sepsis-induced organ dysfunction may be occult; therefore, its presence should be considered in any patient presenting wit infection. Conversely, unrecognized infection may be the cause of new-onset organ dysfunction. Any unexplained organ dysfunction should thus raise the possibility of underlying infection.

A 1991 consensus conference developed initial definitions that focused on the then-prevailing view that sepsis resulted from a host's systemic inflammatory response syndrome (SIRS) to infection. Sepsis complicated by organ dysfunction was termed severe sepsis, which could progress to septic shock, defined as "sepsis-induced hypotension persisting despite adequate fluid resuscitation."

A 2001 task force, recognizing limitations with these definitions, expanded the list of diagnostic criteria but did not offer alternatives because of the lack of supporting evidence. European Society of Intensive Care Medicine and the Society of Critical Care Medicine convened a task force of 19 critical care, infectious disease, surgical, and pulmonary specialists in January 2014 to differentiate sepsis from uncomplicated infection and to update definitions of sepsis and septic shock to be consistent with improved understanding of the pathobiology.

The current use of 2 or more SIRS criteria to identify sepsis was unanimously considered by the task force to be unhelpful. The SIRS criteria do not necessarily indicate a dysregulated life-threatening response. SIRS criteria are present in many hospitalized patients

A large number of health care systems now use Paediatric Early Warning Scores (PEWS) in both ED as well as on the ward, Paediatric Early Warning Systems (PEWS) aim to identify hospitalized children at increased risk of deterioration by assigning a score based on vital signs and clinical status and guiding interventions using a response algorithm to improve outcomes. The early warning score tool for children, the PEWS, consists of three items related to the patient's behaviour, cardiovascular status, and respiratory status scores for the PEWS scale can range from 0 to 9, with a higher number representing a higher risk of clinical deterioration. A score of above 6 needs further assessment.

	0	1	2	3
Behaviour	**Playing/ appropriate**	**Sleeping**	**Irritable**	**Lethargic/confused *or* reduced response to pain**
Cardiovascular	Pink or capillary refill 1–2 seconds	Pale or capillary refill 3 seconds	Gray or capillary refill 4 seconds *or* tachycardia of 20 above normal rate	Gray and mottled or capillary refill 5 seconds or above *or* tachycardia of 30 above normal rate *or* bradycardia
Respiratory	Within normal parameters, no retractions	>10 above normal parameters using accessory muscles *or* 30+ % FiO_2 or 3+	>20 above normal parameters and retractions *or* 40+ % FiO_2 or 6+ L/min	Five below-normal parameters with retractions and grunting *or* 50% FiO_2 or 8+ L/min

Paediatric Early Warning Scores PEWS

There are also associated challenges in identifying which children meeting SIRS criteria may be at risk for sepsis, and so it is essential to review a thorough history to ascertain whether the patient has risk factors for sepsis.

2005 INTERNATIONAL PEDIATRIC SEPSIS CONCENSUS	
SIRS	Meets ≥ 2 of the following criteria, 1 of which must be temperature or WBC count: • Pyrexia (> 38.5 °C) or hypothermia (< 36 °C) • Age-dependent tachycardia or bradycardia • Tachypnea or need for mechanical ventilation • Abnormal WBC count or > 10% immature neutrophils
SEPSIS	**SIRS and suspected or confirmed infection**
SEVERE SEPSIS	**Sepsis and cardiovascular**
	dysfunction, respiratory dysfunction, or ≥ 2 non-cardiorespiratory organ system dysfunctions
SEPSIS 3 (ADULT) 2016 CONCENSUS	**Suspected or confirmed infection** and presence of organ dysfunction

Organ dysfunction can be identified as an acute change in total SOFA score 2 points consequent to the infection. The baseline SOFA (Sequential Organ Failure Assessment) score can be assumed to be zero in patients not known to have pre-existing organ dysfunction.

Although application of Sepsis-3 to children has been attempted formal revisions to the 2005 paediatric sepsis definitions remain pending. Therefore, the majority of studies used to establish evidence for these guidelines referred to the 2005 nomenclature.

The most recent meta-analysis reviewing the criteria for paediatric sepsis was published in 2021 by the Paediatric Sepsis Definition Taskforce. It revealed strong associations of several markers of organ dysfunction with outcomes; in children with sepsis/severe sepsis/septic shock, chronic conditions, oncologic diagnosis, use of vasoactive/inotropic agents, mechanical ventilation, serum lactate, platelet count, fibrinogen, procalcitonin, multi-organ dysfunction syndrome, Paediatric Logistic Organ Dysfunction score, Paediatric Index of Mortality-3, and Paediatric Risk of mortality score each demonstrated significant and consistent associations with mortality.

Barriers to recognition include age-related variation in vital signs, a relatively low prevalence of pediatric sepsis in high-income countries, and alternative more common explanations for abnormal vital signs (fever or crying contributing to tachycardia or tachypnea). Studies have reported that increasing

lactate levels are associated with a higher risk of MODS and mortality in children with infection, in particular, if > 4 mmol/L (> 36 mg/dL). Though, normal lactate does not exclude a sepsis diagnosis in children.

SOFA Sequential Organ Failure Assessment score is not intended to be used as a tool for patient management but as a means to clinically characterize a septic patient.

The pSOFA score was developed by adapting the original SOFA score through 2 approaches. First, the age-dependent cardiovascular and renal variables of the original SOFA score were modified using validated cutoff Second, the respiratory subscore was expanded to include the SpO2:FiO2 ratio as an alternative surrogate of lung injury

Variables	Score				
	0	1	2	3	4
Respiratory					
PaO2:FiO2 [b] or	≥400	300-399	200-299	100-199 With respiratory support	<100 With respiratory support
SpO2:FiO2 [c]	≥292	264-291	221-264	148-220 With respiratory support	<148 With respiratory support
Coagulation					
Platelet count, $\times 10^3$ /µL	≥150	100-149	50-99	20-49	<20
Hepatic					
Bilirubin, mg/dL	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular					
MAP by age group or vasoactive infusion, mm Hg or µg/kg/min [d]					

<1 mo	≥46	<46	Dopamine hydrochloride ≤5 or dobutamine hydrochloride (any)	Dopamine hydrochloride >5 or epinephrine ≤0.1 or norepinephrine bitartrate ≤0.1	Dopamine hydrochloride >15 or epinephrine >0.1 or norepinephrine bitartrate >0.1
1-11 mo	≥55	<55			
12-23 mo	≥60	<60			
24-59 mo	≥62	<62			
60-143 mo	≥65	<65			
144-216 mo	≥67	<67			
>216 mo	≥70	<70			
Neurologic					
Glasgow Coma Score	15	13-14	10-12	6-9	<6
Renal					
Creatinine by age group, mg/dL					
<1 mo	<0.8	0.8-0.9	1.0-1.1	1.2-1.5	≥1.6
1-11 mo	<0.3	0.3-0.4	0.5-0.7	0.8-1.1	≥1.2
12-23 mo	<0.4	0.4-0.5	0.6-1.0	1.1-1.4	≥1.5
24-59 mo	<0.6	0.6-0.8	0.9-1.5	1.6-2.2	≥2.3
60-143 mo	<0.7	0.7-1.0	1.1-1.7	1.8-2.5	≥2.6
144-216 mo	<1.0	1.0-1.6	1.7-2.8	2.9-4.1	≥4.2
>216 mo[e]	<1.2	1.2-1.9	2.0-3.4	3.5-4.9	≥5

The quick SOFA (qSOFA) score, incorporating only altered mentation, systolic blood pressure and respiratory rate, has been suggested as manageable bed-side tool to promptly identify infectious patients prone to poor outcomes. The currently proposed bedside risk-stratification tool of Sepsis-3 criteria, qSOFA, shows moderate prognostic accuracy for PICU transfer and/or mortality in children visiting the ED with suspected bacterial infection. (Front Pediatr. 2018; 6: 266).

2020 Surviving Sepsis Campaign Guideline

Screening, diagnosis, and systematic management	1. Implement systematic screening for timely recognition 2. Consider using blood lactate values to stratify into low- versus high-risk of septic shock or sepsis 2. Implement a protocol/guideline for management of sepsis-related organ dysfunction 3. Obtain blood cultures before starting antimicrobial therapy if this does not delay antimicrobial administration
Antimicrobial therapy	1. Administer antibiotics within 1 h of recognition to children with septic shock and within 3 h of recognition in children with sepsis-associated organ dysfunction without shock 2. Start with empiric broad-spectrum antibiotics to cover all likely pathogens 3. Narrow antimicrobial coverage after culture and susceptibility results 4. Narrow coverage or discontinue antimicrobials if no pathogen is identified, considering site of infection, host risk factors and clinical improvement 5. In children with immune compromise and/or at high risk for multidrug-resistant pathogens, use empiric multi-drug therapy 6. Optimize antimicrobial drug dosing based on pharmacokinetic data 7. Reassess daily for antimicrobial de-escalation 8. Determine antimicrobial duration based on site of infection, microbial etiology, clinical response, and ability to obtain source control
Source control	1. Emergently attain source control if possible 2. Remove intravascular access devices if confirmed to be source of sepsis

Empiric broad-spectrum parenteral antibiotic therapy should be initiated ideally within an hour of the recognition of septic shock, as evidence suggests improvement in survival. In children with sepsis without shock, the 2020 SSC recommends starting antimicrobial therapy after appropriate evaluation and within 3 h of recognition.

Suggested empiric antimicrobial coverage in children with sepsis

Clinical situation	Antibiotic regimen
Sepsis without a defined focus	Ceftriaxone
Sepsis without a defined focus of nosocomial origin	Associate vancomycin
Neonates	Ampicillin + third generation cephalosporin (cefotaxime) + acyclovir (if suspicion of HSV infection)
Suspected genitourinary source	Associate aminoglycoside (e.g., gentamicin)
Suspected atypical pneumonia	Associate azithromycin
Suspected staphylococcal toxic shock syndrome	Associate clindamycin
Suspected encephalitis	Associate acyclovir
Suspected intra-abdominal source	Associate piperacillin with tazobactam, clindamycin, or metronidazole
Suspected COVID-19-related illness (PIMS-TS/MIS-C)	Ceftriaxone. Associate clindamycin if shock
Central venous catheter	Vancomycin + anti-pseudomonal cephalosporin (e.g., cefepime) or piperacillin-tazobactam) or meropenem
Immunocompromise or at risk for infection with Pseudomonas species	Anti-pseudomonal cephalosporin (e.g., cefepime) or meropenem in settings where bacterial organisms with extended-spectrum beta-lactamase (ESBL) resistance are prevalent or for patients who have been recently (within 2 weeks) treated with broad-spectrum antibiotics (e.g. third-generation cephalosporin or fluoroquinolone) Associate vancomycin if risk factors for MRSA are present Increased risk of fungal infection (e.g. immunocompromised with persistent fever on broad-spectrum antibiotics):Associate liposomal amphotericin B or an echinocandin (e.g., caspofungin and micafungin)Risk factors for rickettsial infection (e.g. travel to or reside in an endemic region):Associate tetracycline antibiotic (e.g., doxycycline)Allergic to penicillin or recently received broad-spectrum antibiotics Meropenem Associate vancomycin if risk factors for MRSA are present

Conclusion:

Even though there has been great progress in the recognition, sepsis remains a condition with high morbidity and mortality worldwide. A more accurate definition is required for the paediatric population, to help with correct and timely diagnosis, definition of disease stages and identification of specific therapies for each disease evolution stage.

Haematopoietic Stem Cell Transplantation: A Brief Review

Dr. Ngangbam Sonamani
MD
FNB (Pediatric Haematology Oncology)
Senior Consultant, Mother's Care Children Hospital, Imphal

Introduction

Haematopoietic stem cell transplantation(HSCT) remains the only curative treatment modality for many benign haematological conditions which include bone marrow failure syndromes, congenital haematological defects, immunodeficiency disorders, inborn errors of metabolism and can offer a potential cure for many haematological malignancies and select solid tumours at risk of progression or relapse (Tab 1). HSCT involves a conditioning/preparative phase which includes administration of chemotherapy with or without radiation therapy followed by infusion of autologous (self) or allogenic (other) haematopoietic stem and progenitor cells(HSCs). The allogenic stem cell source, historically donor bone marrow, now includes peripheral haematopoietic stem cells collected by leukapheresis following mobilisation and, more recently umbilical cord blood. The allogenic stem cell donor can be matched related, matched unrelated or haploidentical donor. The conditioning regimen can eradicate any residual malignant cells in case of malignancies and destroys the immune and haematopoietic system of the recipient thereby allowing the HSCs to home in the recipient's haematopoietic microenvironment and engraft. However, it can also cause severe and sometimes life threatening organ toxicities related to chemotherapy. It also takes varying degree of time period following transplantation for the recipient to attain a normally functioning immune system(immune reconstitution). The patients are extremely immunocompromised as a result and are at risk of acquiring severe, sometimes fatal, opportunistic infections. Apart from these, they also experience other transplant related complications such as graft rejection, graft versus host disease and disease relapse. The

transplant related complications along with infectious risks and conditioning related organ toxicities act as barrier to a successful HSCT. However, advancement in understanding of transplant biology, increased knowledge of 44 genetic basis of histocompatibility, improved peri-transplant supportive care and advances in armamentarium to deal with treatment related complications has made it possible for HSCT to emerge as a feasible treatment modality for a wide range of hematologic conditions.

Historical background:

The first ever attempt to restore haematopoiesis was reported in 1939 in a patient of aplastic anaemia by repeatedly infusing fraternal bone marrow. Jacobson et al in 1949 reported that protecting spleen from irradiation allowed lethally irradiated mice to recover normal haematopoiesis. Ford CE et al later on proved that spleen or bone marrow produced elements that were responsible for haematopoietic recovery in these irradiated animals. Barne and Loutit observed an anti-leukaemic effect of transplanted spleen cells in experimental murine models and on the basis of this, they proposed that bone marrow transplant might exert therapeutic effect against malignancies. They further demonstrated that animals who were infused with allogeneic (histo-incompatible) rather than syngeneic (histocompatible) HSCs succumbed to what was termed as 'wasting disease' which would later on be recognised as graft versus host disease (GvHD). ED Thomas performed the first transplants in dogs using high dose radiation and a parallel transplant model in monkeys was established by J Van Bekkum. A major landmark in the field of HSCT was achieved with the observation that administration of methotrexate, an anti metabolite, was effective in treatment as well as prophylaxis of GvHD. Coupled with advancement in knowledge of immune response mediators in transplant biology, the first semi-successful stem cell transplant in human was attempted in 1957 by ED Thomas. A transient graft capable of sustaining haematopoiesis was established in these patients who received chemotherapy followed by intravenous bone marrow infusion. Unfortunately, all the patients experienced disease progression and died. In 1959, ED Thomas made another attempt at transplant in two patients of acute lymphoblastic leukaemia by giving lethal doses of total body irradiation (TBI)

and infusing bone marrow from an identical twin. Graft function was established, however, both the patients succumbed to relapse. Mathe in 1967 reported the first case of sustained hematopoiesis with long term engraftment, acceptable chimerism and anti-leukemic effect in a patient of acute leukaemia. The patient had a successful transplant, however, he died of varicella infection with chronic GvHD on follow up. The first successful allogeneic transplants were done in 1968 and 1969 with three patients of primary immunodeficiency surviving following HSCT. M Bortin in 1970 reported the outcomes of 203 transplants with only 3 survivors at the time of reporting. Majority of the deaths were attributed to GvHD, graft rejection and disease relapse. Following the publication of this not so encouraging results, frequency of HSCT dipped with only a few centres persisting with transplant. Another major breakthrough occurred with the discovery of the HLA system by J Dausset and JJ Van Rood. GvHD and rejection rates decreased drastically by using HLA identical siblings as bone marrow donor. From these pioneer works, HSCT has evolved by leaps and bounds through the years into an attractive therapeutic option for many benign and malignant hematologic conditions.

Table 1 Indications of HSCT in Children:

Conditioning regimens:

Malignant conditions	Non-malignant conditions
Acute lymphoblastic leukaemia	Hemoglobinopathies Thalassemia Sickle cell disease
Acute myelogenous leukaemia	Primary immunodeficiencies SCID Wiscott Aldrich syndrome Leukocyte adhesion defect
Myelodysplasia	Bone marrow failure Fanconi anemia and other inherited bone marrow failure syndromes PNH Aplastic anemia
Myeloproliferative disorders	Neutrophil disorders CGD
Chronic myeloid leukaemia	Inborn errors of metabolism
Hodgkin lymphoma	Hemophagocytic lymphohistiocytosis
Non-Hodgkin lymphoma	

SCID, Severe combined immunodeficiency
PNH, Paroxysmal nocturnal hemoglobinuria

Conditioning regimen refers to the chemotherapy with or without radiation therapy administered to the HSCT recipient before infusion of autologous or allogeneic stem cells. It 46 has distinctive roles in autologous and allogeneic transplants. In autologous transplants, it serves to allow dose intensification of chemotherapy agents which increases its tumour kill capacity, but at the cost of significant haematological toxicities. In allogeneic transplants, it serves three purposes. First, it induces an aplastic marrow thereby creating an empty space for the infused graft to home and gain a competitive advantage in hematopoiesis. Second, it causes enough immunosuppression and prevents the host immune system to mount an effective immune response and reject the infused HSCs. Third, in case of malignancies, it serves to destroy any residual malignant cells. Conditioning regimens which can serve all the three purposes are associated with an increased risk of regimen related toxicities. Organ toxicities can be minimised without compromising on efficacy by pharmacological monitoring of certain drugs such as busulfan so as to maintain a safe therapeutic serum levels. Reducing the dose intensity of the conditioning regimen can also decrease regimen related toxicity. This strategy involves using lower doses of chemotherapy agents and radiation and relies on graft versus leukaemia effect to mount an offensive against malignant cells. The challenge with conditioning is to strike a fine balance between maintaining an optimum myeloablation and manageable toxicities.The introduction of reduced intensity conditioning regimen has made allogeneic HSCT acceptable in many patients who are otherwise ineligible for HSCT with conventional conditioning regimens because of age or presence of co-morbidities.

CIBMTR has published the latest consensus criteria for myeloablative, reduced-intensity and non-myeloablative conditioning regimens based on the severity of bone marrow suppression produced by the regimen(Table 2). Myeloablative regimens produce irreversible myelosuppression within 1 to 3 weeks in the absence of stem cell rescue. Non-myeloablative conditioning produces a transient and minimal myelosuppression and hematopoietic recovery is expected even without stem cell rescue. Reduced-intensity conditioning(RIC) does not fit into either category, as it causes a prolonged pancytopenia that may recover without stem cells support, but in practicality, requires stem cell

rescue. In recent years, RIC has generated a lot of interest in transplant biology, especially in adult population, because of certain features. First, reduced organ toxicity has enabled patients with significant co-morbidities ineligible for conventional high dose conditioning regimen to undergo HSCT. Second, because the duration and intensity of neutropenia produced by RIC is less and host derived immunocompetent T cells are not eliminated immediately, the HSCT recipient does not acquire profound immune compromise in the immediate post transplant phase which may translate into a lower non relapse mortality. The current challenge is to balance these advantages with the risk of potentially reduced anti-leukaemic effects in patients eligible for conventional high dose conditioning regimen. Data from various bone marrow transplant registries has failed to show a definite survival advantage in patients using RIC. Moreover, these patients seem to have a higher risk of disease relapse.

The first hand experience of TBI based conditioning was illustrated in the first human BMT by ED Thomas and colleagues in 1957 and subsequent treatment of victims of radiation accident 47 with bone marrow from unrelated donors in Belgrade in 1959. Cyclophosphamide was introduced as a conditioning agent in 1960s with pioneer works by Santos, Owens and Sensenbrenner. Cyclophosphamide along with TBI became the standard conditioning regimen for HSCT. Recently busulfan, an alkylating agent has gained widespread use as an alternative to TBI, in part because of limited access to radiation facilities.

Myeloablative Conditioning (MAC)
Nonmyeloablative
REDUCED-INTENSITY CONDITIONING

Table 2 Consensus criteria of conditioning regimen intensity:

Adapted from Bacigalupo A, Ballen K, Rizzo D, et al: Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant 15:1628, 2009.

The design of conditioning regimen has evolved over the years to encompass the wide range of applicability of HSCT. For instance, to address

the problem of graft rejection or failure in recipients of T cell depleted graft or mismatched graft, the conditioning regimen has to be devised so as to exert more immunosuppression. In the setting of malignancies, other high dose chemotherapeutic agents have been incorporated in place of or in addition to cyclophosphamide to give more teeth to anti tumour effect. On the other hand, conditioning regimens in aplastic anaemia or primary immunodeficiencies do not require extreme myeloablation as these conditions already have marrow aplasia. Similarly, in benign haematological conditions such as thalassemia or sickle cell disease, the regimens have been designed which do not have much anti tumour effects. In autologous transplants, primacy is accorded to the tumour killing effect of the regimen.

