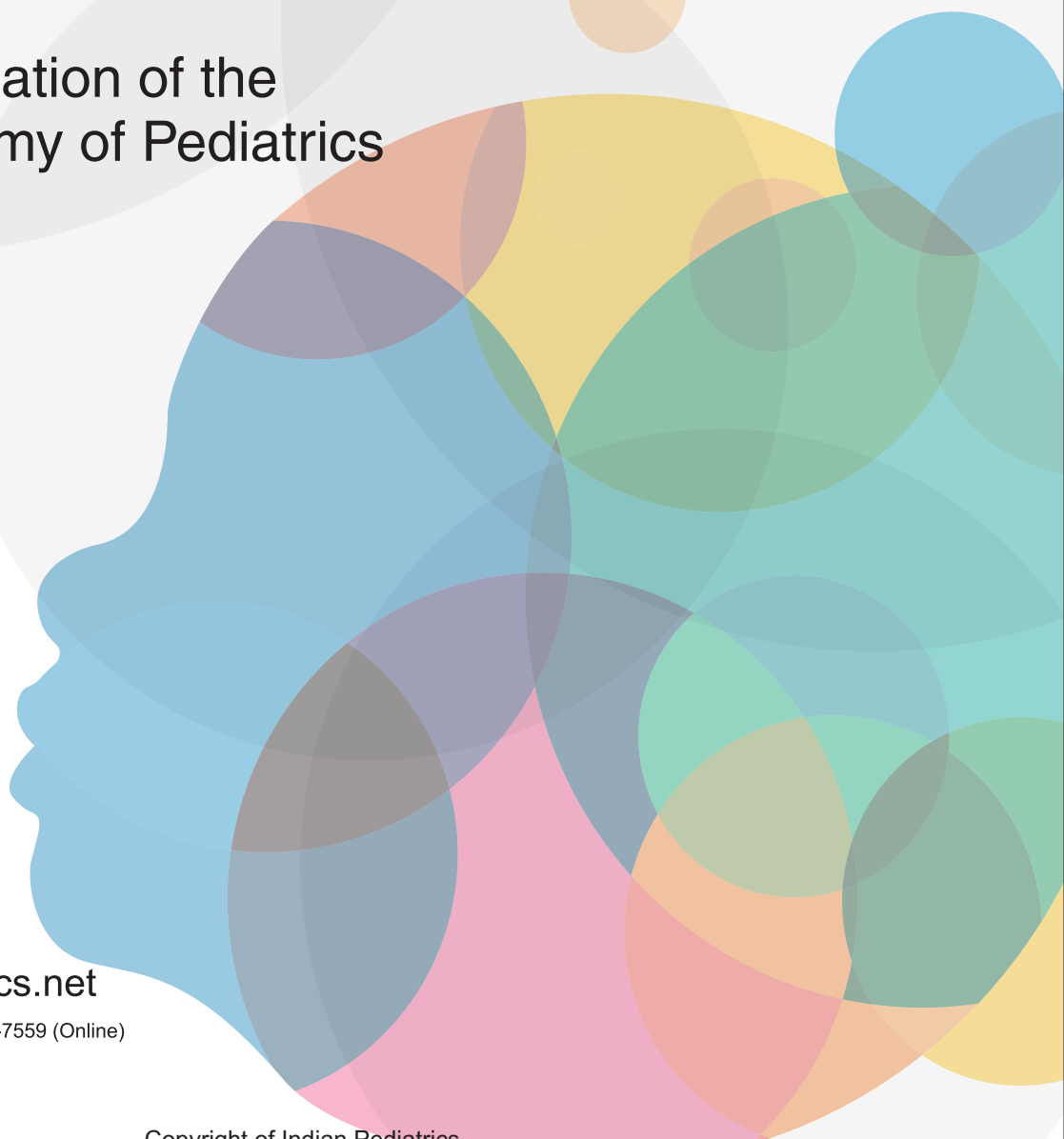
E-copy

Indian Pediatrics

Official Publication of the
Indian Academy of Pediatrics

VOLUME 59
NUMBER 6
June 2022



www.indianpediatrics.net
ISSN0019-6061 (Print) | ISSN0974-7559 (Online)

IAP Guidelines for pediatric skincare state that ideal care of newborn skin comprises of:[1]

- Gentle cleansing[1]
- Protection of barrier function[1]
- Prevention of skin dryness[1]
- Promotion of normal development of the skin[1]

Designed for newborn's sensitive skin

NEW Johnson's® cottontouch®

To promote normal development of the skin: Clinically proven to nurture infant skin microbiome[2,3]

- Ideal for infant massage[4]
- Gentle cleansing with HMP technology[5]
- Protection of barrier function with unique delivery system[6]
- Prevention of skin dryness[2]

RA/JB/BMar2021/05

IAP: Indian Academy of Pediatrics, HMP: Hydrophobically Modified Polymer

References: 1. Madhu R, et al. Indian Academy of Pediatrics Guidelines for Pediatric Skin Care. Indian Pediatr. 2021;58(2):153-161. 2. Data on File. 3. DOF3 Capone AAD 2017. 4. Johnson & Johnson Consumer Products Worldwide. Claim support and data summary for Johnson's Cottontouch Baby Oil. 5. Anwar S, et al. A scoring method to assess the gentleness of cleansers. Presented at the American Academy of Dermatology Annual Meeting, March 30–24, 2020, Denver, CO, USA. 6. Data on file. 6. Capone K, et al. Effects of Emollient Use on the Developing Infant Skin Microbiome. Presented at the American Academy of Dermatology Annual Meeting, March 1–5, 2019, Washington, DC, USA.

Indian Pediatrics

June 2022 Volume 59 Number 6

CONTENTS

Impact Factor 2020 of *Indian Pediatrics* is 1.411

Indian Pediatrics, the official journal of the Indian Academy of Pediatrics, is a peer-reviewed journal with monthly circulation of print/e-copies to over 34,000 pediatrician members. The journal is indexed in Current Contents/Clinical Medicine, Science Citation Index Expanded, Medline, PubMed, Indian Science Abstracts, getCITED, POPLINE, CANCERLIT, TOXLINE, Psych Line, and Dermline. The journal gives priority to reports of outstanding clinical and experimental work, as well as important contributions related to common and topical problems related to children and adolescents. Information for Authors is available on the website of the journal. *Indian Pediatrics* is available free online at *www.indianpediatrics.net.* There are no charges for publishing in the journal.

Address for ordinary letters: **The Editor-in-Chief, Indian Pediatrics, P. Box No. 3889, New Delhi-110 049, India.**
Address for registered/speed post letters: **Dr. Devendra Mishra, Editor-in-Chief, Indian Pediatrics, 115/4, Ground Floor, Gautam Nagar, New Delhi 110 049, India. Tel: (011) 46052593**
E-mail: *jiap@iapindia.org*; Website: *www.indianpediatrics.net*; LinkedIn: *indianpediatrics*
Facebook: *www.facebook.com/Indianpediatrics;* Twitter: *@EditorIndPed;* Instagram: *@indianpediatrics*

Future of Pediatric Practice - Artificial Intelligence Beckoning?

REMESH KUMAR R
President, Indian Academy of Pediatrics 2022
drremesh2006@yahoo.com

Human intelligence has been the most defining quality of our species. It is this single attribute which has distinguished us from all other forms of life and enabled the advancement of civilization over the millennia. Today, the advent of artificial intelligence (AI) promises to take humankind to a new level and deliver results that would have been considered impossible hardly a few decades ago. The smart phones and other everyday gadgets we use, internet search engines, facial and voice recognition softwares, cloud kitchen services, virtual assistants like Alexa and Siri as well the digital advertising that we see while navigating the internet all use AI technologies. Driverless cars are one of the revolutionary products that are presently being developed by Google using AI. From the discernible trends, it certainly looks like life will never be the same again with AI.

From a professional perspective, healthcare is one of the fields where AI is expected to have the highest impact. Already we are seeing AI being extensively used in large hospital settings in diagnostics, records keeping and even in the performance of procedures such as robot assisted surgery. In all such cases where AI is already being used in healthcare, greater efficiency, accuracy and better success rate is seen. With AI assuming centre-stage as the most happening thing these days, with large-scale research and product development taking place backed by unimaginable levels of funds, AI-enabled tools are expected to become more economical, user friendly and widespread in all walks of life. It will not be long before AI enters day-to-day clinical practice. Hence it is high time that we become acquainted with AI and prepare ourselves to utilize its potential for delivering better quality of care to patients.

What is AI?

Intelligence is commonly understood to be the "ability to acquire and apply knowledge and skills". Being intelligent beings, humans are able to perform various 'mental' tasks like observe, infer, acquire, analyze and classify knowledge and also to learn, apply, make decisions and manipulate our surroundings to solve problems or achieve desired results. Artificial intelligence refers to the creation of machines which are endowed with the ability to carry out many of these functions autonomously and without any external inputs. Machines having AI are able to learn from their past usage and update or improve their capability to perform the same function with better efficiency during future usage. It is basically about creating intelligent machines which can think like humans and are able to make decisions.

For example, when you are reading a particular article in a particular website and develop a doubt, you may go to Google search to resolve it. At this point you may be surprised to see that even as you type the first word, Google is able to furnish the entire sentence concerning your doubt with a near cent percent accuracy. This is because the AI used in Google search engine has taken note of the article you are reading in that website and by comparing your personal profile which it has developed from your past internet usage patterns with other similar profiles, it has already decided that you are likely to search this topic. Google search engine has learnt this on its own without being prodded by anyone else working from behind the scenes.

More About AI

This extraordinary capability of information technology to process information on an unimaginably large scale is what has led to the present level of sophistication of AI. Though AI as an academic discipline was first mooted as early as in 1956, it did not really make much headway till the digital revolution and advent of the internet in the last two decades. This is when AI found its voice and began to revolutionize our lives in never before ways.

AI generally falls into two broad categories: Weak or Narrow AI, and Strong or Generic AI. Weak or narrow AI refers to machines that operate within a limited context and are able to simulate human intelligence (even basic human intelligence) to quite a high degree. Such machines are designed for performing only specific functions or tasks. Such machines cannot perform tasks that fall outside the domain they are designed for. All the currently available AI applications fall into this category. On the other hand

Strong or generic AI refers to machines which can substitute a human being and perform any task or tasks that humans are capable of and perhaps even more. This type of AI is presently not available and can be seen only in the realms of science fiction, such as the robots that we see in Star Wars movies.

Machine learning, data science, deep learning and robotics are some of the domains relating to AI. These refer to different levels of processing data and functions performed.

AI and Healthcare

Though the advent of AI cast a widespread influence on all aspects of life, its usefulness in the field of healthcare was particularly felt, thanks to its superhuman ability to process data and achieve high accuracy of result in any given task. AI began to make its presence strongly felt initially in the arena of diagnostics when new machines began to be invented in imaging and related fields. Today, AI-enabled imaging facilities can accurately recognize diseases like pneumonia or breast cancer from an *X*-ray scan even at their earliest stages when experienced doctors too might miss seeing the weak tell tale signs.

It also came to be widely applied in medical records keeping and administrative work. Voice recognition/ dictation software is an example of AI that is currently used in pediatric practice. The ability of AI to recognize, analyze and predict health trajectory will make it an invaluable tool in preventive medicine and community medicine. Wearable AI enabled devices will greatly help in monitoring patient activity and collecting useful data. AI is also making headway in surgery with the help of robotics. The availability of AI tools was one of the reasons why scientists were able to develop effective vaccines for COVID-19 in a fraction of the time that is otherwise need for developing a vaccine. The process of vaccine development could be speeded up mainly because scientists were able to collate and analyze large data quickly and efficiently to arrive at accurate conclusions.

Challenges of AI

Like all new technologies, AI too has been viewed with fear and suspicion. There is a fear that it could lead to job loss and make doctors redundant. There are horror scenarios enacting in people's mind regarding what to do if the AI goes out of human control and takes a life of its own. The famous scientist Stephen Hawking had warned regarding humans losing control to robots, saying, "Whereas the short-term impact of AI depends on who controls it, the long-term impact depends on whether it can be controlled at all." Similarly Microsoft founder Bill Gates and Tesla founder Elon Musk too have warned that AI could pose a long term threat to humanity.

So, will AI prove to be a Frankenstein's Monster? Will it push the doctor out of the clinic? No, say most experts. At the present level of AI technology, machines still operate on the basis of manmade algorithms – albeit highly advanced algorithms – and they only perform functions that humans already do, but with greater degree of efficiency and accuracy, and that too in specific niche activities. We still need humans to back up AI for getting a 360 degree view. AI only makes our job easier and less strenuous without encroaching on any of our expertise. If anything, the efficiency and accuracy AI brings to the table makes it a boon to the patients and doctors alike. Those who see the devil in AI ignore the fact that it lacks other key human attributes like emotional and social sensitivity as well as creativity. These are unique functions that cannot be easily broken down to logic.

The more practical issues of concern with regard to AI are subjects like privacy violation, misuse of data, algorithmic bias caused by bad data and regulatory approval. But with the advantages far outweighing the cons, these issues will surely be sorted out with effective remedies. In fact, UNICEF's Office of Global Insight and Policy has launched a major project to better understand how AI systems can protect, provide for, and empower children, as also for exploring how to embed child rights in the governing policies of artificial intelligence. Every great change calls for a corresponding change in human mindset. AI is a revolutionary new change that is sweeping the world. It is a positive change that can transform healthcare into a more vibrant and successful discipline. Hence, it is time for the medical fraternity to embrace this change by understanding AI and making the best use of it.

REFERENCES

1. *https://sitn.hms.harvard.edu/flash/2019/artificial-intelligence-in-medicine-applications-implications-and-limitations/*
2. *https://www.contemporarypediatrics.com/view/artificial-intelligence-enters-pediatric-practice*
3. *https://www.unicef.org/globalinsight/featured-projects/ai-children*

INVITED COMMENTARY

Pulmonary Functions in Transfusion-Dependent Thalassemia

JAGDISH CHANDRA,* SMRITI ROHATGI
Department of Pediatrics, PGIMSR and ESIC Model Hospital, Basaidarapur, New Delhi 110015.
*jchandra55@gmail.com

Thalassemia is a genetic disorder which starts as a hemolytic anemia but during its course attains the dimension of a 'multi-system disease' [1]. Blood transfusion is the mainstay of treatment for individuals with transfusion-dependant thalassemia (TDT) as it improves anemia and also suppresses ineffective erythropoiesis. Each unit of packed red cells delivers an estimated 200 mg of iron. As body cannot excrete extra iron, complications resulting from iron overload become a major source of morbidity. Studies have highlighted importance of appropriate iron chelation in improving survival. A 2004 study of 977 patients from Italy had reported 68% patients being over 35 years. Patients with lower serum ferritin had a better probability of survival [2]. From India, a recent study of over one thousand patients reported 50% actuarial survival at 26.9 years with poor iron chelation being a significant risk factor for morbidity and mortality [3].

Iron overload in TDT is associated with saturation of transferrin in the body. The excess iron remains in the form of non-transferrin-bound iron. This labile iron is the predominant form of iron that causes tissue damage [4]. The involvement of other organ systems primarily results from iron overload related tissue injury; although, chronic hypoxia is also incriminated [5]. Involvement of heart, endocrine glands and liver has been extensively studied. Well stated guidelines are in place for assessment of these organs in patients with TDT and studies have shown their reversibility with intensive iron chelation with desfrrioxamine or combined oral chelation [6].

Pulmonary dysfunction resulting from iron overload has not been studied extensively in children. Studies done on adult TDT patients suggest that lung fibrosis and/or interstitial edema related to iron overload are the main cause of pulmonary dysfunction [7]. Pulmonary dysfunction in children with TDT is described to be restrictive, large airway obstruction, diffusion impairment, or small airway disease [8-12]. The differences among the published data may be due to the heterogeneity of the studies (different patient age, different iron chelation regimen), duration of transfusion received and level of serum ferritin along with the multifactorial nature of the pathogenesis of pulmonary dysfunction in these patients. Some have also reported that pulmonary dysfunction may be due insufficient anatomic and functional development of the lung during early infancy in patients with TDT. Reduced lung volumes in thalassemic patients were explained by the upward pressure on the diaphragm by the enlarged liver and spleen, when present, which is supported by an increase in the vital capacity and the expiratory reserve volume seen in patients after splenectomy [13]. Fortunately, moderate or massive splenic enlargement is not seen in adequately transfused patients. Although changes in pulmonary function have been attributed to iron overload, those that examined the link between somatic iron stores and pulmonary function had varying conclusions. On autopsy, iron was concentrated in bronchiolar epithelial and mucous glands. Hemosiderin-laden macrophages are present in bronchoalveolar lavage, often in quantities similar to those observed in idiopathic pulmonary hemosiderosis, as well as lymphocytic infiltrates suggestive of alveolitis [14,15].

This issue of the journal has two studies on the subject [16,17]. None of the patient in both the studies had any respiratory symptoms. In the study by Panwar, et al. [16], 50 (68.8%) patients had lung dysfunction, most commonly diffusional impairment (48; 96%), and 9 (81.8%) patients had restrictive defect with moderate to severely deranged DLCO. Baruah and Bhattacharjee [17] observed restrictive pattern on pulmonary function test in 71.2%. They did not perform tests for diffusion abnormalities. Both the studies stress upon adequate iron chelation. The cases had high prevalence of pulmonary dysfunction. Patients in the study by Panwar, et al. [16] were better chelated. However, pulmonary dysfunction was observed by them also in two-third of the patients. They have also shown reversibility in pulmonary dysfunction with intensive iron chelation as has been described for reversal of cardiac and endocrine dysfunction with intensive chelation [6,18,19]. Cases in study from Assam had high serum ferritin as only a few of the patients were on regular iron chelation. High serum ferritin resulting from poor compliance to chelation needs to be addressed through proper focused counseling. Non-

availability of iron chelators can be mitigated by organizing funds through National Health Mission [20].

Funding: None. *Competing interests*: None stated.

REFERENCES

1. Adamkiewicz TV, Abboud MR, Paley C et al. Serum ferritin level changes in children with sickle cell disease on chronic blood transfusion are nonlinear and are associated with iron load and liver injury. Blood. 2009;114,4632-8.
2. Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica. 2004; 89:1187-93.
3. Dhanya R, Sedai A, Ankita K, et al. Life expectancy and risk factors for early deaths in patients with severe thalassemia syndrome in South India. Blood Adv. 2020;4:1448-57.
4. Aydinok Y, Evans P, Manz CY, Porter JB. Timed non-transferrin bound iron determinations probe the origin of chelatable iron pools during deferiprone regimens and predict chelation response. Haematologica. 2012;97:835-841.
5. Piga A, Longo F, Duca L, Roggero S, et al. High nontransferrin bound iron levels and heart disease in thalassemia major. Amer J Hematol. 2009;84:29-33.
6. Cappellini MD, Farmakis D, Porter J, Taher A, eds. Guidelines for management of transfusion dependent thalassemia (TDT). 4th Ed. Nicocia Cy. Thalassemia International Federation. 2021.
7. Carnelli V, D'Angelo E, Pecchiari M, et al. Pulmonary dysfunction in transfusion-dependent patients with thalassemia major. Am J Respir Crit Care Med. 2003; 168:180-184
8. Arora M, Chandra J, Suri JC, et al. Pulmonary function tests in beta thalassemia. Indian J Pediatr. 2001;68:239-242.
9. Azarkeivan A, Mehrvar A, Pour HS, et al. Pulmonary function test in transfusion-dependent beta-thalassemia patients. Pediatr Hematol Oncol. 2008;25:598-606
10. Li AM, Chan D, Li CK, et al. Respiratory function in patients with thalassaemia major: relation with iron overload. Arch Dis Child. 2002;87:328-330
11. Alyasin S, Moghtaderi M, Amin R, et al. Pulmonary function test in transfusion-dependent beta-thalassemia major patients: a pilot study. Pediatr Hematol Oncol. 2011;28:329-333.
12. Keens TG, O'Neal MH, Ortega JA, et al. Pulmonary function abnormalities in thalassemia patients on a hypertransfusion program. Pediatrics. 1980;65:1013-1017.
13. Grant GP, Mansell AL, Graziano JH, et al. The effect of transfusion on lung capacity, diffusing capacity, and arterial oxygen saturation in patients with thalassemia major. Pediatr Res. 1986;20:20-23
14. Cooper DM, Mansel AL, Weiner MA et aI. Low lung capacity and hypoxemia in children with thalassemia major. Amer Rev Resp Dis. 1980; 121:639-646.
15. Priftis KN, Anthracopoulos MB, Tsakanika C, et al. Quantification of siderophages in bronchoalveolar fluid in transfusional and primary pulmonary hemosiderosis. Pediatr Pulmonol. 2006; 41:972-7.
16. Panwar N, Gomber S, Dewan P, Kumar R. Pulmonary dysfunction in transfusion-dependent thalassemia and response to intensive chelation therapy. Indian Pediatr. 2022;59:451-54.
17. Baruah A, Bhattacharjee J. Pulmonary function in children with transfusion-dependent thalassemia and its correlation with iron overload. Indian Pediatr. 2022;59:455-8.
18. Anderson LJ, Westwood MA, Holden S. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. British J Haematol. 2002:127: 348-355.
19. Kontoghiorghes GJ, Kontoghiorghes C. Iron and chelation in Biochemistry and Medicine: New approaches to controlling iron metabolism and treating related diseases. Cells. 2020;9:1456.
20. Ministry of Health and Family Welfare, Government of India. Prevention and control of Hemoglobinopathies in India-Thalassemias, Sickle Cell Disease and other variant hemoglobins. National Health Mission Guidelines on Hemoglobinopathies in India. GoI,2016.

NEWS IN BRIEF

Acute severe hepatitis of unknown origin in children

Mystifying cases of severe acute hepatitis in children have foxed epidemiologists in the last couple of months. Of the 169 cases reported till 21 April, 2022, most of the cases were in the Europe and the USA with UK topping the list at 114 cases. Ten percent (17 children) required a liver transplant and one child died.

Strikingly, fever was uncommon though other symptoms of viral hepatitis like abdominal pain, diarrhoea, vomiting and jaundice were seen. Most had transaminase levels above 500 U/L. Children were negative for hepatitis virus A to E. An intensive search for the culprit has unearthed the Adeno virus in 74 children, SARS-CoV-2 in 20 and coinfection of SARS-CoV-2 and adenovirus in another 19.

The outbreak has engendered anxiety because such severe symptoms have not previously been documented with adeno viral infections. One thought is an increased susceptibility in children due to lack of exposure in the COVID years. The other possibilities being floated are a coinfection with SARS-CoV-2, which has aggravated the illness or a new mutated variant of adeno virus. Prior vaccination against SARS-CoV-2 is not being considered a risk factor, since that most of the affected children were not immunized.

(https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON376 23 April 2022; MMWR 29 April 2022)

GOURI RAO PASSI
gouripassi@hotmail.com

PERSPECTIVE

Impact of Air Pollution on Child Health in India and the Way Forward

SAGNIK DEY

From the Centre for Atmospheric Sciences and Centre of Excellence for Research on Clean Air, Indian Institute of Technology, Delhi. Correspondence to: Dr Sagnik Dey, Center for Atmospheric Sciences and Centre of Excellence for Research on Clean Air, IIT Delhi, New Delhi. sagnik@cas.iitd.ac.in

Recent research in epidemiological modelling reveals that air pollution affects child health in various ways resulting in low birthweight, stillbirth, preterm birth, developmental delay, growth failure, poor respiratory and cardiovascular health, and a higher risk of anemia. India has embarked on the national clean air program, but a much stronger coordinated multi-sectoral approach is required to minimize the child health burden caused by air pollution. Air pollution should be treated as a public health crisis that can only be managed with policy backed by science, gradual transition to clean energy use, emission reduction supported by clean air technologies, long-term commitment from the Government, and cooperation of the citizens.

Keywords: *Clear air, National clean air program, PM2.5, Under-5 mortality*.

India has made remarkable progress in reducing the under-five child mortality rate (U5MR) from 83.1 in 2000 to 42.4 per 1000 livebirths in 2017. Yet, the country had the largest proportion of the global under-five deaths in 2017 [1], with 68.2% being attributed to malnutrition, followed by 10.8% to unsafe water and sanitation, and approximately 8.8% to exposure to air pollution [1].

CHILD HEALTH BURDEN ATTRIBUTABLE TO AIR POLLUTION

The Global Burden of Disease (GBD) India study led by the Indian Council of Medical Research (ICMR) and the Public Health Foundation of India (PHFI) has estimated the U5MR of male and female children at the state level from 1990 to 2019 [2]. In the earlier GBD exercises, the child mortality burden attributable to air pollution was only estimated in terms of acute respiratory infection, while low birthweight was added later as another manifestation. **Fig. 1** shows the under-five deaths per 100,000 population attributable to air pollution in 2019. The top three states with the highest burden were Uttar Pradesh, Rajasthan, and Madhya Pradesh. The states with the least burden were Kerala, Goa, and Tamil Nadu. If the trends from 2000 to 2017 were to continue, India would be on track to meet the United Nations Sustainable Develop-ment Goal 3.2 target of reducing the U5MR below 25 deaths per 1000 live births by 2030 [3] but will fall short of meeting the National Health Mission target of reducing the U5MR below 23 deaths per 1000 live births by 2025 [1].

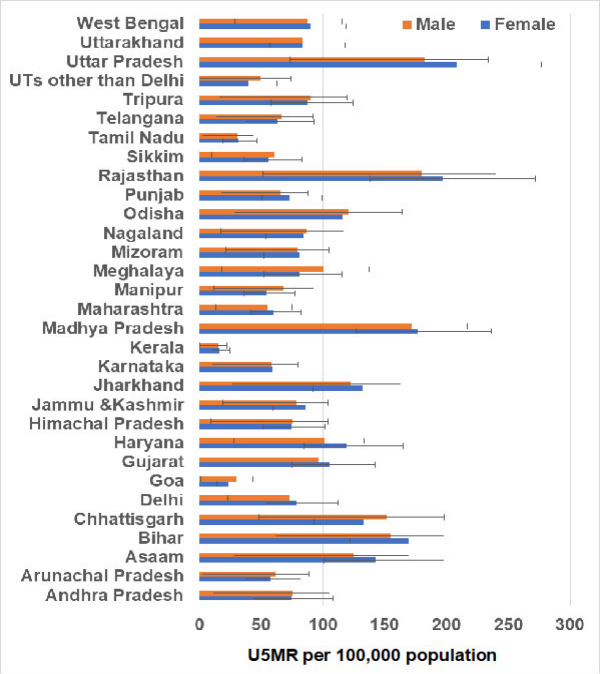
HARMFUL EFFECTS OF AIR POLLUTION ON CHILD HEALTH

The effect of air pollution on the health of a child begins from the intrauterine stage and continues through the early years (**Fig. 2**). Exposure during pregnancy has been found to result in low birthweight, preterm delivery, and stillbirth [1,4,5]. A 10 $\mu g/m^3$ increase in fine particulate matter (PM size <2.5 μm) exposure during pregnancy was reported to be associated with a 2% increase in the prevalence of low birthweight (OR 1.02; 95%CI:1.005, 1.041) after adjusting for other confounders [4]. Another study found ambient PM2.5 exposure in the third trimester to have a significant association with neonatal mortality (OR: 1.016; 95% CI 1.003-1.030, for every 10 $\mu g/m^3$ increase in ambient PM2.5 exposure) [6]. In comparison to those having in-utero exposure to ambient PM2.5 of less than 26.7 $\mu g/m^3$, the fetuses having higher in utero PM2.5 exposures showed a non-linear increase in the risk of low birthweight from PM2.5 levels of 39.3-44.7 $\mu g/m^3$ to greater than 77.3 $\mu g/m^3$ [7]. Besides these, several studies have shown robust associations of air pollution with various other child health outcomes like stunting, wasting, underweight [8,1], childhood anemia [9], allergic rhinitis, asthma [10,11], pneumonia [12], abnormalities in lung development [13], acute respiratory infection [14-16], atherosclerosis [17] behavioral and developmental delay [18,19] as well as the impact on academic performance [20].

LACUNAE IN RESEARCH AND NEW DIRECTIONS

Though studies have convincingly demonstrated air pollution as a major risk to child health, not just in the National capital region (NCR) Delhi but in the entire country, still several critical knowledge gaps exist.

The lack of an adequate ground-based monitoring network in India poses a challenge for optimal assessment



Error bars show 95% UI. Amongst the union territories (UTs), statistics were given separately for Delhi, and the data were combined for all other UTs. The data are compiled from the GBD India database.

Fig. 1 Annual mortality burden of children under the age of five years in India in 2019 attributable to air pollution per 100,000 population.

of ambient PM2.5 exposure to children before and after birth. To address this issue, studies have followed two approaches. In a cross-sectional design, the studies either assumed limited ground-based measurements as representative of the exposure in the entire city [21] or used satellite-derived PM2.5 as a proxy for personal exposure [6]. Each of these approaches has its strengths and weaknesses. The statistical approach relies heavily on the dependency of PM2.5 on a few dominant predictor variables and the availability of data of all critical variables at the desired spatio-temporal scale [22,23]. The physical approach relies on the representativeness of the vertical distributions of PM and the accuracy of the available information about its composition [24]. The scaling factor-based approach relies on the accuracy of the model or reanalysis-derived scaling factors that are used to convert aerosol optical depth to PM2.5 [25]. Nonetheless, recent studies have shown that the algorithms can be trained to a very high level of accuracy with proper calibration [25]. Since satellite-derived exposure has been available for over two decades in India, they are also used in retrospective cohort design [23]. On the other hand, prospective cohorts and studies focusing on household exposure rely on personal exposure assessment using portable sensors [4]. While this is expected to provide the best exposure estimates, it is challenging to scale it up at a population level, and the sensors need robust calibration with reference-grade monitors.

So far, epidemiological studies in India have examined the impacts of exposure to total PM2.5 or PM10 mass. The differential impacts of individual PM types on child health outcomes are not known. Exposure models need to be developed for individual species, tagged to sectoral emissions. Statistical models based on machine learning techniques [23] and chemical transport models [26], being the two most effective tools to develop sector- and species-specific exposure data. Currently, India does not have any chemical speciation network that can be utilized to evaluate modelled exposure. Although measurements of PM composition are carried out intermittently [27], and measurements of oxidative potential (a proxy for quantifying toxicity of PM species) have recently started in India [28], this must be organized in a coordinated way. This was one of the key recommendations of the environmental science panel of the VAIBHAV India summit in 2020 (*innovate.mygov.in*).

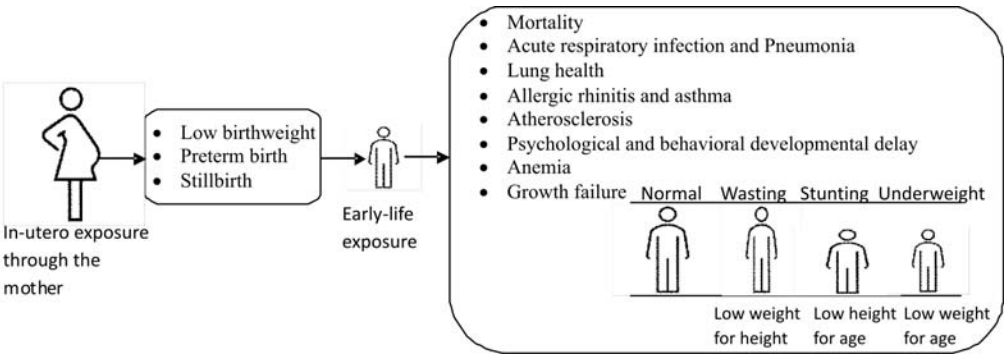


Fig. 2 In utero exposure of the mother to air pollution during pregnancy is a major risk factor for low birthweight, stillbirth, and preterm birth, which can lead to child mortality. Early-life exposure of the child to air pollution can also lead to mortality and negative impacts on multiple health outcomes (for those who survive) and delay in developmental milestones.

Laboratory-based studies done at the cellular level [29] are needed to understand the biological pathways through which exposure to air pollution impacts child health. Further, exposure assessment confined to only ambient or household PM2.5 always has the chance of exposure misclassification and overlaps, and may not capture the true exposure disparity [30]. The personal exposure data collected during recent cohorts (e.g., TAPHE, DAPHNE, CHAI, APPLE, etc.) would be valuable in evaluating such models.

Finally, air pollution is a deadly cocktail of various pollutants, including particulates and gases. Exposure databases do not exist for other criteria pollutants on a national scale, which restricts multi-pollutant epidemiological modelling. Efforts like the creation of the national ambient PM2.5 exposure database [25] are required to address this issue.

WHAT SHOULD WE DO?

India has taken the first step in the right direction by acknowledging air pollution as a national problem, thus gathering momentum for implementing clean air action plans under the National Clean Air Program. However, this is not enough, given the magnitude of the problem.

Evolve a hybrid monitoring approach for improved exposure assessment: Lack of institutional resources and financial constraints prevent India from expanding the ground-based reference-grade monitoring network as per World Health Organization norms of having at least one monitor per million population. So, India can evolve a hybrid monitoring network including reference-grade monitors providing the benchmark data, satellites providing the required spatial coverage, portable sensors (after properly calibrated against reference-grade monitors) providing hyper-local information, and measurements of PM species at strategically suited locations within air sheds [31].

Include health as an integral part of the air pollution management system: Health, to date, is not included in the air pollution management plan as a core indicator. Given that children are more vulnerable to air pollution than adults, environmental policies should urgently be linked to the national health mission.

Strengthen social awareness: General awareness about air pollution is still very poor outside major cities. Though the entire Indo-Gangetic plain, including the rural areas, has PM2.5 levels exceeding the national standards, citizens are not aware of their ill impacts. Physicians would be the ideal ambassadors for clean air advocacy. Unfortunately, most physicians in India are not aware of the multiple health hazards of air pollution beyond respiratory health. The medical curriculum could be more inclusive of environmental health risks.

Invest in air pollution epidemiology research: In the last decade, a plethora of studies came out showcasing the systemic impacts of air pollution on child (and adult) health in India. However, prospective multicentric cohorts with multi-year follow-ups are the need of the hour with a sustained funding commitment from the government.

Focused capacity building promoting interdisciplinary skills: Advancing air pollution epidemiology research in India requires adequately trained human resources. To cater to the need for a focused capacity-building exercise in air pollution epidemiology, the CAPHER-India network has been launched jointly by All India Institute of Medical Science, Delhi, and Indian Institute of Techno-logy Delhi in partnership with the Health Effects Institute, USA. The primary mandate of this network is to provide a platform for researchers from atmospheric chemistry, biostatistics, atmospheric measurement and modelling, epidemiology, medicine, and the public health community to collaborate and provide hands-on training to early-career researchers. The Consortium for climate, health, and air pollution research in India (CHAIR-India), coordinated by the PHFI, is also involved in capacity building.

We must remember that the fight against air pollution is a long-drawn process. Contrary to the general belief that cleaning air could have a negative impact on economic progress, data from developed countries suggest otherwise [32]. We should accept the seriousness of the problem, be prepared mentally, and act with strategic planning, ably supported by science.

Note: The data that is used to generate Fig. 1 has been provided at the GBD India data portal for free use.

Funding: IIT Delhi, Institute Chair Fellowship; Research funding from the Central Pollution Control Board and the Ministry of Environment, Forest, and Climate Change, and SERB, Government of India through various projects; and DST-FIST for computing support; *Competing interests*: None stated.