Myeloablative conditioning (MAC)

Total body irradiation

In a TBI based conditioning regimen, irradiation is planned to achieve three goals, namely, to achieve optimum myeloablation, to provide sufficient immunosuppression and in case of malignancies, to maximise eradication of any residual malignant cells. In order to achieve these, various parameters such as total dose, dose rate, fraction size, inter fraction size and shielding are manipulated while planning a schedule. In theory, a higher total dose, higher dose rate and larger fraction size will produce a greater anti leukaemic and immunosuppressive effect, but it is limited by acute and chronic organ toxicities. Strategy to increase non-hematopoietic organ tolerance to high dose irradiation includes hyper-fractionation which refers to division of the total dose of radiation over time. It is usually done on a twice daily basis and can decrease acute toxicities and late side effects of radiation, but at the cost of higher risk of decreased anti-leukaemic effect. Table 2 Consensus criteria of conditioning regimen intensity

Busulfan

Busulfan is an alkylating agent developed in mid 1970s and it gained widespread popularity as an myeloablative agent as an alternative to TBI. Historically, for acute myeloid leukaemia, the most commonly used regimen with busulfan is a combination with cyclophosphamide. The original regimen

was known as ebig BU/CY f and consisted of BU 4mg/kg/day for 4 days and CY 50 mg/kg/day for 4 days. Dose adjustment in the original regimen led to the development of elittle BU/CY f in which dose of CY was reduced to 60 mg/kg/day for 2 days. The BU/CY regimen has become a standard conditioning regimen in paediatric non-malignant conditions as it avoids the radiation associated toxicities. However, busulfan predisposes to an increased risk of veno-occlusive disease (VOD), one of dreaded complications of stem cell transplant. One strategy to decrease toxicity without compromising on efficacy is to monitor serum levels and adjusting the dose. N-acetyl cysteine (NAC), a glutathione repleter, has been used in few studies to minimise busulfan associated VOD, but without a definitive answer. Treosulfan, a closely related compound, has been incorporated as an alternative to busulfan in certain regimens, also known as eReduced toxicity regimen f in an attempt to decrease non haematological toxicities. Regimen related toxicity and outcomes have been compared between BU based and TBI based regimens in many RCTs and meta-analyses. These results seem to identify a higher risk of VOD and disease relapse rates in BU based regimen, although making a definite generalisation can be difficult because of potentially different anti-leukaemic efficacies of big BU/CY and little BU/CY. Accurate monitoring of BU pharmacokinetics may allow individualisation of BU dosing which may translate into a higher risk-benefit ratio.

Many alternative conditioning regimens have been developed by addition or substitution of various chemotherapeutic agents to the BU/CY or BU/TBI regimen. Etoposide, cytarabine and melphalan have either been incorporated or replaced CY in CY/TBI regimen with mixed results. Fludarabine, a purine analog, is being increasingly used in paediatric allogeneic transplants. It has the advantage of being a strong immunosuppressant and produces little non-haematological toxicities and therefore graft failure/rejection rates can be decreased by its addition. Studies have demonstrated a definitive reduced graft failure rates in Fanconi anaemia patients undergoing allogeneic transplant with fludarabine based conditioning. FLU/BU can achieve adequate myeloablation and reasonable immunosuppression along with added advantage of less organ toxicities. Another new entrant is clofarabine, a purine nucleoside analog. Recent

studies have found it to be promising in the settings of relapsed or refractory acute leukaemia. In combination with BU, it has been reported to produce decreased relapse rates in advanced acute leukaemia.

Non myeloablative conditioning and RIC

Non myeloablative conditioning and RIC use the principle of immunological eradication of malignant cells and to create an empty marrow for incoming HSCs to engraft. However, it does not provide adequately strong immunosuppression to prevent graft rejection. A low dose TBI or a purine analogue is usually sufficient to achieve this. Because the dose intensity of both chemotherapeutic agents and irradiation is decreased, the regimen related toxicities are accordingly reduced and therefore many patients ineligible for conventional high dose conditioning regimen due to presence of co-morbities can be taken up for HSCT. Non relapse mortality has been reported to be reduced in non myeloablative or RIC in comparison to MAC. However, caution must be exercised while interpreting in view of increased risk of relapse rates in malignancies and chronic GvHD in non malignant conditions. In paediatric populations, RIC has not gained widespread acceptance, especially in non malignant conditions such as thalassemia and sickle cell disease, due to high rejection rates following standard myeloablative HSCT.

Haematopoietic Stem cells sources

Historically, bone marrow (BM) was the most frequently used stem cell source. It is the primary source of allogeneic stem cells in children. It can be collected from a normal healthy donor from posterior superior iliac crest by repeated aspiration while the donor is under general anaesthesia. The maximum amount of bone marrow that can be collected is approximately 20 ml/kg of donor weight. It is well tolerated procedure and causes minimal side effects which includes risk of anaesthesia, blood loss and transfusion, pain at aspiration site, neurological deficit and psychological complications. In case of ABO incompatibility between the recipient and donor, the bone marrow collected undergoes RBC depletion to prevent haemolytic reaction.

In recent years, HSCs are increasingly being collected from peripheral blood (PB) and neonatal umbilical cord blood. Peripheral blood stem cell collection

is performed through leukapheresis, a procedure wherein the donor receives granulocyte colony-growth factor (G-CSF) at 5 micrograms/kg/day for 4 days prior to collection. Administration of G-CSF causes the HSCs to detach from the stem cell niches and egress into the peripheral circulation thereby increasing their frequency. The cells with surface marker CD34 are identified and thus helps in enumeration of stem cells. Another approach to increase stem cell yield is to concurrently administer plerixafor and G-CSF. Plerixafor is a reversible antagonist of CXCR4 receptor which is expressed on HSCs surface and promotes adhesion of HSCs to bone marrow milieu. Complications arising out of peripheral stem cell collection are few and far and include bone pain, and those related to central venous access in case of difficult peripheral venous access. A very rare complication is splenic rupture. Use of umbilical cord blood (UCB) as a stem cell source has gained popularity recently. In this, placental blood is collected from umbilical blood and cryopreserved for future use. It offers distinct advantages as allogeneic stem cell source. It is readily available and stringent criteria for HLA typing need not apply thereby enlarging the donor pool. Major drawback is the limited number of stem cells in each cord blood unit which may delay engraftment. The primary difference between stem cells from BM, PB and UCB is the frequency of stem cells, the proportion of pluripotent to lineage-committed progenitor cells and constitution of immune reactive cells.

Selection of Stem cell donor

The selection of stem cell donor in allogeneic HSCT depends on many factors. First and foremost is the availability of HLA matched sibling donor. Risks of graft rejection, acute and chronic GvHD increase as the genetic disparity between the recipient and donor (histoincompatibility) increases. Even in cases of HLA identical siblings, donor selection can still be refined according to donor age, sex, parity and infectious disease status. Second, donor selection is also influenced by speed at which stem cells can be accessed, especially in case of aggressive malignancies.

Unrelated donor

A major obstacle to allogeneic HSCT is the unavailability of matched sibling donor in majority of the patients requiring transplant (70%). For these

patients, potential donors include family members with phenotypical match at HLA locus but distinctive genotype or unrelated donors who match the recipient at majority of the allele. The probability of finding an unrelated matched donor depends on two factors, namely, the ethnicity of the patient because it determines the histocompatibility antigen and haplotype frequency, and the composition of donor pool. The rapid growth of unrelated HSCT in recent years has been facilitated by establishment of volunteer donor registries worldwide. Anthony Nolan Research Institute was the first registry to promote access to HLA matched bone marrow donors for patients. The National Marrow Donor Program (NMDP), a national registry in US was established in 1986 through a federal act. Concurrently, in Europe, Europdonor Foundation led by Jon J van Rood formed a database of HLA phenotypes known as Bone Marrow Donors Worldwide (BMWD). It takes a median time of 51 days from initiation of unrelated donor search to make a formal request for donation. Therefore, conducting an efficient unrelated donor search becomes critical for all patients.

Factorsthat should be taken into consideration while selecting unrelated donors are the degree of HLA type match and presence of anti-donor antibodies in the recipient. Presence of donor specific antibodies puts the recipient at high risk of graft rejection. When a patient has more than one HLA identical match, other criteria to select donor are applied. First, stem cells from a younger donor is preferred because of lower risks of developing acute GvHD, chronic GvHD and improved disease free survival (DFS). Second, donor sex do not seem to influence incidence of acute or chronic GvHD, rejection rate or survival outcomes. Third, transplantation done using parous female donor results in increased risk of developing chronic GvHD with the risk increasing proportionally to number of parity. Of all the factors, donor-recipient HLA match and younger donor are two most significant factors which influence transplant outcome. The concept of eback-up f donor and its importance is being realised of late because in certain cases, collection of stem cells may be deferred. In case where back-up donor was identified, the median delay to rescheduling transplant is significantly shorter as compared to cases where no back-up donor was available.

Genetics of Human leucocyte antigen

In transplant biology, a group of genes known as the major histocompatibility complex (MHC), located at a region of short arm of chromosome 6 plays the most crucial role in deciding transplant outcomes. Complications as graft rejection and GvHD increase as genetic disparity between donor and recipient increases. Antigens most associated with these complications are encoded by genes located at MHC. The most studied and characterised products of these genes are MHC class I and II antigens. Class I molecules include HLA-A, HLA-B and HLA-C, while class II antigens include HLA-D, of which there are five distinct subregions including DR, DQ and DP. The function of class I and II molecules is to present partially degraded products of intracellular proteins to T cells so as to enable them to be identified by the immune system. However, in HSCT, recognition of MHC molecules by the T cells, termed as alloimmunity, lead to graft rejection or GvHD. Some of the definitions commonly used in HLA genetics are as follows:

a. Allele: Unique sequence of an HLA gene defined by molecular methods

b. Antigen: HLA molecule that is recognized by an antibody

c. Haplotype: HLA genes inherited as a chromosomal unit

d. Genotype: Molecularly defined HLA allele or sequence

e. Phenotype: Serologically defined HLA protein or antigen

HLA typing

Serology, cellular and DNA methods

HLA typing by serological method using complement dependent microcytotoxicity assay with alloantisera containing antibodies against polymorphic HLA specificities used to be gold standard in the 60 fs. Different HLA molecules can share a public specificity, whereas a private specificity is unique to a single HLA molecule. The combination of HLA antigens of an individual defines their phenotype, and the alleles define their genotype. The most commonly used cellular assay for the class II region is the mixed lymphocyte culture (MLC), wherein disparity between the antigens encoded by the HLA

class II genes in the donor and recipient leads to lymphocyte activation and proliferation. However, MLC has limited clinical utility for donor selection as it is not predictive of GVHD. Another technique, known as limiting dilution assay, for determining the frequency of donor cytotoxic T-lymphocyte and helper T-lymphocyte precursors against recipient targets is predictive of risk of acute GVHD in settings of unrelated donor transplants with T cell depletion as GVHD prophylaxis. The advent of polymerase chain reaction (PCR) has revolutionised HLA typing approaches and many PCR based methods are in current practice. These include sequence-specific primer method, sequence-specific oligonucleotide probe hybridization (SSOPH) method and oligonucleotide array technology. The term g6/6 h matched refers to recipients and donors who share the same low-resolution-defined HLA-A, HLA-B, and HLA-DR gene. The term g8/8 h and g10/10 h refer to high-resolution-defined matching at the four loci (HLA-A, HLA-B, HLA-C, and HLA-DRB1) and the five loci (HLA-A, HLA-B, HLA-C, and HLA-DRB1 and HLA-DQB1) respectively.

Concept of vector of mismatching

The gvector h or gdirection h of HLA compatibility between a donor and a recipient assumes much clinical significance in transplant biology because it predicts the risk of graft rejection and GVHD. The presence of donor alleles not shared by the recipient is the genetic basis of Host-Versus-Graft (HVG) allorecognition, whereas the presence of recipient alleles not shared by the donor determines Graft-Versus-Host (GVH) allorecognition. gBidirectional h mismatching refers to the condition in which a given HLA locus has both GVH and HVG vectors. A gunidirectional h mismatch in the direction of GVH vector is said to happen when the donor is homozygous and the recipient is heterozygous and shares one allele with the donor at the mismatched locus. A gunidirectional h mismatch in the direction of HVG vector occurs when the recipient is homozygous and the donor is heterozygous and shares one allele with the recipient at the mismatched locus.

Graft versus leukaemia effects

There exists substantial clinical evidences backed by laboratory findings that alloreactive cells possess potent anti tumor activities. The term eadoptive

immunotherapy f was coined by Mathe and colleagues for the anti malignant effects of allogeneic stem cells. It can be either specific or non- specific. Specific adoptive immunotherapy is the one one derived after sensitisation of donor fs cells to antigens present on the leukaemic cells, whereas non-specific adoptive immunotherapy follows an immune reaction of donor lymphocytes against both normal and malignant host cells. It is triggered by differences in histocompatibility antigens and associated with GVHD. Bortin and coworkers coined the term ggraft-versus-leukaemia h for the adoptive immunotherapeutic effect of allogeneic hematopoietic cells against leukemia cells. The role of natural killer (NK) cells in decreasing risk of relapse is being increasingly understood. The KIR (killer cell immunoglobulin like receptor) family can impact transplant outcomes independent of HLA, especially relapse-free survival in AML. Non-expression of the inhibitory KIRs f ligand on the recipient fs leukemic cells can trigger donor NK cell alloreactivity against leukemia thereby reducing risk of leukaemia relapse. It has been suggested to incorporate KIR genotyping in addition to HLA typing for selecting donors with favourable KIR haplotype, particularly in cases of AML transplants.

Haploidentical hematopoietic transplantation

Only 25% of patients requiring HSCT have HLA identical sibling donors. Alternative sources of HSCs include matched unrelated donors (MUDs), unrelated donor umbilical cord blood (UD-UCB), and full haplotype-mismatched related donor. Transplantation from a full haplotype- mismatched family member has many distinct advantages. These include universal availability for almost all patients, luxury of choosing best donor from a panel of candidate family members, no undue delay in obtaining the graft and finally, easy access to donor for cellular therapies after transplantation, if required. Because transplantation is done across HLA barrier, high frequency of T cells that recognize major class I or II HLA disparities between donor and recipient induces very strong graft-versus-host and host-versus-graft alloresponses. Consequently, the major obstacles to a successful haploidentical hematopoietic transplantation are graft rejection, delayed immune reconstitution, significant GVHD and leukaemia relapse.

Two distinct and opposing modalities for haploidentical hematopoietic transplantation have been been developed and evaluated in great details with each of them having their own intrinsic challenges.

1. T cell.replete haplotype-mismatched HSCT

In this method, the high T cell content of the graft induces significant GVHD related mortality and morbidity, although these alloreactive cells can also potentiate the GVL effect. In circumstances where strategies have been used to prevent GVHD in T cell replete HSCT such as post transplantation high dose cyclophosphamide, the weakened graft has led to high incidence of leukaemia relapse. The current challenges in this method are to reduce the incidence and severity of GVHD and to prevent leukemia relapse. Some of modalities currently in practice include:

A. G-CSF.primed graft with robust posttransplantation GVHD prophylaxis

B. Rapamycin-based GVHD prophylaxis

C. High-dose cyclophosphamide-based GVHD prophylaxis

2. T cell.depleted haplotype-mismatched HSCT

The incidence of significant GVHD is greatly reduced even without any posttransplantation immunosuppression because of the minimal residual T lymphocytes in the depleted graft. However, immune recovery is delayed due to the small number of infused T cells which may lead to increased risk of severe and sometimes lethal opportunistic infections. Efforts are being made to accelerate immune reconstitution by adoptive transfer of non-alloreactive pathogen-specific or broad repertoire T lymphocytes, depleted of alloreactive clones. Various strategies for T cell depletion or graft manipulation include:

A. Mega-dose HSCT after myeloablative TBI-based conditioning

B. CD3/CD19 depletion

C. TCR ../.. and CD 19 depletion

D. Alemtuzumab-based T-cell depletion

Engraftment

After infusion of allogeneic haematopoietic stem cells into the recipient, these HSCs begin to repopulate in the stem cell niches, proliferate and differentiate into mature blood cells of different lineages. Because the donor stem cells are subject to increased replication, HSCT recipients have accelerated telomere shortening in peripheral blood. Haematopoietic recovery after HSCT can be assessed by two methods, namely, count recovery of peripheral blood cells and chimerism studies. Chimerism studies are carried out using chromosomal FISH technique for sex mismatched pairs and DNA finger printing of PCR amplified short tandem repeats (STR) for sex matched pairs.

Many factors influence the rate and probability of engraftment after HSCT. They include source of HSCs, the dose of HSCs infused, graft manipulation, and the GVHD prophylactic regimen. In general, neutrophil engraftment occurs by 2 to 3 weeks and platelet recovery is achieved 1 to 2 weeks later. Neutrophil engraftment occurs much more rapidly in PBSC transplants as compared to BM transplants, possibly because of higher stem cell number collected. It usually occurs by approximately 2 weeks post transplantation in case of peripheral blood (PB) stem cell source and by around 3 weeks in BM transplants. Platelet recovery is achieved 4 to 7 days earlier with PBSC transplants in comparison with BMT. Umbilical cord transplantation is associated with significantly delayed haematological recovery due to lower nucleated cell and stem cell content in UC products.

Delayed engraftment usually follows administration of methotrexate as GVHD prophylaxis, whereas count recovery is robust with T cell depleted BM transplant. Using G-CSF is also associated with rapid haematological recovery in settings of related BM transplants. Incidence rates of GVHD varies with the number and quality of HSCs from different sources (peripheral blood, bone marrow, and UCB). PBSC transplants are associated with higher incidence of GVHD, especially chronic, than with BM transplants. This risk is, however, compensated by the more potent GVL seen in PBSC transplants. Incidence of GVHD is lower with UCB transplants as compared with PBSC or BM transplants and this allows for less stringent criteria for HLA typing in UCB transplants.

Immune reconstitution

HSCT results in a state of profound immune incompetence resulting from loss of immunity conferring cells, both innate and adaptive. Therefore, HSCT recipients are at risk of acquiring severe and, sometimes, lethal opportunistic infections for several weeks following transplantation and infectious complications related mortality remains one of the major obstacles to a successful transplantation. Although the degree of myelosuppression varies with type of conditioning regimen, haematopoiesis usually occurs within weeks of transplant. However, the depth and extent of immune incompetence are similar in recipients of both myeloablative and non-myeloablative regimens and it takes a much longer time for recovery of a fully functional immune system.

Several factors such as conditioning regimen, HSCs source, presence of GVHD and/or its treatment, T-cell content of the graft and patient age determine the degree, extent and duration of immune incompetence experienced by the individual HCT recipient. In general, natural killer (NK) cells are the first lymphocyte subset to show recovery followed by CD8 T cells, B cells and ultimately CD4 T cells in that order. Two distinct pathways are involved for regeneration of lymphocytes. In the first pathway, lymphocytes regenerate from BM lymphoid progenitors regenerating a naive immune system. NK cells recover though this pathway exclusively. NK cells usually show full recovery in the first 1 to 2 months post transplantation. Although B cells are also regenerated from lymphoid progenitors, there exists a fundamental difference between regeneration of B cells from that of NK cells. B cell lymphoid regeneration requires a specialized marrow microenvironment termed the ebursal equivalent f which is highly sensitive to toxic effects of GVHD/ or its treatment and regimen related toxicities. In the second pathway, termed homeostatic peripheral expansion, mature T cells contained within the graft expand in vivo in response to T-cell lymphopenia. Various factors drive this process including availability of homeostatic cytokines (IL-7 and IL-15) produced in response to lymphopenia; inflammatory cytokines induced by the preparative regimen; and exposure to viral antigens during the period of profound lymphopenia. This pathway seems to contribute insignificantly to B cell lymphopoiesis as compared to lymphoid progenitors regeneration.

Within the T cell subsets, homeostatic peripheral expansion is much more effective for CD8 T cells than for CD4 T cells. T cell lymphopoiesis may also occur through thymic- dependent pathway wherein a substantial rise in CD4 T cell numbers with diversification of the T cell repertoire can be observed. However, thymic-dependent T-cell regeneration is generally delayed for months to years as factors such as GVHD and treatment have a profound toxic effect on thymic micro-environment. CD4 counts is the most readily available and dependable predictive marker for the recovery of immune competence after HCT.

Infections and treatment

The risk of acquiring life threatening infections are greatest in allogeneic and T cell depleted HSCT. Different infectious complications occurring during different post transplantation phases have been identified. The time from transplant and the presence or absence of GVHD/ or its treatment primarily determine the risk of infection. Other factors are donor/host histocompatibility, disease status, graft source, graft contents, conditioning regimen and neutrophil engraftment.

Post transplant period can be divided into three distinct phases:

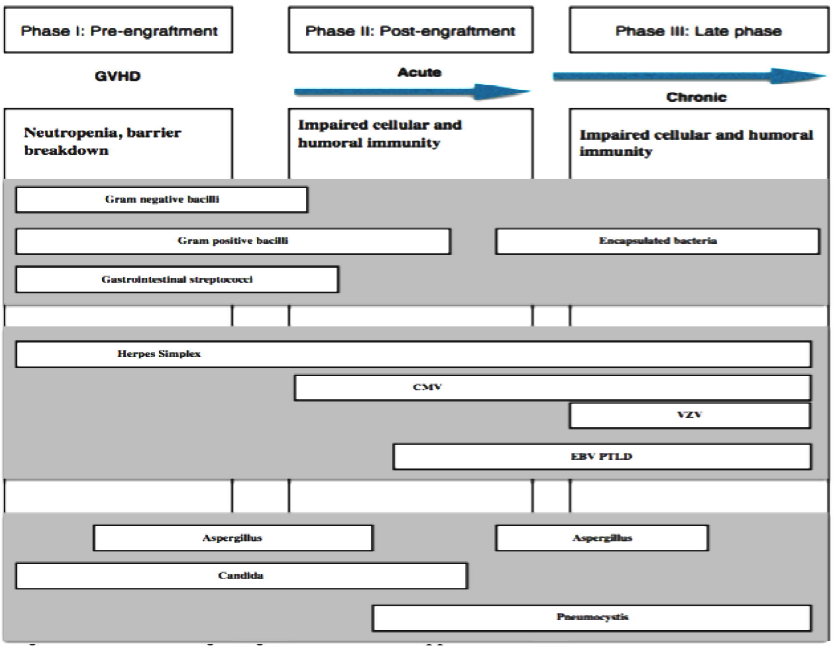
1. Phase I : Pre-engraftment phase (<15.45 days after HCT)
2. Phase II: Post-engraftment phase (30.100 days after HCT)
3. Phase III : Late phase (>100 days after HCT)

Phase I is characterised by prolonged neutropenia and compromised muco-cutaneous barrier resulting in greater risk of bacteria and fungal infections including Candida species and later on Aspergillus species. HSV reactivation also occurs during this period. Administration of prophylactic fluconazole after allogeneic HSCT confers protection against disseminated candidal infections and improved survival, but at the cost of emergence of resistant species. Other agents that have been tried as prophylactic fungal agents include low-dose amphotericin B, liposomal amphotericin and voriconazole.

During phase II, infections related primarily to impaired cell-mediated immunity dominate. Various factors including extent of GVHD and the immunosuppressive therapy for it play a significant role in determining the scope of this defect. Herpesviruses, particularly CMV, Pneumocystis jiroveci and

Aspergillus species are the major infectious threats during this period. The clinical manifestations of CMV after HSCT include pneumonia/ interstitial pneumonitis, colitis, hepatitis, and CMV- related cytopenias. Acyclovir, valacyclovir, and ganciclovir prophylaxis initiated at engraftment have greatly reduced incidence of CMV disease before day 100. Pre-emptive CMV treatment based on the results of positive culture, polymerase chain reaction assay, antigen testing, or bronchoalveolar lavage fluid evaluation have been studied in multiple clinical trials without definitive advantage over prophylactic treatment. Ganciclovir and foscarnet are both equally effective and considered first line therapy for CMV activation and CMV disease.

Common pathogens during phase III include CMV, VZV and infections with encapsulated bacteria (e.g. Streptococcus pneumoniae). Patients at risk of developing infections in Phase III are HSCT recipients of alternative donor and those with chronic GVHD. For prevention of VZV, current recommendation is to administer prophylactic acyclovir for 1 year after HSCT for all patients with a history of varicella infection prior to transplant.



Sinusoidal obstruction syndrome (SOS)/Veno-occlusive disease (VOD)

Veno-occlusive disease (VOD), also termed as Sinusoidal obstruction syndrome (SOS) is a potentially life threatening condition that can develop after HSCT. The condition usually occurs by day 30 post transplantation, although it may develop later. The historical incidence rates ranging from 5% to 60% relate to many factors which include intensity of the conditioning regimen, presence of risk factors and importantly the clinical criteria used for diagnosis. SOS/VOD develops more commonly in allogeneic HSCT with myelo-ablative conditioning, with incidence rates of 10 -15%, as compared to reduced intensity conditioning and autologous transplants with incidence rate of less than 5%. Presentation of SOS/VOD varies widely from mild forms, which usually resolves within a few weeks, to a severe form, defined by the presence of multi-organ dysfunction, with extremely high mortality rate (>80%).

The sinusoidal endothelial cells are first to show affected in SOS/VOD, resulting in obstruction of the hepatic sinusoids in the zone 3 of the hepatic acinus. The same underlying pathology can lead to other endothelial syndromes post transplantation such as capillary leak syndrome, engraftment syndrome, transplant-associated microangiopathy or diffuse alveolar haemorrhage. The postulated theory to explain SOS/VOD is that during HSCT, there occurs intense activation of sinusoidal endothelial cells by various factors such as chemotherapy or radiotherapy included in the conditioning regimen, cytokines released from the injured tissues, endogenous microbial products translocated through damaged mucosal barriers and the complex process of engraftment, which can evolve into endothelial damage leading to appearance of gaps in sinusoidal barrier. This process then facilitates egress of RBCs, leucocytes and cellular debris into the space of Disse and thereby dissecting the endothelial lining. The end result is sloughed sinusoidal lining which embolizes and obstructs sinusoidal flow. The increased resistance to blood flow leads to post sinusoidal portal hypertension, progressive hepatic dysfunction and finally to multi organ failure and death. Along with the physical damage, a procoagulant state follows with low levels of antithrombin, protein C (which may be a useful marker) and factor VII, and increased levels of plasminogen activator inhibitor 1. Other factor which contributes to development of SOS/VOD is that of low constitutive levels of GSH

(glutathione) in centrilobular hepatocytes together with further depletion of intracellular glutathione store by cytotoxic agents leading to endothelial damage.

Various risk factors for the development of SOS/VOD have been studied extensively and validated as listed below.

Table 3 Risk factors for SOS/VOmD

Transplant related	Hepatic related risk factors
Allo-HSCT Unrelated donor HLA-mismatched donor Myeloablative conditioning regimen BU-based conditioning regimen TBI-based conditioning regimen Non-T-cell-depleted graft Second HSCT	Transaminase > 2.5 ULN Serum bilirubin > 1.5 ULN Cirrhosis Hepatic fibrosis Active viral hepatitis Hepatic irradiation Previous use of gemtuzumab ozogamicin Iron overload
Patient and disease related	

Transplant related	Hepatic related risk factors
Young age Low weight Gene polymorphism (GSTM1, GSMTT1, heparanase) Advanced disease (beyond second CR or relapse) Deficit of AT III, t-PA and resistance to activated protein C Thalassemia Hemophagocytic lymphohistiocytosis Adrenoleucodystrophy Osteopetrosis High-dose auto-HSCT in neuroblastoma Juvenile myelo-monocytic chronic leukemia	

ULN: upper limit of normal

Adapted from M Mohty, F Malard, M Abecassis, et al: Sinusoidal obstruction syndrome/veno-occlusive disease: current situation and perspectives—a position statement from the European Society for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplantation (2015) 50, 781–789

SOS/VOD is a clinical entity consisting of a constellation of syndromes including jaundice, ascites and/or unexplained weight gain, and hepatomegaly and/or upper-quadrant abdominal pain. Daily strict monitoring for fluid retention, weight gain, oedema, ascites, hepatomegaly, and jaundice should be ensured from the start of conditioning upto at least 2 weeks after HSCT to detect early signs and symptoms of SOS/VOD, particularly in patients at high risk of developing VOD. The diagnosis is based on the presence of established clinical criteria as defined in modified Seattle and Baltimore criteria.

Table 4 Clinical criteria for diagnosis of SOS/VOD

Modified Seattle Criteria	Baltimore Criteria
Two of the following within 20 days of transplant	Bilirubin > 2mg/dL within 21 days of transplant and two of the following criteria
Bilirubin > 2mg/dL	Hepatomegaly
Hepatomegaly or right upper quadrant pain	Ascites
Weight gain (> 2% from pre-transplant weight)	Weight gain (> 5% from pre-transplant weight)

SOS/VOD can also present as new onset transfusion refractory thrombocytopenia with rapid consumption of transfused platelets not explained by other conditions like sepsis during early period following HSCT. This may be one of the earliest signs of SOS/VOD and is in conjunction with the postulated theory of endothelial nature of SOS/VOD pathobiology. Ultrasonographic evidence of attenuated or reversed hepatic venous flow has high specificity, whereas ultrasound-confirmed ascites and/or hepatomegaly and gall bladder wall thickening may act as useful adjuncts for SOS/VOD diagnosis. Confirmatory diagnosis of SOS/VOD can be made by measurement of the hepatic venous gradient pressure through the jugular vein and liver biopsy, though these are invasive and not in routine practice.

Strategies to prevent SOS/VOD are twofold: reversal of SOS/VOD risk factors and pharmacological intervention. Transplant related risk factors are

amenable to modifications. Certain measures such as pharmacokinetic monitoring of busulfan allowing individualised dose modification ; a change in the order of the drugs (CY/BU instead of BU/CY); and hyper- fractionated TBI in case of TBI based MAC may decrease the risk of SOS/VOD. Pharmacological measures employed for prevention of SOS include heparin, both unfractionated and low-molecular-weight; ursodeoxycholic acid; and defibrotide (DF).

Symptomatic management is the first step in treatment of SOS/VOD. A high index of suspicion is required to identify the condition and immediate measures including fluid and sodium balance, judicious use of diuretics should be started, when diagnosis of SOS/VOD is still only probable. Other symptomatic measures include peritoneocentesis for massive ascites, hemodialysis for renal insufficiency, transjugular intrahepatic portosystemic shunt and liver transplant for severe syndrome. The only proven therapeutic agent till now is defibrotide. DF is a polydisperse oligonucleotide with local anti-thrombotic, anti-ischemic and anti-inflammatory activity and has protective effects on the small vessel endothelium. It seems to act through two distinct mechanisms: protection of endothelial cells and restoration of the thrombotic-fibrinolytic balance. Future perspectives for improved management of SOS/VOD are defining new accurate criteria for diagnosis and grading; more precise identification of risk factors and identification of potential biomarkers.

Graft-versus-host disease

Acute Graft-versus-host disease

Acute Graft-versus-host disease (aGVHD) is a frequent and unpredictable severe inflammatory complication of allogeneic hematopoietic cell transplantation caused when immune competent cells of the graft recognize host tissues, resulting in inflammation and tissue damage. It is one of the most important barriers to a successful HSCT. Several advancements in aGVHD detection, prophylaxis, and treatment modalities might usher an era of multimodal, personalized immunomodulation in management of aGVHD. Several risk factors have been identified for the development of aGVHD and its severity. Increasing genetic disparity between the donor and host is by far the most important single predictor for development of aGVHD. Other risk factors are unrelated donor (UD) transplants, mis-matched related or unrelated donor transplants, TBI-based conditioning regimen, and transplants using female donors. On the other hand,

certain genetic risk factors such as specific polymorphisms in the recipient interleukin (IL)-10 promoter region and IL-10 receptor beta gene (IL10RB) are associated with a lower risk of developing aGVHD.

The pathophysiology of aGVHD consists of three distinct phases: initiation phase; expansion, trafficking, and effector phase; and treatment phase.

The initiation phase involves triggers and sensors of GVH reactions. The genetic triggers include histocompatibility disparities in HLA, miHAs (minor histocompatibility antigens) in case of HLA-matched sibling and KIR (killer immunoglobulin-like receptor). Several non-genetic triggers have also been identified, particularly the danger signals: DAMPs (damage-associated molecular patterns) and/or PAMPs ((pathogen-associated molecular patterns). DAMPs include extracellular matrix components, adenosine triphosphate (ATP), and uric acid. The antigen-presenting cells (APCs) act as the predominant sensors of these GVH triggers. APCs which contribute to GHVD pathophysiology include residual host hematopoietic APCs, host non-hematopoietic APCs, and donor APCs from the graft. Activated APCs then present host antigens to donor T cells resulting in recruitment, activation and proliferation of donor T cells. The donor T cells play the central role in the effector phase while neutrophils, natural killer cells (NK), NK T cells (NKT), and macrophages also contribute to the end-organ damage. Suppressor cells produce a tolerance promoting response thereby reducing inflammation and overall organ damage in GVHD relates to the delicate balance between the effectors and the suppressors. The major suppressors include regulatory T cells (Tregs), myeloid-derived 62

suppressor cells (MDSCs), NK cells and NKT cells. Some of the biomarkers that are released into the circulation during the effector phase include soluble CD30, elafin (skin-specific), regenerating islet-derived 3-a (REG3a; gut-specific), suppressor of tumorigenicity 2 (ST2) and microRNA. In the treatment phase, after initiation of treatment, three primary immunologic outcomes are possible:

1. complete resolution after therapy

2. partially clinical response, but incomplete immunological resolution, leading to state of “injurious resolution”

3. progression despite therapy

The clinical syndrome of aGVHD usually manifests within 100 days after transplant. The classical organs involved in aGVHD are skin (severity ranging from maculo-papular rash to erythroderma and bullae formation), the gastrointestinal tract (resulting in nausea, vomiting, abdominal cramps, and/or diarrhea), and the liver (resulting in hyperbilirubinemia, jaundice, and/or elevated enzymes). Hematopoietic system, endothelium, lungs, CNS and other organs can also be affected. Laboratory findings include lymphopenia, eosinophilia, thrombocytopenia, and hypoalbuminemia.

Staging and overall grade of aGVHD

The severity of aGVHD takes into consideration the stage of individual organ involved, clinical status of the patient and overall grade for the disease. The modified Glucksberg criteria (table 5 and 6) is historically used for both staging of individual organs involved and overall grading. The International Bone Marrow Transplant Registry (IBMTR) severity index (table 7) is based on organ involvement alone with no subjective assessment of performance.

Table 5 Glucksberg criteria for staging of aGVHD :

Organ involved	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4
Skin	No rash	Rash <25% body surface area	25-50%	>50% generalized erythroderma	Bullae and desquamation
Intestines	<10 mL/kg/day	10-19.9 mL/kg/day	20-30 mL/kg/day	>30 mL/kg/day	Severe abdominal pain, ileus, blood or melena
Upper GI	Severe nausea/ vomiting				
Liver	Bilirubin ≤2 mg/dL	Bilirubin 2.1-3 mg/ dL	Bilirubin 3.1-6 mg/ dL	Bilirubin 6.1-15 mg/ dL	Bilirubin > 15 mg/ dL

Table 6 Glucksberg Criteria for Staging and Grading of Acute Graft-Versus-Host Disease - Overall Grade

Overall Clinical Grade	Organ System	Clinical Stage
I (mild)	Skin Liver Gastrointestinal	1-2 1 0
II (moderate)	Skin Liver Gastrointestinal	1-3 1-2 1
III (severe)	Skin Liver Gastrointestinal	2-4 2-4 2-4
IV (life threatening)	Skin Liver Gastrointestinal	2-4 2-4 2-4

Table 7 International Bone Marrow Transplant Registry Severity Index for Acute Graft-Versus-Host Disease

Severity Index	Skin	Liver	Gastrointestinal	Upper gastrointestinal
A	1	0	0	0
B	2	1-2	1-2	1
C	3	3	3	
D	4	4	4	

Prevention and treatment of aGVHD

For most T cell replete transplants, the typical GVHD prophylaxis consists of a calcineurin inhibitor and methotrexate or mycophenylate mofetil (MMF). Steroids do not have a proven role in GVHD prophylaxis. Another method

employed as GVHD prophylaxis is T-cell depletion, either ex vivo, ie, CD34 positive selection or CD3, TCR 5ØüÞ/5ØýÞ depletion, or in vivo via administration of antithymocyte globulin or alemtuzumab. Novel therapeutic agents have been studied in recent clinical trials which have shown early promises. These include maraviroc, a CCR5 inhibitor; bortezomib, an NF-kB–inhibiting immunomodulator and vorinostat, which has tolerogenic effects on APCs.

The standard first line therapy for aGVHD is systemic corticosteroids at a dose of 2mg/kg/day of methylprednisolone. For grade I-II aGVHD, a lower daily dose of 1mg/kg/day seems to confer equal efficacy and systemic side effects of high dose steroid can be avoided with this strategy. Within a few days of initial therapy, a decision regarding steroid-responsiveness or steroid refractoriness should be made. For steroid reponders, corticosteroids are tapered by 10% every 3 to 5 days and once doses are decreased to less than 1 mg/kg/day, they are tapered more slowly. Steroid refractoriness is recognised after 7 to 10 days and progression even sooner if the patient worsens 3 to 4 days after initiation of high-dose steroids. Approximately 35% of patients do not show response to steroid, these patients are candidates for second line immunosuppressive therapy. Various pharmacological agents have been tried, including methotrexate, MMF, IL-2 receptor–targeting agents (etanercept, infliximab), basiliximab, antithymocyte globulin, sirolimus and mesenchymal stem cells. However, none has a proven superiority to the other.

Chronic Graft-versus-host disease

Chronic Graft-versus-host disease presents as a constellation of clinical features and histopathological findings and resembles autoimmune systemic collagen vascular diseases with protean manifestations virtually involving almost every organ (table 8). Although it generally develops after day 100 post transplantation, it can also present as early as 50 days after transplant. Staging of cGVHD is done based on degree of organ involvement.

Table 8 Clinicopathologic Features of Chronic Graft-Versus-Host Disease

Organ system	Clinical features	Histopathological findings
Systemic	Recurrent infections with immunodeficiency	
Skin	Lichen planus, scleroderma, hyperpigmentation or hypopigmentation, dry scale, ulcerated, erythema, freckling Flexion contractures	Epithelial cell damage, basal cell degeneration and necrosis, epidermal atrophy and dermal fibrosis
Hair	Alopecia	
Mouth	Sicca syndrome, depapillation of tongue with variegations, scalloping of lateral margins	Lichenoid changes with mononuclear infiltrates, epithelial cell necrosis, salivary gland inflammation, lymphocyte infiltration, fibrosis
Joints	Decreased range of motion, diffuse myositis/tendonitis	
Eyes	Decreased tearing, injected sclerae, conjunctivae	
Liver	Increased alkaline phosphatase > aminotransferases and bilirubin Cholestasis, cirrhosis	Focal portal inflammation with bile ductile obliteration, chronic aggressive hepatitis, bridging necrosis, cirrhosis
Gastrointestinal	Failure to thrive (children), weight loss (adults) Esophageal strictures, malabsorption, chronic diarrhea	Crypt destruction, single-cell dropout, fibrosis of lamina propria
Lung	Cough, dyspnea, wheezing Bronchiolitis obliterans, chronic rales, pneumothorax	
Hematology	Refractory thrombocytopenia, eosinophilia, Howell-Jolly bodies	

Organ toxicity associated with hematopoietic stem cell transplantation

Organ system	Toxicities
Eye	Decreased lacrimation secondary to cGVHD leading to ocular sicca syndrome, photophobia, punctate keratopathy, scar formation, and corneal perforation Transplant-related retinopathy: optic disc edema Retinal hemorrhages Occlusive microvascular retinopathy Cataracts
Dental	Oral sicca syndrome
Lungs	Idiopathic pneumonia syndrome (IPS) Diffuse alveolar hemorrhage (DAH) Bronchiolitis obliterans syndrome (BOS) Bronchiolitis obliterans organizing pneumonia (BOOP) Periengraftment respiratory distress syndrome (PERDS)
Hematologic	Immune hemolytic anemias Autoimmune hemolytic anemia Autoimmune thrombocytopenia Autoimmune neutropenia
Renal	Chronic kidney disease Thrombotic microangiopathy
Neurologic	Essential tremor and paresthesias Seizures, visual disturbances, and encephalopathy Posterior leukoencephalopathy Cerebrovascular accidents Central nervous system (CNS) infections Leukoencephalopathy
Endocrine	Thyroid dysfunction Growth failure Gonadal failure
Bone	Decreased bone mineral density (BMD) Osteonecrosis Osteochondromas and metaphyseal growth abnormalities

References:

1. W. Nicholas Haining, Christine N. Duncan, Alaa El-haddad et al. Principles of Bone Marrow and Stem Cell Transplantation. In: Stuart H. Orkin, David E. Fisher, David Ginsburg (eds). Nathan and Oski's Hematology and Oncology of Children and Infancy, 8th edition, Elsevier, 2015: 254-289
2. Effie W. Petersdorf, Claudio Anasetti. Unrelated donor hematopoietic stem cell transplantation. In: Ronald Hoffman, Edward J.Benz, Leslie E Silberstein (eds). Hematology Basic principles and practice, 6th edition. Elsevier, 2013: pp 1557- 1568
3. Yair Reisner, David Hagin, Massimo F. Martelli: Haploidentical hematopoietic transplantation: current status and future perspectives. Blood 2011 118: 6006-6017
4. Kirsten M. Williams, Ronald E. Gress: Immune reconstitution and implications for immunotherapy following haematopoietic stem cell transplantation. Best Pract Res Clin Haematol. 2008 September ; 21(3): 579–596
5. C Mackall, T Fry, R Gress, et al: Background to hematopoietic cell transplantation, including post transplant immune recovery. Bone Marrow Transplantation (2009) 44, 457–462
6. M Mohty, F Malard, M Abecassis, et al: Sinusoidal obstruction syndrome/veno-occlusive disease: current situation and perspectives—a position statement from the European Society for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplantation (2015) 50, 781–789
7. Shernan G. Holtan, Marcelo Pasquini, and Daniel J. Weisdorf: Acute graft-versus-host disease: a bench-to-bedside update. Blood 2014 124: 363-373
8. LM Ball and RM Egeler: Acute GvHD: pathogenesis and classification. Bone Marrow Transplantation (2008) 41, S58–S64
9. Paul J. Martin, J. Douglas Rizzo, John R. Wingard et al: First- and Second-Line Systemic Treatment of Acute Graft-versus-Host Disease:

   Recommendations of the American Society of Blood and Marrow Transplantation. Biol Blood Marrow Transplant 18: 1150-1163 (2012)

Calming the cytokine storm

Dr. Nameirakpam Johnson
DM Pediatric Clinical Immunology & Rheumatology
Consultant Pediatric Rheumatology,
Dept. of Pediatrics, JNIMS, Imphal

Introduction: The term Cytokine storm (CS) has been steadily gaining scientific and public interest, with the outbreak of coronavirus disease 2019 (COVID-19) pandemic. CS is generally used to describe the hyperactive inflammatory condition characterised by unremitting fever, cytopenias, hepatosplenomegaly, and elevation of inflammatory biomarkers. The terminology Hemophagocytic Lymphohistiocytosis (HLH) is being used for various CS syndromes. Patients can develop hepatitis, coagulopathy, liver failure, central nervous system involvement, multiorgan failure, and other manifestations. There is utmost need to make prompt recognition and treatment of this condition as the mortality rate is very high if left untreated. Recent UK data showed 1- year survival of 74% in a rheumatological cohort, compared with 21% in patients with a haematological malignancy related HLH(1).

Etiopathology: HLH is characterised by persistently activated cytotoxic T cells and natural killer (NK) cells, which drives activation of macrophages and histiocytes, resulting in excessive proinflammatory cytokine production. It can be broadly classified as primary HLH (driven by genetic inborn errors of immunity which include HLH as a main feature of the disease), familial HLH (driven by genetic defects in PRF1, UNC13D, STX11, or STXBP2), secondary HLH (predominantly driven by environmental/ acquired mechanisms eg, infection, malignancy, rheumatic disease), macrophage activation syndrome HLH occurring due to a rheumatic disease (usually systemic JIA) or autoinflammatory mutation, and cytokine release syndrome HLH due to CAR T-cell or BiTE therapy(2).