REFERENCES

1. Dandona R, Anil Kumar G, Henry NJ, et al. Subnational mapping of under-5 and neonatal mortality trends in India: The Global Burden of Disease Study. 2000-2017. Lancet. 2020;395:1640-58.
2. Dandona L, Dandona R, Anil Kumar G, et al. Nations within a nation: Variations in epidemiological transition across the states of India, 1990-2016 in the Global Burden of Disease Study. Lancet. 2017;390:2437-60.
3. Burstein R, Henry NJ, Collison ML, et al. Mapping 123 million neonatal, infant, and child deaths between 2000 and 2017. Nature. 2019;574:353-74.
4. Balakrishnan K, Ghosh S, Thangavel G, et al. Exposure to fine particulate matter ($PM_{2.5}$) and birthweight in a rural-urban, mother-child cohort in Tamil Nadu, India. Environ Res. 2018;161:524-31.

5. Balakrishnan K, Dey S, Gupta T, et al. The impact of air pollution on deaths, disease burden, and life expectancy across the states of India: the Global Burden of Disease Study 2017. Lancet Plan Health. 2019;3:26-39.
6. deSouza PN, Dey S, Mwenda KM, et al. Robust relationship between ambient air pollution and infant mortality in India. Sci Tot Environ. 2022;815:152755.
7. Goyal N, Canning D. The association of in-utero exposure to ambient fine particulate air pollution with low birth weight in India. Environ Res Lett. 2021;16:054034.
8. Spears D, Dey S, Chowdhury S, et al. The association of early-life exposure to ambient $PM_{2.5}$ and later-childhood height-for-age in India: An observational study. Environ Health. 2019;18:62.
9. Mehta U, Dey S, Chowdhury S, et al. The association between ambient $PM_{2.5}$ exposure and anemia outcomes amongst children under five years of age in India. Environ Epidem. 2021;5:e125.
10. Reddy KRBK, Gupta N, Bhattacharya BG, et al. Impact of air pollution on allergic rhinitis and asthma: consensus statement by Indian Academy of Pediatrics, Indian Pediatr. 2021;58:765-70.
11. Salvi SS, Kumar A, Puri H, et al. Association between air pollution, body mass index, respiratory symptoms, and asthma among adolescent school children living in Delhi, India, Lung India. 2021;38:408-15.
12. Pandey A, Galvani AP. The burden of childhood pneumonia in India and prospects for control, Lancet Child Adol Health. 2020;4:643-45.
13. Saha P, Johny E, Dangi A, et al. Impact of maternal air pollution exposure on children's lung health: An Indian perspective. Toxics. 2018;6:68.
14. Odo D, Yang IA, Dey S, et al. Ambient air pollution and acute respiratory infection in children aged under 5 years living in 35 developing countries. Environ Int. 2022;159:107019.
15. Mondal D, Paul P. Effects of indoor pollution on acute respiratory infections among under-five children in India: Evidence from a nationally representative population-based study, PLoS One. 2020; 15;e0237611.
16. De S. Long-term ambient air pollution exposure and respiratory impedance in children: A cross-sectional study. Respir Med. 2020; 170:105795.
17. Ranzani OT, Mila C, Sanchez M, et al. Association between ambient and household air pollution with carotid-intima-media thickness in peri-urban South India: CHAI-Project. Int J Epidem. 2020;49:69-79.
18. Brabhukumr A, Malhi P, Khaiwal R, Lakshmi PVM. Exposure to household air pollution during first 3 years of life and IQ level among 6-8-year-old children in India – A cross-sectional study. Sci Tot Environ. 2020;709:135110.
19. Geetha B, Sukumar C, Dhivyadeepa E, et al. Autism in India: A case-control study to understand the association between socio-economic and environmental risk factors, Acta Neurol Belg. 2019;119:393-401.
20. Balakrishnan U, Tsaneva M. Air pollution and academic performance: Evidence from India. World Develop. 2021;146: 105553.
21. Mahapatra B, Walia M, Avis WR, Saggurti N. Effect of exposure to PM_{10} on child health: Evidence based on a large-scale survey from 184 cities in India. BMJ Global Health. 2020;5;e002597.
22. Ma Z, Dey S, Sundar C, et al. A review of statistical methods used for developing large-scale and long-term $PM_{2.5}$ models from satellite data. Rem Sens Environ. 2022;269:112827.
23. Liao J, Liu Y, Steenland K, et al. Child survival and early lifetime exposures to ambient fine particulate matter in India: A retrospective cohort study. Environ Health Pers. 2022;130: 17009.
24. Pande, P, Dey S, Chowdhury S, et al. Seasonal transition in PM_{10} exposure and associated all-cause mortality risks in India. Environ Sci Technol. 2022;52:8756-63.
25. Dey S, Purohit B, Balyan P, et al. A satellite-based high-resolution (1-km) ambient $PM_{2.5}$ database for India over two decades (2000-2019): Applications for air quality management. Rem Sens. 2020;12: 3872.
26. Chowdhury S, Dey S, Guttikunda S, et al. Indian annual ambient air quality standard is achievable by completely mitigating emissions from household sources, Proc Natl Acad Sci. 2019; 116:10711-16.
27. Lalchandani V, Kumar V, Tobler A, et al. Real-time characterization and source apportionment of fine particulate matter in the Delhi megacity area during late winter. Sci Total Environ. 2021;770:145324.
28. Puthussery JV, Singh A, Rai P, et al. Real-time measurements of $PM_{2.5}$ oxidative potential using a dithiothreitol assay in Delhi, India. Environ Sci Technol Lett. 2020;7:504-10.
29. Das A, Kumar, A, Habib, G, Vivekanandan, P. Insights on the biolo-gical role of ultrafine particles of size $PM_{<0.25}$: A prospective study from New Delhi. Environ Pollut. 2021;268: 115638.
30. Hajat A, Hsia C, O'Neill MS. Socioeconomic disparities and air pollution exposure: A global review. Curr Environ Health Rep. 2015;2:440-450.
31. Gani S, Pant P, Sarkar S, et al. Systematizing the approach to air quality measurement and analysis in low- and middle-income countries. Environ Res Lett. 2022;17:021004.
32. United States Environmental Protection Agency. The clean act and the economy. Accessed April 06, 2022. Available from: *https://www.epa.gov/clean-air-act-overview/clean-air-act-and-economy*

NEWS IN BRIEF

Guns: the leading cause of death in children in the US

In a Kafakaesque turn of events, firearm-related deaths rapidly spiked during the early COVID pandemic to become the the leading cause of death in children in the United States. There was a 33.5% rise in crude rates of firearm related homicides and 1.1% increase in firearm related suicides in 2019-2020 according to recent data released by the CDC. Prior to 2020, motor vehicle crashes were the predominant cause of death. The second dismaying data is the 83.6% rise in deaths due to drug overdose and poisoning in children making it the number four killer in 2019-2020. The other causes of death have not been much influenced. Even COVID-19 infections per se were a mere 0.2/ 100,000 children. The reasons need to be teased out, understood and addressed quickly. India needs to watch global trends if we want to protect our children.

(https://www.nejm.org/doi/full/10.1056/NEJMc2201761)

GOURI RAO PASSI
gouripassi@hotmail.com

RESEARCH PAPER

Pulmonary Dysfunction in Transfusion-Dependent Thalassemia and Response to Intensive Chelation Therapy

NEHA PANWAR,[1] SUNIL GOMBER,[1] POOJA DEWAN,[1] RAJ KUMAR[2]

From [1]Department of Pediatrics, University College of Medical Sciences; and [2]Vallabh Bhai Patel Chest Institute; Delhi.

Correspondence to: Dr Neha Panwar, Senior Resident, Department of Pediatrics, University College of Medical Sciences, Delhi 110 095. panwarneha64@gmail.com
Received: October 17, 2021; Initial review: November 25, 2021; Accepted: April 08, 2022.

Objectives: To evaluate pulmonary functions in children with transfusion-dependent thalassemia, and its reversal (lung dysfunction) using intensive intravenous chelation with desferrioxamine (DFO) (4 weeks). **Methods**: This descriptive study enrolled 77 children with transfusion-dependent thalassemia. Pulmonary function test (PFT) and iron load (serum ferritin (SF) & T2* MRI of heart and liver) were done. PFT included spirometry, total lung capacity (TLC) by helium dilution test and diffusion capacity by carbon monoxide (DLCO). Follow-up PFT was available for 13 children with moderate to severe lung dysfunction given intravenous DFO. **Results**: 50 (68.8%) patients had lung dysfunction, most commonly diffusional impairment (48; 96%), and reduced TLC (11; 22%); and none had obstructive pattern. 9 (81.8%) patients with restrictive defect had moderate to severely deranged DLCO. PFT and T2* MRI values were inversely correlated with serum ferritin. Among 13 patients receiving intensive chelation for 4 weeks, significant improvement was noticed in forced expiratory volume in one minute/ forced vital capacity ratio (ΔFEV1/FVC) (P=0.009), ΔDLCO (P=0.006) and ΔSF (P=0.01). **Conclusions**: Pulmonary dysfunction is common in children with multi-transfused thalassemia, and routine screening by PFT needs to be part of the management guidelines.

Keywords: *Desferrioxamine, Iron, Hemosiderosis, Pulmonary function tests.*

Published online: April 26, 2022; ***PII***: S097475591600422

Pulmonary dysfunction is being increasingly recognized as one of the sequelae affecting a large proportion of patients with transfusion-dependent thalassemia (TDT)[1-5]. The mechanisms of lung injury in TDT include complications of regular blood transfusions and chronic hypoxia, pulmonary hemosiderosis, pulmonary hyper-tension as well as associated cardiac dysfunction. Restrictive lung injury has been reported as a common pattern of lung dysfunction in TDT in a few previous studies [1,2], although obstructive [3] and diffusional impairment [4,5] have also been described as other patterns of lung injury.

Intensive chelation therapy (ICT) using intravenous desferrioxamine (DFO) is an effective treatment for reversal of cardiac dysfunction in TDT [6,7]. However, the role of the same in reversing lung damage has not been studied. This study was done to evaluate the pattern of pulmonary dysfunction in patients with transfusion-dependent thalassemia, and its reversal using ICT with DFO.

METHODS

This descriptive study was conducted after institutional ethics committee clearance at the thalassemia day care center of a public tertiary care hospital in Delhi, between November, 2017 and April, 2019. We included children older than 7 years with TDT, who were registered at our center and had been receiving regular blood transfusions and chelation therapy as per institutional criteria. We excluded patients with acute respiratory infections, pulmonary tuberculosis, HIV infection, congestive cardiac failure and history of smoking. Informed written consent was obtained from parents (in case of minor participants) or participants aged ≥18 years.

Invited Commentary: Page 445-6.

Taking the prevalence of restrictive lung dysfunction in patients with TDT as 70% [1,5] with 15% relative precision and 95% confidence level, a sample size of 74 was calculated.

A detailed history and physical examination were recorded for all participants. Details like age at which transfusions were started, number of blood transfusions received, annual blood requirement over the preceding one year (mL/kg), and details of chelation therapy were recorded. Mean pretransfusion hemoglobin over the preceding one year was computed from records. Chest radiograph was performed to rule out any infection and evidence of interstitial lung disease.

All participants underwent pulmonary function tests (PFTs) that included spirometry, total lung capacity (TLC) and diffusion capacity (DLCO) (Benchmark-PK Morgan device); best of three technically acceptable values was used. These tests were performed in the respiratory laboratory of Vallabh Bhai Patel Chest Institute, Delhi, by a single technician under the supervision of a respiratory specialist for all patients. Spirometry measured forced expiratory volume in first one second of expiration (FEV1) and forced vital capacity (FVC). Helium dilution technique and carbon monoxide diffusion technique were used to measure TLC and DLCO of patients, respectively. DLCO measurements were adjusted for the degree of anemia. PFT values were compared with predicted values (for age, sex and height) and expressed as percentage of predicted normal values. Reference standards for Indian children [8] were used for interpretation of predicted values for spirometry indices. For interpretation of predicted values of DLCO and TLC, standards given by American Thoracic Society [9] were used. PFT was reported as normal, restrictive, obstruc-tive, or diffusional impairment. Restrictive disease [10] was defined as reduction in TLC to less than 80% and diffusional impairment [10] as a reduction in DLCO to less than 80%. Obstructive pattern [9] was defined as decrease in ratio of FEV1 and forced vital capacity (FVC) to a value below 80%; grading of severity of obstructive lung dysfunction was done based on the percentage of FEV1 of the predicted normal value.

Iron load assessment of participants was done by serum ferritin levels (chemiluminescence immunoassay method), and cardiac and hepatic T_2*MRI. Cardiac and hepatic hemosiderosis were estimated using T_2*MRI (GE Signa HD XTh 1.5 Tesla Vol MR). Patients were categorized into four groups based on their T_2^*MRI results according to following cut-off points: hepatic hemosiderosis: normal >6.3 milliseconds (ms), mild 6.3-2.7 ms, moderate 2.7-1.4 ms and severe <1.4 ms; and cardiac hemosiderosis was classified as normal >20ms, mild 12-20 ms, moderate 8-12 ms and severe <8 ms [11].

Deranged lung function was described as follows: DLCO <40% of predicted – severe dysfunction, 40-59% - moderate dysfunction, and 60-79% mild dysfunction. Restrictive pattern was categorized as mild – 70-79%, moderate – 60-69% and severe <60% of predicted TLC. Obstructive pattern was categorized as mild – 70-100%. moderate – 60-69% and severe <60% of predicted FEV_1. Children with moderate to severe dysfunction were administered ICT (daily IV DFO, 40 mg/kg infused over 6 hours, with mid-infusion 2 mg/kg oral vitamin C) for four weeks duration, along with their usual chelation therapy. PFT and serum ferritin levels were repeated after four weeks of ICT to ascertain improvement.

Statistical analysis: Data were entered in Microsoft Excel file and analyzed using SPSS Version 20.0. Wilcoxon-signed rank test was used to evaluate improvement in PFT following intervention. We estimated Spearman rank correlation coefficient between PFT and iron status as measured by serum ferritin, T_2*MRI of heart and liver. *P* value <0.05 was considered as significant.

RESULTS

We enrolled 77 patients with TDT (73 β-thalassemia major, one heterozygous sickle cell-β thalassemia, and three heterozygous Hb Eβ-thalassemia) aged 7 years - 30 years (83% aged <18 year, 58.4% males). As per age-appropriate criteria (<18 years) [12], 61 (95%) had normal BMI, three had thinness, while none had severe thinness. Similarly, for age >18 years (*n*=13), 8 had normal BMI, two had mild thinness, and one had moderate thinness, as per standard criteria [15]. The mean (SD) pre-transfusion hemoglobin and the annual blood requirement was 8.6 (0.4) g/dL and 239.6 (32.9) mL/kg/year, respectively. All participants were receiving iron chelation therapy. Eight patients were receiving deferasirox (DFX) alone, and 18 deferiprone (DFP) alone; with 51 receiving combinations of chelating drugs (DFP+DFX, 29; DFO+DFP, 13; DFO+DFX, 8; DFO+DFP+DFX, 1). The median (IQR) SF of the study participants was 2735 (2735-4470.5) mg/dL.

The PFT and serum ferritin values were available for all 77 patients but results of T2*MRI of heart and liver were available for only 40. Pulmonary dysfunction was noticed in 50 (64.9%) participants. Diffusional impairment alone was detected in 39 patients, restrictive defect alone was seen in 11 patients, while nine patients had both. Diffusional impairment was severe in three patients and moderate in 18 patients. The patient with heterozygous sickle cell-β thalassemia had mild impairment of diffusional capacity. Amongst the participants with restrictive lung dysfunction, nine patients had mild decrease, and one each had moderate and severe reduction in TLC. None of the participants had obstructive defect. The mean (SD) FEV1/FVC, TLC and DLCO were 101 (6.4), 96.1 (13.0) and 73.1 (21.8), respectively. No child had FEV1/FVC <80% of predicted; whereas, 11 and 48 children had <80% predicted values for TLC and DLCO, respectively.

Chest radiographs were unremarkable in all patients, and echocardiography revealed impaired left ventricular ejection fraction (LVEF) in one child. None of the patients had evidence of pulmonary hypertension on echocardiography. Amongst 40 children in whom T2* MRI was done, hepatic iron overload alone was seen in 22 (55%) participants, and 16 (40%) of them had impaired PFT. Both cardiac and hepatic iron overload was seen in 11 participants and 8 of them had impaired PFT; seven

participants did not have evidence of cardiac or hepatic iron overload on T2* MRI but three of them had impaired PFT. There was inverse correlation present between all pulmonary function indices (P>0.05). Serum ferritin had low but significant correlation with cardiac iron overload (r = -0.33, P=0.04) but not with hepatic iron load (r =- 0.27, P=0.10) (**Table I**).

Of the 17 patients given ICT, post-ICT PFT results were available only for 13 (76.5%) patients (1 non-compliant, 3 refused for repeat PFT). The median (IQR) serum ferritin of these 13 patients decreased significantly after ICT [2634 (2318-4386) vs 2177 (1691.5-3330.5) mg/dL; P=0.016]. There was also a significant improve-ment in PFT after four weeks of ICT (**Web Table I**, **Fig. 1**).

Table I Correlation Between Pulmonary Function Tests, T2*MRI Values and Serum Ferritin in Children With Transfusion Dependent Thalassemia (N=40)

Parameter	*Hepatic T2* MRI values*	*Cardiac T_2* MRI values*	*Serum ferritin*
FEV1	0.13	0.05	-0.07
FVC	0.08	0.08	-0.07
FEV1/FVC	0.15	-0.11	-0.06
TLC	0.32	0.14	-0.04
DLCO	0.10	-0.14	-0.008

Values are in Pearson correlation coefficients (r). All [a]P>0.05. FEV1: forced expiratory volume in one second; FVC: forced vital capacity; FEV1/FVC: forced expiratory volume in one second to forced vital capacity; TLC: total lung capacity; DLCO: single breath diffusing capacity of the lung by carbon monoxide. [a]Spearman rank correlation coefficient used to estimate P value.

DISCUSSION

We found diffusional impairment to be the most common pattern of lung dysfunction in TDT patients. A much lower prevalence of the diffusional impairment (3%) and a higher prevalence of restrictive dysfunction (16%) was reported by Sohn, et al. [3]. These differences may have been due to differences in race, or because they adjusted the diffusion capacity values for alveolar volumes, which is a non-standard practice [10] and only preferred when there is large lung volume reduction due to pneumonectomy.

Restrictive lung disease in TDT as measured by decreased TLC has been reported in 35-79% of patients with thalassemia [1,2]. The lower incidence of restrictive lung disease in present study group could be due to adequate chelation of registered patients in our centre since an early age. The difference may also have been due to different parameters used to define restrictive lung pattern; while Boddu, et al. [1] used spirometry parameters to define restrictive lung dysfunction, we used TLC. A much higher incidence of restrictive dysfunction as reported by Arora, et al. [13] could be due to inclusion of diffusional impairment as part of restrictive defect, whereas most of the researchers attribute diffusional defect to be due to parenchymal damage rather than restrictive defect. Absence of obstructive defect in this study is similar to 3.2% having obstructive defect from another cohort from Delhi [5]. Obstructive lung dysfunction has been described in a few other studies [3] as the most common pattern depending on decrease in FEV 25%-75% values, which again, is not a recommended method for spirometry reporting [9].

We did not find a significant correlation between TLC and DLCO although majority of patients with restrictive defect also had diffusional impairment. This lack of statistical significance may be explained by a lesser number of patients with restrictive defect in our study group. We believe that most of our patients were detected early as pulmonary diffusion defect was seen in the majority while reduced lung volume was seen only in a few patients. Unless, intervened at early stage, it is possible that several of them might develop restrictive pattern in future.

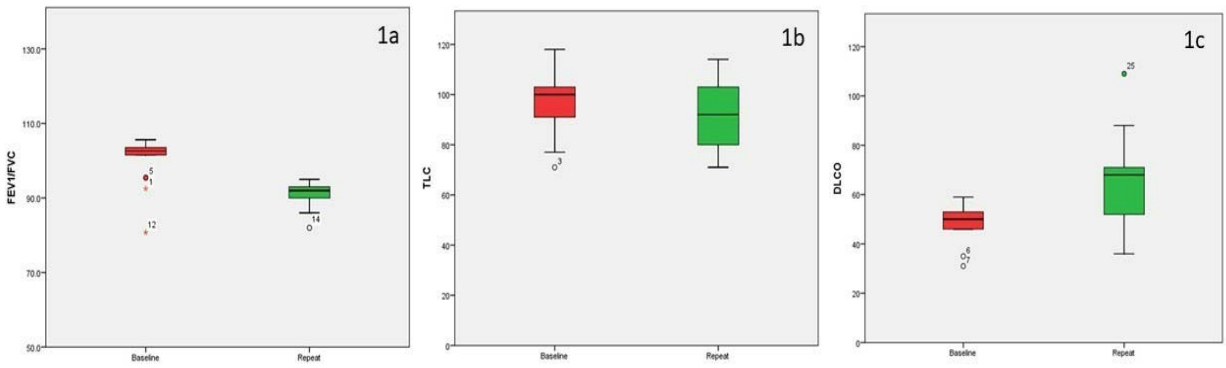


Fig. 1 Effect of intensive intravenous chelation therapy with desferrioxamine on lung function tests (n=13). *a*) Change in FEV1/FVC; *b*) Change in TLC; *c*) Change in DLCO.

WHAT THIS STUDY ADDS?

- Diffusional impairment was the most common pulmonary dysfunction seen in children with transfusion-dependent thalassemia.
- The pulmonary dysfunction did not correlate with cardiac or hepatic iron load as measured by T2*MRI.

The use of intravenous DFO has been shown to cause life-threatening pulmonary syndrome in patients with TDT receiving prolonged intravenous infusion (lasting more than 24 hour) [14]. A dose-dependent toxicity (>10 mg/kg/h) has also been suggested [14] as a possible mechanism. In this study, we infused DFO intravenously over 4 hours without any untoward effect in any patient. The number of patients treated with ICT was small and follow-up PFT were not available for almost a quarter of these, which suggests the need for confirmation with a larger sample.

Our results suggest early diffusional impairment in TDT and its reversibility by intensive chelation therapy in a few patients. We suggest considering annual screening for pulmonary dysfunction in patients with TDT.

Ethics clearance: Institutional Ethics Committee for Human Research, UCMS; No. IEC-HR/2017/32/97 dated Oct 17, 2017.
Contributors: SG: conceptualized the study; NP, PD,RK: were involved in data collection; RK: provided laboratory support; PD,NP: drafted the manuscript; SG,RK: provided critical input. All authors approved the final manuscript and are accountable for the manuscript.
Funding: None; *Competing interest*: None stated.

REFERENCES

1. Boddu A, Kumble A, Mahalingam S, et al. Pulmonary dysfunction in children with beta thalassemia major in relation with iron overload - a cross sectional hospital based study. Asian Journal of Medical Sciences. 2015; 6:47-50.
2. Guidotti F, Piatti G, Marcon A, et al. Pulmonary dysfunction in thalassaemia major: is there any relationship with body iron stores? Br J Haematol. 2017;176:309-14.
3. Sohn EY, Noetzli LJ, Gera A, et al. Pulmonary function in thalassaemia major and its correlation with body iron stores. Br J Haematol. 2011;155:102-105.
4. Li AM, Chan D, Li CK, et al. Respiratory function in patients with thalassaemia major: relation with iron overload. Arch Dis Child. 2002;87:328-30.
5. Parakh A, Dubey AP, Chowdhury V, et al. Study of pulmonary function tests in thalassemic children. J Pediatr Hematol Oncol. 2007;29:151-55.
6. Aldouri MA, Wonke B, Hoffbrand AV, et al. High incidence of cardiomyopathy in beta-thalassaemia patients receiving regular transfusion and iron chelation: reversal by intensified chelation. Acta Haematol. 1990;84:113-17.
7. Davis BA, Porter JB. Long-term outcome of continuous 24-hour deferoxamine infusion via indwelling intravenous catheters in high-risk beta-thalassemia. Blood. 2000;95: 1229-36.
8. Chhabra SK, Kumar R, Mittal V. Prediction equations for spirometry for children from northern India. Indian Pediatr. 2016;53:781-85.
9. Lung function testing: selection of reference values and interpretative strategies. American Thoracic Society. Am Rev Respir Dis. 1991;144:1202-18.
10. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. Eur Respir J. 2005;26: 948-68.
11. Valizadeh N, Alinejad V, Hejazi S, et al. Cardiac and hepatic T2*-weighted magnetic resonance imaging in transfusion dependent hemoglobinopathy in North West of Iran. Iran J Ped Hematol Oncol. 2015;5:206-10.
12. de Onis M, Onyango AW, Borghi E, et al. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ. 2007;85:660-67.
13. Arora M, Chandra J, Suri JC, et al. Pulmonary function tests in beta thalassemia. Indian J Pediatr. 2001;68:239-42.
14. Khaled A, Ezzat DA, Salem HA, et al. Effective method of evaluating myocardial iron concentration in pediatric patients with thalassemia major. J Blood Med. 2019;10: 227-33.
15. Weir CB, Jan A. BMI Classification Percentile and Cut Off Points. [Updated 2021 Jun 29]. In: StatPearls [Internet]. StatPearls Publishing; 2022.

Web Table I Effect of 4-Week Intensive Chelation Therapy (ICT) With Intravenous Desferrioxamine in Multi-transfused Children with Pulmonary Dysfunction (*N*=13)

Parameters	*Before ICT*	*After ICT*	*P value*
FEV1 (%)	78.3 (69.2-84.2)	78 (70.5-89.5)	0.07
FVC (%)	77.1 (67.1-81.7)	78 (69-85.5)	0.34
FEV1/FVC	102.6 (98.5-104)	92 (89-93.5)	0.009
TLC (%)	100 (88.5-103.5)	92 (78.5-108.5)	0.23
DLCO (%)	50 (46-53)	68 (50-76)	0.006

Values in median (IQR). [a]P<0.01. FEV1: forced expiratory volume in one second; FVC: forced vital capacity; FEV1/FVC: forced expiratory volume in one second to forced vital capacity; TLC: total lung capacity; DLCO: single breath diffusing capacity of the lung by carbon monoxide.

[a] Wilcoxan-signed rank test was used to estimate p value

RESEARCH PAPER

Pulmonary Function in Children With Transfusion-Dependent Thalassemia and Its Correlation With Iron Overload

ADITI BARUAH, JONALI BHATTACHARJEE
From Department of Paediatrics, Assam Medical College and Hospital, Dibrugarh, Assam.

Correspondence to: Dr Jonali Bhattacharjee, Registrar, Department of Paediatrics, Tezpur Medical College, Tumuki, Bihaguri, Tezpur, Sonitpur 784 010, Assam.
jonali.bhatta2804@gmail.com
Received: December 27, 2021;
Initial review: February 05, 2022;
Accepted: April 24, 2022.

Objective: To assess the pulmonary function of children with transfusion-dependent thalassemia, and to correlate its pattern with serum iron status. **Methods:** Cross-sectional study done in the pediatrics department of a tertiary care hospital from June, 2018 to May, 2019. 66 children aged 5-18 years with β-thalassemia and HbE/β-thalassemia, admitted for blood transfusion, and with a history of minimum 20 transfusions, were enrolled. Estimation of forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and FEV1/FVC ratio by spirometer, estimation of serum ferritin and CRP, and chest *X*-rays were done in all the participants. **Results**: 53 (80.3%) children had HbE/β-thalassemia, and 47 (71.2%) showed restrictive pulmonary dysfunction. The mean serum ferritin with impaired pulmonary function was 5616 (70.34) ng/mL and serum ferritin level had significant correlation with pulmonary function (P<0.001). **Conclusion**: Restrictive pattern of pulmonary dysfunction was common in children with thalassemia, and body iron status had a significant association with pulmonary impairment.

Keywords: HbE/β-thalassemia, Serum ferritin, Spirometry.

Published online: April 26, 2022; ***PII***: S097475591600421

Hemoglobin E (HbE)/β-thalassemia, a double heterozygote for HbE and β-thalassemia, is the genotype responsible for approximately one-half of all severe β-thalassemia worldwide [1]. Both in β-thalassemia and HbE/β-thalassemia, repeated transfusions lead to iron deposition in the pulmonary interstitium causing pulmonary hemosiderosis, resulting in slowly worsening pulmonary function [2]. Most studies have reported a restrictive pattern of pulmonary dysfunction [2-10]. Previous studies have shown a positive correlation between iron overload and pulmonary dysfunction, but some others have shown no such association [2-10].

The iron overload associated with chronic transfusions in patients with HbE/β-thalassemia is similar to that observed in patients with β-thalassemia [11], but not much information is available on pulmonary impairment due to iron overload in HbE/β-thalassemia. Studies done from India on this topic is scarce and there is no published data from the eastern part of the country, which has a high prevalence of hemoglobinopathies and thalassemia [12]. The present study was conducted with the objectives of assessing the presence, type and extent of pulmonary impairment in children with transfusion-dependent thalassemia and HbE/β-thalassemia, and to correlate it with the body iron status.

METHODS

This cross-sectional study was done in the pediatrics department of a tertiary care teaching hospital of North East India, from June, 2018 to May, 2019. Ethical clearance was taken from the institutional ethics committee before the start of the study.

Invited Commentary: Page 445-6.

Study subjects were children aged 5-18 year, diagnosed as β-thalassemia and HbE/β-thalassemia (by High performance liquid chromatography), who were on regular blood transfusions i.e., two-to-five weekly since the diagnosis of the disease. Inclusion criterion was children who had received a minimum of 20 blood transfusions. Children who had undergone bone marrow transplantation and those who could not perform spirometry were excluded. Children were enrolled in the study after taking informed consent from the parents, and assent from the children. In our hospital, deferasirox is started at the dose of 20-40 mg/kg in children under regular follow-up, once serum ferritin exceeds 1000 µg/mL; however, it is not provided free of charge at our institution.

After recording detailed history and clinical examination, investigations done were *i*) estimation of

serum ferritin, hemoglobin and C-reactive protein (CRP) level, *ii*) lung function tests and *iii*) chest *X*-ray. CRP was estimated to rule out the possibility of any underlying inflammation or infection which can also cause an increase in serum ferritin level and impairment in pulmonary function.

Outcome measures were serum ferritin level (to assess the body iron status) and lung function tests [forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and FEV1/FVC ratio] by a desktop spirometer, MIR Spirolab II (Medical International Research). Spirometry was repeated thrice and the best among three values was taken as final. Interpretation of spirometry findings was done by the authors in consultation with a pulmonologist.

A FEV1/FVC ratio <70%, where FEV1 is reduced more than FVC signifies an obstructive defect like chronic obstructive pulmonary disease and asthma [13]. A FEV1/FVC ratio >70% where FVC is reduced more than FEV1, is seen in restrictive defects such as interstitial lung diseases and chest wall deformities [13]. In restrictive disorders; however, FEV1/FVC ratio is normal or high (normal value is above 0.75-0.85, which is age dependent) and the total lung capacity (TLC) is less than 80% of predicted. According to American Thoracic Society [14] grading for the severity of restrictive disorders in the absence of TLC, mild is >70% of predicted value, moderate is 60-69 %, moderately severe is 50-59%, severe is 35-49 % and very severe is <35 % of predicted value.

Statistical analysis: Data were analyzed in MS Excel 2010. Pearson correlation coefficient was used to find out the correlation of pulmonary dysfunction with age, height, serum ferritin level and number of blood transfusions. *P* value < 0.05 was taken as significant.

RESULTS

Out of 76 children identified; 10 children were excluded (9 children could not perform spirometry and 1 underwent bone marrow transplantation) and 66 children (35 males, mean (SD) age 9.55 (2.81) year) were enrolled (**Table I**); 29 (43.9%) of children were diagnosed before the age of two year. The mean number of blood transfusions was 78.5 (43.1), with children in the 9-12 year age group having had maximum blood transfusions [mean (SD) 80.8 (39.6)].

Only ten children were taking oral iron chelators (regularly or irregularly). Mean (SD) serum ferritin level was 3017.65 (2020.88) ng/mL. As expected, the mean (SD) serum ferritin values were lower in children receiving iron chelators as compared to those not receiving iron chela-tors (*n*=56) [743.0 (122.2) vs 3423.8 (1927.5) (*P*<0.001)]. Majority of children with thalassemia had decreased FVC [mean (SD) 74.2% (12.6%)] and normal or comparatively higher FEV1 [mean (SD) 80.8% (12.5%)]. Thus FEV1/FVC was high in all the participants [mean (SD) 1.04 (0.08)], indicating a restrictive pattern of pulmonary dysfunction.

Table I Baseline Characteristics of Children With Thalassemia Enrolled in the Study (*N*=66)

Parameter	*No. (%)*
Age (y)	
5-8	25 (37.9)
9-12	32 (48.5)
13-18	9 (13.6)
HbE/β-thalassemia	53 (80.3)
Stunting	
Moderate	22 (33.3)
Severe	3 (4.5)
Wasting	
Moderate	3 (19.7)
Severe	6 (9.1)
Intake of iron chelator	10 (15.1)

Majority (40.91%) of children had mild restrictive pattern of pulmonary impairment. The mean serum ferritin in children with pulmonary impairment was significantly higher than those with normal pulmonary function (*P*<0.001) (**Table II**). There was a significant negative correlation between serum ferritin level and FVC (r=-0.89; *P*<0.001) (**Fig. 1**). There was a significant correlation of pulmonary function with number of blood transfusions (r=-0.61, *P*<0.001) but not with height (r=0.09, *P*=0.495376) or age (r=0.23, *P*=0.06). Chest *X*-rays were normal in all the participants.

DISCUSSION

This study to assess lung function in 66 transfusion-dependent children (80.3% with HbE/β-thalassemia) with thalassemia found restrictive pulmonary dysfunction in 47

Table II Severity of Pulmonary Impairment and Serum Ferritin Levels in Children With Thalassemia (*N*=66)

Pulmonary impairment	*No. (%)*	*Serum ferritin*
Normal	19 (28.8)	735.83 (118.1)
Mild	27 (40.9)	2719.19 (150.4)
Moderate	15 (22.7)	4943.73 (562.6)
Moderately severe	2 (3.0)	6795.00 (7.1)
Severe	3 (4.5)	8006.67(5.8)

Ferritin values in ng/mL. P<0.001.

WHAT THIS STUDY ADDS?

- Iron overload is associated with restrictive type of pulmonary impairment in children with β-thalassemia and HbE/β-thalassemia.

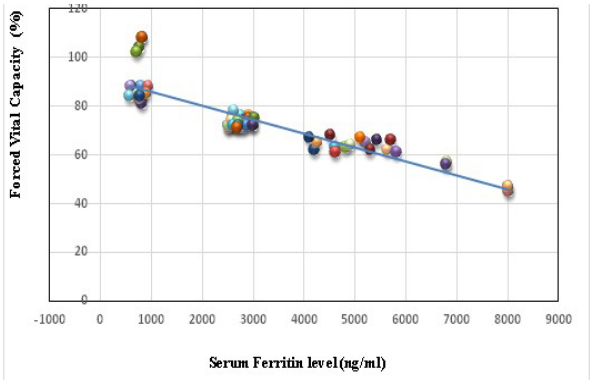


Fig. 1 Scatter plot depicting a negative correlation between serum ferritin level and forced vital capacity (r=-0.89; P<0.001).

(71.2%) children. Serum ferritin level had significant correlation with pulmonary function.