Familial HLH include pathologic changes in PRF1, UNC13D, STX11, and STXBP2 (familial HLH2-HLH5, respectively). HLH is also a common manifestation of several other genetic diseases, including certain pigmentary

disorders, X-linked lymphoproliferative diseases, Epstein-Barr virus (EBV) susceptibility disorders, certain CDC42 mutations, and activating mutations in NLRC4. Infections like cytomegalovirus (CMV), Epstein–Barr virus (EBV)-associated hemophagocytic lymphohistiocystosis (HLH), group A streptococcus (GAS), influenza virus, variola virus, severe acute respiratory syndrome coronavirus (SARS-CoV), human H5N1 avian influenza virus infection. Rheumatic diseases such as systemic lupus erythematosus (SLE) and systemic juvenile idiopathic arthritis (sJIA), Kawasaki disease are also known to have complication with CS where it is called as Macrophage Activation Syndrome (MAS). Furthermore, CS have been reported in response to therapeutic interventions, such as Graft versus Host Disease (GvHD) during hematopoietic stem cell transplantation (HSCT) or hyperinflammation following the administration of immunotherapeutic agents, including antibodies and chimeric antigen receptor (CAR) T cells(2).

Clinical manifestations: CS syndrome has varied manifestations. It is being summarised in table 1.

Table 1:

Constitutional Symptoms	Nervous System	Gastro-intestinal System	Vascular/Lymphatic System	Rheumatologic System
Fever Headache Fatigue Anorexia	Confusion Delirium Ataxia Seizures Anosmia	Nausea Vomiting Diarrhea Abdominal pain	Anemia, cytopenia Coagulopathy Increased ferritin, cytokines, CRP and D-dimer Hemorrhage, stroke Lymphadenopathy Endothelial damage	Vasculitis Arthritis Arthralgia
Heart	Skin	Lungs	Liver	Kidney
Hypotension Arrhythmias Cardiomyopathy Ischemia Cardiogenic shock	Urticaria Rash Edema Vesicles	Pneumonitis Pulmonary edema Dyspnea Hypoxemia ARDS	Hepatomegaly Elevated bilirubin Liver failure Increased AST, ALT and LDH	Acute kidney injury Proteinuria Hematuria Kidney failure

Diagnosis of HLH:

The threshold for assessment for possible HLH should be low. Consider HLH in an unwell child who has persistent fever, falling blood counts and raised ferritin. Send fibrinogen, electrolytes, renal profile, liver function tests, triglycerides, LDH. Do Imaging to assess for hepatosplenomegaly and lymphadenopathy. Further extensive investigations should be done to find out the driver of HLH and possible organ involvement(3). Some of the sensitive and specific investigations for diagnosing HLH are described below:

Ferritin :

Ferritin is the most widely used marker to screen for and monitor HLH; it is reasonably inexpensive and widely available. In paediatric patients, ferritin is highly sensitive and specific for HLH, with one study showing that ferritin concentrations of more than 10000 ìg/L have a 90% sensitivity and 98% specificity for HLH(4).

Soluble CD25:

Soluble CD25, also known as soluble interleukin (IL)-2 receptor, is one of the disease markers specified in HLH -2004 and was elevated in 97% of paediatric patients(5). Clinically significantly raised soluble CD25 makes HLH increasingly probable, but can also be seen in other conditions associated with T- cell activation, such as rheumatological diseases and haematological malignancies(6). However, routine testing for soluble CD25 is not readily available in many parts of the world.

Cytokine profiling, lymphocyte panels, and NK cell activity:

Persistent activation of macrophages, NK cells, and cytotoxic T lymphocytes in patients with HLH leads to excessive cytokine production. In paediatric populations, elevated interferon (IFN)ã and IL-10 are associated with HLH, with increased concentrations of these cytokines predicting disease severity(7).

Bone marrow biopsy :

The presence of haemophagocytosis on bone marrow biopsy is neither sufficient nor required to make a diagnosis of HLH. In the HLH-2004 study,

haemophagocytosis was found on bone marrow smears in 82% of paediatric patients(5).

Diagnostic criteria

The Histiocyte Society established a set of clinical and laboratory criteria to help formalize the diagnosis of the syndrome of HLH for its HLH-94 and HLH-2004 clinical trials.

A diagnosis of HLH was met in the HLH-2004 study if patients had 5 out of 8 clinical criteria(5).

1. Fever.
2. Splenomegaly.
3. Cytopenias that affect at least 2 of 3 lineages in the peripheral blood and are not caused by hypocellular bone or marrow (neutrophils < 1 × 109/L, hemoglobin < 9 g/dL, platelets count < 100 × 10^9/L).
4. Serum Triglycerides e" 3.0 mmol/L (e" 265 mg/dl) or serum fibrinogen < 150 mg/dL.
5. Hemophagocytosis in bone marrow (BM), spleen, or lymph nodes with no evidence of malignancy.
6. Ferritin e" 500mcg/L.
7. Soluble CD 25 e" 2,400 U/m
8. Reduced or absent NK cell activity.

For rheumatologists who encounter HLH/MAS in the setting of known or suspected systemic juvenile idiopathic arthritis, criteria proposed by the European League Against Rheumatism, American College of Rheumatology, and Pediatric Rheumatology International Trials Organization are often used (Table 2)(8).

Table 2. Criteria used to diagnose MAS in patients with known or suspected systemic juvenile idiopathic arthritis

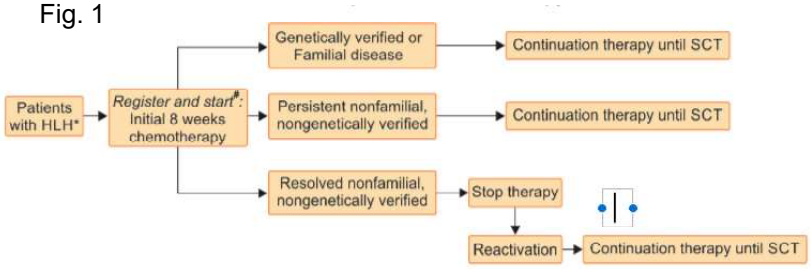
Ferritin >684 ng/mL and 2 of the following:
Platelet count ≤ 181 3 10^9/L Aspartate aminotransferase > 48 U/L Triglycerides > 156 mg/dL Fibrinogen ≤ 360 mg/dL

Treatment of HLH:

Treatment of HLH should begin as soon as the syndrome has been recognized, with the caveat that steroids or chemotherapy should not begin until after evaluating for malignancy (with bone marrow, lymph node, tissue biopsy, etc.) so as not to interfere with diagnosis.[97,108] Assessment for hemophagocytosis and staging for CNS involvement should not delay therapy. The mainstays of HLH treatment consist of immunosuppressive and chemotherapeutic drugs and biologics that aim to dampen the cytokine storm and eliminate activated T-cell and macrophage populations.

- The Histiocytic Society has laid down guidelines for treatment of HLH with its two successive trials—HLH 1994 and HLH 2004 studies (Flowchart 1). Patients with central nervous system (CNS) disease need further intrathecal treatment with steroids and methotrexate (Fig. 1).

Fig. 1



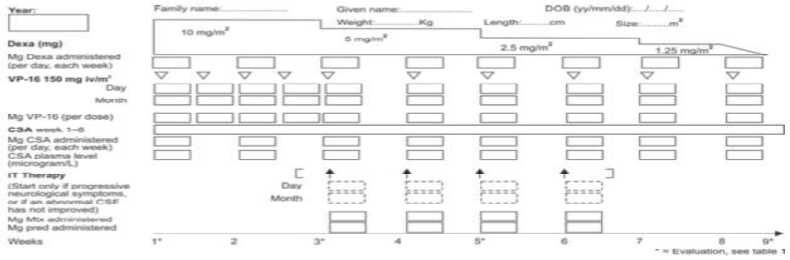


Fig. 1: Initial course—week 1–8.
(CSA: cyclosporin A; CSF: cerebrospinal fluid; IT: intrathecal)

- *Dexamethasone*: 0 mg/m² week 1–2, 5 mg/m² week 3–4, 2.5 mg/m² week 5–6, 1.25 mg/m² week 7, steroids are tapered and discontinued in week 8
- *Injection etoposide:* 150 mg/m² twice weekly for week 1–2, 150 mg/m² once weekly till week 8
  Contraindication: Absolute neutrophil count < 500/mm³ or hypocellular marrow
- *Cyclosporin A*: 6 mg/kg/day with monitoring of levels, target trough levels 200 µg/L
- *Intrathecal therapy:* Cerebrospinal fluid (CSF) evaluated at diagnosis and after 2 weeks. Persistence of progressive neurological symptoms or if an abnormal CSF (cell count and protein) has not improved, intrathecal therapy with 4 weekly injections is done.

There are currently several agents in clinical trials or in the planning stages. Emapalumab, a monoclonal antibody directed against IFN-g, was recently approved by the Food and Drug Administration for HLH that is refractory, recurrent, or progressive or for patients who cannot tolerate conventional treatment(9), Anakinra (recombinant IL-1 receptor antagonist) was recently found to be beneficial in a retrospective case series of secondary HLH, particularly when given early and to patients with underlying rheumatic disease(10). Agents targeting IL-1, tumor necrosis factor, and IL-6 have been reported in case reports and case series, especially for MAS, but no large studies have been performed. Ruxolitinib is a Janus kinase inhibitor that inhibits the signaling of several cytokines, including IFN-g, and may hold promise for treatment of HLH based on murine efficacy data, a few human case reports, and a study of refractory/ relapsed HLH(11).

Treatment of HLH should be coupled with prompt treatment of any identified underlying trigger. Rituximab can be helpful in the treatment of EBV-HLH(12). Most patients should also receive aggressive antimicrobial prophylaxis directed against Pneumocystis jirovecii, general fungal organisms, and also viruses depending on previous exposures. IV immunoglobulin replacement is generally warranted.

Allogeneic HCT is indicated in many pediatric patients with genetic HLH if a suitable donor is available, including patients with pathologic variants in PRF1, UNC13D, STX11, STXBP2, RAB27A, LYST, and SH2D1A and some patients with XIAP deficiency(13).

Concluding remarks

Pediatric HLH remains a challenge, but significant advances have been made in the last 20 years. Advances in rapid screening diagnostics makes it possible to quickly evaluate patients for many inherited diseases, and newer biomarkers are helping to shed light on the driving physiologic processes in individual patients. Novel targeted treatment agents are being developed. Early inclusion of HLH in differential diagnoses could reduce its insufficient and late diagnosis and improve patient outcomes. Diagnosis can be made with clinical and paraclinical criteria while delays in genetic testing should not delay treatment.

References:

1. West J, Stilwell P, Liu H, Ban L, Bythell M, Card T, et al. 1-year survival in haemophagocytic lymphohistiocytosis: a nationwide cohort study from England 2003-2018. J Hematol OncolJ Hematol Oncol. 2023 May 26;16(1):56.
2. Karki R, Kanneganti TD. The 'cytokine storm': molecular mechanisms and therapeutic prospects. Trends Immunol. 2021 Aug;42(8):681–705.
3. Cox MF, Mackenzie S, Low R, Brown M, Sanchez E, Carr A, et al. Diagnosis and investigation of suspected haemophagocytic lymphohistiocytosis in adults: 2023 Hyperinflammation and HLH Across Speciality Collaboration (HiHASC) consensus guideline. Lancet Rheumatol. 2023 Nov;S2665991323002734.
4. Allen CE, Yu X, Kozinetz CA, McClain KL. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2008 Jun;50(6):1227–35.
5. Henter J, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH 2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007 Feb;48(2):124–31.
6. Lin M, Park S, Hayden A, Giustini D, Trinkaus M, Pudek M, et al. Clinical utility of soluble interleukin-2 receptor in hemophagocytic syndromes: a systematic scoping review. Ann Hematol. 2017 Aug;96(8):1241–51.
7. Yang SL, Xu XJ, Tang YM, Song H, Xu WQ, Zhao FY, et al. Associations between inflammatory cytokines and organ damage in pediatric patients with hemophagocytic lymphohistiocytosis. Cytokine. 2016 Sep;85:14–7.
8. Ravelli A, Minoia F, Davì S, Horne A, Bovis F, Pistorio A, et al. 2016 Classification Criteria for Macrophage Activation Syndrome Complicating Systemic Juvenile Idiopathic Arthritis: A European League Against Rheumatism/American College of Rheumatology/Paediatric Rheumatology International Trials Organisation Collaborative Initiative. Arthritis Rheumatol Hoboken NJ. 2016 Mar;68(3):566–76.
9. Al-Salama ZT. Emapalumab: First Global Approval. Drugs. 2019 Jan;79(1):99–103.
10. Eloseily EM, Weiser P, Crayne CB, Haines H, Mannion ML, Stoll ML, et al. Benefit of Anakinra in Treating Pediatric Secondary Hemophagocytic Lymphohistiocytosis. Arthritis Rheumatol Hoboken NJ. 2020 Feb;72(2):326–34.
11. Das R, Guan P, Sprague L, Verbist K, Tedrick P, An QA, et al. Janus kinase inhibition lessens inflammation and ameliorates disease in murine models of hemophagocytic lymphohistiocytosis. Blood. 2016 Mar 31;127(13):1666–75.
12. Milone MC. Treatment of primary Epstein-Barr virus infection in patients with X-linked lymphoproliferative disease using B-cell-directed therapy. Blood. 2004 Sep 28;105(3):994–6.
13. Marsh RA, Jordan MB, Filipovich AH. Reduced-intensity conditioning haematopoietic cell transplantation for haemophagocytic lymphohistiocytosis: an important step forward. Br J Haematol. 2011 Sep;154(5):556–63.

Antenatal Magnesium Sulfate: Does it prevent cerebral palsy?

Dr. Rameshwor Yengkhom
MD (Pediatrics), DM (Neonatology)
Associate Professor, Department of Pediatrics, JNIMS, Imphal

Over the past five decades, the development and adoption of key evidence-based care practices and interventions have improved important outcomes for preterm infants substantially. In particular, the use of antenatal corticosteroids and postnatal surfactant replacement has reduced dramatically early mortality due to respiratory failure in very preterm (<32 weeks' gestation) neonates. However, major long-term neurological morbidities occur in surviving infants. Developing, assessing and implementing strategies to reduce the incidence and severity of neuro-disability, particularly cerebral palsy, the most common physical disability in childhood, is a priority in perinatal care.[1–4]

Cerebral palsy and preterm birth

Infants born preterm have a higher risk of neurologic impairments, cerebral palsy (CP) and cognitive dysfunction being the most frequent, and of substantial disability as a result of these impairments. CP describes a group of disorders affecting the development of movement and posture, causing activity limitation, secondary to nonprogressive disturbances that occurred in the developing fetal or infant brain. The motor disorders are often accompanied by neurodevelopmental or sensory problems, such as seizures, hearing or vision impairments, or behavioral, communicative, and/or cognitive deficits.[1] The risk of serious medical disabilities such as CP, mental retardation and other developmental and behavioural disorders, as well as of other disabilities, such as blindness, hearing loss and epilepsy increases significantly with decreasing gestational age at birth (Table 1).[2] The survival rate of very preterm infants who

are at higher risk has continued to increase overtime due to advances in perinatal care, and whereas some data suggest that the rate of CP among survivors has declined, other data suggest that the rate is unchanged or even increasing.[2-4] The prevalence of CP ranges between 1.5 and 3.6 cases per 1,000 live births.[5]

Table 1: Birth prevalence of cerebral palsy by gestational age bands.

Gestational age (weeks)	*Cerebral palsy per 1000 live births*
22–27	82.3
28–31	43.2
32–36	6.8
Term	1.4

Etiology of Cerebral Palsy

A full understanding of casual pathways and mechanisms leading to CP remains elusive in many cases at the present time.[1] In the pathophysiology of brain lesions associated with CP were described prenatal, perinatal and postnatal factors that include hypoxic-ischemic insults, maternal infection with production of proinflammatory cytokines, excessive glutamate release initiating the excitotoxic cascade, oxidative stress, growth factor deficiency, specific drugs and maternal stress.[6] It is believed that the timing of that insult is in the prenatal or perinatal period in about 70% of the cases in infants born preterm and 85% in those born at term, with only 10 to 28% cases of CP due to birth asphyxia.[7] Preterm delivery, low birth weight, intrauterine infection/inflammation, multiple gestation, maternal or fetal coagulation disorders, antepartum hemorrhage, and preeclampsia have all been associated with CP.[7,8] Moreover, recent studies support the existence of genetic susceptibility factors.[6] Approximately one-third of cases of CP are associated with early preterm birth.[3] The risk of intraventricular hemorrhage increases with the lower the gestational age at birth.[9] The most prevalent pathological lesion seen in CP is periventricular white matter injury

resulting from vulnerability of the immature preoligodendrocytes before 32 weeks of gestation.[5] Preterm birth is enormous public health concern since many of these preterm infants survive with neurobehavioral, cognitive and motor disabilities (Table 2).[10] Both economically and emotionally, the burden of CP is enormous.[11]

Table 2: Prevalence of co-existing impairments, diseases and functional limitations in children with cerebral palsy

Co-morbidity	*Prevalence*
Learning difficulty	1 in 2
Unable to walk	1 in 3
Unable to talk	1 in 4
Epilepsy	1 in 4
Visual impairment	1 in 10
Hearing impairment	1 in 25

Magnesium sulfate in peripartum care

Tocolysis

Magnesium tocolysis (intravenous magnesium sulfate) has been assessed in clinical trials since the 1970s.[12] The recent Cochrane review of magnesium sulfate for preventing preterm birth in threatened preterm labour identified 37 eligible randomised controlled trials (in which a total of 3571 women participated) but concluded that magnesium sulfate is "ineffective at delaying birth or preventing preterm birth, [and] has no apparent advantages for a range of neonatal and maternal outcomes as a tocolytic agent".[13] Despite this conclusive evidence, magnesium tocolysis remains widely used in obstetric care in several countries including the USA.

Pre-eclampsia and eclampsia

Magnesium is well established as an effective intervention for treating women with pre-eclampsia. Meta-analysis of data from six trials (11444 women

with pre-eclampsia) demonstrates that magnesium sulfate versus placebo or no anticonvulsant reduces the risk of eclampsia by more than half, without affecting the incidence of stillbirth or neonatal death.[18] There is also strong trial evidence that magnesium sulfate is more effective that other anticonvulsants (such as diazepam or phenytoin) in treating women with eclampsia.[14] Following the dissemination of these findings, and their incorporation into national and international policy statements and guidelines, magnesium sulfate has become the drug of choice for the peripartum care of women with pre-eclampsia and eclampsia.[15]

Magnesium Sulfate: A Possible Role for neuroprotection

An association between antenatal exposure to magnesium sulfate (MgSO4) and a reduction in the risk of CP in infants born very low birth weights was first suggested by a case control study published in 1995.[12] In this observational study children with CP were significantly less likely to have been exposed to MgSO4 *in utero* during labor than children without CP, suggesting a protective effect of MgSO4 against CP in these very low birth weight infants [odds ratio (OR) of CP in magnesium exposed fetuses 0.14; 95% confidence interval (CI) 0.05-0.51]. MgSO4 had been administered for seizure prophylaxis in pre-eclampsia or as a tocolytic agent.

Magnesium Sulfate: How does it Work?