The proportion with HbE/β-thalassemia in this study was similar to a previous study from Assam [12]. In most studies done on pulmonary function in patients of thalassemia, majority of study participants were β-thalassemia major. Despite having a large number of Hb E-β thalassemia cases in this study, the pattern of pulmonary dysfunction did not significantly differ from previous studies [2-10] where most patients were β-thalassemia major, as Hb E-β thalassemia behaves similar to β-thalassemia, both phenotypically and in iron overloading pattern. [11]. Three Indian studies found serum ferritin levels similar to us among children with thalassemia [4,6,10], though a few others reported lower levels ranging from 1180-1594 ng/mL [3,5,7].

Previous studies have shown restrictive type of lung impairment in the range of 35-95% [2-10]. Two pediatric studies reported mild degree of impairment in 23.8% [3] and 42% [7] of children, similar to our study (40.9%). But another study reported severe restrictive type in 14 (73.5%) children [10]. Correlation between pulmonary dysfunction and iron overload was stated by some authors [2-5]. According to one study, fibrosis and interstitial edema, which are due to iron overload, cause lung dysfunction [3]. We found a significant negative correlation between serum ferritin level and pulmonary impairment. On the other hand, few have found no association between iron overload and severity of pulmonary dysfunction [6]. We found significant correlation with number of blood transfusions but not with age, similar to a previous report [2]. However, a few studies have reported correlation with age, height and duration of chelation [6,10].

Our study had the limitations of non-availability of TLC measurement, small numbers, and only 15.1% children receiving chelation therapy.

In conclusion, we have found restrictive pattern of pulmonary dysfunction of varying severity in children with thalassemia, including HbE/β-thalassemia, with significant association between severity of pulmonary impairment and body iron stores. Pulmonary function tests should be done regularly like other organ function monitoring as deranged lung function shows very few or no symptoms.

Ethics clearance: Institutional Ethics Committee, Assam Medical College; No. ECR/636/Inst/AS/2014 dated Sep 24, 2019.
Contributors: AB: conceptualized the study, analyzed data, reviewed literature, revised the manuscript and critically reviewed; JB: conceptualized the study, collected data, searched literature, analyzed data, drafted the manuscript. Both the authors approved the final manuscript, and are accountable for all aspects related to the study.
Funding: None; *Competing interest*: None stated.

REFERENCES

1. Olivieri NF, Pakbaz Z, Vichinsky E. Hb E/β-thalassemia: a common and clinically diverse disorder. Indian J Med Res. 2011;134:522-31.
2. Boddu A, Kumble A, Mahalingam S, et al. Pulmonary dysfunction in children with β thalassemia major in relation with iron overload - a cross sectional hospital based study. Asian J Med Sci. 2015;6:47-50.
3. Rahim F, Keikhaei B, Ebadi A. Clinical manifestation of pulmonary dysfunction in β-thalassemic major. J Med Sci. 2008;8:484-90.
4. Kandhari A, Sachdeva A, Jain N, et al. Pulmonary dysfunction in β-thalassemia. Blood. 2005;106:3851.
5. Guidotti F, Piatti G, Marcon A, et al. Pulmonary dysfunction in thalassemia major: is there any relationship with body iron stores? Br J Haematol. 2017;176:309-14.
6. Harsoor J, Ratageri VH, Shilpa C, et al. Pulmonary function tests in children with β-thalassemia major. Karnataka Paediatr J. 2020;35:52-6.
7. Ahmed HM, Meabed MH, Ibraheem HK, Elsherif HM. The effect of iron overload on pulmonary function tests in

children with thalassemia major. Menoufia Med J. 2021; 34:1009-13.
8. Parakh A, Dubey AP, Chowdhury V, et al. Study of pulmonary function tests in thalassemic children. J Pediatr Hematol Oncol. 2007;29:151-5.
9. Shivaswamy K, Chikkanna S, Singh J, Gubbari K TN. Study of pulmonary function tests in multitransfused children with β-thalassemia major: an observational study. Int J Contemp Pediatr. 2020;7:2307-11.
10. Gadiparthi M, Bhaskaranand N, Kini PG, et al. Pulmonary function tests in β-thalassemia major and its correlation with serum ferritin levels. Int J Contemp Pediatr. 2019;6:306-9.
11. Olivieri NF, De Silva S, Premawardena A, et al. Iron overload and iron-chelating therapy in hemoglobin E-β thalassemia. J Pediatr Hematol Oncol. 2000; 22:593-7.
12. Baruah MK, Saikia M, Baruah A. Pattern of hemoglobinopathies and thalassemias in upper Assam region of North Eastern India: high performance liquid chromatography studies in 9000 patients. Indian J Pathol Microbiol. 2014; 57:236-43.
13. Ranu H, Wilde M, Madden B. Pulmonary function tests. Ulster Med J. 2011; 80:84-90.
14. Brusasco V, Crapo R, Viegi G. Series" ATS/ERS task force: Standardisation of lung function testing"-Editorial: Coming together: The ATS/ERS consensus on clinical pulmonary function testing. Revue des Maladies Respiratoires. 2006; 23:17S7-10.
15. Alyasin S, Moghtaderi M, Amin R, Kashef S, Karimi M. Pulmonary function test in transfusion-dependent β-thalassemia major patients: A pilot study. Pediatr Hematol Oncol. 2011;28:329-33.

CLIPPINGS

OnasemnogeneAbeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy in patients with two copies of *SMN2* (STR1VE) (Lancet Neurol. 2021;20:284-93)

Spinal muscular atrophy (SMA) is caused due to biallelic variations in SMN1 gene. The results of an open-labelled single-arm multicentric phase 3 trial (STR1VE) to evaluate the safety and efficacy of OnasemnogeneAbeparvovec (marketed as Zolgensma), for treatment of infantile SMA, were published recently. This drug was approved by the Food and Drug Administration (FDA) in 2019. Twenty-two patients fulfilled the inclusion criteria for the study. All of them were younger than 6 months and had biallelic variants in SMN1 and one or two copies of SMN2. All of them received a single dose of OnasemnogeneAbeparvovec ($1{\cdot}1\times10^{14}$ vector genomes per kg) as intravenous infusion. The patients were monitored regularly till 18 months of age. The ability to sit independently for 30 seconds and survival at 14 months of age were the outcomes assessed. The results were compared with a natural cohort of untreated children with SMA from Pediatric Neuro-muscular Clinical Research (PNCR) dataset. Thirteen out of twenty-two patients showed a statistically significant improve-ment in sitting independently for 30 seconds or more when compared to the untreated group. In the treatment group, survival at 14 months without ventilation was significant when compared to the untreated group. Respiratory infection, elevated hepatic aminotransferases and hydrocephalus were the serious adverse events that were noted. The study concluded that gene therapy for SMA showed a clinically significant response and the safety profile supported the clinical use of gene therapy for treatment of SMA.

CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia (N Engl J Med. 2021;384:252-60)

Beta thalassemia and sickle cell anemia are common Mendelian disorders caused by biallelic variants in HBB gene. The available treatment options for these disorders include regular blood transfusion, hematopoietic stem cell transplantation, hydroxyurea and other supportive measures. BCL11A, encoded by BCL11A gene, is a transcription factor that represses the production of gamma globin chains, which constitutes the fetal hemoglobin. Some single nucleotide variations in BCL11A gene are known to cause down regulation of BCL11A and thus an increase in gamma chain and fetal hemoglobin level. The clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 nuclease system helps in creating specific alterations in specific sites in DNA. In this study, patient 1 was a 19-year old female with transfusion dependent beta thalassemia. Patient 2 was a 33 year old female with sickle cell disease. Both these patients underwent autologous hematopoetic stem cell transplantation with CRISPR-Cas9- edited CD34+ hematopeotic stem cells. These cells were edited by using a single guide RNA which directed the CRISPR-cas9 to silence the enhancer region of BCL11A and thus cause an increase in gamma chain production and fetal hemoglobin. The fetal hemoglobin levels showed an early and sustained increase in both the patients during a 12-month follow up period. The authors concluded that CRISPR-cas9-edited hematopeotic stem cells underwent good engrafment and resulted in a phenotype mimicking heriditary persistent fetal hemoglobin.

DHANYA LAKSHMI N
dhanya.lakshmi@manipal.edu

RESEARCH PAPER

Outcomes of Very Preterm Neonates Born by Assisted Reproductive Techniques (ART): A Propensity Score Matched Retrospective Cohort Study

VENKATESHWARLU VARDHELLI,[1] RAJENDRA PRASAD ANNE,[1] SRINIVAS MURKI,[2] GOPIREDDY MURALI MOHAN REDDY,[3] SAIKIRAN DESHABHOTLA,[1] TEJO PRATAP OLETI[1]

From [1]Department of Neonatology, Fernandez Foundation, Hyderabad, Telangana; [2]Department of Neonatology, Paramitha Children's Hospital, Hyderabad, Telangana; [3]Department of Epidemiology, Evidencian Research Associates, Bengaluru, Karnataka.

Correspondence to: Dr Rajendra Prasad Anne, Consultant Neonatologist, NICU, Unit 2, Fernandez Foundation, Opposite Old MLA Quarters, Hyderguda, Hyderabad, Telangana, 500 029.
rajendra.omc@gmail.com
Received: October 08, 2021;
Initial review: December 09,2021;
Accepted: April 16, 2022.

Objective: To compare outcomes of preterm neonates born through assisted reproduction techniques (ART) and non-ART conception. **Methods:** This retrospective cohort study included very preterm neonates (26 weeks to 31 weeks) admitted to our neonatal unit over a six year period from 2014 to 2019. The primary outcome was composite adverse outcome of mortality or any of the major morbidities i.e., intraventricular hemorrhage (IVH) grade ≤3, periventricular leukomalacia (PVL) grade ≤2, bronchopulmonary dysplasia (BPD) at 36 weeks, and retinopathy of prematurity (ROP) requiring treatment. **Results:** Total of 759 neonates (253 in ART group, 506 in non-ART group) were included after propensity score matching for gestational age, sex, and small for gestational age (SGA). Neonates in ART group had similar rates of composite adverse outcome [aOR (95% CI) 0.86 (0.55 – 1.36)], mortality [0.93, (0.53-1.64)] BPD [1.18, (0.37 – 3.76)]; ROP requiring treatment [0.49 (0.14-1.71], and other morbidities. **Conclusion:** Very preterm neonates born through ART were not at increased risk of adverse neonatal outcomes.

Key words: *Chromosomal microarray analysis, Etiology, Exome sequencing.*

Published online: April 26, 2022; ***PII***: S097475591600419

In pregnancies resulting from assisted reproductive techniques (ART), the risk of spontaneous preterm birth, low birthweight [1], obstetric and perinatal complications were higher [2]. World Health Organization (WHO) definition of ART includes all treatments or procedures of invitro handling of both human oocytes and sperm, or of embryos for the purpose of establishing a pregnancy [3]. This definition by WHO does not include artificial insemination. Along with the maternal age, invitro manipulation, epigenetic disorders including abnormal methylation, cryopreservation of gamete or embryo, and invitro culture environment may influence the outcomes of ART [4], which may potentially contribute to adverse outcomes. It is important to understand this pathophysiologic plausibility for the inclusion of neonates into ART group while investigating their outcomes. Studies on the outcomes of very preterm neonates born through ART are limited and have conflicting results with no data from low- and middle-income countries [5-14]. Most of the previous studies didn't adhere to WHO definition and included major congenital anomalies, which might independently affect the results. In this study, we planned to evaluate the outcomes of very preterm neonates born by ART, defined as per the WHO definition.

METHODS

This retrospective cohort study was conducted in level III NICU of a tertiary care neonatal unit in South India after the approval by the Institute's ethics committee. Data of all consecutively born very preterm neonates (26 weeks, 0 days to 31 weeks, 6 days) over a 6-year period (January, 2014 to December, 2019) was retrieved from the electronic medical records of the unit. Neonates with major congenital anomalies, those transferred early to other centers, and those who did not receive active treatment were excluded. Neonates born before 26 weeks and below 600 g birthweight were excluded, as they were not routinely treated in our unit.

As per the WHO definition, infants born through invitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) methods were included in the ART group [4]. Neonates born through ovulation induction (OI) and intrauterine insemination (IUI) were included in non-ART group. Gestational age was estimated based on the fertilization date (oocyte retrieval+14 days) and first trimester ultrasound, and was confirmed by new Ballard score postnatally. Small for gestational age (SGA) and severe SGA were defined as birthweight less than 10th centile and

3rd centile for gestational age, respectively as per Fenton 2013 growth charts. Bronchopulmonary dysplasia (BPD) was defined as requirement of any respiratory support at 36 weeks post-menstrual age (PMA). Intra-ventricular hemorrhage (IVH), periventricular leukomala-cia (PVL) and retinopathy of prematurity (ROP) treat-ment were followed as per Volpe classification, DeVries classification and ETROP recommendations, respectively. Extrauterine growth restriction (EUGR) was defined as discharge weight <10th centile for discharge PMA and sex on Fenton growth charts. The primary outcome was to compare the composite adverse outcome (defined as in-hospital mortality or one of the major morbidities i.e., IVH grade 3 or more, PVL grade 2 or more, BPD, and ROP requiring treatment) between very preterm neonates born through ART and non-ART conception.

Statistical analysis: The incidence of adverse composite outcome in the very preterm group from the previous hospital data of 5 years was 20% [15]. Assuming that the exposed group (ART) might have 10% higher incidence in the adverse composite outcome, with the ratio of exposed to unexposed group of 1:2, 80% power and 5% two-sided alpha error, the required minimum sample size was 231 subjects in the exposed group and 461 subjects in unexposed group. Propensity matching was done by matching for gestational age, sex and SGA by using nearest neighbour matching (NNM) method without replacement, at a 1:2 ratio. Univariable and multivariable binary logistic regression and linear regression adjusting for maternal age, gestational diabetes and gestation type (multifetal pregnancy) was performed to assess the association of ART and non-ART groups with various binary and numeric outcomes, respectively. IBM SPSS version 22.0 was used for statistical analyses.

RESULTS

Among the total of 1371 neonates born during the study period, twenty five (1.8%) neonates had major congenital anomalies and 279 (20.3%) neonates were either transferred to another hospital or did not undergo complete treatment at study hospital or had incomplete data, leaving 1067 neonates for analysis. The distribution of excluded neonates was similar in ART (64/317, 20%) and non-ART groups (230/1,057, 22%). The mean (SD) gestational age and mean birthweight of excluded infants were 28.6 (1.6) weeks and 1034 (250) g, respectively. The reasons for transfer to other hospitals before completion of treatment were financial (availability of insurance at other hospitals in 69.7%) and unsure outcomes due to extreme prematurity (30.3%).

After the propensity score matching, a total of 759 neonates (253 in ART group and 506 in non-ART group) were included in the final analysis. The mean (SD) gestational age of the study population was 29.1 (1.5) weeks and mean (SD) birthweight was 1172 (285) g. The overall incidence of in-hospital mortality and the composite adverse outcome were 11.7% (n=89) and 22.9% (n=174), respectively. After propensity score matching, the groups were comparable in neonatal baseline characteristics but the maternal characteristics of maternal age, primiparity, multifetal gestation and gestational diabetes, were higher in the ART group (**Table I**).

Neonates in ART group had similar rates of composite adverse outcome, mortality, morbidities and discharge characteristics compared to neonates conceived by non-ART conception (**Tables II**). After multivariable logistic regression analysis adjusted for maternal age, type of gestation (multifetal pregnancy) and gestational diabetes, the major outcomes remained similar (**Table II**). On multivariate analysis, only the incidence of hemodynamically significant patent ductus arteriosus (HsPDA) requiring treatment was higher in non-ART group neonates (**Table III**).

Table I Characteristics of Neonates Born By ART and Non-ART

Characteristic	*ART (n=253)*	*Non-ART (n=506)*	*P value*
Maternal age (y)[a]	32.73 (4.43)	28.86 (4.15)	<0.001
Primigravida	155 (61.3)	242 (47.8)	<0.001
Primipara	232 (91.7)	328 (64.8)	<0.001
Multifetal gestational	186 (73.5)	128 (25.3)	<0.001
PIH	112 (44.3)	203 (40.1)	0.27
Gestational diabetes	51 (20.2)	62 (12.3)	0.004
Gestational age (wk)[a]	29.1 (1.66)	29.2 (1.53)	0.27
Birthweight (g)[a]	1175.81 (310.3)	1170.85 (271.8)	0.82
Male sex	131 (51.8)	249 (49.2)	0.5
SGA	35 (13.8)	64 (12.7)	0.65
Severe SGA	2 (0.8)	7 (1.4)	0.73
Doppler abnormalities	38 (15)	105 (20.8)	0.06
No antenatal steroids	10 (4)	21 (4.2)	0.9
Chorioamnionitis	21 (8.3)	42 (8.3)	1.0
PPROM	84 (33.2)	157 (31)	0.54
Cesarean delivery	216 (85.4)	413 (81.6)	0.2
APGAR <7 at 5 min	28 (11.1%)	55 (10.9)	0.93
Resuscitation at birth	66 (26.1%)	138 (27.3)	0.73

All values in no. (%) or [a]mean (SD). ART: artificial reproductive techniques; PIH: pregnancy induced hypertension; SGA: small for gestational age; PPROM: preterm premature rupture of membranes.

Table II Mortality and Major Morbidities in Infants Born Through ART and Non-ART

Outcome	*ART (n=253)*	*Non-ART (n =506)*	*aOR (95% CI)*	*P value*
BPD (respiratory support at 36 wk PMA)	8 (3.2)	12 (2.4)	1.18 (0.37-3.76)	0.78
IVH grade ≥3	16 (6.3)	22 (4.3)	1.5 (0.62-3.6)	0.36
PVL grade ≥2	8 (3.2)	11 (2.2)	1.7 (0.52-5.57)	0.38
ROP requiring treatment	4 (1.6)	18 (3.6)	0.49 (0.14-1.71)	0.26
Mortality	35 (13.8)	54 (10.7)	0.93 (0.53-1.64)	0.8
Composite adverse outcome (any of the above 5)	56 (22.1)	118 (23.3)	0.86 (0.55-1.36)	0.53

All values in no. (%). aOR=adjusted odds ratio, adjusted for maternal age, gestation type, gestation diabetes. ART: artificial reproductive techniques; BPD: bronchopulmonary dysplasia; IVH: intraventricular hemorrhage; PVL: periventricular leukomalacia; ROP: retinopathy of prematurity.

DISCUSSION

In our study, very preterm neonates born through ART conception had no increase in mortality, major morbidities and composite adverse outcome as compared to those born through non-ART conception, after propensity score matching. The higher incidence of HsPDA requiring treatment on multivariate analysis in neonates born through non-ART did not result in increased duration of respiratory support and need for surgical ligation.

A large study by Heo, et al. [11] from Korean Neo-natal Network (KNN) registry investigated the outcomes of very low birth weight (VLBW) neonates born after IVF conception concluded that VLBW neonates born through IVF conception had comparable or better outcomes. These differences were attributed to likely better access to high quality health care for women who underwent IVF conception as they belonged to high socioeconomic status and probable differences in chorionicity. The study included neonates with congenital anomalies and did not adjust the analysis for the variability of participating centers. Picaud, et al. [12] from France reported higher survival without severe morbidity and less BPD in very preterm neonates born through ART which were described by the differences in pregnancy care (close monitoring antenatally) and neonatal characteristics (more inborn, better mature and more birthweight in ART group). This study included the neonates born through OI conception (where oocyte handling is not done) into ART group.

In a very large multicentric propensity matched study on neonates less than 34 weeks by Ahmed, et al. [13], BPD

Table III Outcomes in Infants Born Through ART and Non-ART

Outcome	*ART (n=253)*	*Non-ART (n =506)*	*aOR (95% CI)*	*P value*
RDS	171 (67.6)	349 (69)	0.72 (0.48-1.07)	0.14
Surfactant	161 (63.6)	330 (65)	1.41 (0.95-2.1)	0.09
Duration of respiratory support (total)[b]	6 (2-15)	7 (3-14)	0.99 (0.99-1.01)	0.92
Duration of respiratory support (invasive)[b]	0 (0-2)	0 (0-2)	0.98 (0.94-1.03)	0.46
More than 28 d of oxygen requirement	43 (17)	79 (15.6)	0.88 (0.51-1.51)	0.65
Hs-PDA requiring treatment	66 (26.1)	132 (26.1)	0.64 (0.41-0.99)	0.046
PDA-surgical ligation	5 (2)	4 (0.8)	2.5 (0.45-13.9)	0.3
NEC stage 2A or more	17 (6.7)	43 (8.5)	0.57 (0.27-1.2)	0.14
Culture positive sepsis	62 (24.5)	138 (27.3)	0.65 (0.42-1.02)	0.06
Any Grade IVH	58 (22.9)	146 (28.9)	0.81 (0.52-1.25)	0.33
Any ROP	22 (8.7%)	61 (12.1)	0.63 (0.33-1.21)	0.16
Discharge PMA (wk)[b]	34 (32-35)	34 (32-35)	1.02 (0.97-1.08)	0.52
Discharge weight (g)[b]	1520 (1420-1660)	1480 (1420-1620)	1 (1-1.001)	0.39
EUGR	217 (70)	328 (72.7)	0.93(0.65-1.37)	0.69

All values are in no. (%) or [b]median (IQR). aOR=adjusted odds ratio, adjusted for maternal age, gestation type, gestational diabetes; ART: artificial reproductive techniques; RDS: respiratory distress syndrome; HsPDA: hemodynamically significant patent ductus arteriosus; NEC: necrotizing enterocolitis; IVH: intraventricular hemorrhage; ROP: retinopathy of prematurity; PMA: post menstrual age; EUGR: extrauterine growth restriction.

WHAT THIS STUDY ADDS?

- Very preterm neonates born through assisted reproduction techniques (ART) are not at increased risk of short term adverse neonatal outcomes.

and exposure to chronic respiratory medications were higher in neonates born through IVF conception, which was possibly postulated to epigenetic differences. The inclusion of neonates born by ICSI and IUI conception was not mentioned. Compared to this study, oxygen requirement for >28 days, duration of respiratory support and BPD were similar in both the groups in our study. A metanalysis by Gao, et al. [14] on the effect of ART on ROP showed that the use of IVF was associated with higher risk of ROP occurrence. Our study, with a relatively smaller sample size, did not show any increase in incidence of ROP and severe ROP.

Other studies have shown no differences in outcomes of preterm neonates born through ART but these had differences in the inclusion criteria used [5-9], with some studies including neonates born by IUI and OI conception in ART group [8], and other studies excluding OI conception from the analysis [5,6,9]; whereas, one of those included neonates with congenital anomalies [5]. Including congenital anomalies might affect the reliability of outcomes as these can increase the adverse outcomes of preterm neonates due to their increased need for interventions.

The key strengths of the study were using WHO-ART definition, minimizing the potential confounding by key parameters using propensity score matching, and restriction of the study to infants without major anomalies. Residual confounding was also addressed by presenting adjusted estimates during analysis. Major limitation was exclusion of nearly 20% of eligible infants but this exclusion was not different between the two study groups, so the amount selection bias it could have introduced was expected to be minimal. Being a single center data may be a limitation but conversely, single center data may also decrease the variability of the practices. Also, we need more long-term follow-up studies on this important issue.

Very preterm neonates born after ART did not have increased risk of short-term neonatal outcomes compared to neonates born without ART.

REFERENCES

1. Cavoretto P, Candiani M, Giorgione V, et al. Risk of spontaneous preterm birth in singleton pregnancies conceived after IVF/ICSI treatment: Meta analysis of cohort studies. Ultrasound Obstet Gynecol. 2018;51:43-53.
2. Pandey S, Shetty A, Hamilton M, et al. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: A systematic review and meta-analysis. Hum Reprod Update. 2012;18:485-503.
3. Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. The international committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary on ART termino-logy, 2009. Hum Reprod. 2009;24:2683-7.
4. Tarin JJ, García Pérez MA, Cano A. Assisted reproductive technology results: Why are live birth percentages so low? Mol Reprod Dev. 2014;81:568-83.
5. Al-Hathlol K. Relationship between in vitro fertilization and neonatal outcomes in very low birth weight preterm infants. Am J Perinatol. 2018;35:1113-8.
6. Schimmel MS, Hammerman C, Lusky A, Reichman B. Very low-birth-weight-infants conceived by in vitro fertilization are not at higher risk for mortality and morbidity: a population-based study. Fertil Steril. 2006;85:907-12.
7. Chiarelli L, Mirea L, Yang J, Lee SK, Shah PS, Canadian Neonatal Network. Neonatal outcomes in very preterm singleton infants conceived using assisted reproductive technologies. Am J Perinatol. 2015;32:515-22.
8. Corchia C, Da Frè M, Di Lallo D, et al. Mortality and major morbidities in very preterm infants born from assisted conception or naturally conceived: results of the area-based ACTION study. BMC Pregnancy Childbirth. 2014;14:307.
9. Turker G, Doger E, Arýsoy AE, et al. The effect of IVF pregnancies on mortality and morbidity in tertiary unit. Ital J Pediatr. 2013;39:17.
10. Messerschmidt A, Olischar M, Birnbacher R, et al. Perinatal outcome of preterm infants <1500g after IVF pregnancies compared with natural conception. Arch Dis Child Fetal Neonatal Ed. 2010;95:F225-9.
11. Heo JS, Lee HJ, Lee MH, Choi CW. Comparison of neonatal outcomes of very low birth weight infants by mode of conception: in vitro fertilization versus natural preg-nancy. Fertil Steril. 2019;111:962-70.
12. Picaud JC, Chalies S, Combes C, et al. Neonatal mortality and morbidity in preterm infants born from assisted reproductive technologies. Acta Paediatr. 2012;101:846-51.
13. Ahmad KA, Bennett MM, Rayburn P, et al. Outcomes of preterm infants conceived with in vitro fertilization. J Perinatol. 2019;39:717-22.
14. Gao L, Shao W, Li N, et al. The risk of retinopathy of prematurity in the infants following assisted reproductive technology: A meta-analysis. Biomed Res Int. 2019; 2095730.
15. Anne RP, Vardhelli V, Oleti TP, et al. Propensity-matched comparison of very preterm small-and appropriate-for-gestational-age neonates. Indian J Pediatr. 2022;89:59-66.

RESEARCH PAPER

Clinical and Genetic Profile of Children With Short Stature Presenting to a Genetic Clinic in Northern India

KANIKA SINGH,[1] RATNA DUA PURI,[1] SUNITA BIJARNIA-MAHAY,[1] MEENA LALL,[2] JYOTSNA VERMA,[3] RENU SAXENA,[4] SUDHA KOHLI,[4] DIVYA THOMAS,[3] PUSHPA SAVIOUR,[2] IC VERMA[1]

From Institute of [1]Medical Genetics and Genomics, [2]Cytogenetics, [3]Biochemical Genetics, [4]Molecular Genetics, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi.

Correspondence to:
Dr Ratna Dua Puri, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi 110 060.
ratnadpuri@yahoo.com
Received: November 21,2021;
Initial review: December 29,2021;
Accepted: April 26,2022.

Objective: To define the spectrum of genetic disorders in patients with short stature visiting the genetic out-patient department in a tertiary care hospital. **Methods:** A chart review was done for 455 individuals (10 months-16 yrs) with short stature, who were evaluated at the genetic clinic from 1 January, 2017 upto 31 October, 2018. 226 patients who needed detailed evaluation, the spectrum of genetic diagnosis is presented. **Results**: Proportionate short stature was identified in 63% individuals (*n*=142) of which 93 (65%) were recognizable syndromes such as Turner syndrome, and William syndrome, and RASopathies. In clinically undefined syndromes (39, 27%), a diagnosis could be made by karyotype (*n*=3/10), chromosomal microarray (6/12) and exome sequencing (1/6). In the 84 children in the disproportionate short stature group (37%), lysosomal storage disorders (LSDs) (45%, *n*=38) were identified by enzyme analysis in 86.8% and skeletal dysplasias (44%, *n*=37) identified by skeletal survey in 89% cases. **Conclusions:** In undefined syndromic short stature, chromosomal microarray may be the first investigation of choice if phenotyping is not suggestive of a specific genetic syndrome. Exome sequencing can be useful in identifying newer genes among idiopathic and familial short stature cohorts.

Key words: *Chromosomal microarray analysis, Etiology, Exome sequencing.*

Linear growth is a complex polygenic trait due to the combined effect of many different genes as well as influenced by several environmental factors prenatally and postnatally [1]. However, single genes contribute to the etiology in many patients with significant short stature. The guidelines for genetic testing in short stature have been recently updated by the American College of Medical Genetics (ACMG) [2], along with the likely yield of different genetic tests. Till recently, genetic testing in short stature was limited to testing for Achondroplasia, Turner syndrome and Russell Silver syndrome. The availability of exome sequencing has allowed many new genes like *ACAN, NPR2, FBN1, IHH* and *BMP2* amongst others to be identified and many individuals with milder phenotypes of known monogenic disorders like RASopathies [3] are being diagnosed. We analysed the spectrum of genetic diagnosis in patients with short stature presenting to a tertiary care hospital in northern India.

METHODS

This is a chart review of patients with height less than 3rd centile referred for genetic evaluation of short stature from 1st January, 2017 through 31st October, 2018 (22 months) to the genetic clinic. In addition to short stature, many patients also had additional phenotypes such as developmental delay, cardiac defects, hepatosplenomegaly or bony deformities. Clinical data and investigations including genetic tests were tabulated on a structured, predefined format and analyzed. The anthropometric details were measured as per guidelines [4,5], and data was collated. Two cohorts were defined - patients with proportionate short stature and those with disproportionate short stature on the basis of upper segment and lower segment ratios (US:LS) [6] and were then assessed for etiology. Where standing height could not be taken, length was measured. Patients with incomplete/missing data were excluded from the study.

All patients with disproportionate short stature were clinically evaluated and skeletal survey (antero-posterior views of long bones, hands and feet, pelvis with hip joint, and lateral spine radiographs) was done. In some patients, additionally, a skull radiograph was also taken. Patients with coarse facial features with or without hepatosplenomegaly and dysostosis multiplex were evaluated for lysosomal storage disorders using urine glycosaminoglycans/oligosaccharides and enzyme assay in blood. For those suspected to have achondroplasia or

hypochondroplasia, targeted *FGFR3* gene study was done. In the others, exome sequencing was carried out to evaluate genes of the different skeletal dysplasias based on the clinical and radiographic differential diagnosis [7].

Patients with proportionate short stature were assessed for dysmorphism and associated abnormalities of eye/ear/skin/hair, which could help in assigning the syndrome. Assessment for developmental delay was done by evaluating age dependent skills attained in the gross motor/fine motor, cognitive, language and personal/ social domains. Echocardiography was performed as necessitated on clinical evaluation. Syndrome search was done as per standard practice [8-10]. Karyotyping (50 metaphases counted and five analysed) was done in girls suspected to have Turner syndrome. In patients with features suggestive of William syndrome/22 q deletion syndrome, fluorescence in situ hybridization (FISH) for the specific microdeletion was the first investigation of choice. In children who were small for gestation with a clinical diagnosis of Russell-Silver syndrome (RSS), methylation sensitive multiplex ligation-dependent probe amplification (MS-MLPA) for 11p15 was performed. Maternal uniparental disomy for chromosome 7 was not tested in this study. For the other short stature syndromes, exome sequencing was performed where feasible. Neonates and infants with hypotonia and feeding difficulties with a clinical suspicion of Prader-Willi syndrome (PWS) or those with the characteristic phenotype were evaluated by MS-MLPA. Children suspected of RASopathies and other single gene syndromes were mostly advised clinical or whole exome sequencing to evaluate the genes linked to this pathway, except one who had undergone single gene *PTPN11* sequencing, the commonest gene for Noonan syndrome.

In patients with clinically undefined dysmorphic syndromes chromosomal microarray (CMA) was done to examine chromosomal microdeletions and microduplications. Karyotyping was done if the microarray was not possible. All tests were performed at the in-house laboratory except urine oligosaccharide test and exome sequencing, which was outsourced. Institutional ethics committee cleared the study, and informed written consent was obtained from the legal guardians/adults before genetic testing and photography when done.

Statistical analysis: Data recording and analysing was done in MS Excel format. Descriptive statistics have been used to present the data.

RESULTS

Of a total of 455 patients with short stature in this study period, 226 patients required detailed phenotyping and genetic testing for confirmation of the etiology while 229 were identified on preliminary history/examination and investigations. Of these, 63 % (n=142) had proportionate short stature (**Fig. 1**).

The height of 84 patients with disproportionate short stature ranged from -2 to -3 z-score (60.7%, n=51), -3 to -4 z-score (33.3%, n=28) -4 to -5 z-score (5.9%, n=5). Mean (SD) age was 3.8 (1.9) years and mean (SD) height was 88.8 (10.6) cm. A lysosomal storage disorder (LSD) (45%, n=38) or a skeletal dysplasia (44%, n=37) was present in an almost equal proportion of patients. In patients with LSDs, enzyme analysis confirmed the disorder in 86.8% (n=33) and molecular confirmation was available in 73.6% (n= 28) (**Table I**). Skeletal survey helped to define 89% (n=33) cases of skeletal dysplasia and molecular confirmation could be done in 86.4% (n=32). Nine patients could not be classified (exome sequencing had been done in three of these). Ten patients confirmed with a skeletal dysplasia had proportionate short stature. Overall, a molecular diagnosis was available in 71% (n=60) in this group.

The height of the 142 patients in proportionate short stature group ranged from -2 to -3 z-scores (52.1%, n=74) -3 to -4 z-scores (47.8%, n=68), mean (SD) age was 4.1 (2.5) year and mean (SD) height was 90 (11.5) cms. Recognizable syndromes (excluding Downs syndrome) constituted 65.5% (n=93); 27.4% (n=39) could not be identified on phenotyping and ten cases had skeletal dysplasia (**Fig. 1**). Among the recognizable syndromes, 59% (n=55) were confirmed by the respective cytogene-tic/molecular test and the remaining could not be tested. The molecular and cytogenetic profile of children with a confirmed genetic diagnosis is shown in **Table I**. Testing by MS-MLPA confirmed hypermethylation with 15q11.2 deletion in seven patients with PWS, hypermethylation without deletion in one patient with PWS, hypomethy-lation of 11p15.5 in one patient with RSS. One patient with RSS could not be confirmed on MS-MLPA and karyotype. Two phenotypically male children with DSD and 46 XX karyotype who underwent CMA were found to have deletion of Xp involving *SHOX* and other genes with presence of translocated *SRY* gene.

A stepwise testing identified an etiology in ten patients with a clinically undefined syndrome. Karyotype was done in ten patients and identified an etiology in three (yield = 30%), CMA was done in twelve and identified an etiology in six patients (yield=50%) and exome sequencing was performed in six patients with a definite diagnosis in one patient.