MgSO4 is commonly used worldwide in obstetric practice as an anticonvulsant for treatment of eclampsia and for seizure prophylaxis in pre-eclampsia, and is no longer recommended for tocolysis because it is ineffective.[13] The exact mechanism of action for the neuroprotective effect of MgSO4 in preterm infants is not known. MgSO4 has been proposed to act as a neuroprotectant through one or many of the following mechanisms: Reduction of inflammatory cytokines or free radicals produced during hypoxic-ischemic reperfusion, prevention of excitotoxic calcium-induced injury, membrane stabilization by preventing the membrane depolarization, inhibition of the glutamate receptors involved in injury to preoligodendrocytes, stabilization of fluctuations in blood pressure that occur in neonates, and an increase in cerebral blood flow.[5,7,14,15]

Other animal data suggest that MgSO4 may serve an antiapoptotic role and prevent neuronal cell loss.[10] During asphyxia, there is excessive release and reduced uptake of glutamate in the brain. Glutamate acts on the N-methyl-D-aspartate (NMDA) receptor, a postsynaptic ion channel, allowing excessive calcium influx into the neurons and inducing neuronal injury.[16] Fetal and newborn brains seem to be more susceptible to damage from glutamate release.[17] Magnesium is a naturally occurring NMDA receptor antagonist that blocks neuronal influx of calcium within the ion channels, preventing post-hypoxic brain injury.[16] By its peripheral vasodilator effects, MgSO4 can produce flushing, sweating and a sensation of warmth. Reported side effects include nausea, vomiting, headache, palpitations and rarely pulmonary edema. Administration above the recommended therapeutic levels can lead to respiratory depression, respiratory arrest, cardiac arrest and death. Hypermagnesemia at neonate can lead to hyporeflexia, poor sucking, and rarely, respiratory depression needing mechanical ventilation.[13,17] Magnesium readily crosses the placenta, and can be detected in the fetal serum within 1 hour of maternal infusion, and in the amniotic fluid within 3 hours.[11] Contraindications to MgSO4 therapy are: Respiratory rate <16/min, absent patellar reflexes, urine output <100 ml during the previous 4 hours, renal failure, hypocalcemia.[9]

Randomized Controlled Trials

A number of randomized controlled trials were performed to assess the efficacy of MgSO4 specifically for fetal neuroprotection in women at risk for preterm delivery. The characteristics of these trials are outlined in Table 3. Magnesium and neurologic endpoints trial (MagNET)[18] enrolled 149 women in preterm labor, 25 to 33 weeks of gestation, at a single center from United States. The aim of this study was to determine whether the use of antenatal MgSO4 prevents neonatal intraventricular hemorrhage, periventricular leukomalacia, death and CP. There were two treatment strategies: The tocolytic arm and the neuroprotective arm. For the neuroprotective arm women were randomly allocated to either a 4 gm MgSO4 bolus or saline placebo. Contrary to original hypotheses, they concluded that the use of antenatal magnesium sulfate was associated with worse, not better, perinatal outcome in a dose-response fashion. The

Australasian Collaborative Trial of Magnesium Sulfate (ACTOMgSO4),[9] a placebo controlled trial, included 1,062 women (16 tertiary hospitals from Australia and New Zealand) less than 30 weeks of gestation for whom birth was anticipated within 24 hours. Women were randomly assigned to receive either a loading dose of 4 gm intravenous MgSO4 followed by a maintenance infusion of 1 gm/h (magnesium group) or isotonic sodium chloride solution (placebo group) until delivery or for up to 24 hours. MgSO4 was given as a neuroprotective agent only and not for tocolysis. The primary outcomes were rates of total pediatric mortality, CP in survivors, and the combined outcome of death and CP at 2-year follow-up. Compared to controls, the MgSO4 group had lower rates of total pediatric mortality [13.8 *vs* 17.1%; relative risk (RR): 0.83; 95% confidence interval (CI): 0.64-1.09], CP (6.8 *vs* 8.2%; RR: 0.83; 95% CI: 0.54-1.27), and combined death and CP (19.8 *vs* 24.0%; RR: 0.83; 95% CI: 0.66-1.03), but none of the differences were statistically significant, although the average size of the reductions in these adverse outcomes are potentially clinically important. Substantial gross motor dysfunction (3.4 *vs* 6.6%; RR 0.51; 95% CI: 0.29-0.91) and combined outcome of death or substantial gross motor dysfunction (17.0 *vs* 22.7%; RR: 0.75; 95% CI: 0.59-0.96) were significantly less frequent among surviving children in the MgSO4 group. There was little evidence in this trial of any effect of MgSO4 on the rate of intraventricular hemorrhage or on the rate of cystic periventricular leukomalacia. The magnesium sulfate for prevention of eclampsia trial (MAGPIE)[19,20] was conducted to assess the effects of *in utero* exposure to MgSO4 for children whose mothers had pre-eclampsia, and did not look initially at MgSO4's effect on neurodevelopmental disorders specifically. This study included 125 centers from 19 countries. Women were randomly allocated to receive either MgSO4 or placebo as an intravenous loading dose followed by 24 hours of maintenance therapy. The primary outcome was the composite measure of death or neurosensory disability at age of 18 months. Of the children whose mothers were allocated MgSO4, 15.0% had the primary outcome of death or neurosensory disability compared with 14.1% allocated placebo (RR: 1.06; 95% CI: 0.90-1.25), and of survivors, 1.3% had neurosensory disability at 18 months compared with 1.9% (RR: 0.72; 95% CI: 0.40-1.29). Fifteen children were identified as having CP (five in magnesium group *vs* 10 in placebo group). The PREMAG

trial[21,22] enrolled 573 women less than 33 weeks of gestation who were expected to deliver within 24 hours at 18 collaborating centers in France. The aim of the study was to determine if MgSO4 given to women at risk of very-preterm birth would be neuroprotective in preterm newborns and would prevent neonatal mortality and severe white-matter injury (detected on neonatal cranial ultrasound). Women were randomly assigned to receive a single 4 gm loading dose of MgSO4 or placebo, without maintenance infusion. The rates of total mortality before hospital discharge (9.4 *vs* 10.4%; OR: 0.79; 95% CI: 0.44- 1.44), severe white-matter injury (10.0 *vs* 11.7%; OR: 0.78; 95% CI: 0.47-1.31), and their combined outcome (16.5 *vs* 17.9%; OR: 0.86; 95% CI: 0.55-1.34) were all lower for the MgSO4 compared with placebo group, but no differences were statistically significant.[21] In the 2-year follow-up of the PREMAG trial 472 children were assessed through clinical examination. There was a strong trend toward a neuroprotective effect of MgSO4 against combined CP or death (OR 0.65; 95% CI 0.42-1.03). Also, exposure to MgSO4 was protective against combined gross motor dysfunction or death (OR: 0.62; 95% CI: 0.41-0.93) and achieved significance.[22] The largest trial, beneficial effects of antenatal magnesium sulfate (BEAM)[3] enrolled 2,241 women at imminent risk for delivery between 24 and 31 weeks of gestation (20 centers from United States). Women were randomly assigned to either MgSO4, 6 gm loading dose followed by an infusion of 2 gm/h, or identical placebo. Follow-up was accomplished in 95.6% of children. The primary study outcome was the composite of stillbirth or infant death by 1 year corrected age or moderate or severe CP at or beyond 2 years of corrected age. The rate of the primary outcome was not significantly different in the magnesium group and the placebo group (11.3 *vs* 11.7%; RR: 0.97; 95% CI: 0.77-1.23). They observed significant decrease in the risk of moderate or severe CP (a prespecified secondary outcome) among surviving children in the MgSO4 group (1.9 *vs* 3.5%; RR: 0.55; 95% CI: 0.32-0.95; p = 0.03). In terms of gestational age, at randomization (<28 weeks *vs* > 28 weeks), only infants of pregnancies randomized at less than 28 weeks of gestation showed a significant reduction in moderate or severe CP (2.7 *vs* 6.0%; RR: 0.45; 95% CI: 0.23-0.87). The risk of death was slightly higher in the magnesium group (9.5 *vs* 8.5%; RR: 1.12; 95% CI: 0.85-1.47), but nonsignificant. Exclusion of children with major congenital anomalies discovered after birth slightly lowered the relative risk of the primary

outcome and attenuated the relative risk of death but had minimal effect on the relative risk of CP.

Table 3: Randomized controlled trials: Characteristics and neuroprotective conclusions

Study	*Centers*	*Number of participants*	*Gestational age*	*Magnesium regimen*	*Neuroprotective outcomes*
MagNET, Mittendoff et al[18]	1 (United States)	149 mothers	25-33 weeks	4 gm bolus (neuroprotective arm)	▪ Antenatal MgSO4 was associated with worse perinatal otucome
ACTOMgSO4, Crowther et al[9]	16 (Australia and New Zealand)	1,062 mothers	<30 weeks	4 gm bolus, 1 gm/h maintenance	▪ CP RR: 0.83; 95% CI:0.54-1.27 ▪ Combined outcome of death or CP RR: 0.83; 95% CI: 0.66-1.03 ▪ Differences were not statistically significant
Magpie trial, Duley et al[19,20]	125 (International)	3,283 children	<37 weeks	4 gm bolus, 1 gm/h IV maintenance or, 4 gm bolus combined with10 gm IM, then 5 gm/4 hrs IM maintenance	▪ Combined death or neurosensory disability RR: 1.06; 95% CI: 0.90-1.25 ▪ Neurosensory disability at 18 months RR: 0.72; 95% CI: 0.40-1.29
PreMAG trial + follow-up trial, Marret et al[21,22]	18 (France)	573 mothers (original trial) 472 children (follow-up trial)	<33 weeks	4 gm bolus, no maintenance	▪ Original trial: Nonsignificant decrease in risk of short-term severe white matter injury, mortality before hospital discharge ▪ Follow-up trial (2 years): Combined death or cerebral palsy OR: 0.65; 95% CI: 0.42-1.03 ▪ Combined death or gross motor dysfunction OR: 0.62; 95% CI: 0.41-0.93) (statistically significant)
BEAM, Rouse et al[3]	20 (United States)	2,241 mothers	24-31 weeks	6 gm bolus, 2 gm/h maintenance	▪ Significant decrease in the risk of moderate or severe CP (RR: 0.55; 95% CI: 0.32-0.92) among surviving children in the MgSO4 group ▪ Death and CP RR: 0.97; 95% CI: 0.77-1.23

CI: Confidence interval; CP: Cerebral palsy; OR: Odds ratio; RR: Relative risk

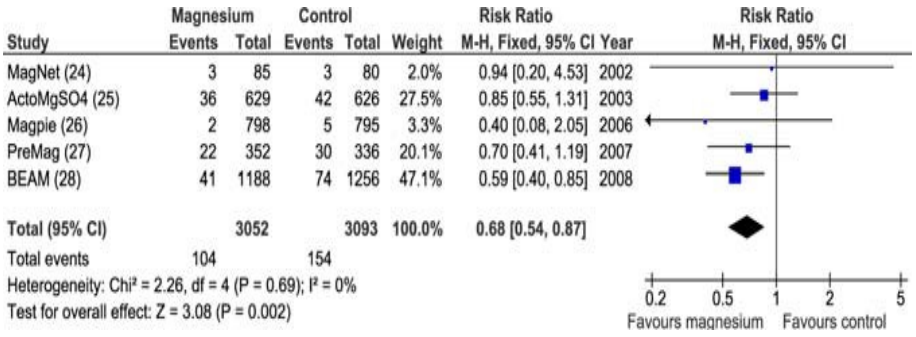
Systematic Reviews/Meta-analyses

A systematic review (Doyle et al)[23] that assessed the effects of MgSO4 as a fetal neuroprotective agent when given to women (less than 37 weeks of gestation) considered at risk of preterm birth included five randomized controlled trials (MagNET, BEAM, ACTOMgSO4, PREMAG and MAGPIE) with 6,145 fetuses. The first four trials were specifically designed to evaluate the neuroprotective effect of MgSO4, although one study (MagNET) also had a tocolytic arm. The fifth study (MAGPIE) was designed to assess the efficacy of MgSO4 for prevention of seizures in women with pre-eclampsia. The primary outcomes were pediatric mortality, CP, and the combination of mortality or CP.

Antenatal MgSO4 therapy given to women at risk of preterm birth substantially reduced the risk of CP (overall RR: 0.69; 95% CI: 0.54-0.87; p = 0.002; neuroprotection trials subgroup RR: 0.71; 95% CI: 0.55-0.91; p = 0.006). The overall absolute risk of CP was 3.7% for fetuses exposed to MgSO4 *vs* 5.4% for unexposed fetuses, giving an absolute risk reduction of 1.7%. The number needed to treat (NNT) to prevent one case of CP was 63 (95% CI: 43-155). There was a significant reduction in the rate of substantial gross motor dysfunction (overall RR: 0.61; 95% CI: 0.44-0.85; p = 0.003; neuroprotection trials group RR: 0.60; 95% CI: 0.43-0.83; p = 0.002). There was no significant effect of antenatal MgSO4 on total pediatric mortality (RR: 1.01; 95% CI: 0.82-1.23; five trials), or on other neurologic impairments or disabilities in the first few years of life. For the overall group of five trials, antenatal MgSO4 had no significant effect on the combined rates of mortality or CP, except in the studies where the primary intent was neuroprotection, where there was a significant reduction in death or CP (RR: 0.85; 95% CI: 0.74-0.98; four trials 4,446 infants). The meta-analysis published by Conde-Agudelo and Romero[5] included the same trials as the Cochrane review, with 5,357 infants. The aim was to determine whether MgSO4 administered to women at risk of preterm delivery before 34 weeks of gestation may reduce the risk of CP. Like the Cochrane review, this study came to similar conclusions. Antenatal MgSO4 was associated with a significant reduction in the risk of CP (RR: 0.69; 95% CI: 0.55-0.88), moderate or severe CP (RR: 0.64; 95% CI: 0.44-0.92), and substantial gross motor dysfunction (RR: 0.60; 95% CI: 0.43-0.83). The absolute risk of CP was 3.9% for fetuses exposed to MgSO4 *vs* 5.6% for unexposed fetuses. The number of women at risk of preterm delivery less than 34 weeks of gestation who needed to be treated with magnesium rather than with placebo to prevent one case of CP in their children was 52 (95% CI: 31-154). There was no significant effect on pediatric mortality (RR: 1.01; 95% CI: 0.89-1.14). This last finding suggests the reduced risk of CP does not appear to be due to selective mortality of MgSO4-exposed infants. MgSO4 therapy had no significant effect on the risk of major maternal complications, adverse neonatal outcomes and other infant neurodevelopmental outcome. The meta-analysis published by Costantine[11] included the same five trials, but separate analyses were performed according to the gestational age at randomization [less than 32

to 34 (5 trials, 5,235 infants) and less than 30 weeks (3 trials, 3,107 infants)]. Antenatal exposure to MgSO4 at less than 32 to 34 weeks significantly reduced the rates of CP (RR: 0.70; 95% CI: 0.55-0.89), and moderate-severe CP (RR: 0.60; 95% CI: 0.43-0.84), without an evident increase in the risk of death (RR: 1.01; 95% CI: 0.89-1.14). For less than 30 weeks group similar results were obtained. The primary outcome of this meta-analysis was a composite outcome of perinatal or infant death or CP among survivors. This combination is necessary since CP and death are competing outcomes. Overall, *in utero* exposure to MgSO4 did not reduce the rate of death or CP (RR: 0.92; 95% CI: 0.83-1.03), but when only neuroprotection trials were analyzed (4 trials, 4,324 infants) they observed that MgSO4 significantly reduces this outcome. The NNT was 46 (95% CI: 26-287) for infants exposed to magnesium *in utero* before 30 weeks, and 56 (95% CI: 34-164) for less than 32 to 34 weeks. This review provides reassurance for obstetricians that antenatal exposure to MgSO4 did not increase the risk of perinatal/infant death. Moreover, it demonstrated beneficial effects of *in utero* exposure for those before 32 to 34 weeks as well as before 30 weeks. So, it does not appear that treatment should be restricted to the latter. The findings of these three systematic reviews/metaanalyses are summarized in Table 4. Although minor maternal complications were more common associated with MgSO4 therapy, there were no significant differences between magnesium and placebo control groups in severe maternal complications, including death, cardiac arrest and respiratory failure in trials or metaanalysis (Table 5). [25]

Table: 4 Meta-analysis of the effect of antenatal magnesium sulfate on the risk of cerebral palsy in preterm infants.



Side effects of magnesium sulfate

Magnesium sulfate infusion is associated with several maternal unpleasant side effects. Women commonly experience a feeling of warmth, facial flushing and discomfort at the intravenous cannula site. In the trials, women receiving magnesium sulphate were three times more likely than controls to decline to continue receiving the intervention because of these effects, but this was uncommon (8% of participants). At high serum levels, magnesium can cause muscle weakness and more serious side effects including respiratory depression are possible, but this is rare and is mainly associated with excessive doses.[32] In infants, the effect of magnesium blockage of calcium entry into cells may theoretically be hypotonia, respiratory depression and apnoea requiring respiratory support. However, analyses of data from BEAM (Beneficial Effects of Antenatal Magnesium Sulfate), the largest trial of magnesium for neuro-protection, did not find a difference in the rates of respiratory depression at birth. More recent data from large cohort studies have not demonstrated an increased need for intensive delivery room resuscitation in very preterm infants antenatally exposed to magnesium sulfate.[32 33]

Costs of treatment

Magnesium sulfate is inexpensive but other cost implications for prophylactic use include the requirement for administration via an infusion pump as well as the need for staff to accompany and monitor women receiving magnesium sulfate. Formal analyses indicate that antenatal magnesium sulfate for neuro-protection of very preterm infants is a highly cost-effective intervention in the prevention of cerebral palsy and improving the quality of life.[34]

International Guidelines

No standard approach has been established for use of MgSO4 for neuroprotection. The American College of Obstetricians and Gynecologists encourages physicians electing to use MgSO4 for fetal neuroprotection to develop

specific guidelines around the issues of inclusion criteria, dosage, concurrent tocolysis, and monitoring in accordance with one of the larger trials.[25] The Antenatal Magnesium Sulfate for Neuroprotection Guideline Development Panel recommends MgSO4 only at less than 30 weeks of gestation. They use a 4 gm loading intravenous dose (20-30 minutes) and a 1 gm/h maintenance dose, with no immediate repeat, until birth or for 24 hours.[26] The Society of Obstetricians and Gynecologists of Canada recommends that antenatal MgSO4 administration should be considered for fetal neuroprotection when women present at < 31 + 6 weeks with imminent preterm birth (active labor with cervical dilatation > 4 cm, with or without preterm prelabor rupture of membranes) and/or planned preterm birth for fetal or maternal indications. They use a 4 gm intravenous loading dose, over 30 minutes, followed by a 1 gm/h maintenance infusion until birth.[27]

In 2010, authorities in the USA and Australasia introduced guidelines recommending the use of magnesium sulfate for neuro-protection of very preterm infants, a process expedited perhaps because most of the primary trials and systematic reviews were developed and delivered in those countries.[39 40] More recently, several other national bodies, including those in Canada and the Republic of Ireland, have developed similar practice guidelines and recommendations. The Australasian guidelines recommend that magnesium sulfate for neuro-protection is restricted to women at imminent risk of delivery before 30 weeks' gestation, arguing that uncertainty on the effects for infants born after longer gestations still remains. Currently, the Australasian investigators are undertaking a new trial to assess whether magnesium sulphate versus placebo given to women with threatened, imminent delivery between 30 and 34 weeks' gestation affects rates of mortality and neuro-disability, including cerebral palsy. The RCOG endorses the Australasian recommendations, and suggests that these are appropriate sources for developing local protocols (Table 5). However, this advice has not yet translated into an RCOG formal 'Green-top' guideline which would lead to an expectation of the practice being adopted.

Table 5: Australasian binational clinical practice guideline: Key recommendations

Indication	Delivery before 30 weeks' gestation planned* or definitely expected within 24 h, regardless of: ‣ cause of preterm delivery ‣ plurality ‣ parity ‣ planned mode of delivery ‣ receipt of antenatal corticosteroids
Regimen	Magnesium sulfate infused intravenously: ‣ 4 g loading dose (over 20–30 min) ‣ 1 g/h maintenance dose Continue regimen until birth or for 24 h, whichever comes first
Repeat doses	If delivery does not occur within 24 h, but remains imminent, consider a repeat dose
Urgent delivery:	Do not delay urgent delivery for maternal or foetal compromise to administer magnesium sulfate

*As close as possible to 4 h before a planned delivery

Conclusion

Preterm birth is a risk factor for CP, and the magnitude of the risk is inversely correlated with gestational age at birth. CP is a leading cause of chronic childhood disability, with profound medical, emotional, and economic consequences. CP is permanent and can result in severe sequelae for the infant, significantly affecting the family and the society. In order to reduce the impact of CP from very preterm birth, efforts must be focused on primary prevention. Brain protection remains a challenge in infants who are born very preterm. MgSO4 has been one of the rare pharmacologic interventions used in randomized control trials for its potential neuroprotective properties. The mechanisms by which magnesium might reduce or prevent neuronal damage have not been fully elucidated. While it is plausible that MgSO4 could provide neuroprotection through mechanisms, such as reduced vascular instability and hypoxic damage, and reductions in cytokine/excitatory amino acid-induced damage, further study is needed to clarify its role in these processes.[15] Given the relative safety of MgSO4 for the mother, the lack of evident risk regarding infant mortality, and the familiarity of most obstetricians with its use, MgSO4 can be considered for neuroprophylaxis in the setting of preterm birth. The ideal candidate and the specific protocol

should be decided upon at the institution level. Comparisons between the published trials are made difficult by differences in inclusion criteria, population studied, magnesium sulfate regimens, gestational age and evaluated outcomes, and therefore, conclusions need to be interpreted cautiously. The appropriate total dosage, infusion period, need for retreatment and therapeutic window for neuroprotection are still not known. Diagnostic techniques, such as magnetic resonance imaging of the brain might help to determine how magnesium affects the brain, particularly the motor system, to reduce the rate of adverse motor outcomes. A reported economic analysis of magnesium neuroprophylaxis for women at risk for preterm birth less than 32 weeks of gestation concluded that MgSO4 therapy is cost effective. [28] Encouraging results from systematic reviews and metaanalyses using several randomized controlled trials confirmed that administration of MgSO4 improves the neurodevelopmental future of preterm infants. These results suggest that antenatal MgSO4 could be used for the primary prevention of CP in preterm infants. However, since CP is a result of multiple interacting risk factors rather than of a single cause, it is unlikely that antenatal MgSO4 administration alone can prevent all cases of this illness in preterm infants.

Table 6: Systematic review/meta-analyses: Results

Study	*Gestational age*	*RR of cerebral palsy*	*NNT*	*RR of gross motor dysfunction*	*RR of total pediatric mortality*
Doyle et al[23]	<37 weeks	0.69 (95% CI: 0.54-0.87)	63 (95% CI: 43-155)	0.61 (95% CI: 0.44-0.85)	1.01 (95% CI: 0.82-1.23)
Conde-Agudelo and Romero[5]	<34 weeks	0.69 (95% CI: 0.55-0.88)	52 (95% CI: 31-154)	0.60 (95% CI: 0.43-0.83)	1.01 (95% CI: 0.89-1.14)
Costantine et al[11]	<32-34 weeks <30 weeks	0.70 (95% CI: 0.55-0.89) 0.69 (95% CI: 0.52-0.92)	56 (95% CI: 34-164) 46 (95% CI: 26-287)	— —	1.01 (95% CI: 0.89-1.14) 1.00 (95% CI: 0.87-1.15)

CI: Confidence interval; RR: Relative risk

References

1. Bax M, Goldstein M, Rosenbaum, et al. Proposed definition and classification of cerebral palsy. Dev Med Child Neurol 2005;47(8):571-76.
2. Moster D, Lie RT, Markestad T. Long-term medical and social consequences of preterm birth. N Engl J Med 2008;359(3): 262-73.
3. Rouse DJ, Hirtz DG, Thom E, et al. A randomized, controlled trial of magnesium sulfate for the prevention of cerebral palsy. N Engl J Med 2008;359(9):895-905.
4. Vincer MJ, Allen AC, Joseph KS, et al. Increasing prevalence of cerebral palsy among very preterm infants: A populationbased study. Pediatrics 2006; 118(6):1621-26.
5. Conde-Agudelo A, Romero R. Antenatal magnesium sulfate for the prevention of cerebral palsy in preterm infants less than 34 weeks' gestation: A systematic review and meta-analysis. Am J Obstet Gynecol 2009;200(6):595-609.
6. Degos V, Loron G, Mantz J, et al. Neuroprotective strategies for the neonatal brain. Anesth Analg 2008;106(6):1670-80.
7. Costantine MM, Drever N. Antenatal exposure to magnesium sulfate and neuroprotection in preterm infants. Obstet Gynecol Clin North Am 2011; 38(2):351-66.
8. Hankins GD, Speer M. Defining the pathogenesis and pathophysiology of neonatal encephalopathy and cerebral palsy. Obstet Gynecol 2003;102(3):628-36.
9. Crowther CA, Hiller JE, Doyle LW, et al. Effect of magnesium sulfate given for neuroprotection before preterm birth: A randomized controlled trial. JAMA 2003;290(20):2669-76.
10. Burd I, Breen K, Friedman A, et al. Magnesium sulfate reduces inflammation-associated brain injury in fetal mice. Am J Obstet Gynecol 2010;202(3):292.e1-9.

11. Costantine MM, Weiner SJ. Effects of antenatal exposure to magnesium sulfate on neuroprotection and mortality in preterm infants: A meta-analysis. Obstet Gynecol 2009;114(2 Pt 1): 354-64.
12. Nelson KB, Grether JK. Can magnesium sulfate reduce the risk of cerebral palsy in very low birthweight infants? Pediatrics 1995; 95(2):263-69.
13. Costa Fda S, Lopes L, Brennecke S. Magnesium sulphate for fetal neuroprotection. Rev Bras Ginecol Obstet 2011;33(6): 265-70.
14. Heyborne K, Bowes WA. The use of antenatal magnesium sulfate for neuroprotection for infants born prematurely. F1000 Med Rep 2010;2:78.
15. Mercer BM, Merlino AA. Magnesium sulfate for preterm labor and preterm birth. Obstet Gynecol 2009;114(3):650-68.
16. Bhat MA, Charoo BA, Bhat JI, et al. Magnesium sulfate in severe perinatal asphyxia: A randomized, placebo-controlled trial. Pediatrics 2009;123(5):764-69.
17. Doyle LW, Crowther CA, Middleton P, et al. Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus. Cochrane Database Syst Rev 2009(1):CD004661.
18. Mittendorf R, Dambrosia J, Pryde PG, et al. Association between the use of antenatal magnesium sulfate in preterm labor and adverse health outcomes in infants. Am J Obstet Gynecol 2002;186(6):1111-18.
19. Magpie Trial Follow-Up Study Collaborative Group. The Magpie Trial: A randomised trial comparing magnesium sulphate with placebo for pre-eclampsia. Outcome for women at 2 years. BJOG 2007;114(3):300-09.
20. Magpie Trial Follow-Up Study Collaborative Group. The Magpie Trial: A randomised trial comparing magnesium sulphate with placebo for pre-eclampsia. Outcome for children at 18 months. BJOG 2007;114(3):289-99.
21. Marret S, Marpeau L, Zupan-Simunek V, et al. Magnesium sulphate given before very-preterm birth to protect infant brain: The randomised controlled PREMAG trial BJOG 2007;114(3):310-18.
22. Marret S, Marpeau L, Benichou J. Benefit of magnesium sulphate given before very preterm birth to protect infant brain. Pediatrics 2008;121(1):225-26.

23. Doyle LW, Crowther CA, Middleton P, et al. Antenatal magnesium sulfate and neurologic outcome in preterm infants: A systematic review. Obstet Gynecol 2009;113(6):1327-33.
24. Goojha C. Can magnesium sulphate provide neuroprotection in preterm infants? A literature review. RCSIsmj 2011;4(1): 46-52.
25. American College of Obstetricians and Gynecologists Comitee on Obstetric Practise, Society for Maternal-Fetal Medicine. Comittee Opinion No. 455. Magnesium sulfate before anticipated preterm birth for neuroprotection. Obstet Gynecol 2010;115:669.
26. The Antenatal Magnesium Sulphate for Neuroprotection Guideline Development Panel. Antenatal magnesium sulphate prior to preterm birth for neuroprotection of the fetus, infant and child: National clinical practice guidelines. The University of Adelaide. 2010.
27. Magee L, Sawchuck D, Synnes A, et al. SOGC Clinical Practice Guideline. Magnesium sulphate for fetal neuroprotection. J Obstet Gynaecol Can 2011;33(5):516-29.
28. Cahill AG, Odibo AO, Stout MJ, et al. Magnesium sulphate therapy for the prevention of cerebral palsy in preterm infants: A decision-analytic and economic analysis. Am J Obstet Gynecol 2011;205(6):542.e1-7.

Does Zinc supplementation prevent febrile seizure and its recurrence? An Insight Perspective!

Dr. Lourembam Radhapyari
Senior Resident, Department of Paediatrics
RIMS, Imphal, Manipur.

Febrile seizure (FS) is a commonly encountered neurological source of hospital attendance in children with a global incidence of 2 to 8%. It is the most common seizure type in children aged 6 months to 5 years. The peak age for the occurrence is 18 to 24 months, and the majority (90%) of the children report the first episode before the age of 3 years. FS account for approximately 30% of all seizure disorders in children.[1]

The "International League Against Epilepsy," defines a febrile seizure as "a seizure occurring in childhood after one month of age, associated with a febrile illness not caused by an infection of the central nervous system, without previous neonatal seizures or a previous unprovoked seizure, and not meeting criteria for other acute symptomatic seizures".[2]

The pathophysiology and age-specific predilection of FS is still unclear, and a complex interplay of genetic and environ-mental factors has been postulated. A robust relationship with febrile seizures and genetic epilepsy has been established in one-third of children with positive family history. However, a significant pedigree chart is absent in several children with FS, and routine genetic testing is not warranted. Additional factors considered in the pathogenesis include dyselec-trolytemia, pyridoxine deficiency, human herpes virus 6, and disorders of trace elements, primarily iron and zinc. Recently, deficiencies of

trace elements in chil-dren with febrile seizure have drawn substantial attention, given their high concentration in the brain and potential role in synaptic neurotransmission. [3,4]

Zinc modulates the activity of glutamic acid decarboxylase, the rate limiting enzyme in the synthesis of gamma-aminobutyric acid (GABA), which is a major inhibitory neurotransmitter. Zinc activates pyridoxal kinase, an enzyme which helps in synthesis of pyridoxal phosphate, which in turn activates glutamic acid decarboxylase which involved in synthesis of GABA Thus, it has got anti-convulsive effect. It also increases affinity for neurotransmitter receptors like glutamate receptors and mediates calcium's inhibitory effects on the excitatory N-methyl-d-aspartate receptors receptors. Low zinc level activates these receptors and induces an epileptic discharge in children with high fever.[5,6]Additionally, zinc prevents opiate-induced inhibition of GABAergic neurons by blocking opiate binding in the hippocampal mossy fiber system. Moreover, Zinc plays a regulatory role in the immune system, and Zinc –deficient persons experience increased response to a variety of pathogens. It is still unclear whether low serum zinc concentrations can be due to fever and /or infections.[7]

With this background, many researchers have conducted studies regarding the putative role of zinc in febrile seizures and hypothesized association of low zinc level with increased neuronal discharge. Recent metanalysis of studies documented serum zinc level is lower in patients with febrile seizure as compared to febrile patients without seizure, supporting the evidence of zinc hypothesis in the pathogenesis of FS.[6-9]

However, there is very limited research on the role of zinc supplementation in prevention of recurrence of febrile seizures in children. Fallah et al. (2015) from Iran, assessed seizure recurrence in a randomized single blinded study on 100 children (18-60 months) with a first simple FS, after supplementation of either daily zinc sulfate 2 mg/kg for six consecutive months or placebo. However, they concluded no significant benefit of zinc

supplementation over placebo in prevention of seizure recurrence during one year follow-up.[10] Another randomised, placebo-controlled trial from India by Kulkarni et al. (2020) on 70 children aged 6-60 months with simple FS, also noted no significant difference in recurrence after zinc supplementation (1mg/kg) on follow up.[11]

In contrast to the above studies, Abdelrahman, et al. (2020) from Egypt found low zinc level in children aged 6-60 months with FS as compared with those without seizure. Further, the cases with low zinc level were administered elemental zinc daily (2.5mg) for 3 months. Significant decrease in the frequency of the seizure was documented.[12] In agreement to this result, Shaaban et.al (2023) in their 90 children (30 simple FS,30 complex FS, 30 controls) also concluded supplementing zinc therapy with 2 mg/kg/day zinc dropped the seizure frequency, the difference being higher in the complex group and hypothesised possible prophylactic therapy against recurrence of febrile seizure.[7]

Recently, systemic review and meta-analysis by Kumar M et.al [13] included four randomized/quasi-randomized trials, with total 350 children and did not note any significant difference between recurrence rate of FS in children on zinc supplementation compared to children on placebo. They also concluded that available evidence pertaining to zinc supplementation for prevention of febrile seizures is of low to very low quality and included clinical trials were inadequately powered with high risk of bias.

In conclusion, there is very limited literature available regarding prevention of febrile seizure and its recurrence after zinc supplementation, despite existing robust evidence on the role of zinc in febrile seizure. Further research, in the form of well-designed multicentric randomized controlled trials is highly recommended as current clinical evidence is insufficient to make practice recommendation.

References:

1. Steering Committee on Quality Improvement and Management,Subcommittee on Febrile Seizures American Academy of Pediatrics. Febrile seizures: Clinical Practice Guideline for the Long-Term Management of the Child with Simple Febrile Seizures.Pediatrics.2008;121:1281-6.
2. Capovilla G, Mastrangelo M, Romeo A, Vigevano F. Recommendations for the management of "febrile seizures" Ad hoc Task Force of LICE Guidelines Commission. Epilepsia 2009;50(Suppl 1) :2–6
3. Arul J, Kommu PPK, Kasinathan A, Ray L, Krishnan L. Zinc Status and Febrile Seizures: Results from a Cross-sectional Study. J Neurosci Rural Pract. 2020 Oct;11(4):597-600.
4. Saghazadeh A, Mahmoudi M, Meysamie A, Gharedaghi M, Zamponi GW, Rezaei N. Possible role of trace elements in epilepsy and febrile seizures: a meta-analysis. Nutr Rev 2015;73(11):760–77
5. Cossart R, Bernard C, Ben-Ari Y. Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signalling in epilepsies. Trends Neurosci 2005; 28:108–115
6. Hosseini F, Nikkhah A, Afkhami Goli M. Serum zinc level in children with febrile seizure. Iran J Child Neurol 2020;14(1):43–47
7. Ghada Ahmed Shaaban, Saif El-Deen EM, Bahgat KA, El Attar RS, El Shennawy SI. Role Of Serum Zinc Level and Its Supplementation In Children With Febrile Seizures. J Popl Ther Clin Pharmacol [Internet]. 2023 Apr. 15 [cited 2023 Dec. 8];30(7):118-24. 7.
8. Heydarian F, Nakhaei AA, Majd HM, Bakhtiari E. Zinc deficiency and febrile seizure: A systematic review and meta-analysis. Turk J Pediatr. 2020;62:347-58.
9. Kuntari, S., Soumena, R. Z., Masturina, M., Sari, R. K., Samosir, S. M., Noviandi, R., Wungu, C. D. K., & Gunawan, P. I. (2023). Levels of Zinc and Iron Serum in Children with Febrile Seizures: Systematic Review and Meta-Analysis. Journal of Medicinal and Chemical Sciences, (6), 2812-2823.
9. Fallah R, Sabbaghzadegan S, Karbasi SA, Binesh F. Efficacy of zinc sulfate supplement on febrile seizure recurrence prevention in children with normal serum zinc level: A randomised clinical trial. Nutrition. 2015;31: 1358-61.
10. Kulkarni S, Kulkarni M. Study of zinc supplementation in prevention of recurrence of febrile seizures in children at a tertiary care center. MedPulse Int J Pediatr. 2020;13:1-4.
11. Abdelrahman A , Shokeir A , Abd Elmagid S. Serum zinc level in children presenting with febrile seizures. Alexandria Journal of Pediatrics.2020; 33(3), 144
12. Kumar M, Swarnim S, Khanam S. Zinc Supplementation for Prevention of Febrile Seizures Recurrences in Children: A Systematic Review and Meta-Analysis. Indian Pediatr. 2021 Sep 15;58(9):857-860. Epub 2021 Aug 2. PMID: 34338220.

ARDS IN CHILDREN: An Overview

Dr. Khumanthem John

Introduction:

The Acute Respiratory Distress Syndrome (ARDS) is a common cause of respiratory failure in critically ill patients and is defined by the acute onset of noncardiogenic pulmonary oedema, hypoxaemia and the need for mechanical ventilation1. In the pediatric intensive care unit (PICU), pediatric acute respiratory distress syndrome (PARDS) is a leading source of morbidity and mortality2.

Incidence:

According to the Pediatric Acute Respiratory Distress Syndrome Incidence and Epidemiology (PARDIE) which is an International observational study involving 145 PICUs across 27 countries; new PARDS occurred in 3.2% of PICU patient with 17.1% mortality3. Contemporary epidemiological data from India is mainly in the form of retrospective data from single centers4. A retrospective study of children with ARDS between 2016 and 2020 was performed to study the clinical profile and outcome of pediatric ARDS. Children with ARDS comprised 7.8% of total admissions5.

Etiology6:

The triggers for Pediatric ARDS are summarized in the following table. In children processes like Pneumonia, Bronchiolitis and Sepsis are the major risk factors. Children with pre-existing lung disease, a history of prematurity, those on long term ventilation and/or with congenital heart disease are at a greater risk of PARDS.

Table 1: Mechanisms of injury of PARDS

Direct lung injury	Indirect lung injury
Pneumonia	Severe sepsis
Aspiration of gastric contents	Major trauma
Pulmonary contusion	Hyper transfusion
Toxic gas inhalation	Acute pancreatitis
Near drowning	Drug overdose
Diffuse pulmonary infection	Reperfusion injury
	Post cardiac bypass
	Lung transplant

Pathophysiology7:

Direct injury is thought to cause regional consolidation from destruction of the alveolar architecture, while indirect injury is believed to be associated with pulmonary vascular congestion, interstitial oedema, and less severe alveolar involvement.

Regardless of its inciting factors, ARDS commonly progresses through stages defined by distinct clinical, radiographic, and histopathologic features. **The Exudative phase** is characterized by the acute development of decreased pulmonary compliance and arterial hypoxemia. The chest radiograph reveals diffuse alveolar infiltrates from pulmonary oedema. In **the Fibroproliferative stage**, increased alveolar dead-space fraction and refractory pulmonary hypertension may develop as a result of chronic inflammation and scarring of the alveolar- capillary unit. During **the Recovery phase**, there is restoration of the alveolar epithelial barrier, gradual improvement in pulmonary compliance, resolution of hypoxemia, and eventual return to premorbid pulmonary function.

Edema in ARDS is not caused primarily by cardiac failure but rather by disruption of the structural components that regulate alveolar fluid balance. Normally, attachments between endothelial cells allow movement of fluid, but

not proteins or solutes, into the interstitial space. The rate of fluid movement into the interstitium depends on net difference between hydrostatic and osmotic pressures in the pulmonary capillaries relative to the interstitial environment. Interstitial fluid clearance by the pulmonary lymphatic system is disrupted in ARDS by injury to the alveolar epithelium and/or pulmonary capillary endothelium. These events trigger the host immune response, causing neutrophil activation and elaboration of proinflammatory cytokines.

Surfactant is produced by alveolar epithelial type II cells and contains phospholipid and protein components. It promotes alveolar and small airway stability by lowering surface tension. Surfactant s principal protein constituents also facilitate clearance of infectious organisms. Following lung injury, surfactant production declines, and the activity of what remain is impaired due to alterations in phospholipids and inactivation by alveolar exudates. In the nondiseased state, the interaction of

surfactant with the elastic properties of the lung and chest wall contributes to pulmonary hysteresis, a phenomenon allowing for the maintenance of lung volume at lower transpulmonary pressure during expiration than are required during inspiration. In the injured lung, a higher pressure is require to achieve and maintain lung recruitment, and a decrease in lung compliance throught out the respiratory cycle.

Clinical Presentation7:

Leakage of proteinaceous fluid into the alveolar spaces and regional atelectasis leads to rapidly worsening hypoxia and labored breathing from transpulmonary pressures needed to maintain alveolar patency. Hypocarbia occurs early, when the patient first manifest tachypnea. The PaCO2 subsequently rises as respiratory muscles fatigue ensues. The patient often present rales over atelectasis-prone or congested ling units, decrease air entry over areas of consolidation and wheezes over areas where small airways closure are occurring.

Diagnosis of ARDS8,9:

The Pediatric Acute Lung Injury Consensus Conference (PALICC) developed criteria pediatric-specific definitions for ARDS (PARDS) in 2015 as both the AECC and Berlin definitions were focused on adult lung injury and have limitation when applied to children. The proposed definitions of PARDS (Figure 1) and those children at risk for PARDS (Figure 2) are as follows.

Age	Exclude patients with peri-natal related lung disease			
Timing	Within 7 days of known clinical insult			
Origin of Edema	Respiratory failure not fully explained by cardiac failure or fluid overload			
Chest Imaging	Chest imaging findings of new infiltrate(s) consistent with acute pulmonary parenchymal disease			
Oxygenation	**Non Invasive mechanical ventilation**	**Invasive mechanical ventilation**		
	PARDS (No severity stratification)	Mild	Moderate	Severe
	Full face-mask bi-level ventilation or CPAP ≥5 cm H_20 [2] PF ratio ≤ 300 SF ratio ≤ 264 [1]	4 ≤ OI < 8 5 ≤ OSI < 7.5[1]	8 ≤ OI < 16 7.5 ≤ OSI < 12.3[1]	OI ≥ 16 OSI ≥ 12.3[1]
Special Populations				
Cyanotic Heart Disease	Standard Criteria above for age, timing, origin of edema and chest imaging with an acute deterioration in oxygenation not explained by underlying cardiac disease. [3]			
Chronic Lung Disease	Standard Criteria above for age, timing, and origin of edema with chest imaging consistent with new infiltrate and acute deterioration in oxygenation from baseline which meet oxygenation criteria above.[3]			
Left Ventricular dysfunction	Standard Criteria for age, timing and origin of edema with chest imaging changes consistent with new infiltrate and acute deterioration in oxygenation which meet criteria above not explained by left ventricular dysfunction.			

Figure 1: Pediatric acute respiratory distress syndrome definition. OI = oxygenation index, OSI = oxygen saturation index. [1]Use Pao_2-based metric when available. If Pao_2 not available, wean Fio_2 to maintain Spo_2 ≤ 97% to calculate OSI or oxygen saturation/Fio_2 ratio. [2]For nonintubated patients treated with supplemental oxygen or nasal modes of noninvasive ventilation, see **Figure 2** for at-risk criteria. [3]Acute respiratory distress syndrome severity groups stratified by OI or OSI should not be applied to children with chronic lung disease who normally receive invasive mechanical ventilation or children with cyanotic congenital heart disease. OI = (Fio_2 × mean airway pressure × 100)/Pao_2. OSI = (Fio_2 × mean airway pressure × 100)/Spo_2.

Age	Exclude patients with peri-natal related lung disease		
Timing	Within 7 days of known clinical insult		
Origin of Edema	Respiratory failure not fully explained by cardiac failure or fluid overload		
Chest Imaging	Chest imaging findings of new infiltrate(s) consistent with acute pulmonary parenchymal disease		
Oxygenation	**Non Invasive mechanical ventilation**		**Invasive mechanical Ventilation**
	Nasal mask CPAP or BiPAP	Oxygen via mask, nasal cannula or High Flow	Oxygen supplementation to maintain SpO_2 ≥ 88% but OI < 4 or OSI < 5[1]
	FiO_2 ≥ 40% to attain SpO_2 88-97%	SpO_2 88-97% with oxygen supplementation at minimum flow[2]: < 1 year: 2 L/min 1 – 5 years: 4 L/min 5 – 10 years: 6 L/min >10 years: 8 L/min	

Figure 2: At risk of pediatric acute respiratory distress syndrome definition. [1]Given lack of available data, for patients on an oxygen blender, flow for at-risk calculation = Fio_2 × flow rate (L/min) (e.g., 6 L/min flow at 0.35 Fio_2 = 2.1 L/min). [2]If Pao_2 not available, wean Fio_2 to maintain Spo_2 ≤ 97% to calculate oxygen saturation index.

"At Risk" for ARDS (Figure 2)

These criteria have been developed to help in the early identification of patients who may eventually progress to established ARDS.

Timing and Triggers

To be classified as PARDS, symptoms of hypoxemia and radiographic changes should have occurred within 7 days of a known clinical insult.

Co-existence of ARDS with Left Ventricular (LV) Failure/ Dysfunction.

Left Ventricular dysfunction may coexist with ARDS, or may develop as a complication. Therefore, any new onset changes in chest X-ray findings or worsening oxygenation criteria not explained by fluid overload or worsening qualifies as PARDS.

Radiographic Findings in PARDS

Chest imaging findings of new infiltrate consistent with acute pulmonary parenchymal disease is prerequisite to diagnose ARDS.

Respiratory Criteria for Disease Severity

Oxygenation Index (OI) is recommended as the primary measure to classify lung severity in those patients on mechanical ventilation, whereas PaO2/FiO2 (PF) ratio is recommended to be used for those on noninvasive support [CPAP/BiPAP- minimum CPAP of 5 cm H2O]. In order to decrease dependence on arterial blood gas for calculation of oxygenation index, oxygen saturation index(OSI) may be used for risk stratification for those patient on mechanical ventilation where as oxygen saturation(SF) ratio can be used when PF ratio is not available to diagnose PARDS in patients receiving non-invasive full-face mask ventilation with a minimum CPAP of 5cm H2O.