DISCUSSION

In this study we present the genetic spectrum of patients referred to a genetic clinic who had short stature as one of the presenting phenotypes. The utility of CMA in

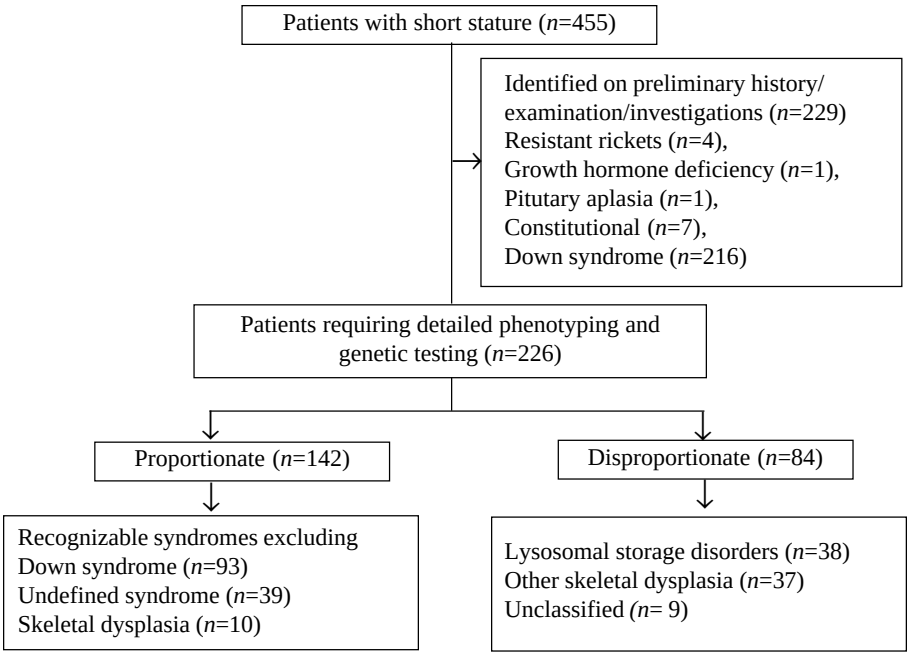


Fig. 1 Study flow chart and results.

syndromic short stature is reported to vary between 10-15% [3]. In the current study the small cohort evaluated by CMA could account for the high yield of 50%. We identified two individuals with *SHOX* gene deletion by CMA as a part of a larger deletion on X chromosome and translocation of the *SRY* gene. Kumar, et al. [11] reported *SHOX* gene deletion in 6.5% of Indian children with idiopathic short stature [11], and worldwide it is reported in 2-15% of such children [1]. However, we could not test all patients of nonsyndromic short stature for *SHOX* gene deletion to derive a diagnostic yield from this study. In this study, a diverse cytogenetic profile including mosai-cism and isochromosome Xq was seen in Turner synd-rome patients, similar to that reported in literature [12]. However, previous studies evaluating karyotype-pheno-type correlations in Turner syndrome have shown conflicting results [12,13].

The yield of exome sequencing in cases of short stature with a negative chromosomal analysis and gene panel testing varies between 16.5% and 46% [2]. In this study, exome sequencing could be done in only six patients with an unexpected diagnosis of histiocytosis and lymphadenopathy syndrome in one patient who presented with only short stature and myopia. The identification of this disorder had important implications for monitoring and comorbid diagnosis in this child. It is possible that decreasing costs of exome sequencing since the time of this study would enable testing of a larger cohort of 'idiopathic' short stature patients and improve patient management.

Traditionally, skeletal dysplasias are diagnosed on clinical examination and skeletal survey as was seen in 89% of the study cohort. However, we feel that with recognition of short stature in heterozygous carriers, exome sequencing could increase the genetic diagnosis, as seen in one patient with familial short stature and pathogenic variant in the recently implicated *ACAN* gene [3]. In this study, a definite molecular diagnosis of skeletal dysplasia (including LSDs) was achieved in 71% cases.

While individuals with short stature and defects in the GH-IGF-1 axis were under represented in our study (two patients), as they were probably referred to endocrinology clinics, it remains an important cause of proportionate short stature to be evaluated. Its incidence of 1.5-5.1% has been reported previously [11,14].

The limitation of this study is that due to its retrospective nature and bias of a genetic clinic referral, patients with hypothyroidism, GH deficiency, celiac disease, constitutional growth delay may be under represented and individuals with idiopathic short stature were not a part of this cohort.

Clinical evaluation within the spectrum of proportion-ate and disproportionate short stature helps to categorize the kind of genetic tests to be performed from the armamen-tarium of urine analysis for glycosaminoglycans and

WHAT THIS STUDY ADDS?

- In clinically undefined syndromes of short stature with developmental delay and/or dysmorphology chromosomal microarray may be the first investigation of choice.
- In familial short stature or idiopathic short stature exome sequencing may identify rare monogenic short stature syndromes.

oligosaccharides, metabolic enzyme testing, karyotype, chromosomal microarray, targeted gene testing and next generation sequencing tests including panel and exome tests. Through this study, we have attempted to represent the appropriate testing indications. This become more relevant with the increasing availability of the tests and decreasing costs since the time of this data. Achieving a definitive diagnosis can help to guide prognosis, explore utility of recombinant human growth hormone therapy [15] and provide genetic counselling to families.

Ethics clearance: EIC;Sir Ganga Ram Hospital; No: EC/04/21/1862, dated June 8, 2021.
Contributors: RDP,KS,SBM,ICV: conceptualization and writing original draft; RDP, KS: methodology; ML,PS: cytogenetic analysis of cases; JV,DT: enzyme analysis of cases; RS,SK: molecular analysis of cases. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
Funding: None; *Competing interests*: None stated

REFERENCES

1. Jee YH, Andrade AC, Baron J, Nilsson O. Genetics of Short Stature. Endocrinol Metab Clin North Am. 2017;46:259-81.
2. Mintz CS, Seaver LH, Irons M, Grimberg A, Lozano R; ACMG Professional Practice and Guidelines Committee. Focused Revision: ACMG practice resource: Genetic evaluation of short stature. Genet Med. 2021;23:813-5.
3. Jee YH, Baron J, Nilsson O. New developments in the genetic diagnosis of short stature. Curr Opin Pediatr. 2018;30:541-7.
4. Khadilkar VV, Khadilkar AV. Revised Indian Academy of Pediatrics 2015 growth charts for height, weight and body mass index for 5-18-year-old Indian children. Indian J Endocrinol Metab. 2015;19:470-6.
5. Khadilkar VV, Khadilkar AV, Choudhury P, et al. IAP Growth Monitoring Guidelines for Children From Birth to 18 Years. Indian Pediatr. 2007;44:187-97.
6. Yadav S, Dabas A. Approach to short stature. Indian J Pediatr. 2015;82:462-70.
7. Spranger JW, Brill PW, Hall C, et al. Bone dysplasias. An atlas of Genetic disorders of skeletal development. 4th ed. New York: Oxford University Press; 2018.
8. Jones KL, Jones MC, Campo MD, editors. Smith's Recognizable Patterns of Human Malformation. 7th ed. Philadelphia: Elsevier Saunders; 2013.
9. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University. Accessed: January 11, 2022. Avai-lable from: *https://omim.org/*
10. Face2gene. FDNA. Accessed: June 12, 2021. Available from: *https://www.face2gene.com*
11. Kumar A, Jain V, Chowdhury MR, et al. Pathogenic/likely pathogenic variants in the SHOX, GHR and IGFALS genes among Indian children with idiopathic short stature. J Pediatr Endocrinol Metab. 2020;33:79-88.
12. Miguel-Neto J, Carvalho AB, Marques-de-Faria AP, et al. New approach to phenotypic variability and karyotype-phenotype correlation in Turner syndrome. J Pediatr Endocrinol Metab. 2016;29:475-79.
13. Mondal S, Bhattacharjee R, Chowdhury S, Mukhopadhyay S. Karyotype-phenotype correlation in Turner syndrome at a single center in Eastern India. Indian Pediatr. 2021;58: 34-37.
14. Hauer NN, Popp B, Schoeller E, et al. Clinical relevance of systematic phenotyping and exome sequencing in patients with short stature. Genet Med. 2018;20:630-8.
15. Kirk J. Indications for growth hormone therapy in children. Arch Dis Child. 2012;97:63-8.

Diagnosis and Management of Acquired Aplastic Anemia: Consensus Statement of Indian Academy of Pediatrics

ARUN DANEWA,[1] MANAS KALRA,[2] ANUPAM SACHDEVA,[2] PALLAVI SACHDEVA,[2] DEEPAK BANSAL,[3] SUNIL BHAT,[4] DIVIJ SACHDEVA,[2] SIRISHA RANI,[5] SATYA P YADAV,[6] SATYENDRA KATEWA,[7] ARCHANA KUMAR,[8] DEENDAYALAN MUNIRATNAM,[9] BHARAT R AGARWAL,[10] TULIKA SETH,[11] AMITA MAHAJAN,[12] VIKAS DUA,[13] GAURAV KHARYA,[12] RUCHIRA MISRA,[14] DHWANEE DESAI,[6] VINOD GUNASEKARAN,[15] VINITA SRIVASTAVA[16]

From [1]Action Balaji Hospital, New Delhi; [2]Sir Ganga Ram Hospital, New Delhi; [3]PGIMER, Chandigarh; [4]Narayana Health City, Bangalore, Karnataka; [5]Rainbow Children's Hospital, Hyderabad, Andhra Pradesh; [6]Medanta Hospitals, Gurugram, Haryana; [7]Manipal Hospital, Jaipur, Rajasthan; [8]KGMU, Lucknow, Uttar Pradesh; [9]Rela Institute and Medical Centre, Chennai, Tamil Nadu; [10]BJ Wadia Hospital for Children, Mumbai, Maharashtra; [11]AIIMS, New Delhi; [12]Indraprastha Apollo Hospitals, New Delhi; [13]Fortis Memorial Research Institute, Gurugram, Haryana; [14]SRCC Children's Hospital, Mumbai, Maharashtra; [15]Kauvery Hospital, Tiruchirappalli, Tamil Nadu; [16]Ministry of Health and Family Welfare, New Delhi.

Correspondence to: Dr Anupam Sachdeva, Director, Pediatric Hematology Oncology and Bone Marrow Transplantation unit, Institute for Child Health, Sir Ganga Ram Hospital, New Delhi 110 060. anupamace@yahoo.co.in

Justification: In India, there is a lack of uniformity of treatment strategies for aplastic anemia (AA), and many children are managed only with supportive care due to non-availability of hematopoietic stem cell transplantation (HSCT). **Process:** Eminent national faculty members were invited to participate in the process of forming a consensus statement in Hyderabad in July, 2016. Draft guidelines were circulated to all members, and comments received in a online meeting in October, 2020 were incorporated into the final draft. These were approved by all experts. **Objective**: To facilitate appropriate management of children with acquired aplastic anemia. **Recommendations**: Key recommendations are: *i*) A bone marrow biopsy is must to make a diagnosis of AA; *ii*) Rule out inherited bone marrow failure syndromes (IBMFS), connective tissue disorders, viral infections, paroxysmal nocturnal hemoglobinuria (PNH), drug or heavy metal induced marrow suppression in all cases of AA; *iii*) Conservative approach to transfusions should be followed, with a target to keep hemoglobin >6 g/dL in children with no co-morbidities; *iv*) HLA-matched sibling donor HSCT is the preferred choice of treatment for newly diagnosed very severe/ severe AA; *v*) In absence of HLA-matched family donor, a matched unrelated donor (MUD) transplant or immunosuppressive therapy (IST) should be considered as alternate choice based on physician expertise; *vi*) Fludarabine, cyclophosphamide and anti-thymocyte globulin (ATG) based conditioning with cyclosporine and methotrexate as graft versus host disease (GvHD) prophylaxis is the preferred regimen; *vii*) Horse ATG and cyclosporine are the recommended drugs for IST. One should wait for 3-6 months for the response assessment and consideration of next line therapy.

Keywords: *Anti-thymocyte globulin, Cyclosporine, Hematopoietic stem cell transplant, Immunosuppressive therapy.*

Published online: February 01, 2022; ***PII:*** S097475591600403

Survival of children with aplastic anemia (AA) has markedly improved in the developed world, because of advances in hematopoietic stem cell transplantation (HSCT), and immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporine, where transplant is not possible. In our country, there is a lack of uniformity of treatment strategies and many children are managed only with supportive care.

OBJECTIVES

The purpose of this guideline is to improve the care of children with acquired aplastic anemia by an early and accurate diagnosis, prompt referral, detailed counseling and definitive therapy in the form of HSCT or IST.

PROCESS

Eminent national faculty members were invited to participate in the process of forming a consensus statement in Hyderabad in July, 2016. Selected members were requested to prepare guidelines on specific subtopics. These guidelines were then incorporated into a draft statement, which was circulated to all members. An online meeting was organized in October, 2020; opinions expressed by the participants were incorporated into the final draft, which was again circulated to all experts and finalized.

RECOMMENDATIONS

Aplastic anemia is defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate and with no increase in reticulin. Diagnostic criteria of AA include: (1) presence of at least two of the following in the peripheral blood counts; *i*) hemoglobin <100 g/L; *ii*) platelet count $<50 \times 10^9$/L; *iii*) neutrophil count $<1.5 \times 10^9$/L, and (2) low bone marrow cellularity (<25%) [1]. Severity of AA is defined as per the criteria depicted in **Box I** [2,3] (1C).

Box I Severity Classification of Aplastic Anemia

Severe aplastic anemia (SAA)

BM cellularity < 25% (or 25-50% if <30% of BM is hematopoietic cells) and

at least two of the following:

- Peripheral blood neutrophil count $<0.5\times10^9$/L
- Peripheral blood platelet count $<20\times10^9$/L
- Peripheral blood reticulocyte count $<20\times10^9$/L

Very severe aplastic anemia (VSAA)

As above, but peripheral blood neutrophil count must be $<0.2\times10^9$/L

Non-severe aplastic anemia

Hypocellular bone marrow with peripheral blood counts not meeting criteria either for SAA or VSAA

Modified from Reference 1 and 2.

This guideline will focus specifically on early diagnosis and treatment of acquired AA, and will not refer to management of inherited bone marrow failure syndromes (IBFMS) and bone marrow aplasia that occurs secondary to chemotherapy and/or radiation exposure.

Diagnosis

The expert panel felt that there are no specific markers and the diagnosis is reached by exclusion of other reasonable entities. There are three diagnostic steps to define AA: *i*) confirming the suspicion of AA; *ii*) defining the severity of AA and; *iii*) excluding IBMFS and identifiable causes of marrow aplasia.

History and Physical Examination

Most children with AA present with a history pertaining to decrease in the blood cell lines (anemia, leucopenia, neutropenia and thrombocytopenia). There may be a history of lethargy, tiredness, poor appetite and increasing pallor secondary to anemia. The child may present with fever with or without a focus because of neutropenia. They may also give a history of bleeding, especially mucocutaneous bleeding, consequent to thrombocytopenia. There may be a history of blood transfusion prior to presentation. Any history of recent illnesses like viral infections or jaundice (approximately 5-10% of patients with SAA have a preceding seronegative hepatitis) should be elicited [4]. A history of joint pains/swelling, skin rash, photosensitivity, hair loss, mouth ulcers etc may point towards a connective tissue disorder. A detailed drug and occupational exposure history should always be taken (1C). A detailed dietary history may enable to investigate for nutritional megaloblastic anemia as the likely etiology of pancytopenia. A similar history in the family can point towards a IBMFS, or a common environmental exposure. It is essential to perform a detailed examination for every patient that presents with bicytopenia or pancytopenia. AA is clinically characterized by anemia and evidence of mucocutaneous bleeds, in the absence of enlarged lymph nodes, splenomegaly and hepatomegaly. One also needs to identify any stigmata of IBMFS. **Web Table I** gives the various signs which point towards the diagnosis.

Investigations

1. Complete blood count with peripheral smear and reticulocyte count

Following findings may be seen:

i) *Pancytopenia/bicytopenia*: There is moderate to severe leucopenia with relative lymphocytosis and variable degree of neutropenia; In early stages, isolated cytopenia, commonly thrombocytopenia may be seen; and anemia, usually macrocytic. Look for dysplastic neutrophils, abnormal platelets, blasts and other abnormal cells to exclude other causes of pancytopenia.

ii) *Reticulocytopenia*

2. Bone marrow aspiration and/or biopsy

Despite severe thrombocytopenia, adequate pressure is sufficient following a bone marrow aspirate and biopsy to prevent bleeding and there is no need of platelet transfusion before procedure [5]. The marrow is usually hypocellular with prominent fat spaces; few residual haemopoietic cells may be present. A bone marrow aspirate may occasionally be a dry tap. Trephine bone marrow biopsy (at least 2 cm length) is necessary in every AA case to assess the overall cellularity (which may be patchy), to look at the morphology of residual hematopoietic cells and to exclude an abnormal infiltrate. Avoid tangential biopsies as subcortical marrow is normally hypocellular. **Box II** shows the differential diagnosis of pancytopenia according to bone marrow cellularity. Bone marrow cellularity can be estimated using a simple formula (100-age = Bone marrow cellularity in %). The appearance of the marrow in inherited and acquired AA syndromes is identical, and the histological distinction between them is blurred.

Erythropoiesis: Erythroid series in marrow is decreased. AA should be differentiated from myelodysplastic syndrome (MDS). Dyserythropoiesis is not a common finding in immune mediated AA [6].

Megakaryopoiesis: In AA, megakaryocytes are either reduced or absent. Megakaryocytes are the most useful element in differentiating MDS from severe aplastic anemia (SAA) [7]. Small mononuclear or aberrant megakaryocytes are typical of MDS, whereas megakaryocytes are markedly reduced or absent in SAA.

Box II Differential Diagnosis of Pancytopenia

Pancytopenia with hypocellular marrow

- Acquired aplastic anemia
- Inherited bone marrow failure like Fanconi anemia (FA), amegakaryocytic thrombocytopenia, dyskeratosis congenita, Shwachman-diamond syndrome
- Paroxysmal nocturnal hemoglobinuria (PNH)
- Hypoplastic myelodysplastic syndrome

Pancytopenia with cellular marrow

- Primary bone marrow disease like- PNH, Myelodysplasia
- Secondary to systemic disease like- SLE, Hypersplenism, Vitamin B_{12} and folate acid deficiency, Infection

Pancytopenia due to infiltrative bone disorder

- Acute lymphoblastic leukemia
- Acute myeloid leukemia
- Myelofibrosis
- Storage disorders

White blood cell (WBC) series: Lymphocytes, macrophages, plasma cells and mast cells appear prominent in bone marrow, while the mature WBC forms, viz granulocytes, are reduced in bone marrow in children with SAA. Dysplastic granulocytes are not seen in AA.

3. Bone marrow cytogenetics

Obtaining a good bone marrow aspirate may be difficult in a child with hypocellular bone marrow as the number of metaphases observed may be insufficient. In such a situation, we should perform a fluorescent in situ hybridization (FISH) analysis for chromosomes 5 and 7 to exclude MDS. Abnormal cytogenetic clones may be present up to 12% of patients with AA, and are not important for diagnosis or prognosis [8]. These may also arise during the course of the disease. Most frequent anomalies are - trisomy 8, trisomy 6, 5q, anomalies of chromosome 7 and 13 [9].

4. Vitamin B12 and folate levels

If a deficiency of vitamin B12 or folate is documented or suspected based on the peripheral smear or bone marrow findings, then correction must be done before diagnosing AA. Bone marrow aplasia due to vitamin deficiency is exceedingly rare. The levels are extremely sensitive to administration of supplements and hence should be done before administration of the same.

5. Tests of liver function and viral studies

The onset of AA often occurs 2-3 months after an acute episode of hepatitis and is more common in young males [10]. Hepatitis associated AA shows poor prognosis [10]. Viral markers for Hepatitis A, B, C, EBV, HIV, and CMV are indicated but the disease is usually seronegative. Parvovirus usually causes only red cell aplasia and not AA. Viral infections require adaptive immunity for viral clearance that is mediated by antibodies and T-cells. Oligoclonal CD8+ T-cells may target the hematopoietic tissue, which can lead to stem cell death secondary to effects of IFNγ and TNFα. Pro-inflammatory cytokines may also damage the hematopoietic stem cell compartment and cause pancytopenia.

6. Chromosomal breakage analysis (1B)

The diagnosis of Fanconi anemia (FA) is very important, because these patients do not respond to IST, require dose-reductions in transplant conditioning and need careful follow-up for development of non-hematologic malignancies.

Spontaneous breakage (chromatid-type aberrations) can occur in standard cytogenetic preparations, although this phenomenon is highly variable and considered unreliable for diagnostic purposes. Extreme sensitivity of FA cells to the chromosome breaking effect of the cross-linking agents mitomycin C (MMC) and diepoxybutane (DEB) is routinely utilized to diagnose FA by a chromosomal breakage test [11,12]. Chromosomal breakage should be done in all patients below 40 years of age before IST/ HSCT, even if no physical features of FA are identified. Chromosomal breakage should also be tested in sibling donors of affected patients in case of FA. 5-7 mL of venous blood in collected in green topped vacutainers (heparinized sample). If the child / patient has been transfused, wait for at least 2 weeks before obtaining the specimen. Skin fibroblasts have been used for diagnosis of IBFMS in case of recent transfusion of difficulty to culture metaphases but it is not available universally.

Next generation sequencing (NGS) for IBMFS: It is very useful to detect IBMFS, especially if the chromosomal breakage has been negative [13]. The panel recommends carrying out this test, but it is not mandatory. Customized panels are now available in India to rule out IBMFS.

7. Tests to detect a PNH clone (1C)

PNH is an uncommon cause of AA in children. The disease may lead to features of thrombosis, hemolysis, hemoglobinuria and AA. Ham test and sucrose lysis test have been abandoned (as they do not detect clones in neutrophils, monocytes and in transfused patients).

Analysis of glycosylphosphatidylinositol (GPI)-anchored proteins (like CD55 and CD59) by flowcytometry (Fluorescein-labeled proaerolysin, FLAER) is the preferred

method. PNH clone lacks these markers and may be seen in small amounts. They occur in up to 50% of AA patients, which may remain stable, diminish, disappear or increase [14].If a PNH clone is present, evaluate for evidence of intravascular hemolysis (urine hemosiderin and LDH). The presence of less than 50% deficient circulating cells without evidence for thrombosis or significant hemolysis generally does not require PNH-specific therapy and responds well to IST[15].

8. Anti-nuclear antibody and anti-dsDNA

Anti-nuclear antibody (ANA) should be done in all patients presenting with pancytopenia as SLE can present with normocellular, hypocellular or myelofibrosis in marrow with variable cytopenia [16]. Children who develop MAS (macropahge activation syndrome), may have presentation like AA. Anti-dsDNA antibody should be done in cases where ANA is positive or if the index of suspicion is very high.

Treatment for Acquired Aplastic Anemia

Supportive Care

The main focus should be on prevention of infections and bleeding and expectant management of treatment-related toxicities and psychological support

i) When to transfuse

Based on concerns with alloimmunization and iron overload, there should be a uniform approach to utilize as few transfusions as possible to keep the patient relatively asymptomatic (1A). Unnecessary transfusion practice should be avoided in AA (1A).

A common problem in multi-transfused patients with AA is that they may develop alloimmunization to leucocytes present in red cell and platelet transfusions by generating HLA or non-HLA (minor histocompatibility) antibodies. This can result in platelet refractoriness, as well as an increased risk of graft rejection and graft versus host disease after allogeneic BMT [17].

It is recommended to give prophylactic platelet transfusions when the platelet count is $<10\times10^9$/L; however, the physician may choose to transfuse only when there is mucosal bleeding (1B). In febrile patients it is desirable to maintain platelet count above 20×10^9/L, however, it may not be possible due to alloimmunization and platelet refractoriness [18] (2C).

During ATG infusion, patients should be transfused optimally to achieve a platelet count of $>30 \times10^9$/L before ATG treatment (2C).

RBC transfusion depends on clinical symptoms, Hb value and quality of life (1A). If Hb levels are less than 6.0 g/L, packed red blood cell (PRBC) transfusion should be given.

Granulocyte apheresis may have an adjunctive role in severe infections in SAA patients as a possible way to bridge the gap between specific treatment and neutrophil recovery [19].

Whenever possible, use leucoreduced blood products to prevent HLA alloimmunization. Irradiated blood product should be used in all AA patients especially who are potential BMT candidates (1C).

Blood and platelet donations from family members should be avoided because the recipient may become sensitized to minor histocompatibility antigens from the potential bone marrow donor resulting in a high risk of graft rejection.

Other practical measures to prevent bleeding include good dental hygiene, the use of oral tranexamic acid and control of menorrhagia with hormone therapy.

ii) Infection control

Patients with AA are at risk of bacterial and fungal infections. The risk of infection depends upon neutrophil count and may vary from patient to patient.

We recommend considering basic principles of asepsis such as: *i*) washing hands before preparing food; *ii*) avoid contamination of food; *iii*) consumption of pasteurized juices and dairy products, and *iv*) consumption of clean, fresh and hygienic home cooked food [20].

Exposure to construction areas should be avoided as this increases the risk of fungal infections. Additionally, it seems reasonable to avoid contact with garbage, compost or potted plants. Hand washing and rubbing with alcohol-based disinfection solution must be used before and after handling the patient by the staff and by visitors. Optimal hand hygiene has been shown to be highly effective in reducing neutropenic infections. Routine prophylactic antimicrobials are not required. Patients with AA are at high risk of fungal infection, including Aspergillus. It is suggested to use prophylactic antifungals for patients with very severe AA (absolute neutrophil count <200/ mm^3). However, definite evidence for this recommendation is lacking [21] (2B). As fluconazole provides no cover against Aspergillus species, the drugs of choice are voriconazole and posaconazole. Azoles are potential inhibitors of cytochrome P450 3A4 enzyme which can lead to increase in the plasma levels of immunosuppressant drugs like cyclosporine thereby resulting in toxicity [22].

Routine prophylaxis against *Pneumocystis jirovecii* or anti-viral prophylaxis is not required [17,20] (2C).

P. jirovecii prophylaxis is essential post BMT for all patients regardless of diagnosis, but not usually required post ATG treatment.

Acyclovir prophylaxis is essential for all transplanted patients and may be used for the first 3-4 weeks after IST with ATG [17,20].

As for all neutropenic patients, fever is an emergency and requires immediate hospitalization and treatment before the results of bacterial investigations are available.

Single anti-pseudomonas antibiotic may be enough in most cases. However, the choice of antibiotic will also depend on whether the infection is hospital acquired or is community acquired. The exact choice depends upon local hospital microbiological sensitivity/resistance pattern. It is recommended that investigations for systemic fungal infection and empirical anti-fungal therapy is introduced into the febrile neutropenia regimen if fever persists for more than 96 hours.

A chest radiograph should be included as part of the investigation of new or persistent fever with respiratory symptoms, and computed tomography (CT) chest may be also done if there is a high index of clinical suspicion for aspergillus pneumonia.

iii) Hemopoietic growth factors

The routine use of recombinant human erythropoietin (EPO) or G-CSF is not recommended. A short course of subcutaneous G-CSF at a dose of 5 microgram/kg per day may be considered for severe systemic infections that are not responding to intravenous antibiotics and antifungals. If there is no response by 1 week, then discontinue G-CSF. GM-CSF is not recommended for the treatment [23]. The addition of eltrombopag to IST may be considered [24]. However, the routine use of eltrombopag cannot be recommended as definite evidence is lacking in children.

iv) Iron chelation

Frequent transfusions in SAA may result in significant iron overload and its complications. Subcutaneous desferrioxamine (DFO) or oral deferasirox should be considered when the serum ferritin is >1000 ng/ml [18].

Definitive Treatment (Fig. 1)

i) When and whom to treat [25]

Severe and very severe AA almost always requires treatment, both immediate and definitive. For patients with non-severe AA, as defined by lack of blood count criteria for SAA, observation is often appropriate, especially when they are not transfusion-dependent. Moderate AA patients who progress to severe pancytopenia and meet the criteria for SAA or become transfusion-dependent can then be treated accordingly.

It is necessary that before specific treatment is given, the patient is stabilized (bleeding controlled and free from infection).

ii) Choice of definitive treatment

Hematopoiesis can be restored in severe AA with HSCT or IST. Transplantation is the preferred treatment when matched sibling donor available.

a) Matched-related HSCT

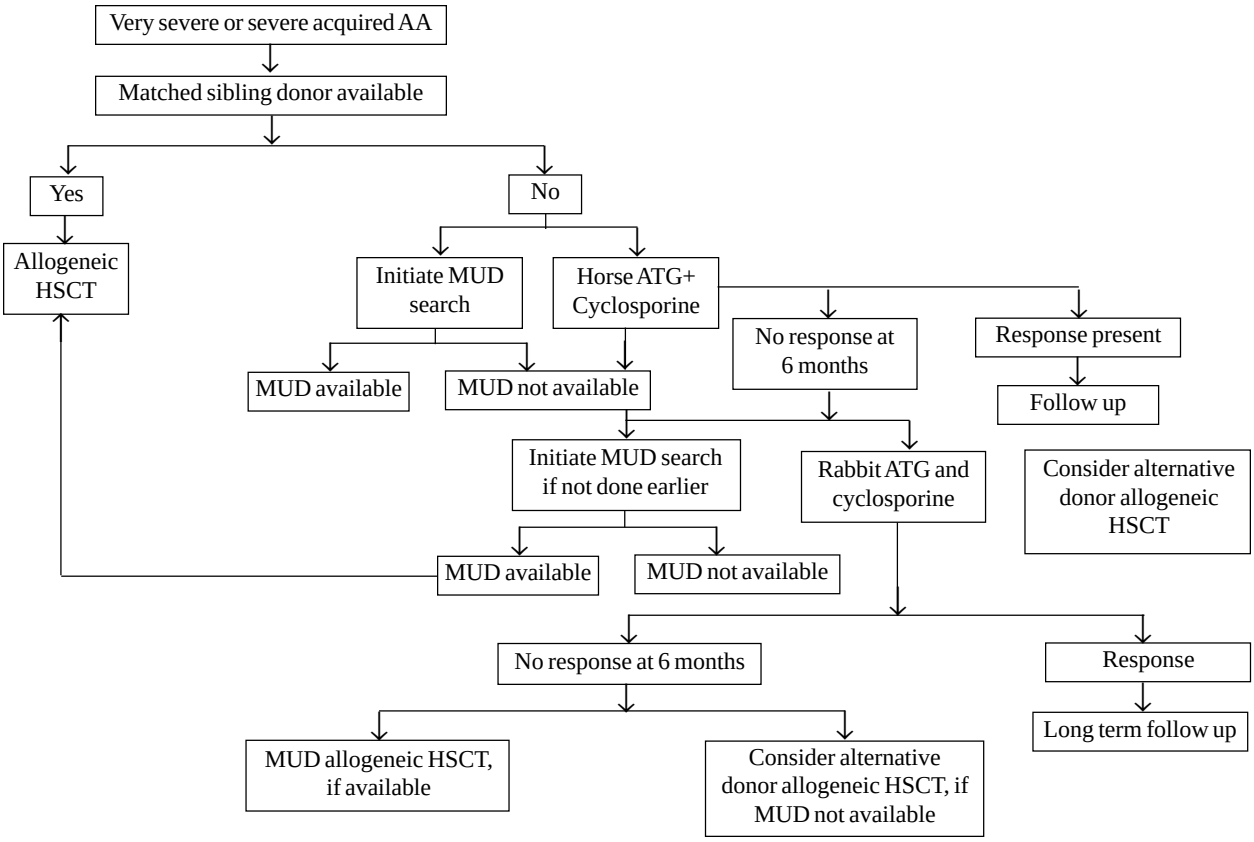
Allogeneic BMT from an HLA-identical sibling donor (matched sibling donor, MSD) is the initial treatment of choice for newly diagnosed patients with AA if they have *i*) Severe or VSAA *ii*) Non-severe AA and in whom treatment is indicated [25] (1A).

Matched-sibling transplantation is always preferred. Ideally patient should be free from infections before transplant. The expected overall response rate with matched related donor HSCT is ~90% [25].

The transplant physician may proceed with BMT in the presence of active infection, particularly fungal infection, as the transplant offers the best chance of early neutrophil recovery. Delaying the transplant may actually risk progression of the fungal infection. The conditioning regimens and GVHD prophylaxis described below refer specifically to patients with acquired AA. Patients with FA and other types of inherited AA need special consideration and should not follow these pathways, as the conditioning regimen and GVHD prophylaxis are completely different. Current guidelines from European Society for Blood and Marrow Transplantation (EBMT) [26] and the British Society for Standards in Hematolgy [27] call for a combination of fludarabine-cyclophospamide with ATG or alemtuzumab.

The recommended post-transplant immunosuppression usually comprises of two drugs, cyclosporine and short course methotrexate. The starting dose of cyclosporine is 3-5 mg/kg/day in 2 divided doses. The cyclosporine levels should be maintained between 150-200 microgram /L, to avoid toxicity. Therapeutic cyclosporine should be continued for at least nine months before gradually reducing the dose to zero over the following three months.

Source and dose of stem cells: It is recommended that bone marrow stem cells should be used [28] but the transplant physician may decide to use peripheral blood stem cells (PBSC) based on the experience or logistics. It is recommended that at least 3×10^6/kg of CD34 stem cells



HSCT: hematopoietic stem cell transplantation; MUD: matched unrelated donor; ATG: anti-thymocyte globulin.

Fig. 1 Management algorithm for pediatric aplastic anemia.

should be given [29]. In the absence of a MSD, a center may choose to proceed with IST or MUD transplant as per the physician expertise. MUD has encouraging outcomes and may be used in case there is a 10/10 match with the recipient and the logistics like finances and timing can be appropriately coordinated [28]. The Indian Stem Cell Registries have made this increasingly possible [30]. The current cost of arranging a MUD donation through an Indian registry is approximately 10 lakhs. If there is no MSD or MUD available, and the IST course has failed, alternative donor transplant options like haploidentical transplant may be considered [31-34].

Complications from HSCT: Post-HSCT, children need regular monitoring. Short-term complications include chemotherapy related side effects (e.g. hemorrhagic cystitis, infections), acute GVHD, and graft failure. Long-term complications include chronic GVHD, delayed immune reconstitution and relapse.

b) Immunosuppressive therapy. Indications are:

i) Patients with non-severe AA who are dependent on red cell and/or platelet transfusions and no matched sibling donor available

ii) Patients with severe or very severe disease who lack an HLA-compatible sibling donor or where HSCT is not feasible

For those patients with non-severe AA who are not dependent on either red cell or platelet transfusions, and maintain safe blood counts, it is reasonable to observe the blood count and monitor the patient regularly without initially starting IST.

Horse (or equine) ATG infusions combined with oral cyclosporine remains the standard first line IST [35] (1A). The expected overall response rate with IST is 60-75% with a long-term survival of 80-90% [25,36].

- Immunosuppression administration

Anti-thymocyte globulin (ATG): It must be given via central line in a hospitalized patient with monitoring for infusion reactions and other side effects. Perform an ATG skin test for hypersensitivity with intradermal testing with

0.02 mL of a 1:1000 v/v (volume/volume) saline dilution of ATG with a separate saline control injection of similar volume. Read the result after 10 minutes: a wheal at the ATG site 3 or more mm larger in diameter than that at the saline control site suggests clinical sensitivity and an increased possibility of a systemic allergic reaction. Increasingly intravenous (IV) test dose has replaced skin testing. We can add 0.1 mL of ATG in 100 mL saline and start the first few drops at a very slow rate, which can be gradually increased to complete the test dose in 30 minutes [18].

Platelets should be maintained at more than $>30\times10^9$/L before ATG treatment and during the ATG administration period, but should not be given concurrently with ATG administration because of the anti-platelet activity of ATG [18].

Horse ATG is administered at a dose of 40 mg/kg as intravenous infusion in a glass ornon-PVC bottle over 12-18 hours, daily for 4 days. ATG can be diluted in normal saline or 5% Dextrose + 0.45% sodium chloride solution (DNS). The concentration of the diluted solution should not exceed 4 mg of ATG/mL. ATG administration should not start late in the day or on weekends when hospitals may be short-staffed as infusion reactions are more common on day 1 of ATG. Premedication before each ATG dose with acetaminophen and diphenhydramine is conventional, and common infusion reactions are managed symptomatically.