Chronic Lung Disease/ Cyanotic Congenital Heart Disease

Patients with pre-existing chronic lung disease who are tested with supplemental oxygen, non-invasive ventilation, or invasive ventilation via tracheostomy should be considered to have PARDS if they have acute deterioration that meets PARDS criteria (acute onset, a known clinical insult,

chest images supporting new onset pulmonary parenchymal disease) and decline oxygenation from baseline.

Similarly, patients with cyanotic congenital heart disease are considered to have PARDS if they have an acute deterioration in oxygenation not explained by the underlying cardiac disease. However, they should not be stratified by OI or OSI risk categories.

Management9

The management strategies of PARDS can be discussed as follows:

I) Control of causative Factor.
II) Respiratory Support.
III) Rescue Therapies.
IV) Non-Respiratory Supportive Care.
V) Other Therapies to improve Oxygenation
VI) Potentially Promising Therapies

I) Control of causative Factor:

Identification of trigger source and source control are important in the management of ARDS. Sepsis being a common trigger of ARDS, early antibiotic therapy is recommended in cases suspected of being infected.

II) **Respiratory Support**

Respiratory support in patients of ARDS ranges from NIV to invasive ventilation. However, it is Complicated by ventilator induced lung injury resulting from alveolar overdistension (volutrauma) due to low lung compliance, high ventilatory pressure combined with repeated alveolar collapse or re-expansion (atelectrauma), and oxygen toxicity. The goal of ventilating children with ARDS is to maintain adequate gas exchange with minimal ventilatory indunced lung injury.

The concept of patient self-inflicted lung injury worsening regional stress in lung tissue is also gathering evidence. It is hypothesized to occur in patients with excessive drive and strong respiratory effort due to abrupt swings in transpulmonary pressure. It is also compounded by steep rise in transvascular pressure favoring negative pressure pulmonary oedema. This has implication on

patients who have good respiratory drive and are given NIV trial, as some studies propose that putting them on noninvasive support may actually worsen ARDS.

A. Non-Invasive ventilation

Non-invasive ventilation (NIV) such as BiPAP in patients with ARDS reduces the intrapulmonary shunt and decreases the work of breathing, but its use in pediatric patients is often debatable due to risk of failure and subsequent delay in starting invasive mechanical ventilation. Because of the high risk of failure, NIV should be reserved for mild ARDS patients who are hemodynamically stable, and do not have multiorgan dysfunction. Oronasal masks and facial masks provide more superior levels of support and synchrony compared to nasal masks. Once initiated, patients should be frequently examined for complications such as pneumothorax, gastric distension, skin breakdown and conjunctivitis. It should be provided under strict monitoring where prompt intubation is possible without delay. If after 1 hour monitoring HR, RR,S/F ratio and level of consciousness there is no significant improvement, NIV should be stopped and invasive mechanical ventilation should be initiated. A dip in S/F ratio at 1 h predicts NIV failure. Approximately 50% of PARDS patient on NIV, show non-response and progress to invasive ventilation. Selected population of children, such as children with immunodeficiency who are at grater risk of complication from invasive mechanical ventilation may benefit from earlier NIV inorder to avoid risks of infection associated with invasive mechanical ventilation.

High Flow nasal cannula (HFNC) system, which can deliver heated and humidified very high flow oxygen through the nose is also an alternative to NIV. HFNC systems are able to increase the end expiratory lung volume, reduces the work of breathing, and improve CO2 clearance and oxygenation. In addition to there beneficial effects, and contrary to NIV, HFNCs do not require any nasal or mask interface, which significantly improve tolerance and use.

Whether HFNC is similar or worse in efficiency compared to NIV is still debatable as there is lack of sufficient pediatric data.

B. Ventilatory Support

1) Endotracheal intubation

- Cuffed endotracheal tubes (ETTs) are recommended when conventionally ventilating a patient with PARDS. Cuffed pressure should preferably be kept </=20cm H2O.
- It is also recommended to allow for an ETT leak during high frequency oscillatory ventilation (HFOV) to augment ventilation, if needed, assuming mean airway pressure can be maintained.

2) Oxygenation.

- Strategy of permissive hypoxemia (as part of lung protective strategy) is followed to minimize the adverse effect of high ventilatory support.
- One may accept a relatively low arterial O2 saturation while maintaining adequate oxygen delivery by optimizing cardiac output.
- In mild PARDS with positive end-expiratory pressure (PEEP) less than 10 cm of H2O, SPO2 is usually targeted in the range of 92-97%.
- Lower SPO2 levels (in the range 88-92%) should be considered for those with PARDS with PEEP greater than or equal to 10 cm H2O. When SpO2 is < 92%, monitoring of central venous saturation and marker of oxygen delivery is recommended.
- As per ventilator management protocol for adults published by the National Institute of Health ARDS Clinical Trials Network (ARDSNet), the recommended PaO2 target is 55 to 80 mmHg (SPO2 target 88%-95%)
- For children with ARDS, PaO2 of 60 to 80 mmHg is usually considered safe as per expert opinion but there are no studies supporting the safety of this target.
- High Fio2 should be avoided to minimize the risk of direct cellular toxicity and avoid reabsorption atelectasis.

3) Ventilation

- While using lung protective strategies, permissive hypercapnia maintaining a pH of atleast 7.15-7.3 with the exceptions that include intracranial

hypertension, severe pulmonary hypertension, select congenital heart disease lesions, hemodynamic instability, and significant ventricular dysfunction.

- Hypercapnic acidosis, apart from protecting against the harmful effects of excessive tidal volume also leads to blunting of many oxidative and inflammatory cascades.
- Hypercapnic acidosis reduces the severity of injury in pre-clinical ARDS models, including ventilator-induced lung injury (VILI). ADAM17 is an important mediator of VILI; its inhibition is one mechanism of hypercapnic protection.

4) Respiratory System Mechanics

- During pressure-regulated ventilation, peak pressure is used to evaluate inspiratory pressure which remains stable through inspiration.
- In volume-controlled ventilator mode, the peak inspiratory is strongly influenced by the resistance of the ETT and of the airways, therefore the plateau pressure is used.
- Plateau pressure, is measured by no-flow end-inspiratory pause (0.2-1s) allowing pressure equilibration, in the absence of patient effort.
- An increased plateau pressure suggests lowered compliance, whereas an increased peak pressure suggests increased resistance in volume control mode.
- The expiratory phase of a flow time scalar helps detection of dynamic hyperinflation (inspiration occurring while expiratory flow is not null) and expiratory flow limitation, which may occur due to high respiratory rate for compensation of low tidal volume, and relatively long inspiratory time.

5) CO2 Monitoring

- End-tidal CO2 monitoring is recommended in patients on invasive ventilation as it provides useful bedside information such as dead space to tidal volume ratio, End Tidal Alveolar Dead Space Fraction (AVDSF) and Ventilation index. These indices are useful markers of severity of lung injury, recruitment vs overdistension and are linked to mortality risk in patient with ARDS.

- The proportion of delivered tidal volume not participating in gas exchange constitutes **VD/VT** (comprising both alveolar and airway dead space) and is calculated using the Enghoff modification of Bohr equation

VD/VT = [PaCO2/PeCO2]/PaCO2

Where PeCO2 is mean expired CO2 measured by volumetric capnometry.

Ventilatory ratio (VR), a simple bedside ratio is described as

VR= [VE measured x PaCO2 measured]/ VE predicted x PaCO2 ideal

Where [VE measured] is measured minute ventilation (ml/min), PaCO2 measured is the measured arterial pressure of carbon dioxide (mmHg), [VE predicted] is the predicted minute ventilation calculated as predicted body weight X 100 (ml/min) & PaCO2 ideal is the expected arterial pressure of carbon dioxide in normal lungs if ventilated with the predicted minute ventilation. PaCO2 ideal is usually set as 37.5mmHg.

Sinha et al concluded in their study that VR correlates with pulmonary dead space and higher values are associated with increased risk of mortality10.

6) Tidal Volume

- Physiologic tidal volume in a normal lung is in the range of 6-8 ml/kg Predicted Body Weight. Predicted body weight (PBW) should preferably be used for targeting tidal volume during lung protective ventilation, as it provides us a better estimate of lung size. For estimating PBW in the ventilated child in the PICU setting, classical growth charts are to be used with gender, length/ height, and head circumference measurement.
- Patient with more severe lung injury, as expressed by poorer oxygenation, lower compliance (i.e. reflecting a small residual inflatable lung volume available for alveolar ventilation and gas exchange due to larger part of lung being collapsed, poorly aerated or hyperinflated-“baby lung“ concept) should receive tidal volume below physiological values i.e. 3-6 ml/kg PBW.
- In the recent guidelines, tidal volumes have been recommended as 3-6ml/kg PBW for patient with poor respiratory system compliance and near

physiological range (5-8ml/kg ideal Body Weight) for patients with better respiratory system compliance.

- To approximate the "correct" tidal volume for mechanical ventilation, the best method recommended is to use the actual body weight if the child s weight is less than or equal to the 50th percentile and ideal body weight (IBW)(i.e predicted from height or ulna length) if above the 50th percentile.
- Despite the advantage of LTV ventilation demonstrated by the ARMA trial, a recent neta-analysis by de Jegar et al. did not show any association with mortality, when low TV ventilation (<7ml/kg) was compared with high TV(>10ml/kg) in children11.

7) Positive End-Expiratory Pressure (PEEP)

- PEEP improves oxygenation by recruitment of small airways and collapse alveoli and an increased in functional residual capacity. It also decreases intrapulmonary shunt, increases mean airway pressure and reduces dead space to tidal volume ratio (VD/VT).
- In severe PARDS, PEEP levels are usually > 10cm H2O, which is titrated to achieve adequate oxygenation and hemodynamic response. When PEEP levels exceed 15 cm H2O, plateau pressure should be monitored and targeted within acceptable limits.
- PEEP changes should be followed by assessment of S/F ratio, dynamic compliance of respiratory system and VD/VT ratio to assess response.
- Smallwood et al. in their study concluded that for an increase in PEEP, only 56% of children with ARDS showed a positive response in terms of oxygenation12.
- In order to quantify the time to equilibration of oxygenation and pulmonary compliance after increase in PEEP [for upto 90 minutes of PEEP changes], Smallwood et al. demonstrated that in responders, the time to 90% of overall improvement required 38 min for compliance and 71 min for oxygenation whereas in nonresponders, both compliance and oxygenation deteriorated at 65 min13.

- Walkey et al. in a recent meta-analysis of 2,728 adult patients with ARDS were not able to demonstrate a benefit in terms of mortality, number of organ failure and ventilator free days in higher PEEP/low VT strategy versus lower PEEP/low VT strategy ventilation. A mortality benefit was seen with in low VT/ High PEEP ventilation against High VT/ low PEEP approach14.
- Eventually, the risk-benefit ratio of PEEP depends on recruitability of lungs, being higher in patients with moderate-severe PARDS and lower in patient with mild PARDS. A simple method of PEEP titration is using the ARDS network PEEP-FiO2 table wherein changes in PEEP(up/down) are made targeting lowest possible driving pressure.15
- Use of esophageal pressure balloon in the distal1/3rd of the esophagus, to approximate the pleural pressure is a physiological method of PEEP estimation. It estimates the end-expiratory transpulmonary pressure (PLexp) PEEP is set to maintain PLexp around 0.

However, a randomized control trial by Beitler et al. in 202 adults with moderate to severe ARDS, esophageal pressure guided PEEP, versus high PEEP-FiO2 table resulted in no significant difference in mortality or ventilator free days16.

8) Inspiratory Time

- Underly lung pathology and its influence on the time constant of the respiratory system (Compliance x Resistance) should be used to set the I time.
- Flow time graphs monitored for end inspiratory or end expiratory flow interruption may help titrate optimum I time/I:E ratio.

9) Mode of ventilation

- No single conventional mode of ventilation mode has been shown to be superior to the other. It is the setting rather than the mode that is important.
- The preferred mode is time-cycled, pressure regulated, volumecontrolled mode to limit peak inspiratory pressure.
- With the exception of severe PARDS on high ventilator settings, spontaneous breathing during mechanical ventilation helps in homogenous distribution of

positive pressure over lung parenchyma. It also prevents disuse muscle atrophy and hence diaphragmatic dysfunction.

10) Inspiratory Pressure

- An inspiratory plateau pressure limit of 28 cm H2O is targeted, allowing for slightly higher plateau pressure (29-32 cm H2O) for patients with increased chest wall elastase (i.e. reduced chest wall compliance).
- As pressure-limited ventilation adjusts tidal volume based on the actual lung comlpliance and amount of residual inflatable lung volume in a sick lung, it can be recommended as lung-protective strategy.

11) Driving Pressure

- In patient with ARDS, respiratory system compliance (Crs) depends upon the amount of remaining "functional" lung size available for ventilation. This "functional" preserved lung is usually not "stiff" and has normal compliance.
- It is this "functional" lung that is most subjected to cyclic parenchymal deformation during mechanical ventilation, described by an index known as driving pressure (P). P is a measure of tidal volume (VT) corrected for Crs [P = VT/ Crs], has been suggested by some studies to be better predictor of survival than VT alone. This important aspect of lung protective ventilation has been highlighted by Amao et. al.17 in adults with ARDS.
- The driving pressure (P= Plateau pressure-PEEP) was strongly associated with survival- a 1-SD increment in driving pressure (approximately 7 cm H2O) was associated with increased mortality with a relative risk of 1.4 (CI 1.31-1.51;P<0.00). Amao et. al.17 also described how individual changes in tidal volume or PEEP are not independently associated with survival. They are associated only if they were among the changes that lead to reduction in driving pressure.
- Although studies in pediatric age group are scanty, it is an important area of research and one should monitor driving pressure while titrating ventilator setting.

12) Recruitment Maneuvers

- Definition: Recruitment maneuvers (RM) can be defined as a voluntary strategy to increase the transpulmonary pressure transiently with the goal to reopen those alveolar units that are not aerated or poorly aerated but reopenable.
- Incremental PEEP titration in moderate-severe PARDS involves:

  --- In PCV- Start with baseline settings of PEEP 10-15 cm H2O, P – 15-18 cm H2O, targeting a TV of 5 ml/kg, sats between 88-92% order to assess recruitability make stepwise increments of PEEP by 2-3 cm H2O every 30-60 secs, keeping P constant, observing stepwise increase in tidal volume, until no increase in tidal Volume occurs (this is the optimal PEEP).

  --- In VCV- Start with baseline settings as above, in this case keep Tidal Volume (Vte) constant during increments of PEEP, observe for increase in plateau pressure and the least reading of driving pressure during these increments (the PEEP level at the least increment of driving pressure is the optimal PEEP)
- Sustained inflation maneuvers by increasing airway pressure to a level of 30-40 cm H2O (or even higher) over a short period of time (generally 30-40 s) is another frequently used recruitment maneuvers.
- Pensier et. al. in their meta-analysis reported the application of RMs do not significantly improve 28-day mortality. It was, however, associated with positive improvement in oxygenation and a lesser requirement of rescue therapy18.
- Recruitment maneuvers can be used in a child with ARDS who desaturate either due to worsening lung condition (maneuver followed by increase in setting) or after disconnection of circuit for suctioning. This can be achieved by a manual inspiratory hold for 20-30 s.
- One should carefully observe the child during the maneuver. It is also important to be ensured that there is no air-leak before starting the maneuver

13) **Ventilator Induced Lung Injury – " Permeability Originating Obstructive response":**

- Ventilating patients with PARDS needs skillful avoidance of atelectotrauma and volutrauma that predispose to ventilator induced lung injury.

- The “POOR becomes POORer hypothesis” proposed by Gaver et. al. described the hypothesis of “Permeability Originated Obstructive Response”19.
- They describe a vicious cycle of VILI propagation in a concentric manner from an initial atelectasis focus.
- In PARDS, the exudation of proteinaceous edema fluid leads to inactivation of surfactant.

This leads to significant heterogeneity in alveoli within the lung parenchyma, causing “ stress concentrators” to develop. These occur due to pressure of oedema fluid from adjacent healthy alveoli onto the surfactant depleted alveoli.

14) Weaning

- A daily assessment of extubation readiness by monitoring certain clinical and physiological variables (i.e.respiratory rate, work of breathing, exhaled tidal volume/kg, progress in underlying etiology of ARDS) should be done to avoid the risks of prolonged mechanical ventilation.
- Most pediatric studies involving Spontaneous breathing trials and extubation readiness tests have in fact shown higher failed extubation rates (approximately 14%) as opposed to clinical judgement alone (4-6%).
- During weaning, the ventilator s contribution to total ventilation is gradually reduced. The weaning is started by simply reducing the frequency of controlled breaths.
- In volume-controlled ventilation, the VT is usually reduced to about 4-6 ml/kg.
- In pressure-controlled ventilation, the PIP is gradually reduced in steps of 1-2 cm H2O. PEEP and FiO2 are reduced while monitoring the PaO2. As the amount of mandatory support is reduced, PSV can be introduced into synchronized intermittent mandatory ventilation (SIMV) mode with pressure support ventilation (PSV) of about 5-10 cm above PEEP.

- Patient on mechanical ventilation are at an increased risk of critical muscle weakness and hence testing the central respiratory drive as well as strength of inspiratory muscles is an important part of weaning. These can be achieved by performing certain maneuvers on the

  ventilator such as P0.1 and Maximal inspiratory pressure (MIP). The respiratory drive can be assessed quantifying the pressure developed against an occlusion during the first 100 ms of inspiration (P0.1). This delay prevents any reaction from the patient in response to occlusion.

III) Rescue Therapies

a) High Frequency Ventilation (HFV)

- High-Frequency oscillatory ventilation uses high-frequency very-low tidal volumes and laminar air flow to protect the lung.
- Consider starting high frequency ventilation in children with moderate to severe ARDS, with Pplat>28 cm H2O or those who fail to improve on conventional ventilation.
- HFV may particularly be useful in children with air leaks-pneumothorax, bronchopleural fistulae.
- Recently, two large randomized trials (in adults with P/F ratio <200), the OSCILLATE20 and OSCAR21 trials have reported an adverse outcome for this ventilator modality when compared to conventional mechanical ventilation. While the OSCAR trial showed no difference in 30-day mortality, the OSCILLATE trials was prematurely stopped (after 500 patient analyses) because of a significantly higher in hospital (47% vs 35%) and 60- day mortality (47% vs 38%) in the HFOVgroup. Further analyses have shed light on the use of lung protective strategies in CMV which were not prevalent during previous HFOV studies leading to better outcome in CMV group.

2. Extracorporeal Membrane Oxygenation9, 22

- Extracorporeal Membrane Oxygenation (ECMO) has been used as a rescue therapy for over two decades in children with ARDS, with reported survival rates of 50%.

- ECMO should be considered to support children with severe ARDS where the cause of respiratory failure is believed to be reversible or the child is likely to be suitable for lung transplantation.
- It is not possible to apply strict criteria for the selection of children who will benefit from ECMO in PARDS. Children with severe PARDS should be considered for ECMO when lung Protective strategies result in inadequate gas exchange.
- The decision to institute ECMO should be based on structured evaluation of case history and clinical status.
- Serial evaluation of ECMO eligibility is more useful than single point assessment. The careful consideration of quality of life and likelihood of benefit should be assessed.
- ECMO should not be deployed in patient in whom life sustaining measures are likely to be limited.

IV) Non-Respiratory Supportive Care.

1. Fluid administration

- Fluid prescription in PARDS patients on mechanical ventilation should be 70% of maintenance fluids, in order to compensate for lesser insensible water losses due to heated humidified ventilator circuit.
- Fluid restriction should only be implemented after children have been resuscitated adequately from septic shock.
- Once hemodynamically stability is achieved, fluid administration should be carefully titrated to minimize capillary leak and control pulmonary oedema.
- Alodaidi et. al. in their recent meta-analysis, describing association between fluid balance and adverse outcomes in critically ill children reported a 6% increase in odds of mortality for every 1% increase in percentage fluid overload23.
- The FACTT trial24 studied the effects of a conservative versus a liberal fluid approach using 2 separate groups of central venous pressure and pulmonary artery occlusion pressure targets in adult ARDS patients.

- Although the trial did not demonstrate advantage of either fluid strategy on 60-day mortality (25.5% mortality in fluid conservative, versus 28.4% in fluid liberal, p=0.30), however, the conservative arm resulted in 2.5 more ventilator free days[VFD](p<0.001) and 2.2 additional ICU free days (p<0.001)
- Cumulative balance should be followed daily in order to prevent fluid overload. Timely removal by using diuretic therapy or continuous renal replacement therapy (CRRT) should be done where positive fluid balance is high.

2. Blood Transfusion

- PARDS patients may require transfusion in order to maintain adequate oxygen delivery, particularly when associated with septic shock.
- Studies have shown RBC transfusion to be associated with worsening oxygenation index and longer duration of mechanical ventilation.
- As per consensus- based guidelines, trigger for packed RBCs transfusion in children with PARDS is hemoglobin of 7g/dl in the absence of severe hypoxemia, cyanotic congenital heart disease, or hemodynamic instability.
- Two distinct post transfusion entities- TRALI (Transfusion associated lung injury) and TACO (Transfusion associated circulatory overload) may complicate the course of PARDS patients.

3. Nutrition

- Initiate enteric nutrition as early as possible. Initially start with small volume (5-10ml) and increase it with monitoring for intolerance.
- In children who are unlikely to tolerate enteral feeds for few days, parenteral nutrition is initiated.
- There is some supportive evidence in adult patients with ARDS that Omega 3 Fatty acid supplementation improves clinical outcomes but there is no evidence to support use of any specific nutritional formula or supplements in children.

4. Supportive Therapies9,22.

- Patient with PARDS should receive effective targeted sedation with minimal possible doses to be able to prevent asynchrony and discomfort during

mechanical ventilation and thus optimize oxygen delivery, oxygen consumption, and work of breathing.