Prednisone (1 mg/kg) or methylprednisolone (2mg/kg) is started on day 1 and continued for 2 weeks, as prophylaxis for serum sickness. In the presence of life-threatening reactions, the ATG infusion is withheld temporarily until alarming signs and symptoms subside. Depending on the severity of reactions, reinitiate ATG at the normal or a slower infusion rate (sometimes over 24 hours) in a monitored setting. Increased liver enzymes tend to normalize over several days, and ATG may be infused despite mild to moderate elevation in transaminases.

Serum sickness typically occurs between day 7 and 14 from the start of ATG treatment. If serum sickness occurs, intravenous hydrocortisone 1-2 mg/kg/dose six hourly (max 100 mg) should be commenced. The common symptoms of serum sickness include arthralgia, myalgia, rash, fever, edema, mild proteinuria and platelet consumption often necessitating increased platelet transfusion support.

Cyclosporine (CsA): Initiate CsA on day 1 of IST or after completion of steroids to a target trough level between 150 and 200 ng/mL, starting at a dose of 5 mg/kg per day. Many patients develop hypertension during CsA treatment and amlodipine is the preferred anti-hypertensive drug because of minimal overlap with CsA toxicities. Bothersome gingival hyperplasia can improve on a short course of azithromycin or metronidazole ointment or dose reduction of CsA. Calcium channel blockers have been associated with worse gingival hyperplasia when combined with CsA [37]. Continue careful monitoring of renal function and adjustment of dosing to achieve target CsA levels. Continue CsA if modest increases in creatinine. More serious compromise of kidney function from baseline (1.5 times the baseline) may require temporary cessation of CsA with later reintroduction at lower doses. Consider withholding CsA in case of microangiopathic anemia and posterior reversible encephalopathy syndrome (PRES). Avoid concomitant use of other nephrotoxic agents.

There is a significant risk of relapse with rapid tapering of CsA and we recommend that CsA should be continued for at least 12 months followed by very slow tapering over 6 months to 12 months [29]. Blood pressure, renal and liver function tests should also be monitored regularly while on CsA.

v) What more can we do?

Eltrombopag has been used in adult AA patients, either alone or with IST. Along with IST, the response rates upto 80% have been reported [24,38]. Eltrombopag can be administered at a dose of 150 mg daily in patients who are 12 years of age or older, at a dose of 75 mg daily in patients who are 6 to 11 years of age, and at a dose of 2.5 mg per kilogram of body weight per day in patients who are 2 to 5 years of age along with ATG and cyclosporine [24]. However, the data in pediatric age group is still not entirely convincing [38]. Therefore, it cannot be recommended as a routine. Romiplostim is being tried for stimulation of hematopoiesis in AA. However, there is no enough data to routinely recommend its usage for this indication. More studies are needed to explore the role of thrombopoietin receptor agonists for patients with AA.

vi) When do we consider for second course of ATG?

A second course of ATG is recommended if there is no response or relapse after the first course. This should not be given earlier than 6 months after the first course because it usually takes around 3 to 6 months before a response occurs [26,27]. Second course of immunosuppression is given with rabbit ATG and cyclosporine [26,27]. Rabbit ATG is given for 5 days as a daily intravenous infusion over 12-18 hours. The daily dose of rabbit ATG is 3.75 mg/kg body weight.

A test dose of 2.5 mg for rabbit ATG, diluted in 100 mL normal saline and infused over 1 hour, is often given beforehand and if a severe systemic reaction or

Box III Response Criteria For Aplastic Anemia

Response criteria for severe aplastic anemia

None: Still severe

Partial response: Transfusion independence and no longer meeting criteria for severe disease

Complete response: Hemoglobin normal for age, neutrophil count >1.5×10^9/L, platelet count >150×10^9/L

Response criteria for non-severe aplastic anemia

None: Worse or not meeting criteria below

Partial response: Transfusion independence (if previously dependent) or doubling or normalization of at least one cell line or increase of baseline hemoglobin of >30 g/L (if initially <60) or increase of baseline neutrophils of >0.5 x10^9/L (if initially <0.5) or increase of baseline platelets of >20x10^9/L (if initially <20)

Complete response: Hemoglobin normal for age, neutrophil count >1.5 x10^9/L, platelet count >150 x10^9/L

Modified from reference 35.

anaphylaxis occurs, further doses of that preparation of ATG must not be given.

vii) How to assess response to IST

Response should be confirmed by two or more blood counts at least 4 weeks apart. [35]. **Box III** shows the response criteria for severe and non-severe AA [35].

viii) Refractory SAA

When child is refractory to IST and no matched sibling donor is available; MUD BMT is indicated in those who have no matched siblings and failed at least one course of ATG and CsA [39] (1B). If MUD is not available/feasible, a second course of IST with rabbit ATG (1B) or haplo-identical HSCT can be considered based on center preference (2B).

What Not to Do in AA

Supportive measures alone, growth factors, androgens or cyclosporine (CsA) alone are not definitive therapies. Patients where ATG or transplant are not possible early in disease course, cyclosporine with or without androgens can be tried, with a response rate of 20-30%.

Corticosteroids are of unproven benefit and inferior in efficacy to conventional immunosuppression regimens, but they are more toxic and should not be used as therapy in SAA. Infectious complications especially fungal infections and other life-threatening complications can occur after unnecessary steroid use.

Follow-Up

Patients with AA should be followed up indefinitely to monitor for relapse and later clonal disorders, such as MDS, leukemia, PNH and solid tumors.

CONCLUSIONS

Acquired aplastic anemia is a disease characterized by immune mediated bone marrow failure leading to pancytopenia and hypocellular marrow. A standard of care should follow in diagnosis and treatment of acquired aplastic anemia. MSD BMT is ideal when available. IST should be combined (ATG + CsA) and not monotherapy, when no matched sibling donor available. MUD transplant can be considered when at least one course of IST fails.

Contributors: AD, MK, AS, PS, DB: designed the manuscript; AS, SB, DS, SR, SPY, SK, AK, DM, BRA, TS, AM, VD, GK, RM, DD, VG, VS: analyzed and critically reviewed the manuscript. All authors approved the final version of manuscript.

REFERENCES

1. Incidence of aplastic anemia: The relevance of diagnostic criteria. By the International Agranulocytosis and Aplastic Anemia Study. Blood.1987;70:1718-21.
2. Camitta BM, Rappeport JM, Parkman R, Nathan DG. Selection of patients for bone marrow transplantation in severe aplastic anemia. Blood.1975;45:355-63.
3. Bacigalupo A, Hows J, Gluckman E, et al. Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anaemia (SAA): a report of the EBMT SAA working party. Br J Haematol. 1988;70:177-82.
4. Locasciulli A, Bacigalupo A, Bruno B, et al; Severe Aplastic Anemia Working Party of the European Blood and Marrow Transplant Group (SAA-WP, EBMT). Hepatitis-associated aplastic anaemia: epidemiology and treatment results obtained in Europe. A report of The EBMT aplastic anaemia working party. Br J Haematol 2010:149:890-5.
5. Kelsey P, Murphy MF, Brown M, et al. Guidelines for the use of platelet transfusions. Br J Haematol,2003:122:10–23.
6. Hong M, He G. The 2016 Revision to the World Health Organization Classification of Myelodysplastic Syndromes. J Transl Int Med.2017;5:139-143.
7. Durrani J, Maciejewski JP. Idiopathic aplastic anemia vs hypocellular myelodysplastic syndrome. Hematology Am Soc Hematol Educ Program.2019;1:97-104.
8. Gupta V, Brooker C, Tooze JA, et al. Clinical relevance of cytogenetic abnormalities at diagnosis of acquired aplastic anaemia in adults. Br J Haematol.2006;134:95-9.
9. Keung YK, Pettenati MJ, Cruz JM, et al. Bone marrow cytogenetic abnormalities of aplastic anemia. Am J Hematol. 2001;66:167-71.
10. Rauff B, Idrees M, Shah SA, et al. Hepatitis associated aplastic anemia: a review. Virol J.2011;8:87.
11. Korgaonkar S, Ghosh K, Jijina F, Vundinti BR. Chromosomal breakage study in children suspected with Fanconi anemia in the Indian population. J Pediatr Hematol Oncol.2010;32:606-10.
12. Talwar R, Choudhry VP, Kucheria K. Differentiation of Fanconi anemia from idiopathic aplastic anemia by induced chromosomal breakage study using mitomycin-C (MMC). Indian Pediatr. 2004;41:473-7.
13. Skibenes ST, Clausen I, Raaschou-Jensen K. Next-generation sequencing in hypoplastic bone marrow failure: What difference does it make? Eur J Haematol. 2021;106:3-13.
14. Sabet K, Panigrahi AR. Pediatric patients with severe aplastic anemia treated with combined immunosuppressive therapy, eltrombopag and eculizumab and their clinical course to stem cell transplant. Blood. 2020;136:13.
15. Brodsky RA. How I treat paroxysmal nocturnal hemo-

globinuria. Blood. 2021;137:1304-9.
16. Fayyaz A, Igoe A, Kurien BT, et al. Haematological manifestations of lupus. Lupus Sci Med. 2015;2:e000078.
17. Hosoba S, Waller EK, Shenvi N, et al. Peritransplantation red blood cell transfusion is associated with increased risk of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2018;24: 973-82.
18. Marsh JC, Ball SE, Cavenagh J, et al. Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol 2009;147:43-70.
19. Quillen K, Wong E, Scheinberg P, et al. Granulocyte transfusions in severe aplastic anemia: an eleven-year experience. Haematologica. 2009; 94:1661-8.
20. Jubelirer SJ. The benefit of the neutropenic diet: fact or fiction? Oncologist. 2011;16:704-7.
21. Höchsmann B, Moicean A, Risitano A, et al. Supportive care in severe and very severe aplastic anemia. Bone Marrow Transplant. 2013;48:168-73.
22. Groll AH, Townsend R, Desai A, et al. Drug-drug interactions between triazole antifungal agents used to treat invasive aspergillosis and immunosuppressants metabolized by cytochrome P450 3A4. Transpl Infect Dis. 201;19:e12751.
23. Young DJ, Dunbar CE. Immunosuppression and growth factors for severe aplastic anemia: new data for old questions. Haematologica. 2020;105:1170-1.
24. Townsley DM, Scheinberg P, Winkler T, et al. Eltrombopag Added to Standard Immunosuppression for Aplastic Anemia. N Engl J Med. 2017; 376:1540-50
25. Samarasinghe S, Veys P, Vora A, Wynn R. Paediatric amendment to adult BSH Guidelines for aplastic anaemia. Br J Haematol. 2018;180:201-5.
26. Aljurf M, Al-Zahrani H, Van Lint MT, Passweg JR. Standard treatment of acquired SAA in adult patients 18-40 years old with an HLA-identical sibling donor. Bone Marrow Transpl 2013;48:178-79.
27. Killick SB, Bown N, Cavenagh J, et al. British Society for Standards in Haematology. Guidelines for the diagnosis and management of adult aplastic anaemia. Br J Haematol. 2016;172:187-207.
28. Samarasinghe S, Steward C, Hiwarkar P, et al. Excellent outcome of matched unrelated donor transplantation in paediatric aplastic anaemia following failure with immunosuppressive therapy: a United Kingdom multicentre retrospective experience. Br J Haematol. 2012;157:339-46.
29. Georges GE, Doney K, Storb R. Severe aplastic anemia: allogeneic bone marrow transplantation as first-line treatment. Blood Adv. 2018; 2: 2020-28.
30. Saikia T. Blood and bone marrow transplantation in India: Past, present, and future. Indian J Med Paediatr Oncol. 2020; 41: 308.
31. George B, Mathews V, Lakshmi KM, et al. The use of a fludarabine-based conditioning regimen in patients with severe aplastic anemia–a retrospective analysis from three Indian centers. Clin Transplant. 2013; 27: 923-9.
32. Ramzan M, Yadav SP, Zafar MS, et al. Outcome of pediatric acquired aplastic anemia: a developing world experience. Pediatr Hematol Oncol. 2014; 31: 29-38.
33. George B, Mathews V, Viswabandya A, et al. Allogeneic hematopoietic stem cell transplantation is superior to immunosuppressive therapy in Indian children with aplastic anemia–a single-center analysis of 100 patients. Pediatr Hematol Oncol. 2010;27:122-31.
34. Sachdev M, Bansal M, Chakraborty S, et al. Allogeneic Hematopoietic Stem Cell Transplant Offer Good Outcomes in Pediatric Aplastic Anemia: Experience From Developing World. J Pediatr Hematol Oncol. 2021;43:e677-e81.
35. Camitta, BM. What is the definition of cure for aplastic anemia? Acta Haematologica, 2000;103:16-8.
36. Scheinberg P, Wu CO, Nunez O, Young NS. Long-term outcome of pediatric patients with severe aplastic anemia treated with antithymocyte globulin and cyclosporine. J Pediatr. 2008;153:814-9.
37. James JA, Marley JJ, Jamal S, et al. The calcium channel blocker used with cyclosporin has an effect on gingival overgrowth. J Clin Periodontol. 2000;27:109-15.
38. Groarke EM, Patel BA, Gutierrez-Rodrigues F, et al. Eltrombopag added to immunosuppression for children with treatment-naïve severe aplastic anaemia. Br J Haematol. 2021; 192: 605-14.
39. Bacigalupo A, Locatelli F, Lanino E, et al. Fludarabine, cyclophosphamide and ATG for alternative donor transplants in acquired severe aplastic anaemia – a report of the EBMT SAA Working Party. Bone Marrow Transplant. 2005;41:45-50.

NEWS IN BRIEF

Overturning of *Roe versus Wade*- a public health calamity

On May 2, the media company Politico created a storm when it published the leaked papers of the first draft of a US Supreme Court judgement. In this, the plan is to overturn a previous 1973 landmark judgement in the *Roe versus Wade* case where abortion was made a constitutional right in the United States. What does this mean? This would mean that abortion laws would be completely in the hands of the State Governments in the US. In almost 22 states, abortion would be banned or extremely restricted. And it is estimated that the number of legal abortions would fall by 14%.

Based on previous judgements, currently women in the US have the right to terminate pregnancy upto the point of fetal viability, considered to be 28 weeks, but with advancing science it has been brought down to 22-24 weeks. There have been many previous attempts to make abortion more restrictive.

Huge amount of non-controvertible evidence exists that banning abortions will have dire public health and economic consequences. It is predicted that maternity related deaths will rise from 1 in 3000 to 1 in 1000 for non Hispanic black women if abortions are banned in the US. Research from economists from the University of Michigan suggests that women who are denied abortions face significantly more financial catastrophes like debt, eviction and bankruptcy than women who have access to it. Banning abortion would fly in the face of reason and wisdom and will be remembered as a black moment in history.
(https://www.nature.com/articles/d41586-022-01249-2)

GOURI RAO PASSI
gouripassi@hotmail.com

Web Table I Signs Pointing Towards Diagnosis of Pancytopenia

Head	Microcephaly – FA
Eye	Pallor, jaundice Microphthalmia, epicanthal fold, strabismus - FA
Ears	Small and dysplastic ears Hearing defects- FA
Face	Elfin face -FA
Skin	Skin bleeds Malar rash – SLE Hyperpigmentation - FA/ DKC café au lait spots - FA Hypopigmented area - FA
Tongue	Oral leukoplakia – DKC
Neck	Sprengel shoulder, Klippel-Fiel anomaly - FA
Upper limb	Absence or hypoplastic radius - FA
Lower Limbs:	Toe syndactyly, abnormal toes Congenital hip dislocation - FA
Hand anomalies	Thenar hypoplasia, clinodactyly of 5th digit, syndactyly, hyperextensible thumbs - FA Knuckle pigmentation - vitamin B 12 and Folic acid deficiency Nail changes (dystrophy) - DKC
Short stature	FA
Cardiac	VSD - FA
Renal	Horseshoe shaped kidney and ureter abnormalities- FA
Gonads	In males: undescended testes, hypospadias, micropenis- FA In females: Hypogenitalia, bicornuate uterus, abnormal menses

FA: Fanconi Anemia, SLE: Systemic lupus erythematosus, DKC: Dyskeratosis congenital, VSD: Ventricular septal defect

Indian Academy of Pediatrics Consensus Guidelines for Adolescent Friendly Health Services

PREETI M GALAGALI,[1] CHANDRIKA RAO,[2] CHITRA DINAKAR,[3] PIYUSH GUPTA,[4] DHEERAJ SHAH,[5] SHILPA CHANDRASHEKARAIAH,[6] JAYASHREE KANTHILA,[7] DIGANT SHASTRI,[8] R REMESH KUMAR,[9] MKC NAIR[10]

From [1]Bengaluru Adolescent Care and Counselling Centre, Bengaluru, Karnataka; [2]Departments of Pediatrics, MS Ramaiah Medical College and Hospital, Bengaluru, Karnataka; [3]St John's Medical College Hospital and St John's National Academy of Health Sciences, Bengaluru, Karnataka; [4,5]University College of Medical Sciences and GTB Hospital, New Delhi; [6]Karanataka Institute of Medical Sciences, Hubballi, Karnataka; [7]Kasturba Medical College, Mangalore, Karnataka; [8]President and [9]Honorary Secretary-General, Indian Academy of Pediatrics, 2019; [10]NIMS-SPECTRUM-Child Development Research Centre, NIMS Medicity, Thiruvananathapuram, Kerala.

Correspondence to: Dr Preeti M Galagali, Director and Adolescent Health Specialist, Bengaluru Adolescent Care and Counselling Centre, 528 2nd Block Rajajinagar, Bengaluru 560 010, Karnataka. drpgalagali@gmail.com

Justification: Adolescent health is critical to the current and future well- being of the world. Pediatricians need country specific guidelines in accordance with international and national standards to establish comprehensive adolescent friendly health services in clinical practice. **Process**: Indian Academy of Pediatrics (IAP) in association with Adolescent Health Academy formed a committee of subject experts in June, 2019 to formulate guidelines for adolescent friendly health services. After a review of current scientific literature and drafting guidelines on each topic, a national consultative meeting was organized on 16 August, 2019 for detailed discussions and deliberations. This was followed by discussions over e-mail and refining of draft recommendations. The final guidelines were approved by the IAP Executive Board in December, 2021. **Objective**: To formulate guidelines to enable pediatricians to establish adolescent friendly health services. **Recommendations**: Pediatricians should coordinate healthcare for adolescents and plan for transition of care to an adult physician by 18 years of age. Pediatricians should establish respectful, confidential and quality adolescent friendly health services for both out-patient and in-patient care. The healthcare facility should provide preventive, therapeutic, and health promoting services. Pediatricians should partner with the multidisciplinary speciality services, community, and adolescents to expand the scope and reach of adolescent friendly health services.

Keywords: *Counseling, Health care transition, Standards, Universal health coverage, Young adults*.

Published online: April 26, 2022; ***PII:*** S097475591600423

India has the largest population of adolescents in the world numbering 251 million [1]. Investment in adolescent well-being will ensure health and progress of the nation. Adolescents need age appropriate information, a safe environment to develop life skills, and a responsive healthcare system [2]. Globally, health services for adolescents are disorganised, poorly coordinated and uneven in quality [3]. Rashtriya Kishor Swasthya Karyakaram (RKSK), the national adolescent health program of India, aims to establish adolescent friendly health services (AFHS) and community services for universal health coverage [1]. Indian Academy of Pediatrics (IAP) has been at the forefront of compre-hensive adolescent health care since more than two decades [4,5], and carried out this activity to further its agenda of providing quality adolescent healthcare.

NEED FOR ADOLESCENT FRIENDLY HEALTH SERVICES (AFHS)

Adolescents have a developmental need for autonomy and usually hesitate to seek help for the fear of being shamed, judged, and reprimanded. They prefer to seek confidential care regarding sensitive issues like sexuality, body image, mental distress, and substance use. Community-based surveys have revealed several barriers to the utilization of healthcare services (**Table I**) [6-8]. Need based AFHS should be designed by partnering with the adolescents and their caregivers to overcome these barriers [9,10].

AFHS are defined as developmentally appropriate comprehensive health services for health promotion, disease prevention and treatment of adolescents [11,12]. At AFHS, the adolescents feel safe, secure, and confident to discuss their problems, confide and seek help. WHO has outlined five essential criteria for qualifying health services as adolescent friendly (**Box I**).

Adolescent Health Policy and Programs

The first national adolescent health policy, Adolescent Reproductive and Sexual Health (ARSH) Strategy was initiated by Ministry of Health and Family Welfare in 2005 that outlined criteria for AFHS in India [13]. This was

Table I Barriers in Utilization of Adolescent Healthcare Services

Adolescents and caregivers	*Healthcare providers*	*Healthcare facility*
Ignorant about the importance of adolescent health and wellness check ups	Inadequate knowledge and training regarding AFHS	Lack of privacy
Lack of knowledge regarding availability of AFHS	Difficulty in implementation of consent and confidentiality as per existing laws	Lack of confidential healthcare services
Discomfort, embarrassment, shame and stigma towards sharing health concerns, especially those related to mental disorders and sexuality	Inability to provide care without parental involvement	Lack of training personnel
Lack of respect, privacy and confidentiality	Lack of clarity regarding policies of healthcare for married adolescents	Unaffordable healthcare
Overcrowding	Time constraints	Inconvenient location and timings
Prolonged waiting time		
Absence of same sex healthcare provider		Services do not cater to needs of
Financial constraints		adolescents

Prepared from material available in the references 32-34.

followed by the Reproductive, Maternal, Newborn Child plus Adolescent Health (RMNCH+A) policy in 2013 with focus on the continuum of healthcare over the entire life span [14]. In 2014, the National Adolescent Health Program called Rashtriya Kishor Swasthya Karyakaram (RKSK) was launched under the National Health Mission [1]. In 2018, under Ayushman Bharat, a school health program was launched to augment RKSK and Rashtriya Bal Swasthya Karyakram (RBSK) programs [15].

OBJECTIVE

To formulate guidelines for organizing adolescent friendly health services and to enable pediatricians to establish these services at the existing pediatric facilities.

PROCESS

The process of forming the IAP guidelines for AFHS was initiated on 1 June, 2019 with the formation of a national committee of subject experts in collaboration with Adolescent Health Academy (AHA). Six subgroups of experts including adolescent health specialists and pediatricians were formed to evaluate scientific evidence regarding existing adolescent health status and services, rationale and concept of AFHS, national adolescent health policy and program, training in adolescent health at undergraduate and postgraduate levels, age of pediatric care, basic AFHS and expanded/specialized adolescent services. Each sub-committee reviewed the published literature using search engines like PUBMED, SCOPUS, EMBASE. After multiple rounds of discussions, the sub-committees prepared draft guidelines pertaining to their respective topics. The draft guidelines were presented and discussed in depth at the National consultative meet conducted at Bangalore on 16 August, 2019. Further suggestions and the latest research were incorporated in the guidelines document, and the final document was prepared after consensus through a series of online and email discussions. The final guidelines were approved by the IAP Executive Board in December, 2021.

ADOLESCENT HEALTH: CURRENT STATUS

Adolescents (10-19 years), comprise 16% of the world population [16]. Their health is essential to achieve the sustainable developmental goals (SDG) by 2030 [17,18]. Adolescence is an age of vulnerability and opportunity [19]. Adolescents are vulnerable to risk taking behavior due to a highly reactive limbic system and reward centre, and an immature prefrontal cortex that controls emotions [19-21]. As the brain continues to mature until the late twenties, individuals from 10-24 years of age face similar risks to health and are grouped together as adolescents and young adults (AYAs) or young people. Young adults are defined as individuals between 18 to 24 years and youth as those from 15 to 24 years [17].

Lifestyle adopted in adolescence is known to track into adulthood. More than 70% of the premature mortality and morbidity in adults is due to diseases that begin in adolescence like malnutrition, substance use and mental disorders [17]. As the brain is still under construction in adolescence, there is a window of opportunity to intervene and motivate for a healthy lifestyle. Investment in adolescent health gives a triple dividend; it ensures the current health of adolescents, their health as adults and also of their children and future generations [20].

Canadian Pediatric Society and American Academy of Pediatrics have included adolescent care under the pediatric speciality [22-24]. In India, comprehensive care for

adolescents is sparse. Routine medical care is provided by general practitioners, physicians, pediatricians, gynecologists etc.

Healthcare transition (HCT) is defined as "a purposeful, planned process that addresses the medical, psychosocial, educational, and vocational needs of adolescents and young adults with chronic medical conditions, as they advance from a pediatric and familycentred to an adult, individual focused health care provider" [25]. There is a need to establish 'transition clinics' that promote shared care between the pediatric and the adult physicians and collaborations with the adolescents, parents, educa-tors, social workers and other healthcare professionals. The precise age for HCT depends on the developmental age of the patients, their health care needs, parental support and the expertise of the treating physician [26]. Studies from India have also reiterated the need for HCT services [27,28].

Indian Scenario

Adolescents comprise 18% of the Indian population [29]. Among young people aged 15 to 24 years, 28% are not in education, employment or training [29], and 27% of adolescent girls are married and 1% have experienced sexual violence [30]. In the last decade, an increase in high-risk sexual behavior from 64 to 70% has been estimated, among adolescent boys [31].

Diarrhea and tuberculosis are prevalent across all age groups and in both sexes. There exists a triple burden of malnutrition. There is high prevalence of short stature, underweight, anemia, and micronutrient deficiencies on one hand; whereas, 5% of adolescents are obese and over 70% have insufficient physical activity [32]. The prevalence of non-communicable diseases like hypertension, diabetes mellitus, mental disorders and smart-phone addiction is 5%, 15%,14% and 42%, respectively [32-34].

GUIDELINES

Age of Adolescence and Pediatric Care in India

Adolescent healthcare should be supervised and coordinated by a pediatrician from 10 to 18 years of age. This is in concurrence with the UN Convention on Child Rights (CRC) [35] and the current Indian laws. The Juvenile Justice Act 2015, Protection of Children from Sexual Offences Act 2012, RBSK and IAP define childhood similarly [4,36-38]. According to the Indian laws, an individual above the age of 18 years is considered as an adult [39]. Pediatricians, who follow most children from the newborn period onwards, and share a rapport with the child/adolescent and their family and are knowledgeable about the growth and development are the most suitable medical professionals to provide integrated health care to adolescents.

Age of Transition of Care to an Adult Physician

The transition to adult care should preferably be accomplished by 18 years of age. It should be conducted in a phased manner over a few months to years with due consideration for the individual patient and family needs [40,41]. A written HCT policy should be framed with roles of the pediatric team, the adult care team and allied health professionals clearly defined including preparing and motivating the adolescents and their families for transition with the help of a dedicated clinical coordinator and peer and family support groups. The adolescents should be ready and mature enough for the change, which involves taking primary responsibility for their own medical needs. They should understand all aspects related to the disease, treatment, adherence, prognosis and implications during adulthood. Prior to the final transfer, a detailed medical summary is made and a combined management plan discussed with the adult physician, adolescent and family. Wherever feasible, it is recommended that a transition clinic be established with continuum of 'shared care' for a few months/years pre- and post-transition with periodic feedback from the patient regarding quality of services provided.

Basic Adolescent Health Services

All pediatric facilities should provide AFHS. In outpatient care, pediatricians should schedule exclusive time for adolescent healthcare. AFHS should be designed according to the WHO's global standards for quality services [3].

Box I World Health Organization Criteria for Adolescent Friendly Health Services

Accessible: Services to be available on all weekdays. To be established in existing health facilities (e.g. hospitals and clinics) or in community settings (e.g. schools and anganwadis)

Acceptable: Respectful and confidential care to be provided

Equitable: Affordable healthcare to attain universal health coverage. The services to cater to the needs of all adolescents including urban, rural, out of school, orphans, disadvantaged, marginalised and married

Appropriate: Need-based preventive, curative and counselling services to be provided with the healthcare personnel adopting a respectful and non-judgemental attitude

Effective: Services to follow a standards driven quality improvement approach with an inbuilt mechanism to assess effectiveness at regular intervals along with a feedback from the adolescent clients

Prepared from material available in references 37 and 38.

In routine care, the pediatrician should spend a few minutes alone with every child above the age of 10 years, with parental consent, to elicit a brief HEEADSSS history and discuss management [5]. This would help in rapport building and convey the importance of confidential adolescent health care to the family. Before establishing AFHS, the pediatricians, should familiarize themselves with management of common adolescent health issues and adolescent care facilities in the community, train the ancillary health care staff (e.g., receptionist, laboratory technician) in the nuances of adolescent communication and establish medical, school and community networks to increase the scope and reach of the services. The existing out-patient pediatric facility can be converted into an adolescent friendly clinic by adopting the following changes in the structure, policy and clinical approach [39,42,43].

Structure of the adolescent clinic

- Waiting area should have an adolescent friendly décor, without baby posters and toys. Age appropriate health education material, posters, pamphlets and booklets should be available.
- Essential health care personnel should be available during the working hours of the clinic. These include a paediatrician, receptionist, a nurse and a laboratory technician (if a laboratory is attached to the clinic)
- Healthcare services that are provided by the clinic should be displayed in the waiting area (**Box II**)
- Consultation and examination rooms should preferably be separate with a door to allow for privacy. If a separate room is not available, the examination area should be cordoned off by a screen
- Equipment and materials at the center include orchidometer, adult sized stethoscope, blood pressure cuff, examination cot and weighing machine, stadiometer, IAP growth reference data, blood pressure centile, Tanners, immunization and Snellen charts, questionnaires like HEEADSSS, Patient Health Questionnaire-2 (PHQ-2), Screening for Childhood Anxiety Related Emotional Disorders (SCARED), Screening to Behaviour Intervention (S2BI) and Ask Suicide- Screening Questionnaire (ASQ) to screen for psychosocial issues, depression, anxiety, drug use and suicidal behavior respectively (**Web Box I**) [44-47]. Teen screening questionnaire-Mental Health (TSQ-M) is a validated Indian tool to screen for mental disorders [48].

Clinic Policy

- Exclusive timings for adolescent clinic should be scheduled during the day as adolescents do not like to be in the company of younger children at a healthcare facility. The timings should be convenient e.g adolescents going to school would prefer to access the healthcare facility in the evening.
- Registration should be easy and quick. The recep-tionist should be sensitive towards adolescents.
- Consultation should be both by walk-in and by appointment, and the waiting period should be minimal.
- Professional charges should be affordable and flexible to cater to the needs of marginalized adolescent.
- Consent and confidentiality policy should be according to the existing Indian laws. Adolescents above the age of 12 years can give consent only for history taking and examination and those above 18 years can give consent for investigation and drug therapy [39]. Assent should be taken from all adolescents for medical interventions in addition to the mandatory parental consent.
- Medical records should be kept confidential and privacy should be protected.
- Feedback from the adolescents and the parents should be taken regarding their satisfaction with healthcare services. Suggestions to improve should also be elicited and implemented [49,50].
- Regular evaluation regarding quality of services should be conducted [50].
- An adolescent friendly referral network comprising of (but not limited to) gynecologists, dermatologists, psychiatrists, psychologists, endocrinologists, orthopedic surgeons, dietitian, social workers, remedial educators, and NGOs should be established with their contact details readily available with the receptionist. The feasibility of conducting weekly specialized clinics at the AFHS should be discussed with the specialists to facilitate multidisciplinary care. [39]

Clinical Approach

Pediatricians should be sensitive and empathetic towards adolescents and their families. A clinical encounter with an adolescent usually takes 30-45 minutes. The first 5-10 minutes are spent with the adolescent and parents, the next 20

Box II Suggested List of Services at Adolescent Friendly Healthcare Center

- Management of physical and mental disorders
- Counselling
- Annual health and wellness check-ups
- Sports pre participation evaluation
- Immunization
- Sexuality and life skill education sessions
- Parental guidance
- Premarital counselling

minutes with the adolescent alone for eliciting a history, doing a physical examination, imparting anticipatory guidance and discussing the management. The last 10 minutes are spent with the patient and his/her parents reviewing the treatment plan. Personal information shared by the adolescent is kept confidential within limits. Confidentiality is broken in cases of child sexual abuse (CSA), suicidal behavior or ideation, conflict with law or hospitalization [39]. Under the Protection of Children from Sexual Offences Act (POCSO), cases of CSA have to be reported mandatorily to the police [37].

History taking: A routine pediatric history should be taken in the presence of parents or caregivers. Later a personal interview is conducted with the adolescent. Confidentiality rules should be expressed clearly before the interview. Ensuring auditory and visual privacy is essential to maintain an adolescent friendly ambience. Patient centered, effective communication is the corner-stone of establishing a therapeutic relationship. A clinician should master the art of verbal, non-verbal and active listening skills and ask open ended questions [39,43]. HEEADSSS is a tool for structuring the medical interview with the adolescents and their parents [51], and is a mnemonic and covers questions to ask in four important domains namely peer, family, self and academic. It indicates problem areas (e.g., substance use), identifies need of additional medico-social support services (e.g., HIV infection) and prioritizes management areas. It also identifies strengths of the adolescents and supportive adults who can partner to provide care. Screening questionnaires (**Web Box I**) and DSM 5 criteria aid in the diagnosis of mental disorders.

Physical examination: A male pediatrician should ensure the presence of a female health professional while examining a female adolescent. The pediatrician should assess weight, height and BMI (with plotting on growth chart), sexual maturity rating (SMR), blood pressure and examine vision, hearing, teeth, skin, thyroid and spine along with the conventional problem directed examination [39]. If an adolescent is reluctant for the genitalia examination during the first visit, SMR can be self-reported by visualizing the Tanner charts. In subsequent visits, after establishing rapport, SMR can be evaluated in privacy. Adolescents should be taught the technique of breast and testicular self-examination. Pelvic examination is conducted, if the adolescent is sexually active.

Anticipatory guidance: Every adolescent visit is an opportunity to provide anticipatory guidance to promote a healthy lifestyle and prevent high risk behavior [39]. (**Box III**) Positive coping strategies and protective factors like participation in hobbies, sports and parental and school connectedness are reinforced and encouraged. Adole-scents and their families are motivated to complete the IAP recommended adolescent immunizations (HPV, TdaP), catch up vaccinations (MMR, hepatitis B, chicken pox, typhoid and hepatitis A) and vaccinations for special situations (influenza, pneumococcal, meningococcal and COVID-19) [52].

Management

- There should be a shared decision making with the adolescent and their parents regarding therapy and follow up [53]. To motivate adherence, the relevance, risks and rewards of the therapy should be highlighted and road blocks, if any should be addressed [54].
- Most of the issues causing mental distress in adolescents can be managed by counselling [43]. During counselling, the pediatrician, should provide accurate scientific information, and consider the cognitive development, psycho-social, financial and spiritual needs of the clients while guiding them through the steps of decision making and behavior change.
- Collaborative care in the form of partnerships with parents, educators, peers, social workers and multi-disciplinary professionals is essential.
- Indications for urgent referral to 'adolescent friendly' mental health professionals are suicidal behavior, severe substance use disorder, psychoses and multiple co-morbidities.

Annual wellness visits: An annual health check-up is recommended for all adolescents to address health concerns and screen for medical disorders [55,56] (**Box IV**).

Inpatient care: Adolescent wards in all hospitals should provide uniform adolescent preventive and curative services as outlined above. A separate ward for boys and girls should be allocated for admitting adolescents from 10 to 18 years.

Box III Components of Anticipatory Guidance

- Information on normal development, nutrition, physical activity, sleep
- Study skills
- Immunization
- Hygiene, handwashing technique and dental care
- Menstrual hygiene
- Injury prevention
- Handling peer pressure and bullying
- Media literacy and addiction, cyber bullying.
- Responsible sexual behavior and safe sexual practices
- Substance use prevention
- Parental guidance
- Life skills
- Important laws e.g., POCSO, cyber laws, Narcotics Drugs Psychotropic Substances Act, Juvenile Justice Act

The wards should be located in the department of pediatrics, and the patient care and medical protocol should be coordinated by pediatricians. All healthcare professionals working in these wards including the doctors from different specialties, trainee doctors, nurses, laboratory and *X*-ray technicians, ward boys, helpers, etc. should undergo training to provide adolescent friendly healthcare.

Expanded AFHS

Expanded AFHS means extending the scope of care beyond the pediatric facility. AFHS can be specialized and expanded according to the medical specialty providing healthcare (e.g., gynecology, pediatric surgery) or related to the site of delivery of services (e.g., schools, community centers, telehealth).

Specialty care: Each pediatric specialty (e.g., pediatric surgery, ophthalmology, endocrinology, etc.) must be enabled and empowered to have access to basic training and protocols to assess adolescent relevant issues. Every adolescent seen in outpatient or in inpatient care must be seen by a pediatrician. The key 'friendly' components of AFHS must be followed in all specialty areas. Each specialty should have networking and partnership with the AFHS in the hospital and in the community. Specialty clinics (e.g. gynecology, dermatology, pulmonology, endocrinology, nephrology) could be conducted for adolescents on a weekly or biweekly basis to enable multidisciplinary care at the existing adolescent clinic in the hospital.

Extended delivery of adolescent services: According to the Lancet Commission on Adolescent Health and Wellbeing, opportunities exist for AFHS to be delivered across various platforms (**Web Table I**) [20]. AFHS could be established at schools, communities' health centers, m-health, media and social marketing, and through structural actions [57,58] Pediatricians should partner with these interdependent platforms and advocate for quality care at all levels.

Some strategies for advocacy related to AFHS are detailed in **Web box II**.

CONCLUSION

The implementation of the guidelines shall meet the need of integrated, accessible, equitable, effective and quality healthcare for adolescents (**Box V**). To provide adequate adolescent care, pediatricians, need to adopt appropriate AFHS measures and modify the existing pediatric facility. They should take care to include adolescents, healthcare professionals, community and the government in planning the services. Benchmarking of services should be done at regular intervals. A change in medical curriculum, reframing of current laws, conducting need-based research, and AFHS innovations on the digital platform is likely to revolutionize adolescent healthcare in the future.

Contributors: All the authors and committee members made important intellectual contribution to the guideline document, and have approved the final manuscript. *Funding*: None; *Competing interest*: None stated.

Note: Additional material related to this article is available at *www.indianpediatrics.net*

REFERENCES

1. Adolescent Health Division, Ministry of Health and Family Welfare Government of India. Strategy Handbook. Rashtriya Kishor Swasthya Karyakram. Government of India; 2014.
2. World Health Organisation. Adolescent health. Accessed December 18, 2021. Available from: *https://www.who.int/health-topics/adolescent-health#tab=tab_1*
3. World Health Organisation. Global standards for quality health-care services for adolescents: a guide to implement a standards-

Box IV Components of Annual Wellness Visits

Screening for psycho social stressors, high risk behavior, mental disorders, immunization status

HEEADSSS psychosocial history

Screening questionnaires: Patient Health Questionnaire-2 (PHQ-2), Screening for Childhood Anxiety Related Emotional Disorders (SCARED), Screening to Behavior Intervention (S2BI), Ask Suicide- Screening Questionnaire (ASQ)

Immunization history

Physical examination

Weight, height, body mass index (BMI), blood pressure, visual acuity, dental and systemic examination

Anticipatory guidance for adolescents and parents

See box III

Laboratory investigation

- Hemoglobin
- In sexually active adolescents – annual screening for HIV and Syphilis. First void urine for leucocytes in boys (screening test for sexually transmitted infections) and swab for gram stain/culture/KOH wet mount for girls.
- Oral glucose tolerance test, and lipid profile if obese and/or family history of a death in a first degree relative due to a cardiovascular event at <55 years of age

HEEADSSS: home, education/employment, eating, activities, drugs, sexuality, suicide/depression, and safety.

Box V Summary: IAP Consensus Guidelines for Adolescent Friendly Health Services

1. *Age of adolescence and pediatric care*: Adolescent healthcare from 10 to 18 years of age should be under the purview of the pediatrician
2. *Transition to adult care*: The transition should be preferably ensured by 18 years of age. It should be conducted in a planned and phased manner.
3. *Basic AFHS*: All pediatric facilities should provide adolescent responsive care, be aware about laws regarding consent and confidentiality, conduct a one on one HEEADSSS psychosocial interview, screen and manage common adolescent health problems, impart anticipatory guidance, do need based counselling for parents and adolescents and foster a multidisciplinary adolescent friendly referral network.
4. *Expanded AFHS*: Based on their experience and expertise, pediatricians should consider setting up multispecialty and multisite adolescent friendly services by partnering and collaborating with other health professionals, adolescents, caregivers and the community.
5. *Advocacy*: Every pediatrician should be an advocate for AFHS. They should implement the guidelines in clinical practice and widely disseminate these amongst health professionals.

driven approach to improve the quality of health care services for adole-scents. Volume 1: Standards and criteria. Geneva: World Health Organization; 2015.
4. John TJ. IAP policy on age of children for pediatric care. Indian Pediatr. 1999; 36:461-3.
5. Shastri D. Respectful adolescent care - A must know concept. Indian Pediatr.2019;56:909-10.
6. Sreekumar S, Ramakrishnan J, Harisankar D, Mannethodi K. Felt needs and expectations of adolescents regarding sexual and reproductive health from schools and health systems: A descriptive study. Indian J Sex Transm Dis AIDS. 2019;40:30-34.
7. Santhya KG, Prakash R, Jejeebhoy SJ, Singh SK, et al. Accessing adolescent friendly health clinics in India: The perspectives of adolescents and youth. Population council; 2014.
8. Kumar T, Pal P, Kaur P. Health seeking behavior and health awareness among rural and urban adolescents in Dehradun District, Uttarakhand, India. Int J Adolesc Med Health. 2017;29(2):/j/ijamh.2017.29.issue-2/ijamh-2015-0046/ijamh-2015-0046.xml. doi: 10.1515/ijamh-2015-0046
9. Mahalakshmy T, Premarajan KC, Soundappan K, et al. A Mixed methods evaluation of adolescent friendly health clinic under National Adolescent Health Program, Puducherry, India. Indian J Pediatr. 2019 ;86:132-9.
10. Sivagurunathan C, Umadevi R, Rama R, Gopalakrishnan S. Adolescent health: Present status and its related programmes in India. Are we in the right direction? J Clin Diagn Res. 2015;9: LE01-6.
11. World Health Organization. Making health services adolescent friendly. Developing national quality standards for adolescent friendly health services. World Health Organization. 2012.
12. Ministry of Health and Family Welfare, Government of India. Implementation Guidelines Rashtriya Kishor Swasthya Karyakram (RKSK). Government of India;2018.
13. Ministry of Health and Family Welfare, Government of India. Implementation Guide on RCH II Adolescent Reproductive Sexual Health Strategy for State and District Programme Managers. Government of India; 2006. Accessed December 18, 2021. Available from: *http://www.searo.who.int/entity/child_ adolescent/topics/adolescent_health/rch_ asrh_ i ndia.pdf*
14. Ministry of Health and Family Welfare Government of India A Strategic Approach to Reproductive, Maternal, Newborn, Child and Adolescent Health (RMNCH+A) in India. Government of India; 2013. Accessed December 18, 2021. Available from: *http:/ /nhm.gov.in/images/pdf/ RMNCH +A/RMNCH+A_Strategy.pdf*
15. Ministry of Health and Family Welfare Ministry of Human Resource Development. Government of India. Operational Guidelines on School Health Programme under Ayushman Bharat Health and Wellness Ambassadors partnering to build a stronger future. Government of India; 2018.
16. World Health Organization. Adolescent data. Accessed December 18, 2021. Available from: *https://platform.who.int/ data/maternal-newborn-child-adolescentageing/adolescent-data*
17. Global Accelerated Action for the Health of Adolescents (AA-HA!): guidance to support country implementation.World Health Organization; 2017. Accessed December 18, 2021. Available from: *https://apps.who.int/iris/bitstream/handle/10665/255 415/9789241512343-eng.pdf?sequence=1*
18. World Health Organisation and UNICEF. A Vision for Primary Health Care in the 21st Century. Towards universal health coverage and the sustainable development goals. Almaty: World Health Organization and UNICEF; 2018.
19. Sawyer SM, Azzopardi PS, Wickremarathne D, Patton GC. The age of adolescence. Lancet Child Adolesc Health. 2018;2:223-28.
20. Patton GC, Sawyer SM, Santelli JS, et al. Our future: A Lancet commission on adolescent health and wellbeing. Lancet. 2016;387: 2423-78.
21. Patton GC, Olsson CA, Skirbekk V, et al. Adolescence and the next generation. Nature. 2018;554:458-66.
22. Hardin AP, Hackell JM, AAP committee on practice and ambulatory medicine. Age Limit of Pediatrics. Pediatrics. 2017; 140:e2017215117.
23. Sacks D; Canadian Pediatric Society. Age limits and adolescents. Paediatr Child Health. 2003;8:577-78.
24. Sawyer SM, McNeil R, Francis KL, et al. The age of pediatrics. Lancet Child Adolesc Health. 2019;3:822-30.
25. Carrizosa J, An I, Appleton R, et al. Models of transition clinics. Epilepsia. 2014;55:46-51.
26. Schmidt A, Ilango SM, McManus MA, et al. Outcomes of pediatric to adult health care transition interventions: An updated systematic review. J Pediatr Nurs. 2020;51:92-107.
27. Verma A, Sahay S. Healthcare needs and programmatic gaps in transition from pediatric to adult care of vertically transmitted HIV infected adolescents in India. PLoS One. 2019;14: e0224490.
28. Menon J, Peter AM, Nayar L, Kannankulangara A. Need and feasibility of a transition clinic for adolescents with chronic illness: A qualitative study. Indian J Pediatr. 2020;87:421-26.
29. World Health Organisation. Country Profile: India. Accessed December 18, 2021. Available from: *https://platform.who.int/ data/maternal-newborn-child-adolescentageing/static-visualizations/adolescent-country-profile*
30. International Institute for Population Sciences (IIPS) and ICF. National Family Health Survey (NFHS-4), 2015-16: India. IIPS. Accessed Dec 18, 2021. Available from: *http://rchiips.org/*

NFHS/NFHS-4Reports/India.pdf
31. Sharma SK, Vishwakarma D. Transitions in adolescent boys and young men's high risk sexual behavior in India. BMC Public Health.2020;20:1089-103.
32. Sethi V, Lahiri A, Bhanot A, et al. Adolescents, Diets and nutrition: Growing well in a Changing World, The Comprehensive national nutrition survey, Thematic Reports, Issue 1, 2019.
33. United Nations Children's Fund. The State of the World's Children 2021: On my mind – promoting, protecting and caring for children's mental health. UNICEF, 2021.
34. Davey S, Davey A. Assessment of smartphone addiction in Indian adolescents: A mixed method study by systematic-review and meta-analysis approach. Int J Prev Med. 2014; 5:1500-11.
35. United Nations. Convention on the Rights of the Child. UNGA resolution 44/25. United Nations; 1989
36. The Juvenile Justice Act 2015. Accessed December 18, 2021. Available from: *http://cara.nic.in/PDF/JJ%20act%202015.pdf*
37. The Protection of Children from Sexual Offences Act 2012. Accessed December 18, 2021. Available from: *https://wcd.nic.in/sites/default/files/POCSO%20Act%2C%202012.pdf*
38. Ministry of Health and Family Welfare, Government of India. Child Health Screening and Early Intervention Services National Rural Health Mission. Government of India; 2013. Accessed Dec 18, 2021. Available from: *http://nhm.gov.in/images/pdf/programmes/RBSK/Resource_Documents/RBSK%20Resource%20Material.pdf*
39. Bhave S editor. Bhave's Textbook of Adolescent Medicine. PeePee Publishers, 2016.
40. Bert F, Camussi E, Gili R, et al. Transitional care: A new model of care from young age to adulthood. Health Policy. 2020;124:1121-28.
41. White PH, Cooley WC; Transitions Clinical Report Authoring Group; American Academy of Pediatrics; American Academy of Family Physicians; American College of Physicians. Supporting the Health Care Transition from Adolescence to Adulthood in the Medical Home. Pediatrics. 2018;142: e20182587.
42. Bali S, Yadav K, Alok Y. A study of physical infrastructure and preparedness of Public Health Institution for providing adolescent friendly health services in Central India. Ind J Prev Soc Med. 2020;51:195-203.
43. Ministry of Health and Family Welfare, Government of India.Training Module for Orientation Program for Medical Officers on Adolescent Friendly Health Services under Rashtriya Kishor Swasthya Karyakram. Government of India; 2015.
44. Russell PS, Basker M, Russell S, et al. Comparison of a self-rated and clinician rated measure for identifying depression among adole-scents in a primary-care setting. Indian J Pediatr. 2012;79: S45-51.
45. Russell PS, Nair MKC. Rationale and study design for anxiety disorders among adolescents in a rural community population in India. Indian J Pediatr. 2013;80:S132-8.
46. Demaso DR, Walter HJ, Wharff EA. Suicide and attempted suicide. *In:* Kleigman RM, Geme JS, editors. Nelson Textbook of Paediatrics. 21st ed, Elsevier; 2019.p.159-62.
47. Levy S, Weitzman ER, Marin AC, et al. Sensitivity and specificity of S2BI for identifying alcohol and cannabis use disorders among adolescents presenting for primary care. Subst Abus. 2021;42:388-395.
48. Nair MKC, Chacko D, Rajaraman V, et al. The diagnostic accuracy and validity of the teen screen questionnaire-mental health for clinical and epidemiological studies in primary-care settings. Indian J Psychol Med. 2014;36:187-91.
49. Ambresin AE, Bennett K, Patton GC, et al. Assessment of youth-friendly health care: a systematic review of indicators drawn from young people's perspectives. J Adolesc Health. 2013;52: 670-81.
50. World Health Organisation. Global standards for quality health-care services for adolescents: guide to implement a standards-driven approach to improve the quality of health care services for adolescents. Vol. 2: Implementation guide. WHO; 2016.
51. Klein M, Goldenring JM, Adelman W. HEEADSSS 3.0: The psychosocial interview for adolescents updated for a new century fuelled by media.Contemp Pediatr. 2014 Accessed December 18, 2021. Available from: *https://www.contemporarypediatrics.com/view/heeadsss-30-psychosocial-interviewadolescents-updated-new-century-fueled-media*
52. Kasi SG, Shivananda S, Marathe S, et al. Indian Academy of Pediatrics (IAP) Advisory Committee on Vaccines and Immunization Practices (ACVIP): Recommended Immunization Schedule (2020-21) and Update on Immunization for Children Aged 0 Through 18 Years. Indian Pediatr. 2021; 58:44-53
53. World Health Organisation. Assessing and supporting adolescents' capacity for autonomous decision-making in healthcare settings: a tool for health-care providers. World Health Organization; 2021.
54. Miller WR, Rollnick S. Motivational interviewing: helping people change. 3rd edition. Guilford Press; 2013.
55. Hagan J, Shaw J, Duncan P, editors. Bright Futures: Guidelines for Health Supervision of Infants, Children, and Adolescents: Pocket Guide. 4th ed. American Academy of Pediatrics; 2017.
56. Webb MJ, Kauer SD, Ozer EM, et al. Does screening for and intervening with multiple health compromising behaviors and mental health disorders amongst young people attending primary care improve health outcomes? BMC Fam Pract. 2016;17:104.
57. Sawyer SM, Reavley N, Bonell C, et al. Platforms for Delivering Adolescent Health Actions. *In*: Bundy DAP, Silva Nd, Horton S, et al., editors. Child and Adolescent Health and Development. 3rd edition. The World Bank; 2017.
58. Galagali PM, Brooks MJ. Psychological care in low-resource settings for adolescents. Clin Child Psychol Psychiatry. 2020; 25:698-711.

ANNEXURE

IAP Guidelines Committee on Adolescent Friendly Health Services

Chairpersons: MKC Nair, Digant D Shastri; ***Convener***: Preeti M Galagali; ***Writing Committee Members***: Shilpa Chandra-shekaraiah, Chitra Dinakar, Preeti M Galagali, Piyush Gupta, Jayashree Kanthila, Chandrika Rao, Dheeraj Shah; ***Members***: Harmesh Bains, CP Bansal, Poonam Bhatia, Swati Y Bhave, Sukanta Chaterjee, JC Garg, Atul Kanikar, Sonia Kanitkar, Latha Ravichandran,, MN Venkiteswaran; ***Rapporteur***: Kritika Agarwal; **Ex-officio**: Remesh Kumar. (*All members attended the National Consultative Meet at Bengaluru on 16 August, 2019 except CPB and S Chaterjee*).

Web Box I Screening Tools for Common Mental Disorders

Depression screening

Patient Health Questionnaire-2 (PHQ-2): *https://aidsetc.org/sites/default/files/resources_files/PHQ-2_English.pdf*

Becks Depression Inventory (BDI): *https://www.ismanet.org/doctoryourspirit/pdfs/Beck-Depression-Inventory-BDI.pdf*

Patient Health Questionnaire-9: Modified for teens (PHQ-9): *https://www.aacap.org/App_Themes/AACAP/docs/member_resources/toolbox_for_clinical_practice_and_outcomes/symptoms/GLAD-PC_PHQ-9.pdf*

Anxiety disorder screening

Screen for Child Anxiety Related Emotional Disorders (SCARED): *https://www.ohsu.edu/sites/default/files/2019-06/SCARED-form-Parent-and-Child-version.pdf*

Substance use disorder screening

Screening to brief intervention tool (S2BI): *https://www.drugabuse.gov/ast/s2bi/#/*

Suicide screening

Adolescent Suicide- Screening Questionnaire (ASQ): *https://www.nimh.nih.gov/research/research-conducted-at-nimh/asq-toolkit-materials/asq-tool/asq-screening-tool*

Web Box II IAP Advocacy – Strategies for AFHS

1.Dissemination of guidelines to all IAP members and share with other professional organizations like Indian Medical Association, Federation of Obstetrics and Gynecology Institutions and Community Medicine.	5. Suggest to government to approve extension of adolescent healthcare up to 18 years under the purview of pediatricians with universal implementation. Extending the adolescent age up to 24 years to be considered as per existing global practice.
2. Conduct training workshops for pediatricians, allied health professionals, parents, teachers and adolescents in AFHS.	6. Emphasize on the need to review certain laws e.g., POCSO and laws regarding consent and confidentiality, especially for married and sexually active adolescents to enable access to health services without legal liabilities. Rigorous enforcement of the Motor Vehicle Act, Narcotic Drugs Psychotropic Act and laws for nutrient labelling of packaged food items. Stronger laws for online safety and to restrict the access of minors to pornography and online child sexual abuse material .A change in the nature of laws from being punitive to more reformatory is recommended.
3. Ensure that AFHS is an integral part of the department of pediatrics at all medical colleges, hospitals and private healthcare establishments. Update the IAP adolescent health card. Encourage research on various adolescent health issues	
4. Suggest to NMC to include hospital and community based AFHS skill training sessions with emphasis on mental health evaluation, digital wellness, counselling, trauma informed care and planning transition to adult care in undergraduate and postgraduate curriculum. Evaluation of medical trainees to include the above. Strive for NMC approved fellowships and a super specialty course in adolescent health.	7. IAP should have a public private partnership with the government to strengthen RKSK and AFHS. Telehealth, tele-counselling, m-Health and digital health services should be integrated into AFHS and RKSK with active participation of all stakeholders including adolescents in its design.

Web Table I Expanded Adolescent Friendly Health Services

Health care Domains Addressed	*Expanded Action Plan at various levels*				
	Health services	*Schools*	*Communities*	*Mobile health*	*Media and social marketing*
Sexual and reproductive health, including HIV/AIDS	Early diagnosis and treatment of HIV/AIDS and STDs Antenatal, delivery, postnatal care, condoms, contraceptives Transition to adult care for HIV/AIDS Centres to handle POCSO cases	Comprehensive sexuality education Safe schools with clean toilets and facilities for menstrual care Peer-led interventions Teen clubs	Positive youth development Peer education Teen clubs	Targeting of knowledge, attitudes, and risk behaviors Promote knowledge of prevention of sexual abuse	Promotion of community support for sexual and reproductive health and HIV/AIDS health access
Malnutrition (under and over nutrition, micronutrient deficiencies)	Screening and micronutrient supplementation Management of co-morbidities	Micronutrient supplements Healthy school meals, physical activity	Micronutrient and protein-energy supplements De-worming Nutrition education	Interactive personalized feedback	Junk food advertising restrictions Campaigns to build community awareness
Vaccine-preventable and infectious diseases	Early identification and treatment Vaccinations De-worming Bed net distribution	Vaccinations De-worming	De-worming Bed net distribution	Vaccine reminders via SMS	Campaigns to build community awareness
Injury and violence	Trauma care, including first responders (ambulances) Screening for mental disorders	Multi component interventions targeting violent behavior and substance use	Promotion of parental skills, communication, gender equality Economic empowerment Police enforcement of traffic rules		Promotion of knowledge of the effects of violence and available services
Tobacco, alcohol, and illicit drugs	Risk screening Motivational interviewing to promote cessation	Alcohol and smoke-free policies Parent and teacher training and monitoring groups.	Promotion of positive parenting skills Mentoring	Targeting of knowledge, attitudes, and risk behaviours Text messaging to encourage quitting	Promotion of adolescent mental health literacy
Mental disorders, including suicide	Practitioner training for management Routine assessment of mental health, including suicide risk	Educational interventions Gatekeeper training Mental health services and life skills education	Gatekeeper training	Suicide and psychosocial wellness help lines.	Promotion of adolescent mental health literacy
Chronic physical disorders	Management of condition Promotion of self-management and transition to adult health care	School-based health services	Peer support initiatives	Monitoring control and providing guidance regarding management	
Structural Actions	Legislation, taxation, and implementation of policies are essential structural actions to improve adolescent health. Indeed, for many health risks, such as tobacco and alcohol, road traffic injuries, violence, unsafe work, and obesity, structural actions are the most effective interventions for adolescent health				

Prepared from material available in the references 10,67-69

Practical Approach to the Interpretation of Complete Blood Count Reports and Histograms

SONALI DIXIT,[1] TANVI JHA,[1] RICHA GUPTA,[1] DHEERAJ SHAH,[2] NITIN DAYAL,[3] MRINALINI KOTRU[1]

From Departments of [1]Pathology and [2]Pediatrics, University College of Medical Sciences and GTB Hospital, Delhi; [3]Department of Hematology, Max Super Speciality Hospital, Saket, New Delhi.

Correspondence to: Dr Mrinalini Kotru, Department of Pathology, University College of Medical Sciences and GTB Hospital, Dilshad Garden, Delhi 110095. mrinalini.kotru@gmail.com

Improvement in technology and inclusion of new parameters in automated hematology analyzers allows for better and faster detection of anemias. These parameters along with histograms provide details and clues that help to diagnose the etiology of anemia and help bridge the time lag in detection and treatment. Timely and expert interpretation of complete blood counts should not be limited to the pathologist but should also interest the clinician to allow for efficient patient care.

Keywords: *Anemias, Platelet indices, Red cell distribution width, Reticulocyte indices.*

Automated hematology analyzer is a cost-effective strategy in resource-limited settings for diagnosis of common blood disorders [5]. Moreover, given the difficulty of blood sampling in the pediatric age group, extracting the maximum possible information from each investigation is essential. Modern-day automated hematology analyzers, with their constantly expanding assessment parameters provide a good, quick overview of the patients' blood picture [6]. We, herein, provide a framework to better interpret complete blood count (CBC) parameters and histograms, which can serve as an invaluable tool towards diagnosing childhood anemias.

INTERPRETATION OF CBC PARAMETERS

CBC is the first investigation routinely performed in both in-patient and out-patient settings. Most automated hematology analyzers generate: numerical and graphical data. Numerical data is in the form of measurement of various WBC, RBC and platelet parameters. The automated analyzers also provide RBC, WBC, and platelet histograms derived by plotting each cell's size on the *X*-axis and their relative number on the *Y*-axis. Interpretation of these CBC parameters and histograms together helps us assess the presentation and etiology of anemia without requiring other expensive investigations.

RBC Parameters

Automated hematology analyzers provide information on RBC parameters like hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), RBC count and hematocrit (HCT), that are used to assess the type of anemia, the treatment response and long-term follow-up of patients (**Table I**) [7,8]. Anemias are classified based on MCV and morphology as normocytic, microcytic and macrocytic.

All macrocytic anemias are evaluated and treated for vitamin B12 and folate deficiency. Non-megaloblastic macrocytic anemia requires further evaluation of reticulocyte count, features of hemolysis and interpretation of bone marrow aspirate smears. Reticulocyte count is necessary for the evaluation of normocytic normochromic anemias. When this is <3%, megaloblastic anemia, liver disease and hypothyroidism, must be ruled out. However, when >3%, further evaluation of WBC and platelet parameters followed by bone marrow evaluation may be required [8]. Further, RDW informs us regarding RBC anisocytosis and helps differentiate between pure micro/normocytic and mixed red cell populations. Recently reticulocyte indices have been introduced in many high-end counters. Newer reticulocyte indices (**Table I**) provide information regarding treatment-response in conditions such as nutritional anemia [9]. They also have a role in diagnosis and monitoring of aplastic anemia [10,11]. Newer parameters, additionally, provide information that has made the detection of type and cause of anemias easier and may, over time, reduce the dependence on peripheral blood smear examination for all cases [12]. They also help distinguish between the various etiologies of anemia such as iron deficiency anemia (IDA), anemia of chronic disease (ACD), anemia of inflammation (AI) and anemias due to

Table I Newer RBC and Reticulocyte Parameters on Automated Analyzer

RBC parameter	*Significance in anemia*
RBC hemoglobin equivalent (RBC-He): Hemoglobin content of all mature RBCs	Along with RET-He helps in detecting onset of anemia and also improvement in erythropoiesis
Fragmented red cell count (FRC): Fragmented RBCs	In detection of microangiopathies, DIC, infections, sepsis, immune disorders, etc.
Red cell size factor (RSf): Cellular hemoglobin content of RBCs and reticulocytes	• Low in IDA • Can be used for IDA screening in pediatric population
Percentage hypochromic cells (%HC) or equivalent low hemoglobin density (LHD%): hypochromic RBCs (%)	• Low in IDA • Can be used for IDA screening in pediatric population • Iron restricted erythropoiesis marker
Percentage unghosted cells: Target cells in peripheral blood	Screening of thalassemia
RBC-Y: Size and contents of the RBCs	Can help distinguish between hemoglobinopathies and IDA.
Reticulocyte hemoglobin equivalent (Ret-He) or Mean Reticulocyte Hemoglobin Content (CHr): Mean content of hemoglobin within reticulocytes	• Differentiate between IDA and FID • Iron restricted erythropoiesis marker
Low fluorescence reticulocyte (LFR), Medium fluorescence reticulocyte (MFR), High fluorescence reticulocyte (HFR): Maturity stages of reticulocytes	• Differentiate between IDA and FID • Iron restricted erythropoiesis marker
Immature reticulocyte fraction (IRF): Sum of HFR and MFR	• Assesses effectiveness of erythropoiesis • Assessment of response to iron or vitamin-B12/folate supplementation in nutritional anemias • Monitoring EPO therapy response
Reticulocyte-Y (RET-Y): Size and contents of the reticulocyte	• Low in IDA and AI

ACD: anemia of chronic disease, AI: anemia of inflammation, DIC: disseminated intravascular coagulation, EPO: erythropoietin, FID: functional iron deficiency, IDA: iron deficiency anemia. All normal values are taken as standard reference values as mentioned in Nathan and Oski's Hematology and Oncology of Infancy and Childhood, 8th Ed [8].

inherited conditions such as thalassemia (**Table I**) [7,8,10,13-17].

The conventional parameters are generated in all automated cell counters, while the newer parameters discussed in **Table I** are available in specific counters and need to be customized to be generated as printouts. Additionally, there are a number of indicators that can be derived from the parameters reported in the automated analyzers such as the Mentzer index (MCV/RBC) that can also help differentiate between IDA and β-thalassemia.

In RBC histograms, the cell-counters count RBCs between 25 and 250 femtoliter (fL). The histograms have two flexible discriminators that help differentiate RBC curves from others: RBC lower discriminator (RL) that fluctuates between 25 and 75 fL and RBC upper discriminator (RU) that fluctuates between 200 and 250 fL. When the cell population is homogeneous, the curve shows a symmetrical bell-shaped or Gaussian distribution. The area of the histogram's peak (60 to 125 fL) helps to calculate MCV and RDW (**Fig. 1**).

In a normal RBC histogram, RBCs are located between 55-125 fL. MCV is calculated using a perpendicular line between the base of the curve and its peak. RDW helps calculate the variation in RBC size and can be of 2 forms: RDW-SD and RDW-CV. RDW-SD is the standard deviation expressed as fL obtained by drawing a line of 20% on the y-axis. Its normal range is between 35-45 fL. RDW-CV is the coefficient of variation percentage and lies within the range of 11.5% to 14.5%. It is calculated as: RDW-CV = SD/MCV ×100 (**Fig. 2**).

To avoid interference of aperture artifacts, giant platelets, RBC agglutinates, the information <20% of the scale on the histogram is excluded. When RBCs are smaller than normal in size, as in microcytic anemia, the curve shifts to the left and when larger than normal, as in macrocytic anemia, the curve shifts to the right. The extension of the lower end of the scale helps in the detection of RBC fragments, WBC fragments and platelets (**Fig. 1**).

RBC Histograms Flags in Childhood Anemia

Flags are signals that occur when automated hematology analyzers detect an abnormal result (**Fig. 2**). Any abnormal flag should always be correlated with the peripheral smear findings.

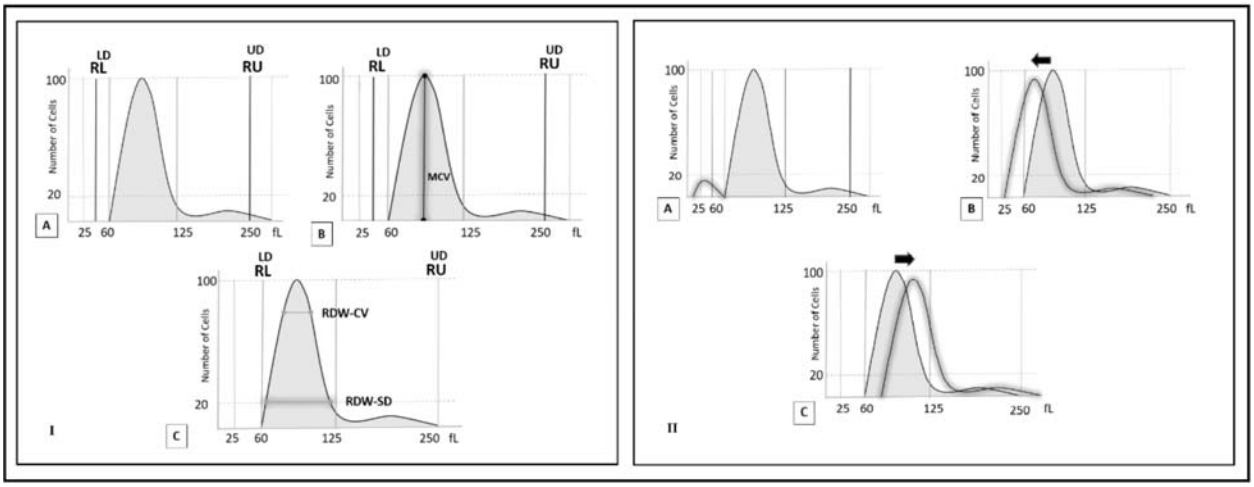


Fig. 1 Interpretation of RBC Histograms (I): normal RBC curve (A), calculation of MCV (B) and RDW (C). Shift in the curve of RBC Histogram (II): extension of lower end of curve as seen in case of normal MCV with flagging (A), leftward shift of curve as seen in the presence of microcytic RBCs (B), and rightward shift of curve as seen in the presence of macrocytic RBCs (C).

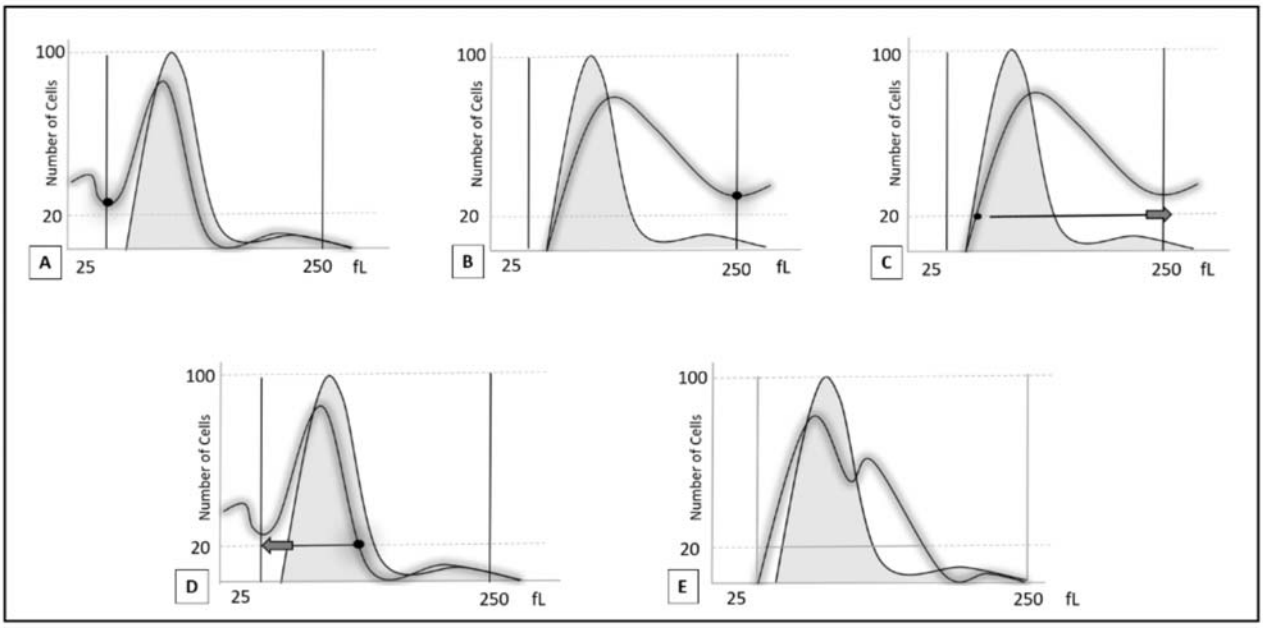


Fig. 2 Flags encountered in RBC histograms: RL Flag (A), RU Flag (B), RDW Flags (C and D) and MP Flag (D).

RL Flag: This occurs due to abnormal height at lower discriminator when it exceeds the preset height by >10%. This is seen in the presence of platelet clumps, RBC fragments, extreme micro-erythrocytosis, giant platelets, micro-RBCs and noise.