- Pediatric specific sedation scores such as the Penn State Sedation algorithm should be used to monitor, target, and titrate sedation, to facilitate interprofessional communication and avoid oversedation.
- Withdrawal assessment tools should be used during tapering of sedation and assessment for Iatrogenic withdrawal syndrome during weaning should be done.
- RESTORE study25 which was a multi-centre cluster-randomised control trial studying the effect of nurse-implemented, goal directed sedation management protocol on clinical outcomes in mechanically ventilated children showed safety of a bedside driven sedation protocol.
- Although the primary outcome did not show significant results, the study showed that mechanically ventilated pediatric subjects can be maintained in a more awake state, with a reduction in iatrogenic withdrawal syndrome.
- When physiologically stable, pediatric patients with PARDS should receive a periodic assessment of their capacity to resume unassisted breathing (eg. extubation) that is synchronized with sedative titration to an aroused state.
- In moderate-severe ARDS, neuromuscular blocking agents(NMB) should be used in minimal yet effective dose to improve tolerance to mechanical ventilation and to optimize oxygen delivery, oxygen consumption, and work of breathing.
- Brisk spontaneous respiratory effort and high respiratory drive in ventilated patients with PARDS, leads to patient-ventilator asynchrony and increased lung injury.
- Care should be taken to prevent nosocomial infection. Early diagnosis and prompt treatment of these infections are crucial to their recovery.
- Monitor the glucose values regularly and consider use of insulin if the values are persistently above 180 mg/dl. Tight glucose control in children with ARDS should not be implemented until further trials confirm its safety and efficacy.

- Coagulopathy and mechanical ventilation are risk factors for clinically important gastrointestinal bleeding in children. Use stress ulcer prophylaxis with intravenous H2 antagonist or proton pump inhibitor.

V) Other Therapies to improve Oxygenation

1. Prone Position9,22

- Several studies have suggested that prone positioning in mechanical ventilated patient results in more homogenous distribution of ventilation/perfusion matching and less stress/strain in the lung.
- At the same time proning may increase the chest wall stiffness or may increase the Pplat.
- Prone Position cannot be recommended as routine therapy in PARDS.
- PROSEVA TRIAL26 was randomized controlled trial (RCT) of prone positioning in adults with severe ARDS in which they randomized 466 adults with severe ARDS to supine or prone positioning.
- Patient randomized to the „prone arm had a duration of proning for 16h a day for upto 28 days.
- Patient randomized to the prone arm of the trial (n=237) experienced a 50% reduction in all-cause mortality at 28 d (the primary outcome of the trial) compared with those who remained in the supine position.
- With recent evidence supporting its use in adults with severe hypoxemia, prone positioning should be considered in children with PARDS characterized by severe hypoxemia.

2. Nitric Oxide 9,22

- Inhaled nitric oxide (iNO) is a potent pulmonary vasodilator and helps to improve ventilation-perfusion matching.
- Routine use of Inhaled nitric oxide is not recommended in PARDS.
- It may be used in patients for temporary rescue where hypoxemia is refractory to more conventional intervention or as a bridge to ECMO.
- Its use may also be considered in patients with documented pulmonary hypertension or severe right ventricular dysfunction.

- In a study by Bhalla et. al., propensity score matched analysis was applied to compare cohort of children with ARDS who were treated with iNO versus those who were not. There was no benefit in terms of mortality or 28d ventilator-free days in those who received iNO27.

VI) Potentially Promising Therapies

1. Surfactant9,22.

- A meta-analysis of Six trials of surfactant therapy in children with Acute respiratory Failure including bronchiolitis, and acute lung injury showed decreased mortality, increased ventilator free days (VFDs) and decreased duration of ventilator free days.
- However, the recent CARDS study (Calfactant in ARDS) conducted in both adult and Pediatric patients, was stopped prematurely due to futility as it failed to show any improvement in oxygenation or mortality as previously reported28.
- Hence, At this time, Surfactant therapy cannot be recommended as routine therapy in PARDS.

2. Corticosteroids

- Corticosteroids decrease the production of a number of inflammatory and profibrotic mediators by many mechanisms.
- Though SCCM/ESICM guidelines29 in adult recommend steroid for patients with ARDS; there is at present inadequate evidence to support routine use of steroids in children with ARDS.

The therapeutic strategies for managing ARDS are summarized in Table 2 and Figure 3 and 4.

Table 2. Therapeutic strategies in ARDS - Control of causative factors (sepsis, shock etc)

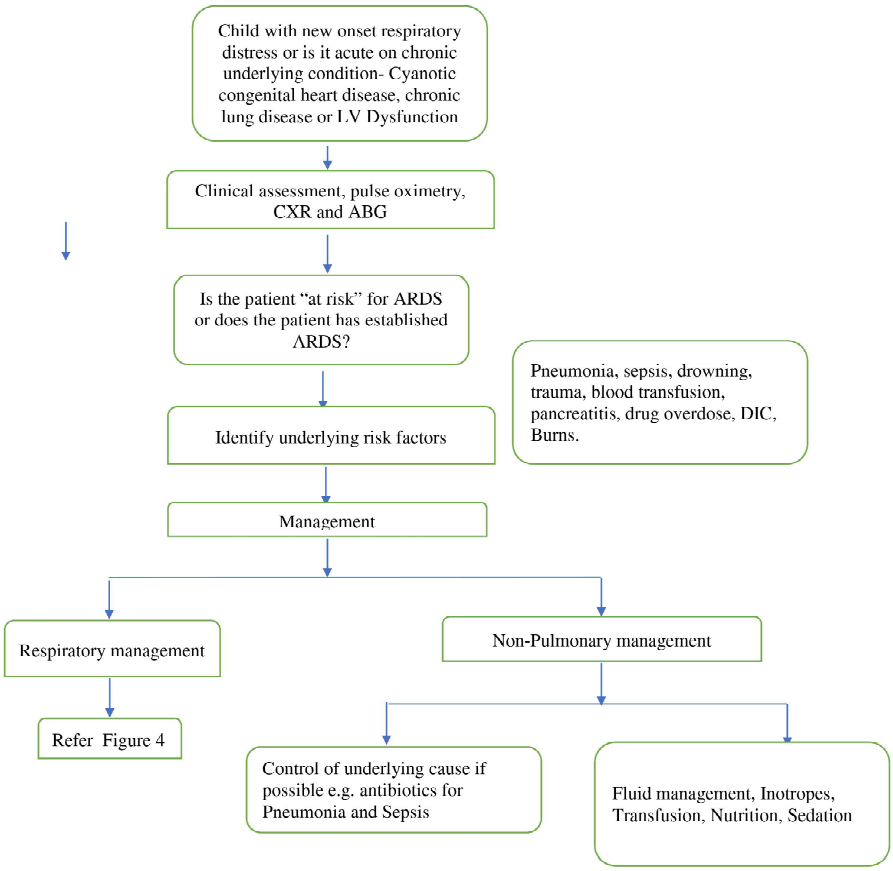
- **Mechanical ventilation**
  - Controlled oxygen exposure (FiO2)
  - Avoidance of volutrauma (low VT) and atelectrauma (appropriate PEEP)
- **Non-conventional ventilation**

- High Frequency Ventilation
- Liquid Ventilation

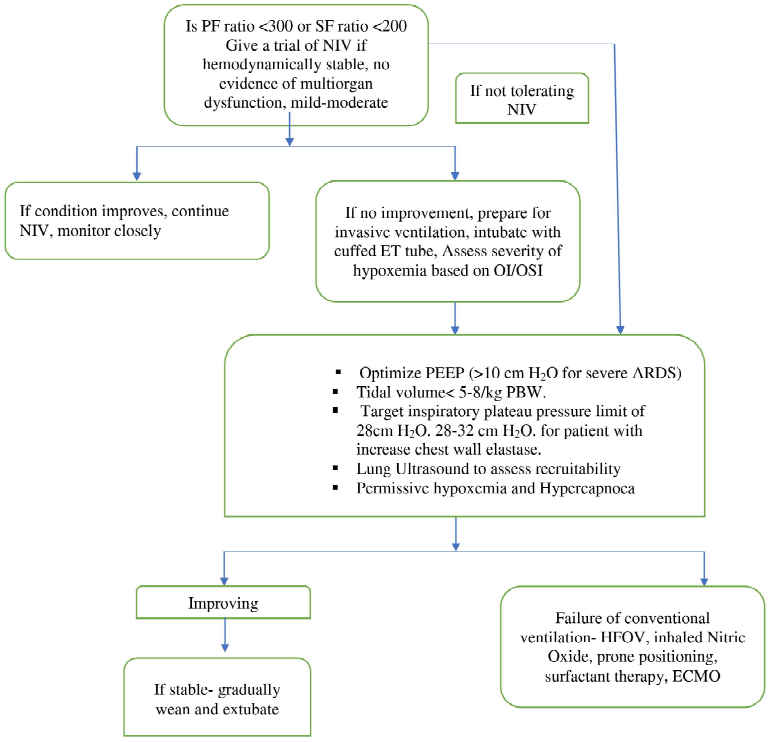
- **Drug based Therapies**
  - Nitric oxide
  - Surfactant
  - Corticosteroids and other anti-inflammatory agents
- **Careful fluid administration**
- **Positioning (Prone ventilation)**
- **Supportive Therapy**
  - Analgesic and sedation, Nutrition
  - Psychosocial support

Fig2: Fraction of oxygen in inspired gas; PEEP Positive end-expiratory pressure; VT Tidal volume :

Figure 4: Respiratory Management in children with ARDS



N.B.: ABG- Arterial Blood Gas, ARDS: Acute respiratory Distress Syndrome, CXR-Chest X-ray, DIC-Disseminated intravascular coagulation, LV-Left Ventricule



N.B. ARDS-Acute respiratory Distress Syndrome; ECMO-Extracorporeal membrane oxygenation;ET-Endotracheal; HFOV-High Frequency oscillatory ventilation;NIV-Non-invasive ventilation; OI-Oxygen Index; OSI-Oxygen saturation Index; PBW-Predicted body weight; PEEP-Positive end-expiratory pressure;PF-PaO2/FiO2 ratio; SF-SaO2/FiO2 ratio.

Prognosis :

In Children with ARDS the severity of hypoxia at presentation and multiorgan failure are strong predictors of mortality. Most of the patients with ARDS succumb to multiorgan failure with <5% of deaths being actually due to respiratory failure. Mortality in pediatric ALI and ARDS seems to be decreasing from as high as 50%-75% 15-20 years ago to <10% more recently. Outcome are improving even for immunocompromised children. It is not clear whether lung protective ventilation or supportive care advances are responsible for this encouraging trend. The Longterm outcomes among survivors of pediatric ARDS appear to be good. In a study with long term follow up, 7 of 28 survivors who are tracked had normal pulmonary and exercise capacity.

References:

1. Matthay MA, Zemans RL, Zimmerman GA, et al. Acute respiratory distress syndrome. *Nat Rev Dis Primers*. 2019;5(1):18. Published 2019 Mar 14. doi:10.1038/s41572-019-0069-0
2. Khemani RG, Smith LS, Zimmerman JJ, Erickson S. Pediatric acute respiratory distress syndrome: definition, incidence, and epidemiology: proceedings from the Pediatric Acute Lung Injury Consensus Conference. *Pediatr Crit Care Med.* 2015;**16**:S23–40. doi: 10.1097/PCC.0000000000000432.
3. Khemani RG, Smith L, Lopez-Fernandez YM, Kwok J, Morzov R, Klein MJ, Yehya N, Willson D, Kneyber MCJ, Lillie J, Fernandez A, Newth CJL, Jouvet P, Thomas NJ; Pediatric Acute Respiratory Distress syndrome Incidence and Epidemiology (PARDIE) Investigators; Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network. Paediatric acute respiratory distress syndrome incidence and epidemiology (PARDIE): an international, observational study. Lancet Respir Med. 2019 Feb;7(2):115-128. doi: 10.1016/S2213-2600(18)30344-8. Epub 2018 Oct 22. Erratum in: Lancet Respir Med. 2018 Nov 13;: Erratum in: Lancet Respir Med. 2019 Mar;7(3):e12. PMID: 30361119; PMCID: PMC7045907.
4. Solomon R. Pediatric Acute Respiratory Distress Syndrome in India: Time for Collaborative Study? Indian J Crit Care Med. 2022 Aug;26(8):896-897. doi: 10.5005/jp-journals-10071-24300. PMID: 36042766; PMCID: PMC9363816.
5. Pujari CG, Lalitha AV, Raj JM, Kavilapurapu A. Epidemiology of Acute Respiratory Distress Syndrome in Pediatric Intensive Care Unit: Single-center Experience. Indian J Crit Care Med. 2022 Aug;26(8):949-955. doi: 10.5005/jp-journals-10071-24285. PMID: 36042772; PMCID: PMC9363796.
6. Paediatrics and Child Health Volume 31, Issue 6, June 2021, Pages 229-232
7. Rogers Handbook of Pediatric Intensive care, 5th Edition.p.156-57.
8. Pediatric Acute Lung Injury Consensus Conference Group. Pediatric acute respiratory distress syndrome: consensus recommendations from the Pediatric Acute Lung Injury Consensus Conference. Pediatr Crit Care Med. 2015 Jun;16(5):428-39. doi: 10.1097/PCC.0000000000000350. PMID: 25647235; PMCID: PMC5253180.
9. Pediatric Intensive Care Protocols of AIIMS, 8th Edition. P.141-167.
10. Sinha P, Sanders RD, Soni N, Vukoja MK, Gajic O. Acute respiratory distress syndrome: the prognostic value of ventilatory ratio—a simple bedside tool to monitor ventilatory efficiency. Am J Respir Crit Care Med. 2013 May 15;187(10):1150-3. doi: 10.1164/rccm.201211-2037LE. PMID: 23675728.
11. de Jager P, Burgerhof JG, van Heerde M, Albers MJ, Markhorst DG, Kneyber MC. Tidal volume and mortality in mechanically ventilated children: a systematic review and meta-analysis of observational studies*. Crit Care Med. 2014 Dec;42(12):2461-72. doi: 10.1097/CCM.0000000000000546. PMID: 25083979.
12. Smallwood CD, Walsh BK, Arnold JH, Gouldstone A. Empirical Probability of Positive Response to PEEP Changes and Mechanical Ventilation Factors Associated With Improved Oxygenation

During Pediatric Ventilation. Respir Care. 2019 Oct;64(10):1193-1198. doi: 10.4187/respcare.06707. Epub 2019 May 14. PMID: 31088988.
13. Smallwood CD, Walsh BK, Arnold JH, Gouldstone A. Equilibration Time Required for Respiratory System Compliance and Oxygenation Response Following Changes in Positive End-Expiratory Pressure in Mechanically Ventilated Children. Crit Care Med. 2018 May;46(5):e375-e379. doi: 10.1097/CCM.0000000000003001. PMID: 29406422.
14. Walkey AJ, Del Sorbo L, Hodgson CL, Adhikari NKJ, Wunsch H, Meade MO, Uleryk E, Hess D, Talmor DS, Thompson BT, Brower RG, Fan E. Higher PEEP versus Lower PEEP Strategies for Patients with Acute Respiratory Distress Syndrome. A Systematic Review and Meta-Analysis. Ann Am Thorac Soc. 2017 Oct;14(Supplement_4):S297-S303. doi: 10.1513/AnnalsATS.201704-338OT. PMID: 29043834.
15. Acute Respiratory Distress Syndrome Network; Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, Wheeler A. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med. 2000 May 4;342(18):1301-8. doi: 10.1056/NEJM200005043421801. PMID: 10793162.
16. Beitler JR, Sarge T, Banner-Goodspeed VM, Gong MN, Cook D, Novack V, Loring SH, Talmor D; EPVent-2 Study Group. Effect of Titrating Positive End-Expiratory Pressure (PEEP) With an Esophageal Pressure-Guided Strategy vs an Empirical High PEEP-Fio2 Strategy on Death and Days Free From Mechanical Ventilation Among Patients With Acute Respiratory Distress Syndrome: A Randomized Clinical Trial. JAMA. 2019 Mar 5;321(9):846-857. doi: 10.1001/jama.2019.0555. PMID: 30776290; PMCID: PMC6439595.
17. Amato MB, Meade MO, Slutsky AS, Brochard L, Costa EL, Schoenfeld DA, Stewart TE, Briel M, Talmor D, Mercat A, Richard JC, Carvalho CR, Brower RG. Driving pressure and survival in the acute respiratory distress syndrome. N Engl J Med. 2015 Feb 19;372(8):747-55. doi: 10.1056/NEJMsa1410639. PMID: 25693014.
18. Pensier J, de Jong A, Hajjej Z, Molinari N, Carr J, Belafia F, Chanques G, Futier E, Azoulay E, Jaber S. Effect of lung recruitment maneuver on oxygenation, physiological parameters and mortality in acute respiratory distress syndrome patients: a systematic review and meta-analysis. Intensive Care Med. 2019 Dec;45(12):1691-1702. doi: 10.1007/s00134-019-05821-9. Epub 2019 Nov 7. PMID: 31701204.
19. Gaver DP 3rd, Nieman GF, Gatto LA, Cereda M, Habashi NM, Bates JHT. The POOR Get POORer: A Hypothesis for the Pathogenesis of Ventilator-induced Lung Injury. Am J Respir Crit Care Med. 2020 Oct 15;202(8):1081-1087. doi: 10.1164/rccm.202002-0453CP. PMID: 33054329; PMCID: PMC7560804.
20. Ferguson ND, Cook DJ, Guyatt GH, Mehta S, Hand L, Austin P, Zhou Q, Matte A, Walter SD, Lamontagne F, Granton JT, Arabi YM, Arroliga AC, Stewart TE, Slutsky AS, Meade MO; OSCILLATE Trial Investigators; Canadian Critical Care Trials Group. High-frequency oscillation in early acute respiratory distress syndrome. N Engl J Med. 2013 Feb 28;368(9):795-805. doi: 10.1056/NEJMoa1215554. Epub 2013 Jan 22. PMID: 23339639.
21. Young D, Lamb SE, Shah S, MacKenzie I, Tunnicliffe W, Lall R, Rowan K, Cuthbertson BH; OSCAR Study Group. High-frequency oscillation for acute respiratory distress syndrome. N

Engl J Med. 2013 Feb 28;368(9):806-13. doi: 10.1056/NEJMoa1215716. Epub 2013 Jan 22. PMID: 23339638.
22. Pediatric Acute Lung Injury Consensus Conference Group. Pediatric acute respiratory distress syndrome: consensus recommendations from the Pediatric Acute Lung Injury Consensus Conference. Pediatr Crit Care Med. 2015 Jun;16(5):428-39. doi: 10.1097/PCC.0000000000000350. PMID: 25647235; PMCID: PMC5253180.
23. Alobaidi R, Morgan C, Basu RK, Stenson E, Featherstone R, Majumdar SR, Bagshaw SM. Association Between Fluid Balance and Outcomes in Critically Ill Children: A Systematic Review and Meta-analysis. JAMA Pediatr. 2018 Mar 1;172(3):257-268. doi: 10.1001/jamapediatrics.2017.4540. PMID: 29356810; PMCID: PMC5885847.
24. National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network; Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B, Connors AF Jr, Hite RD, Harabin AL. Comparison of two fluid-management strategies in acute lung injury. N Engl J Med. 2006 Jun 15;354(24):2564-75. doi: 10.1056/NEJMoa062200. Epub 2006 May 21. PMID: 16714767.
25. Curley MA, Wypij D, Watson RS, Grant MJ, Asaro LA, Cheifetz IM, Dodson BL, Franck LS, Gedeit RG, Angus DC, Matthay MA; RESTORE Study Investigators and the Pediatric Acute Lung Injury and Sepsis Investigators Network. Protocolized sedation vs usual care in pediatric patients mechanically ventilated for acute respiratory failure: a randomized clinical trial. JAMA. 2015 Jan 27;313(4):379-89. doi: 10.1001/jama.2014.18399. PMID: 25602358; PMCID: PMC4955566.
26. Guérin C, Reignier J, Richard JC, Beuret P, Gacouin A, Boulain T, Mercier E, Badet M, Mercat A, Baudin O, Clavel M, Chatellier D, Jaber S, Rosselli S, Mancebo J, Sirodot M, Hilbert G, Bengler C, Richecoeur J, Gainnier M, Bayle F, Bourdin G, Leray V, Girard R, Baboi L, Ayzac L; PROSEVA Study Group. Prone positioning in severe acute respiratory distress syndrome. N Engl J Med. 2013 Jun 6;368(23):2159-68. doi: 10.1056/NEJMoa1214103. Epub 2013 May 20. PMID: 23688302.
27. Bhalla AK, Yehya N, Mack WJ, Wilson ML, Khemani RG, Newth CJL. The Association Between Inhaled Nitric Oxide Treatment and ICU Mortality and 28-Day Ventilator-Free Days in Pediatric Acute Respiratory Distress Syndrome. Crit Care Med. 2018 Nov;46(11):1803-1810. doi: 10.1097/CCM.0000000000003312. PMID: 30028363; PMCID: PMC6185752.
28. Willson DF, Truwit JD, Conaway MR, Traul CS, Egan EE. The Adult Calfactant in Acute Respiratory Distress Syndrome Trial. Chest. 2015 Aug;148(2):356-364. doi: 10.1378/chest.14-1139. PMID: 25855884.
29. Annane D, Pastores SM, Rochwerg B, Arlt W, Balk RA, Beishuizen A, Briegel J, Carcillo J, Christ-Crain M, Cooper MS, Marik PE, Umberto Meduri G, Olsen KM, Rodgers SC, Russell JA, Van den Berghe G. Guidelines for the Diagnosis and Management of Critical Illness-Related Corticosteroid Insufficiency (CIRCI) in Critically Ill Patients (Part I): Society of Critical Care Medicine (SCCM) and European Society of Intensive Care Medicine (ESICM) 2017. Crit Care Med. 2017 Dec;45(12):2078-2088. doi: 10.1097/CCM.0000000000002737. PMID: 28938253.