RU Flag: This is represented by abnormal height at upper discriminator when it exceeds the preset height by >5%. It can be seen in the presence of nucleated RBCs, RBC agglutination, cold agglutinins and chronic lymphocytic leukemia (CLL) when the small lymphoid cells are present in very high numbers. In the presence of cold agglutinins, the flag disappears when the sample is incubated at 37ºC.

RDW Flag: This occurs in the presence of abnormal RDW and is seen when the curve does not match the 20% line twice. The possible causes of this may include those of RL and RU flags.

MP Flag: Here multiple peaks are seen in the presence of increased anisocytosis, i.e., raised RDW. This is also known as bimodal flag. Causes include post-transfusion blood samples, iron-deficiency anemia in recovery and

dimorphic anemias (both iron and vitamin B12/folate deficiency).

Platelet Parameters

Platelets may be abnormal in size or number in various anemias and may help determine the etiology of anemia. Details of newer platelet indices are summarized in **Table II**.

Platelets are counted and represented between 2 and 20 fL in platelet histograms. At 20 fL, there may be interference in counting due to RBC and WBC fragments, while at 2 fL, interference may be due to air, EDTA particles and air bubbles. Here, two flexible discriminators: lower discriminator (LD) or platelet lower discriminator (PL), upper discriminator (UD) or platelet upper discriminator (PU), and a fixed discriminator at 12 fL are used. The platelet histogram curve should lie between LD and UD and it starts and ends at the baseline. The platelet curve is normally left skewed. In thrombocytosis, the curve shifts upwards, while it shifts downwards in thrombocytopenia.

Platelet histogram is used to calculate mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) (**Fig. 3**). MPV, which is analogous to the MCV of RBCs, represents the average volume of the counted platelets. It lies between 8 and 12 fL normally. MPV (fL) = Plateletcrit (%) / platelet count (x 103/ μL)

MPV, i.e., the range of platelet size, varies with platelet count. In physiological conditions, MPV is inversely related to platelet count and is raised in thrombocytopenia [18,19]. Additionally, MPV is used to discriminate between reactive (MPV normal) and malignant thrombocytosis (MPV raised). MPV is raised in conditions such as splenectomy, chronic myeloid leukemia (CML), myelofibrosis, Bernard Soulier syndrome and immune thrombocytopenic purpura (ITP) [19]. It is decreased in hypersplenism, aplastic anemia, megaloblastic anemia, Wiskott Aldrich syndrome and chemotherapy.

PDW is a measure of the variation of platelet size. It is a coefficient of variation calculated as SD / MPV×100 and has a reference range of 9 to 14%. It is expressed in the histogram by drawing an arbitrary line at the height of 20%. PDW is high in aplastic anemia, megaloblastic anemia, chronic myelogenous leukemia (CML), chemotherapy, etc. and is falsely elevated in the presence of platelet clumps, microcytic RBCs and fragments.

P-LCR is the percentage of platelets that exceed the normal value of platelet volume of 12 fL in the total platelet count. It is calculated as platelet cell concentration (P-LCC)/platelet count, where P-LCC refers to the platelets in the volume range of 12 to 30 fL. The normal range of P-LCR is 15 to 35% and it is raised in the presence of platelet clumps, giant platelets and microcytic RBCs.

Platelet Histogram Flags in Childhood Anemia

LD Flag (PL Flag): This occurs when LD exceeds preset height by 10%. This can occur due to the presence of a high blank value, platelet aggregation, cell fragments, contaminated reagents and high numbers of bacteria.

Table II Platelet Parameters on Automated Analyzer

Platelet parameters	*Significance in Anemia*
Platelet volume distribution width (PDW): Variation in platelet size	Raised when platelet anisocytosis present.
Mean platelet volume (MPV): Thrombocyte volume	Act as acute phase reactant;High in anemias associated with myeloproliferative neoplasms and chronic disease, e.g., type I diabetes mellitus; Predictor of higher risk of stroke or myocardial infarction particularly in children with type I diabetes mellitus.
Plateletcrit (Pct): Volume of circulating platelets in unit volume of blood	High in active stages of certain chronic diseases (e.g., Crohn disease)
[a]Reticulated platelets or immature platelet fraction (IPF): Immature platelets	Recovery of thrombopoiesis (dengue);Peripheral destruction of platelets (autoimmune conditions, malaria)
Platelet large cell ratio (P-LCR): Large circulating platelets	Reactive thrombocytosis (IDA, viral infections); Peripheral destruction of platelets (DHF)
Platelet component distribution width (PCDW)	Raised when variation in platelet shape is present (e.g., giant platelets in reactive thrombocytosis)
Mean platelet mass (MPM) or mean platelet component (MPC)	Raised in reactive thrombocytosis (IDA, thalassemia)

IDA: iron deficiency anemia, DHF: dengue hemorrhagic fever. [a]Similar parameters calculated differently in different types of automated cell counters. All normal values are taken as standard reference values as mentioned in Nathan and Oski's Hematology and Oncology of Infancy and Childhood, 8th Ed [8].

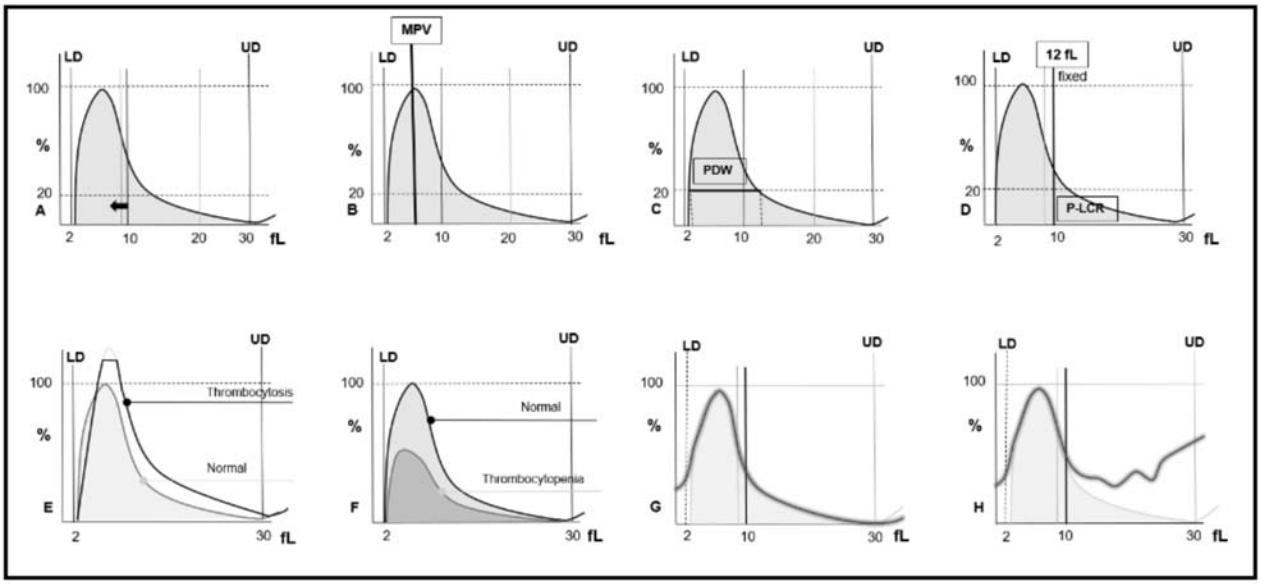


Fig. 3. Interpretation of normal platelet histogram: Normal platelet histogram is left skewed (arrow, A), MPV calculation (B), PDW calculation (C), thrombocytosis vs normal curve (D), thrombocytopenia vs normal curve (E), PL flag (F) and PU flag (G).

UD Flag (PU Flag): This occurs when there is abnormal height at UD and it exceeds the preset height by >40 %. It can be seen in the presence of platelet clumps (clotted sample or EDTA incompatibility), giant platelets and microcytic RBCs.

MP Flag: This occurs when there are multiple peaks present. It can be seen in cases of platelet transfusion, recovery from chemotherapy and platelet aggregation.

WBC Parameters

WBC differential indicates the chronicity of the disease and very high counts may indicate severe infections and malignancies. Further, the presence of leucopenia along with anemia, can point towards more specific etiologies (**Table III**).

WBC histograms generated by automated hemato-logy analyzers plot the size of cells (in fL) on the *X*-axis and the frequency of the cells on *Y*-axis. The counter here also sets a lower discriminator (LD or WL) that fluctuates between 30 and 60 fL and an upper discriminator (UD or WU) that is fixed at 300 fL. The number of cells between these two discriminators is the total WBC count. A normal WBC histogram curve should be within the discri-minators and start and end at the baseline. WBC histograms consist of two troughs or valleys; T1 lies between 78 and 114 fL and T2 <150 fL. These 2 troughs are detected by 2 inner discriminators that separate the WBC populations into 3 groups, based on the size of cells. The peak between LD and T1 represents a small cell popu-lation, i.e., lymphocytes with their volume ranging from 35 to 90 fL. The peak between T1and T2 represents medium cell population comprising of eosinophils, basophils, monocytes, promyelocytes and blasts with their volume ranging between 90 and 160 fL. The peak between T2 and UD denotes neutrophils with their size ranging from 160 to 300 fL. (**Fig. 4**)

Table III White Blood Cell Parameters on Automated Analyzer

WBC parameters	*Significance in anemia*
Immature granulocyte count (IMG): Immature myeloid cells	Systemic inflammation, sepsis, hematological disorders (MPN, AML, bone marrow infiltrative disorder).
High fluorescent lymphocytes (HFL) or Atypical lymphocytes, ALY% or Large unstained cells, %LUC	Reactive lymphocytes, lymphoma cells, blasts; Helps in sepsis monitoring.
Neutrophil granulation (NEUT-X/NEUT-Y): Granularity/nucleic acid and protein content	Raised in sepsis; Low in MDS or MDS/MPN.
Malaria factor (Mf)	More than 3.7 in the absence of a WBC peak in malaria.

AML: acute myeloid leukemia, MDS: myelodysplastic syndrome, MDS/MPN: myelodysplastic syndrome/myeloproliferative neoplasm. All normal values are taken as standard reference values as mentioned in Nathan and Oski's Hematology and Oncology of Infancy and Childhood [8].

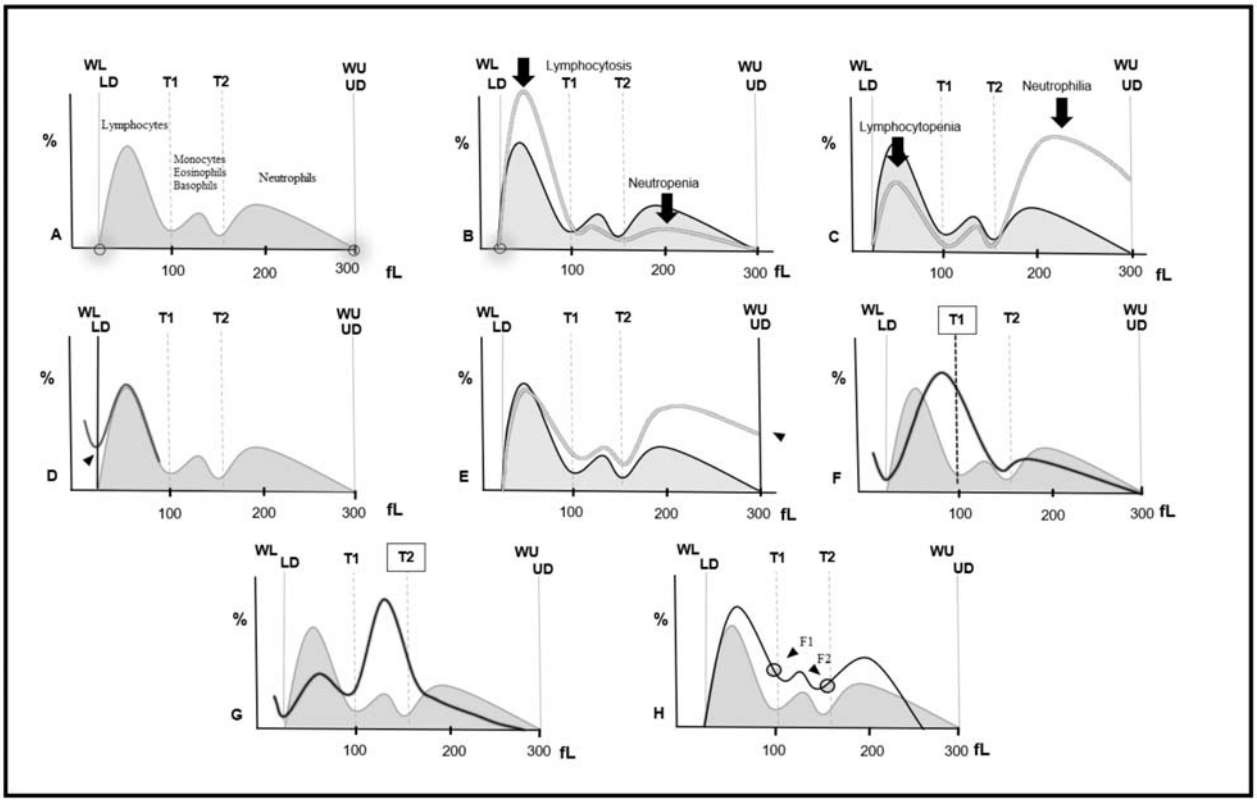


Fig. 4 Interpretation of WBC Histograms: Normal Histogram (A), histograms showing normal distribution of neutrophils and lymphocytes and their distribution when there is an abnormal increase or decrease (B, C), LD/WL Flag (D), UD/WU Flag (E), T1 Flag (F), T2 Flag (G) and FI, F2, F3 Flag (H). In LD Flag, curve does not start at baseline. In UD Flag, the curve does not end at baseline (arrow head). In T1, there is no differentiation between lymphocytes and mixed cell population. In T2, there is no differentiation between mixed cells and neutrophils. In F1, F2, F3 flags, T1 and T2 discriminators are set but there is no clear separation between different populations.

WBC Histogram Flags in Childhood Anemia

WL flag (LD flag): This is an abnormal curve at the LD that occurs when the height of LD exceeds the present 2% of the *Y*-axis. This may be seen in the presence of nucleated RBCs, clotted sample and cold agglutinins.

WU flag (UD flag): This is an abnormal curve at the UD. A WU flag appears when the height of UD is greater than the preset 10% on *Y*-axis. WU flagging occurs in case of inadequate WBC lysing, WBC aggregation and extreme leukocytosis.

T1 flag: This is an abnormal curve at T1 level. T1 and T2 flags appear when discrimination between 3 populations is not possible. A T1 flag appears when differentiation between lymphocytes and medium-sized cell populations is not possible, for example, in cases of chronic myeloid leukemia and leukocytosis.

F1, F2 and F3 flags: This occurs when the height of T1 surpasses the present limit of 40%. The F1 flag denotes that the discrimination between small cell and middle cell populations is not an accurate example in acute lymphoblastic leukemia. An F2 flag occurs when the middle cell data is inaccurate. The T1 and T2 exceed the preset limits of 40% and 50%, respectively. Examples of F2 flags are eosinophilia, acute myeloid leukemia and monocytosis. F3 flag occurs when the T2 exceeds the preset limit of 50%, denoting that the large cells data is inaccurate.

Utility of histograms can be demonstrated by interpreting few common case scenarios (**Web Box I**). Automated hematology analyzers do not provide a complete answer regarding the underlying etiology of anemia and may leave room for misinterpretation. Thus, further testing is required to make a confirmatory diagnosis. However, despite these pitfalls, it plays a role in early decision-making and can help in reducing the time lag between clinical presentation and institution of appropriate therapy.

Note: Additional material related to this article is available at *www.indianpediatrics.net*

Contributors: SD, TJ: concept, design, definition of intellectual

Table IV Other Causes of Abnormal Values in Complete Blood Count (CBC) Reports

Factor	*CBC report*
Nucleated RBCs (nRBCs)	High WBCs, R1 flag
Cryoglobulins	High WBCs, High platelets, High MCV
Hyperlipidemia	High MCHC, High hemoglobin
Hyperbilirubinemia	High MCHC, High hemoglobin
Cold agglutinins	Low RBCs, high MCV. high MCH, high MCHC
Schistocytes	Low RBCs, High platelets, left shift in RBC histogram, Platelet histogram not touching baseline
Fragmented WBCs	High platelets
Large platelet clumps	High WBCs, High RBCs, low platelets, R1 flag, Platelet histogram not touching baseline
Unlysed RBCs	High WBCs, high lymphocytes, R1 flag
Clotted sample	Low counts
Heparinized sample	High WBCs
Very high WBCs	High hemoglobin, High MCHC
Smudge cells	Low WBCs

MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, MCV: mean corpuscular volume, RBC: red blood cell, WBC: white blood cell.

content, literature search, experimental studies, data acquisition, data analysis, manuscript preparation, manuscript editing; SD, TJ, MK: concept, design, definition of intellectual content, manuscript preparation, manuscript editing. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

Funding: None; *Competing interest*: None stated.

REFERENCES

1. Marcdante KJ, Kliegman RM. Nelson essentials of pediatrics. 7th Edition. Elsevier Saunders. 2016.
2. Paul VK. Ghai Essential Pediatrics. 9th Edition. CBS Publishers and Distributors. 2019.
3. Kapur D, Agarwal KN, Sharma S, et al. Iron status of children aged 9-36 months in an urban slum integrated child development services project in Delhi. Indian Pediatr. 2002;39:136-44
4. Kumar T, Taneja S, Yajnik CS, et al. Prevalence and predictors of anemia in a population of North Indian children. Nutrition. 2014;30:531-37.
5. Xie D, Xie Y, Liu P, et al. Performance of a cost effective and automated blood counting system for resource limited settings operated by trained and untrained users. J Biophotonics. 2018;11:e201700030.
6. Gounari E, Tsavdaridou V, Koletsa T, et al. Utility of hematology analyzer and flow cytometry in timely and correct detection of circulating plasma cells: Report of three cases. Cytometry B Clin Cytom. 2016;90:531-37.
7. Kujovich JL. Evaluation of anemia. Obstet Gynecol Clin North Am. 2016;43:247-64.
8. Nathan D, Orkin S, Oski F. Nathan and Oski's hematology and oncology of infancy and childhood. Elsevier. 2015.
9. Balci YI, Akpinar FO, Polat A, et al. Evaluation of reticulocyte parameters in iron deficiency, vitamin B12 deficiency and mixed anemia. Clin Lab. 2016;62:343-47.
10. Piva E, Brugnara C, Spolaore F, Plebani M. Clinical utility of reticulocyte parameters. Clin Lab Med. 2015;35:133-63.
11. Arkin CF, Davis BH, Bessman JD, et al. Methods for reticulocyte counting (automated blood cell counters, flow cytometry, and supravital dyes); approved guideline. The National Committee for Clinical Laboratory Standards. 2004;24:1-34.
12. Briggs C. Quality counts: New parameters in blood cell counting. Int J Lab Hemat. 2009;31:277-97.
13. Markoviæ M, Majkiæ-Singh N, Subota V. Usefulness of soluble transferrin receptor and ferritin in iron deficiency and chronic disease. Scand J Clin Lab Invest. 2005;65: 571-76.
14. Angeles Vázquez López M, Molinos FL, Carmona ML, et al. Serum transferrin receptor in children: usefulness for determinating the nature of anemia in infection. J Pediatr Hematol Oncol. 2006;28:809-15.
15. Urrechaga E, Borque L, Escanero JF. Erythrocyte and reticulocyte indices on the LH 750 as potential markers of functional iron deficiency. Anemia. 2010;2010:625919.
16. Osta V, Caldirola MS, Fernandez M, et al. Utility of new mature erythrocyte and reticulocyte indices in screening for iron-deficiency anemia in a pediatric population. Int J Lab Hematol. 2013;35:400-05.
17. Thomas ETA, Bhagya S, Majeed A. Clinical utility of blood cell histogram interpretation. J Clin Diagn Res. 2017;11:OE01-04.
18. Dastjerdi, MS, Emami, T, Najafian, A, Amini, M. Mean platelet volume measurement, EDTA or citrate? Hematology. 2006;11:317-19.
19. Korniluk A, Koper-Lenkiewicz OM, Kamiñska J, et al. Mean platelet volume (MPV): new perspectives for an old marker in the course and prognosis of inflammatory conditions. Mediators of Inflammation. 2019; 2019: 9213074.

Web Box I Case Based Interpretation of Histograms

a. A 1-year-old female presented with pallor and frequent episodes of breathlessness. CBC parameters show a fall in MCV, MCH, MCHC and a left shift of RBC histograms indicating a microcytic anemia. Raise in RDW and a widening of RBC curve is suggestive of IDA, which was in turn, confirmed by estimating serum iron, total iron binding capacity (TIBC) and serum ferritin.

b. A 2-year-old male presented with pallor, breathlessness and early fatigability. Very low MCV, MCH, MCHC and abundant platelets along with left-shift of RBC curve give a clue for thalassemia trait. On further testing, HPLC showed mildly raised HbA2 and HbF, suggesting a diagnosis of beta thalassemia trait. RDW is usually not raised in beta thalassemia trait. However, here in view of raised RDW, further work-up for other associated conditions such as IDA is suggested.

c. A 5-year-old male presented with low-grade fever, abdominal distension, loss of weight and loss of appetite. Examination revealed hepatosplenomegaly and multiple enlarged palpable lymph nodes. CBC showed high WBC counts. WBC histogram shows lack of differentiation between medium and small cell population, suggestive of blasts. A peripheral smear examination and further characterization using special stains and flow cytometry is required.

d. An 8-year-old male presented with high grade fever and generalized weakness. CBC showed high WBC counts. However, the population can be identified as neutrophils on WBC histogram indicating leukemoid reaction.

e. A 13-year-old female presented with fever for 4 days, associated with one episode of vomiting, severe weakness and dyspnea on exertion. RBC histogram was normal. WBC histogram showed dual peak in small-medium cell population, small peak in large cell population suggestive of atypical cells/activated lymphocytes. Platelet histogram showed downward shift suggestive of thrombocytopenia with increased PDW for giant platelets. Dengue NS1 antigen was found positive.

Study Design: Observational Studies

SIDDARTH RAMJI
From Department of Neonatology, Maulana Azad Medical College, New Delhi.
Correspondence to: Dr Siddarth Ramji, Director-Professor, Department of Neonatology, Maulana Azad Medical College, Delhi.
siddarthramji@gmail.com

Observational study designs are those where the investigator/researcher just observes and does not carry out any intervention(s)/actions to alter the outcome. The three most common types of observational studies are cross-sectional, case control and cohort (or longitudinal). In cross-sectional studies, both the exposure/risk factor(s) and the outcome(s) are determined at a single time point. They can provide information on prevalence of a condition and snapshot of probable associations that can be used to generate hypothesis. Case-control studies are where subjects are selected based on presence/absence of outcome and the risk factors are determined during the study after enrolment of study subjects. The association between exposure and outcome is reported as odds ratio. These studies; however, have high risk of bias, which must be taken care of during study design. Cohort studies are prospective in nature, where subjects are selected based on presence/absence of exposure, and the outcome(s) is determined at the end of study. These studies can provide incidence of disease/outcome and the association between exposure and outcome is reported as relative risk. They are useful to ascertain causality. High dropouts of study participants and confounding can be problems encountered in these studies.

Keywords: *Case-control, Cohort, Cross-sectional, Odds ratio, Relative risk, Survey*

Published online: April 26, 2022; ***PII:*** S097475591600418

Observational studies are research study designs where the investigator/researcher just observes and does not influence either the exposure or the outcome. In contrast, in experimental studies, the intervention/exposure is under the control of the investigator to bring a change in outcome [1]. These types of studies are important as they address many research questions which cannot be answered by experimental studies/clinical trials or where the latter study designs are not possible (e.g., health outcome after natural disasters such as the gas leakage from an industrial unit in Bhopal in 1984 or after a nuclear accident as occurred in Chernobyl Nuclear power plant in 1986), wherein it would be unethical for such events to be purposefully induced for purposes of experimentation. These may be relatively easier and faster to conduct than experimental designs.

Observational studies can either be descriptive or analytical. **Table I** summarizes the various ways in which observational studies can be categorized.

DESCRIPTIVE STUDIES

Descriptive studies generally describe the magnitude of a problem and characteristics of the population/individuals. The various types of such studies include case reports,

Table I Categorization of Observational studies

Classification category	*Qualifier/Explanation*
Relation to the population	• Subjects are selected based on presence of risk factors as in Cohort studies
	• Subjects are selected based on presence/absence of outcome as in case-control studies
Period of observation	• Single time point as in cross sectional and case-control studies
	• Followed longitudinally over time as in cohort studies
Timing of measurements	• Concurrent as in cohort studies
	• Non-concurrently as in case-control studies
	• Both concurrent and non-concurrent as in cross-sectional studies (depends on type of data being recorded)
Direction of investigation	• Prospective when investigation moves from risk factors/exposure to outcome (as in cohort studies)
	• Retrospective when the investigation moves from outcome to determine the risk factors (as in case-control studies)

case series or surveys. A case report generally describes a patient presenting with an unusual disease, or simultaneous occurrence of more than one condition, or uncommon clinical features in a known disease. A case series is a collection of similar cases. Such studies, other than providing some advancement to knowledge of a disease, are of limited value. Another method often used in epidemiological health care research is conducting surveys. Surveys are done during a defined time-period and information on several variables of interest is collected from the target population. They provide estimates of prevalence of the various variables of interest, and their distribution. Such studies could also provide insight into individual opinions and practices. Advantages include ease of conduct and cost efficiency. The disadvantages include low response rates and a variety of biases.

ANALYTICAL STUDIES

An analytical study tests a hypothesis to determine an association between two or more variables, like causation, risk, or effect. Such studies have two or more study groups for comparison. The primary focus of this article will be the three most common types of analytical observational studies - cross-sectional, case control (also known as retrospective) and cohort (or longitudinal, also known as prospective) studies. It may be pertinent to note that the primary objective of most clinical studies is to determine one of the following - burden of disease (prevalence or incidence), cause of disease, prognosis, or effect of treatment/intervention. Each of the study designs should mention the details of participant, exposure and comparison/control group (as applicable), outcome to be analyzed and time as per the PICOT format clearly in the protocol.

Incidence (new events) can be determined from cohort studies and prevalence (old plus new events) from cross sectional studies. Cause/etiology can be ascertained from cohort, case control or cross-sectional studies where there are two or more comparison groups (in their decreasing order of reliability). Prognosis can be provided by cohort studies that prospectively measure outcome. However, effect of treatment cannot be reliably obtained from observational studies and would need a controlled clinical trial (experimental design).

Cross-Sectional Studies

Cross-sectional studies provide a snapshot of both the exposure (or risk factor) and the outcome in the sample. Here the information on exposure and outcome are noted at the same time. They are generally descriptive and provide information on prevalence (the number of cases in a population at a given point/period). However, cross-sectional studies can also be analytical where the strength of association between two variables can be estimated and is reported as an odds ratio (OR), which can be useful for hypothesis generation.

Conducting Cross-Sectional Studies

The first step would be to formulate a research question. The next step is to identify the target population to whom the results would be generalized and then select a study sample as per the logistic considerations. The sampling strategy could be simple random, stratified (e.g., age and gender where outcomes are dependent on these variables), systematic (especially in hospital-based studies), multi-stage (e.g., districts, villages, households) or cluster sampling (the last two sampling methods are useful when large populations are to be included in the study) [2]. Lastly, the variables relevant to the research question must be identified during the study planning stage.

Advantages and Disadvantages

Cross-sectional studies are useful when one wishes to gather information rapidly and in an inexpensive way. It can provide descriptive data and can also determine an association between two or more groups. Another advantage is that multiple outcomes can be studied.

However, cross-sectional studies cannot assess risk but can provide information on association. They would also not be an appropriate choice where one or more of the variables of interest are rare. They do not provide reliable information on causality or the sequence of events. An example is provided in **Box I**.

Case-Control Studies

Case-control studies are retrospective in nature. Subjects are enrolled based on "presence (cases)" or "absence (controls)" of outcome (or disease). Information related to

Box I An Example of a Cross-Sectional Study

Example: A study in Ethiopia aimed to determine the prevalence and associated factors of malaria in under-five children. This was a facility-based cross-sectional study conducted among 585 under-five children who attended public health facilities. Health facilities were selected by stratified cluster sampling, and systematic random sampling was done to select study participants from the selected facilities. Malaria was defined as a positive thin or thick blood film for the *Plasmodium* parasite. It was observed that 51 (8.7%) children had malaria. [3]

Comment: In this example, the study was conducted across multiple hospitals (hence cluster sampling was used) and systematic random sampling was used to select participants within each health facility. The research question required estimation of point prevalence of malaria as per a predefined diagnostic criterion.

potential risk factors is collected by the investigator after the outcome has occurred. As the direction of enquiry is from outcome to exposure, hence is termed as a retrospective study.

Conducting Case-Control Studies

As for all studies a research question must be formulated. Unlike cross sectional studies, case-control studies would require an *apriori* hypothesis and hence one must decide what must be measured and how. The next step is to choose the case and control group with valid and precise definitions. The definition of a case should be objective (e.g., diagnostic criteria of a disease or event) such that there is no ambiguity in type of cases and/or their severity. Cases can be hospital-based or population-based. Defining control is equally important and critical to the outcome of case-control studies. Every effort must be made to ensure absence of outcome in controls. The controls should represent the population from where the cases have been selected and need not always be healthy. However, they should be equally predisposed to develop the outcome under study and are selected from the same source population as the cases. They can be selected from friends, relatives or from the neighbourhood as this can be done with minimum effort. Such controls, however, have the risk of being very similar with respect to exposures and other characteristics (can be overmatched). On the other hand, selecting an appropriate hospital control can be challenging and care must be taken to prevent selection bias. Use of two or more controls increases reliability (e.g., outpatients, inpatients, from general practice, etc.). Selecting an appropriate control is one of the most important steps in a case-control study [4].

The exposure of interest is considered 'a risk factor' and its association with the outcome is reported as odds ratio (OR) (or as adjusted OR after confounder control). The odds ratio from case control studies informs one as to how much higher the odds of exposure are among case-patients than among controls (or if it is associated with reduced risk, it would inform about how much lower is the odds of exposure among case-patients than among control). For rare diseases, such as cancer, the odds ratio is likely to approximate relative risk values obtained from prospective (cohort) studies. An example is provided in **Box II**.

Nested case-control study

This is a variant of a case-control study in which the cases and controls are drawn from a pre-existing cohort study. For every defined case in the cohort a matched control from the cohort is selected. They are particularly useful for studying the biological precursors of disease. The advantage of this design is that it minimizes selection, recall bias and measurement bias (as the variables have been pre-defined and collected concurrently in a standardized manner having been part of a cohort study) in comparison to case-controls studies, and is faster and less expensive than a cohort study. An example is provided in **Box III**.

Advantages and Disadvantages

Case control studies are relatively simple to perform and provide results over a relatively shorter time than cohort studies and require lesser resources. They provide information about predictors of an outcome. The problem of confounding can be overcome using matched controls for a few of the important selected confounding characteristics.

The disadvantages are that they cannot provide information on incidence or prevalence and can look at only one outcome. They are also more prone to bias, particularly selection, observation, recall and measurement bias. Selection bias could be minimized by use of matched or population-based controls. Recall bias could be minimized by using recorded data before the occurrence of the outcome being studied. Because of the risks of confounding and bias, case control studies are less reliable for ascertaining causality but can help in hypothesis generation.

Box II Example of a Case-Control Study

Example: This study attempted to identify the characteristics of malnourished children below five years of age and the risk factors of childhood malnutrition. It hypothesized that risk of childhood malnutrition (outcome) was increased in poor households (exposure). Case was a child with moderate to severe malnutrition (z-scores <-2SD from the median of WHO reference of any anthropometry - weight, length/height, weight/length). Control was a child with z-scores between -2SD and +2SD and was age matched with the cases. The participants were identified from those attending the maternal-child health clinics. The study identified the variables/exposures that could affect nutrition from three domains-socio-economic characteristics, household food security and child's dietary intake, and caregiver practices and resources. The study clearly defined how the above measures were to be collected. There were 137 cases and 137 controls. The study identified the following as significant risk factors for childhood malnutrition – Household poverty (OR 3.15,95% CI: 1.65-6.04),…. [5]

Comment: In the above example there is a clearly defined research question and hypothesis. The cases and controls are clearly defined. Variables to be measured and process of measurement were also clearly defined. The study has reported the association of exposure and outcome as OR. Though not depicted in the example, the study had observed several significant risk factors and hence it also reported adjusted ORs after adjusting for confounding.

Box III Example of a Nested Case-Control Study

Example: This study was designed to determine predictors of tuberculosis recurrence in the UK. Leicester TB service has a cohort of all TB cases reported, managed, and kept under follow up and surveillance in the region in a prospectively maintained database. For this nested case–control study the participants were identified from the entries in the database between 1994 and 2014. All participants who had been cured after their first episode of TB infection were eligible for inclusion. Those who had been reported as new and first recurrence episode of TB in the database were defined as cases. Controls were those with no recurrence and were matched to cases in a ratio of two to one by the date of notification. Clinical, sociodemographic and TB specific risk factors for recurrence were identified and extracted from the database of the cohort. From the original cohort, there were 4764 eligible patients included in the current study. In these 82 TB recurrences were noted (1.8%) (cases). Smoking (OR 3.8; p=0.04), grade 3/4 adverse drug reactions (OR 5.6; p=0.02), ethnicity 'Indian subcontinent' (OR 8.5; *P*=<0.01), ……were predictors of TB recurrence [6].

Comment: Since the cases and controls were selected from an existing cohort of TB patients based on a defined outcome that had already occurred i.e., TB recurrence, it qualifies as a nested case-control study.

Cohort Studies

Cohort studies (or longitudinal studies) are generally prospective but can also be retrospective. However, unless qualified, the term cohort studies imply prospective cohort studies. Cohort studies are the designs of choice for determining incidence and natural course of a disease. The association of risk factors with outcome is generally reported as relative risk (RR) or attributable risk.

Prospective Cohort Studies

In prospective cohort studies, the participants are a group of individuals in whom the outcome of interest (e.g., lung cancer) has not occurred at the time of selection into the study. The investigator identifies all possible relevant variables that may contribute to the development of the outcome and measures them accurately in the participants during follow-up. The participants during follow-up are carefully followed up and observed to see if they develop the outcome of interest. The steps in a prospective cohort are as follows: *i*) identify a research hypothesis, *ii*) define objectively the exposure (risk factors) and outcomes, *iii*) develop a standard data collection tool (to minimize information bias), *iv*) identify steps during follow-up to minimize the drop outs (selection bias), and *v*) define the analysis plan to measure the association between one/ many factors and the outcome.

a. *Descriptive Cohort*: A descriptive cohort study describes outcome over time for a specific group of individuals, without any comparison of groups. Examples are patients with a defined type of cancer(s) who are followed up to describe the epidemiology of the disease.

b. *Single analytic cohort*: In such a study, those who develop the outcome of interest are treated as 'cases' and those who do not develop the outcome of interest are treated as 'controls' (also known as internal controls). The investigator may also opt for an external control when internal controls are not available (when exposure cannot not be verified/ was not measured).

c. *Two group analytic cohort*. When there are two cohorts being followed, one of the groups would have been exposed to the variable of interest while the other would not. Both groups would be followed to observe for the occurrence of the outcome of interest. Here those without exposure to the variable of interest would serve as the comparison/control group (external controls).

Retrospective Cohort Study

This is a type of cohort study where the investigator looks back retrospectively at already collected data (prospectively) after the outcome of interest has occurred i.e., post-hoc. The important distinguishing feature of retrospective from prospective cohort study is that the investigator comes up with the idea of the study and begins to identify variables and subjects after the outcome of interest has occurred. An *ambispective cohort* study design allows the researcher to retrospectively measure the exposure in a cohort and follow them prospectively for a disease outcome. An ambispective design saves resources and time.

Advantages and Disadvantages

Cohort studies are the choice where randomized control trials would be considered unethical e.g., effect of smoking on lung cancer. As the design affords a temporal sequence, it is a very good method to establish cause and effect (hence allows one to measure incidence) and analyze risk factors or predictors (allows to measure relative and attributable risk). They also have the advantage of being able to measure multiple outcomes from a single study e.g., effect of breast-feeding duration on child growth, obesity, diarrhea, and acute illnesses [10] or even multiple exposures e.g., effect of multiple environmental exposures to child health outcomes [11].

The disadvantages could include loss of subjects (loss of cases becomes more important as it can alter incidence rates) during the follow-up, confounding and non-representative nature of the cohort sample (selection bias).

Examples of various cohort studies are provided in **Box IV**.

Comparison of Cohort, Case-control, and Cross-sectional studies

Table II summarizes the comparison of the three types of studies. Let us see an illustration of how each of the study designs can be used to address the same research question. Let us say, if one wanted to assess the association of maternal anemia in pregnancy and low birth weight (LBW), one could address the research question by all three study designs.

Cohort study design: Here, mothers with a hemoglobin below and above a pre-defined level (to define anemia) at a defined gestational period of pregnancy would be enrolled (where the hemoglobin estimation is standardized) and followed till birth of the baby and outcome determined by the weighing the baby and classifying as low or not low birth weight. The study would need each mother to be followed up till outcome. To enrol the required number of women could take a long time. There could be drops outs, there could be other factors that could appear during follow up that could contribute to LBW (e.g., hypertension). But the advantage is that data would be collected concurrently, would be reliable and not only association but causality could also be established.

Case-control design: For this, infants who are born with LBW and normal birth weight would be sampled as cases and controls. Information about the hemoglobin status of the mothers of these infants would be sought from records. However, there could be concerns about the accuracy and reliability of the hemoglobin estimation. In addition, details of other risk factors may be missing/ incomplete. The study can establish an association but causality would have lower reliability than a cohort study.

Cross-sectional study: For this study design, we would sample women who have delivered recently. The hemoglobin could be either estimated at the time of participant selection or the information could be obtained from maternal antenatal records. The weight of baby could be measured at time of mother-baby dyad selection or noted from hospital records. While this method would be rapid, the methods used to obtain information/measure anemia or birth weight could affect the interpretation of the results. The issues related to reliability of data and confounding will remain. Hence, this study design would at best help generate a hypothesis.

CONCLUSION

Observational studies are useful because the associations that these studies provide can be used to generate hypothesis. They are also useful to study rare events. The

Box IV Examples of Cohort Studies

Single group prospective cohort study

Example: A prospective birth cohort focused on atopy and asthma development in children that hypothesized low physical activity as a risk factor of asthma. Asthma was identified between 6-10 years using ISAAC criteria. Physical activity was assessed using questionnaire at 4-5 years age. The children were followed at regular 1-2 year interval till 10 years of age. There were 1957 children who met the inclusion criteria at the age of 4 to 5 years. Of these, 1838 children (94%) were evaluated for asthma symptoms between 6 and 10 years. A total of 186 children (10.1%) met the ISAAC definition of asthma between the age of 6 and 10. No association was found between physical activity and asthma (RR 1.13, 95% CI:0.95-1.34) [7].

Comment: In this birth cohort study the cases were those who developed asthma and the internal controls were those who did not develop asthma. The definitions of outcomes are clearly defined. The exposure variables that needed to be studied and measured at each visit had been identified. The study reported the incidence of asthma and the association between exposure and outcome as RR.

Two-group prospective cohort study

Example: This study evaluated longitudinal changes in cardiac structure and function of patients with Rheumatoid arthritis (RA) compared with persons in the general population. A cohort of 160 patients with RA and 1,391 persons without RA (non-RA cohort), each underwent 2-dimensional, pulsed-wave tissue Doppler echocardiography at baseline and after 4 to 5 years of follow-up. The mean mitral inflow E/A ratio decreased faster in the RA cohort than the non-RA cohort ($p<0.001$), the left atrial volume index increased at a higher rate in the RA cohort than the non-RA cohort ($p<0.001$) [8].

Comment: In this example the exposure was RA/no RA, and outcome was cardiac structure and function. As the outcomes were continuous variables, instead of RRs, the investigators compared the means and their variances between the two groups. It is important to note that the comparison of outcomes will depend on the characteristic of the variable being measured.

Retrospective cohort study

Example: In this retrospective cohort study (from a prospectively followed British birth cohort), the association of life course events with adult irritable bowel syndrome (IBS) was evaluated. Adult subjects were enrolled after the outcome of interest – irritable bowel syndrome - had occurred. The investigators extracted data on life course events data from the birth cohort that was being followed. The outcome was self-reported IBS by the age of 42 years. The prevalence of self-reported IBS in this cohort was 8.4% (95% CI=8.2-8.6). Being female (OR=2.00, 95% CI=1.67-2.3), and having psychopathology at 23 years (OR=1.25, 95% CI=1.01-1.54) were associated with increased odds for IBS [9].

Key Messages

- Observational studies are the option when randomized clinical trials are not feasible or unethical.
- Observational studies are useful for generating hypothesis and studying rare events.
- Main problem with observational studies is confounders and biases; but advanced statistical methods may help in controlling for many confounders.

Table II Comparison of Cohort, Case-control, and Cross-Sectional Studies

Parameters	*Cohort study*	*Case-control study*	*Cross-sectional study*
Direction of investigation	From Exposure to Outcome (forward)	From outcome to exposure (backward)	As it exists
Recruitment of subjects	Based on presence/ absence of exposure	Based on presence/ absence of outcome	Neither exposure nor outcome
What does it measure	Incidence, Relative risk	Odds ratio	Prevalence
Temporal relationship	Good	Difficult	Not possible
Causality assessment	Good	Fair	Poor
Suitability for rare exposures	Good	Poor	poor
Suitability for rare outcomes	Poor	Good	Poor
Biases	Low	High	Low
Confounding	Major problem	Major problem	Major problem
Loss of subjects	High	Low	None
Completeness of data	High	Low	Complete
Range of exposures that can be assessed	Small	Large	Large
Duration and cost	High	Low	Low
Range of outcomes that can be assessed	Large	Small	Large

choice of study design is not only determined by the research question, but also the pros and cons of each study and the feasibility for its implementation (**Table II**).

Acknowledgement: Dr. Aashima Gupta for finalization of the manuscript.
Funding: None; *Competing interests*: None stated.

REFERENCES

1. Khan AM, Gupta P, Mishra D. The 3-question approach: a simplified framework for selecting study designs. Indian Pediatr. 2019;56:669-72.
2. Indrayan A. Medical Statistics,3rd edn. Boca Raton, CRC Press, Taylor and Francis Group, 2013; 83-110.
3. Tsegaye AT, Ayele A, Birhanu S. Prevalence and associated factors of malaria in children under the age of five years in Wogera district, northwest Ethiopia: A cross-sectional study. PloS One. 2021;16:e0257944.
4. Case control and cross-sectional studies. *In*: Coggon D, Rose G, Barker DJP, editors. Epidemiology for the Uninitiated, 5th ed. BMJ publishing group, 2003.
5. Wong HJ, Moy FM, Nair S. Risk factors of malnutrition among preschool children in Terengganu, Malaysia: A case control study. BMC Public Health. 2014;14:785.
6. Rosser A, Richardson M, Wiselka, M.J. et al. A nested case–control study of predictors for tuberculosis recurrence in a large UK Centre. BMC Infect Dis. 2018;18:94.
7. Eijkemans M, Mommers M, Remmers T, et al. Physical activity and asthma development in childhood: Prospective birth cohort study. Pediatr Pulmonol. 2020;55:76-82.
8. Davis JM, Lin G, Oh JK, et al. Five-year changes in cardiac structure and function in patients with rheumatoid arthritis compared with the general population. Int J Cardiol. 2017;240:379-85.
9. Goodwin L, White PD, Hotopf M, et al. Life course study of the etiology of self-reported irritable bowel syndrome in the 1958 British birth cohort. Psychosom Med. 2013;75:202-10.
10. Pattison KL, Kraschnewski JL, Lehman E, et al. Breastfeeding initiation and duration and child health outcomes in the first baby study. Prev Med. 2019;118:1-6.
11. Aasvang GM, Agier L, Andruðaitytë S, et al. Human early life exposome (HELIX) study: A European population-based exposome cohort. BMJ Open. 2018;8:e021311.

Serum (1,3)-β-D-Glucan for Screening of Neonatal Fungemia

This study was conducted to identify the mycological pattern, and to calculate the diagnostic accuracy of serum (1,3)-β-D-glucan for screening fungal sepsis in 351 high-risk neonates. *Candida tropicalis* was the most common isolate (*n*=16, 38.1%). At optimum cut-off (47.2 pg/mL), sensitivity and specificity of serum (1,3)-β-D-glucan were 92.9% and 69.9%, respectively.

Keywords: *Candida, Diagnosis, Sepsis.*

Fungal sepsis is a prime cause of neonatal mortality and neurological impairment, particularly in preterm newborns [1,2]. Isolation of fungus from sterile sites remains the gold standard for diagnosis, but it is time-consuming and associated with high false negativity rates. Serum (1, 3)-β-D-glucan (BDG), a component of cell wall of multiple clinically important fungi, is a promising marker of early neonatal fungemia [3]. However, the existing literature suffers from a few lacunae viz., small sample size, inclusion of only preterm newborns, predominantly developed country setting, and only *Candida* species were studied [3,4]. Hence, we conducted this study to find the microbiological pattern of fungal sepsis in neonates at high risk of fungal sepsis, and to assess the diagnostic performance of serum BDG as a screening test in them.

This cross-sectional study was carried out in the neonatal unit (64 bedded Special newborn care unit, 18 bedded Neonatal intensive care unit (level III), and 10 bedded step-down unit) of a public sector tertiary care center between January, 2018, and December, 2021 after taking approval from Institutional ethics committee. Newborns with ≥3 risk factors for fungal sepsis (birth weight <2.5 kg, hospital stay >3 weeks, invasive respiratory support >1 week, antibiotic therapy >72 hours, central venous/ arterial catheter >72 hours, total parenteral nutrition, persistent severe thrombocytopenia despite second line antibiotics) were consecutively enrolled in the study [5]. Newborns with major congenital anomalies, those with parental refusal to participate, and those exposed to prior antifungal therapy, intravenous immunoglobulin, and amoxicillin-clavulanate, were excluded. Cotten, et al. [6] reported approximately 20% prevalence of fungal sepsis in high-risk newborns. Considering a sensitivity and specificity of 80% and 10% error, the required sample size was calculated as 338 (assuming 10% attrition and 5% β error) [7].

Serum BDG level was estimated by spectrophotometry after enrolment. Blood samples of the selected newborns were incubated in BacT/Alert 3D system (*bioMérieux*) at 37°C for 5 days, and subsequently sub-cultured in appropriate medium, if found positive. Other investigations were performed according to clinical indications/ unit protocol. Newborns included in this study did not receive probiotics.

Shapiro-Wilk test was used to check for normal distribution. Chi square test and Mann-Whitney *U* test were used for checking significance of difference between proportions and medians, respectively. Receiver operator characteristic (ROC) curve was generated and Youden index was used to detect the cut-off value for best diagnostic accuracy. SPSS version 16.0 was used for data analysis. $P<0.05$ was taken as statistically significant.

During the study period, 394 admitted newborns met the inclusion criteria, and 43 were excluded (17- received immunoglobulin, 13- received amoxicillin-clavulanate, 5- prior antifungal therapy, 3- inadequate blood sample, 2- refusal of consent 2- major congenital anomaly, 1- left against medical advice). Thus, a total of 351 newborns (48.1% boys) were enrolled in the study. Baseline characteristics of the two groups are shown in **Table I**. Forty-two newborns (12%) developed culture-positive fungal sepsis. *Candida tropicalis* was most common isolate (*n*=16, 38.1%), followed by *Candida albicans* (*n*=13, 31%). All isolates were sensitive to caspofungin and voriconazole, while 37 (88.1%) and 33 (78.6%) isolates were sensitive to amphotericin-B, and fluconazole, respectively. Median (IQR) serum BDG level of newborns who developed culture-positive fungal sepsis was significantly higher than who did not develop culture-positive fungal sepsis [84.6 (26.9) pg/mL vs 34.3 (48.3) pg/mL, $P<0.001$]. Area under the curve of the ROC curve was 0.897 (95% CI: 0.857 - 0.938) (**Fig. 1**). Optimum cut-off was 47.2 pg/mL with a sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio and accuracy, respectively of 92.9% (95% CI: 80.5-98.5%), 69.9% (95% CI: 64.4-75%), 29.6% (95% CI: 25.8-33.6%), 98.6% (95% CI: 96-99.5%), 3.1 (95% CI: 2.6-3.7), 0.1 (95% CI: 0.03-0.3) and 72.6% (95% CI: 67.7-77.2%).

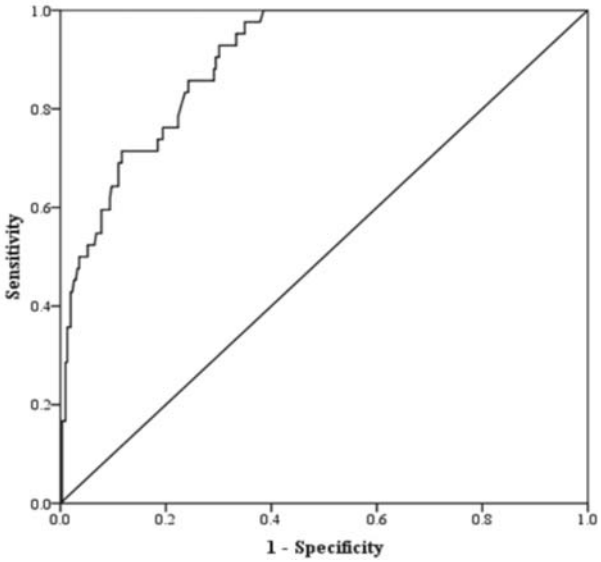


Fig. 1 ROC curve of serum (1,3)–β-D-glucan for discrimination between neonates with and without culture positive fungal sepsis.

Table I Baseline Characteristics of the Study Participants

Variables	Fungal sepsis developed (*n*=42)	Fungal sepsis did not develop (*n*=309)
Birthweight (kg)[a]	1.588 (1.470-2.100)	1.560 (1.280-1.820)
Gestational age (wk)[a]	34.2 (33.1-35)	34.1 (33.2-34.8)
Male sex	22 (52.4)	147 (47.6)
Preterm	41 (97.6)	307 (99.4)
Antenatal corticosteroid[b]	13 (68.4)	104 (70.3)
Multiple pregnancy	12 (28.6)	72 (23.3)
Cesarean Section	23 (54.8)	178 (57.6)
Prolonged rupture of membrane	25 (59.5)	189 (61.2)
Invasive respiratory support > 1wk	13 (31.0)	133 (43.0)
Hospital stay >3 wk	6 (14.3)	88 (28.5)
Antibiotic exposure >72 h	17 (40.5)	158 (51.1)
Arterial/ venous catheter >72 h	21 (50.0)	168 (54.4)
Total parenteral nutrition	9 (21.4)	71 (23.0)
Persistent thrombocytopenia (<100×10^9L)	19 (45.2)	185 (59.9)

Values in no. (%) or [a]median (IQR). [b]denominators were 19 (in fungal sepsis group) and 148 (without fungal sepsis group). All P≥0.05.

Similar to previous observation, *C. tropicalis* was the predominant fungal pathogen in the current study, followed by *C. albicans* [8]. Sensitivity to antifungal agents was also similar to the previous reports [8,9]. Juyal, *et al.* [9] noted *C. parapsilosis* as the most common species, which might be due to difference in microbiological pattern between tertiary centers. Area under curve of the ROC curve was comparable to previous observations [5,10] and other characteristics of serum BDG were similar to the findings of Mackay, et al. [5] and met the criteria of an excellent screening test [7]. Lower sensitivity and higher specificity was reported by Shabaan, et al. [10], possibly due to use of a higher cut-off value. Variation of BDG between different species of Candida could not be evaluated due to two unidentified isolates. Effect of combining other markers with BDG was also not checked.

Serum BDG level could be used as a diagnostic marker of fungal sepsis in high-risk newborns; however, its use can could only be recommended after further evaluation in multiple settings.

Ethics Clearance: IEC, Burdwan Medical College; No. BMC-B-1058/C, dated July 11, 2017.
Contributors: KI: writing manuscript, collection of data, analysis of data; NK: collection of data, analysis of data, designing study; UM: writing and revising manuscript; KKD: collection of data, analysis of data; KN: planning study, revising manuscript. All authors approved the final version of manuscript.
Funding: None; *Competing interest*: None stated.

KAMIRUL ISLAM, NAZIMA KHATUN, UJJAL MONDAL, KUNTALKANTI DAS,* KAUSTAV NAYEK
Department of Pediatrics, Burdwan Medical College, Burdwan, West Bengal.
**kuntalkantidas1986@gmail.com*

REFERENCES

1. Benjamin DK, Stoll BJ, Fanaroff AA, et al. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics. 2006;117:84-92.
2. Makhoul IR, Kassis I, Smolkin T, et al. Review of 49 neonates with acquired fungal sepsis: further characterization. Pediatrics. 2001;107:61-6.
3. Jaijakul S, Vazquez JA, Swanson RN, et al. (1,3)-β-D-glucan as a prognostic marker of treatment response in invasive candidiasis. Clin Infect Dis. 2012;55:521-6.
4. Guo J, Wu Y, Lai W, et al. The diagnostic value of (1,3)-β-D-glucan alone or combined with traditional inflammatory markers in neonatal invasive candidiasis. BMC Infect Dis. 2019;19:716.
5. Mackay CA, Ballot DE, Perovic O. Serum 1,3-β-D-glucan assay in the diagnosis of invasive fungal disease in neonates. Pediatr Rep. 2011;3:e14.
6. Cotten CM, McDonald S, Stoll B, et al. The association of third-generation cephalosporin use and invasive candidiasis in extremely low birth-weight infants. Pediatrics. 2006;118: 717-22.
7. Kumar R, Indrayan A. Receiver operating characteristic (ROC) curve for medical researchers. Indian Pediatr. 2011;48: 277-87.
8. Basu S, Kumar R, Tilak R, Kumar A. Candida blood stream infection in neonates: Experience from a tertiary care teaching hospital of central India. Indian Pediatr. 2017;54:556-9.
9. Juyal D, Sharma M, Pal S, et al. Emergence of non-albicans Candida species in neonatal candidemia. N Am J Med Sci. 2013;5:541-5.
10. Shabaan AE, Elbaz LM, El-Emshaty WM, et al. Role of serum (1,3)-β-d-glucan assay in early diagnosis of invasive fungal infections in a neonatal intensive care unit. J Pediatr (Rio J). 2018;94:559-65.

Juvenile Xanthogranuloma of Subglottis: Rare, Recurrent and Refractory

Juvenile xanthogranuloma is a benign, proliferative disorder seen in early childhood. It is the most common form of non-Langerhans cell histiocytosis (LCH) and usually presents with self-resolving cutaneous lesions [1]. Laryngeal xanthogranuloma is extremely rare and only seven cases have been reported in literature. These cases presented with airway obstruction requiring either surgical excision or tracheostomy. Recurrences are not uncommon [2]. A 4-year-old girl presented with acute onset stridor and respiratory distress. She was initially managed as croup with no improvement. On examination, there were no cutaneous lesions, organomegaly or any other abnormality. Contrast-enhanced computed tomography (CECT) neck revealed a well-defined uniformly enhancing tracheal mass at C5-C6 level with marked narrowing of subglottic airway (**Fig. 1)**. Bronchoscopy showed subglottic mass causing significant airway obstruction. Histopathological examination of the mass showed sheets of foamy histiocytic, numerous multinucleated giant cells, plasma cells and areas of hemorrhage. Immunohistochemistry confirmed positivity for CD68 and was negative for CD1a, consistent with the diagnosis of juvenile xanthogranuloma. Following histopathological confirmation, she underwent microlaryngeal surgery. Tracheal mass was excised followed by laser and mitomycin C ablation. She improved and was discharged on short course of steroids.

After five months, she had a recurrence, which was confirmed on bronchoscopy and imaging. She was given steroids and vinblastine as per LCH protocol, but had partial response. On follow-up, child has mild snoring while sleeping; however, there is no respiratory difficulty and she continues to be under close surveillance.

Airway obstruction in young children is usually secondary to foreign bodies or infectious/ inflammatory triggers. Tumorous growths are relatively rare in this age group. Diagnosis in such cases may therefore be delayed. Timely intervention with CT imaging and bronchoscopy is crucial in these scenarios as definitive diagnosis requires histopathological confirmation. Juvenile xanthogranuloma, a benign proliferative disorder of dendritic cell phenotype, is known to have varied biological behaviour from spontaneously regressive cutaneous lesion to rare but fatal central nervous system involvement. The pathogenesis is unknown and the initiating stimuli may be one of many infectious or physical factors [3]. Cutaneous manifestations are most common presenting with solitary yellowish nodules. Extracutaneous manifestations have been reported in 4% of children [4]. Most systemic lesions undergo spontaneous resolution; however, serious complications are known to be associated with few systemic juvenile xanthogranuloma cases [5].

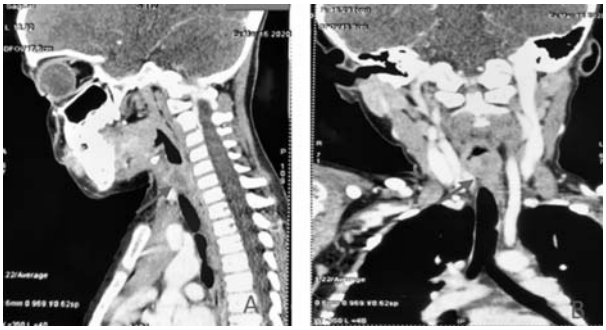


Fig. 1 Contrast-enhanced computed tomography images (sagittal view: A and coronal view: B) showing an elongated mildly enhancing soft tissue mass seen in the glottic and subglottic larynx, extending from the level of posterior commissure at true glottis level and extending inferiorly into the posterior subglottic region, causing marked narrowing of subglottic airway.

Diagnosis of the condition is confirmed by histological features and immunohistochemistry. Treatment of laryngeal juvenile xanthogranuloma includes local excision with or without tracheostomy. In case of recurrence in present case, we achieved partial; though satisfactory, response with steroids and vinblastine as per LCH protocol.

In conclusion, juvenile xanthogranuloma should be considered as a differential diagnosis for subglottic masses causing airway obstruction. Considering its favorable prognosis and tendency of spontaneous regression, decision for reductive surgery with or without tracheostomy needs to be taken judiciously. Close follow-up is advised as recurrence is not uncommon and may require further intervention.

CHANDRIKA VERMA, KUSHAGRA TANEJA,* AMITA MAHAJAN
Department of Pediatric Hematology Oncology, Indraprastha Apollo Hospital, New Delhi.
**kushagrataneja321@gmail.com*

REFERENCES

1. Weitzman S, Jaffe R. Uncommon histiocytic disorders: the non-Langerhans cell histiocytoses. Pediatr Blood Cancer. 2005;45: 256-64.
2. Kawamoto A, Katori Y, Honkura Y, et al. Isolated juvenile xantho-granuloma in the larynx of a three-year-old child. Tohoku J Exp Med. 2013;230:123-7.
3. Chang MW. Update on juvenile xanthogranuloma: Unusual cutaneous and systemic variants. Semin Cutan Med Surg. 1999; 18:195-205.
4. Dehner LP. Juvenile xanthogranulomas in the first two decades of life: A clinicopathologic study of 174 cases with cutaneous and extracutaneous manifestations. Am J Surg Pathol. 2003;27:579-93.
5. Freyer DR, Kennedy R, Bostrom BC, et al. Juvenile xanthogranuloma: Forms of systemic disease and their clinical implications. J Pediatr. 1996;129:227-37.

Neonatal Multisystem Inflammatory Syndrome (MIS-N) presenting as Necrotizing Enterocolitis and Cardiac Dysfunction

Multisystem inflammatory syndrome in neonates (MIS-N) associated with perinatal severe acute respiratory syndrome 2 (SARS-COV-2) exposure is increasingly being reported in recent times [1-3]. MIS-N presents with a variety of clinical presentations, requires a high index of suspicion, and is a diagnosis of exclusion.

We report a female neonate of 2.64 kg, born late preterm at 35 weeks gestation, by cesarean section due to placenta previa and preterm labor. The baby was vigorous at birth, treated initially for transient tachypnea of the newborn (TTNB), and shifted to the mother's side on day 2 with exclusive breastfeeding. The baby developed abdominal distension with bilious vomiting on day 3 of birth and was shifted to NICU. She developed fever spikes of 38.5°C and shock within 12 hours of abdominal symptoms. On examination, she was febrile, drowsy, had tachycardia (heart rate, 202/min), had hypotension (mean blood pressure:32 mm of Hg) with cool peripheries, tachypnea (respiratory rate:72/min) with chest retractions, and oxygen saturation of 96% on 30% FiO2 with 5 cm of CPAP support. The precordium was hyperdynamic with a systolic murmur of grade 3/6 over the left infraclavicular area, hepatomegaly of 3 cm below right costal margin and tender abdomen. *X*-ray abdomen showed dilated bowel, right iliac soap bubble appearance and left sided pneumatosis intestinalis (**Fig. 1**). An echocardiogram revealed moderate biventricular dysfunction with poor contractility (left ventricular ejection fraction of 42%), dilated right atrium and right ventricle, large patent ductus arteriosus (4 mm) with a left to right shunt, dilated inferior vena cava, and normal coronaries. The sepsis screen was positive with elevated CRP (61.6 mg/dL) and neutrophilic leukocytosis (total 22×10^9/L, 78% neutro-phils). A diagnosis of probable sepsis with hemodynamically significant PDA (HsPDA) and necrotizing enterocolitis (NEC) was made. Treatment included nil per oral, intravenous fluids, orogastric decompression, antibiotics, frusemide, inotropic support, and mechanical ventilation. On reviewing the history, mother was unvaccinated for coronavirus disease 2019 (CoVID-19) and had a SARS-CoV-2 infection three weeks before delivery.

The baby had high titers of IgG antibodies 241.13 AU/mL (<10.0 non-reactive) with negative IgM titers for SARS-CoV-2. The SARS-CoV-2 reverse transcriptase – polymerase chain reaction (RT-PCR) of the neonate was negative. Inflammatory markers done on day 4 of birth were elevated, (IL6 19.9 pg/mL, ferritin:244 ng/mL, NT-Pro BNP: >35000 pg/mL), serum LDH 1086.00 U/L, D-dimer: 4259 ng FEU/mL. Blood culture was sterile. The baby was treated with dexamethasone (0.15 mg/kg/dose 12-hourly for 3 days followed by oral prednisolone 1 mg/kg/day for 4 days) and intravenous immunoglobulins (1 g/kg/day of IVIG for 2 days). The baby gradually improved in the next 48 hours with improvement in cardiac function, closure of the PDA, resolution of abdominal distension, shock, and normalization of inflammatory markers. The baby was extubated on day 5, full feeds were achieved on day 8, and was successfully discharged on day 10.

Acute deterioration on day 3, shock needing inotrope support and ventilation, NEC requiring supportive care, cardiac dysfunction, negative blood cultures, high inflammatory markers, positive COVID serology with maternal COVID history led us to the possibility of MIS-N in this newborn, which meets the modified CDC criteria for MIS-N.

The exact pathogenesis for MIS-N remains elusive with the most possible hypothesis being immune dysregulation and endothelial injury. Neonates with MIS-N present with respiratory (respiratory distress), cardiac (cardiac dysfunction, coronary aneurysms, thrombus, conduction abnormalities), gastrointestinal (NEC), central nervous system (encephalopathy, stroke), dermatological (vasculitis rash), and sepsis-like (fever, hypothermia, shock) manifestations. Immunomodulator therapy (IVIG, steroids) forms the crux of management of MIS-N [5].

We conclude that MIS-N should be considered in any neonate with unexplained necrotizing enterocolitis and/or cardiac dysfunction, after ruling out the common causes.

SAI KIRAN VODDAPELLI, SRINIVAS MURKI, VADIJE PRAVEEN RAO*
Department of Neonatology, Paramitha Children's Hospital, Hyderabad, Telangana.
**pravinv2107@gmail.com*

REFERENCES

1. Diggikar S, Nanjegowda R, Kumar A, et al. Neonatal multisystem inflammatory syndrome secondary to SARS CoV 2 infection. J Paediatr Child Health. 2021. Aug 13:10.1111/jpc.1569.
2. Lakshminrusimha S, Hudak ML, Dimitriades VR, Higgins RD. Multisystem inflammatory syndrome in neonates following

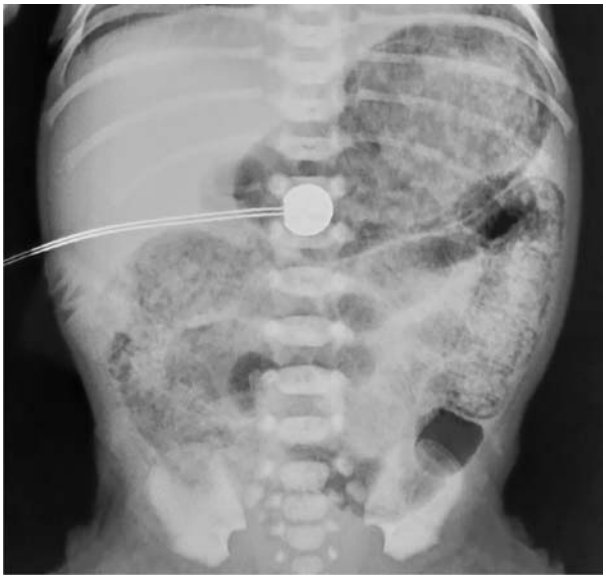


Fig. 1 Abdominal *X*-ray showing dilated bowel, soap bubble appearance in the right iliac fossa, and possible pneumatosis intestinalis in left lower abdominal quadrant.

maternal SARS-CoV-2 infection. Am J Perinatol. 2021. Oct 28. [Epub ahead of print].
3. Pawar R, Gavade V, Patil N, et al. Neonatal multisystem inflammatory syndrome (MIS-N) associated with prenatal maternal SARS-CoV-2: A case series. Children. 2021;8:572.
4. More K, Aiyer S, Goti A, et al. Multisystem inflammatory syndrome in neonates (MIS-N) associated with SARS-CoV-2 infection: a case series. Eur J Pediatr. 2022;81;1889-98.
5. Kappanayil M, Balan S, Alawani S, et al. Multisystem inflammatory syndrome in a neonate, temporally associated with prenatal exposure to SARS-CoV-2: A case report. Lancet Child Adolesc. Health. 2021;5:304-8.

Rapunzel Syndrome: Diagnosed After Laparotomy in a Young Girl

Rapunzel syndrome is an unusual and rare form of trichobezoar found in patients with the habit of hair pulling (trichotillomania) and swallowing it (trichophagia), consequently leading to collection of bezoars in stomach and intestines. We describe this syndrome in a very young girl who presented to us as a case of moderate acute malnutrition with intestinal obstruction.

Bezoars have been known to occur in the form of undigested masses found in the stomach but Rapunzel syndrome involves the presence of a gastric trichobezoar with a long tail extending beyond the duodenum and till terminal ileum [1]. Around 100 cases have been described in the literature since then, with a mean age of presentation of 10.8 years [2,3]. It is usually seen in young girls with or without known psychiatric disorders [4].

A 5-year-old girl presented to us with poor growth and not eating well. There was history of pain abdomen and occasional vomiting after meals. On examination, the child looked pale, emaciated, and stunted. Her hemoglobin level was 9 g/dL. Her weight was 11.2 kg (< 1st centile as per WHO weight-for-age chart and her height was 92.5 cm (<1st centile WHO height-for-age chart). The abdomen was mildly distended with normal bowel sounds. The patient's mother admitted that she had a habit of picking hair from floor and secretly swallowing it. A diagnosis of moderate acute malnutrition with moderate anemia with sub-acute intestinal obstruction was made. The patient was kept nil per orally and received intravenous fluids, and surgical opinion was sought. After two days, the child passed stool mixed with hair strands with relief of abdominal distention. The child was allowed to eat semi-solid food, which resulted in vomiting of strands of hair and recurrence of abdominal distention. CBC showed moderate anemia with dimorphic picture. *X*-ray abdomen showed dilated gut with multiple air fluid level at various level. Serial USG abdomen failed to detect any intra luminal mass in the stomach or duodenum and ileum. Computed tomography was not available at that time.

Exploratory laparotomy was performed through a supraumbilical midline abdominal incision. A longitudinal 4 cm gastrotomy made on the anterior surface of the corpus of the stomach revealed an intraluminal smooth contour mass occupying bulk of stomach with post pyloric extension. There was a continuous thin strand of trichobezoar in duodenum with thick tail of 2-3 cm diameter along jejunum and terminal ileum, which were removed with separate enterotomy.

After discharge child was provided nutritional rehabilitation. Five months post-surgery, the child has gained 3 kg weight and is on iron supplements. She is receiving behavioural modification advice but not on any psychiatric treatment.

Rapunzel syndrome is rare but should be taken into consideration while investigating a malnourished child with intestinal obstruction. Trichotillomania and trichophagia as a diagnosis are considered on finding some bald patches on scalp or history of habit of pulling out hair and swallowing it [4]. This child did not have bald patches on her scalp.

Upper gastrointestinal endoscopy is the gold standard diagnostic modality but was not available at this hospital [3]. USG abdomen, although is said to be specific for trichobezoar, but in our case serial ultrasound abdomen failed to detect the nature or presence of any intra luminal mass. Ultrasound as an imaging modality has often missed subtle finding of a trichobezoar [5]. CT scan reveals the nature and the extent of the trichobezoar, and is regarded as the best modality for diagnosis. Obstruction of the upper digestive tract is the most common clinical manifestation of this disorder [1].

In the present case, surgery was attempted as an exploratory method due to uncertain cause of intestinal obstruction. Psychiatric follow-up is important, and care should be extended to family members, who should be vigilant with patients since recurrences of the problem have been described [4]. The need for adequate follow-up should be emphasized to avoid recurrences, although these are rare since the trauma of surgery may prevent the patient from provoking another episode.

SPALCHEN GONBO,* TSEWANG NAMGYAL, YANGZIN DOLMA
Pediatric and Surgery Unit District Hospital Leh, SNM Hospital Leh, Ladakh.
**spalchen@gmail.com*

REFERENCES

1. CL Andrus,Ponsky JL. Bezoars: classification, pathophysiology, and treatment. Amer J Gastroenterol. 1988;83:476-78.
2. West WM, Duncan ND. CT appearances of the Rapunzel syndrome: An unusual form of bezoar and gastrointestinal obstruction. Pediatric Radiol. 1998;28:315-6.
3. Gupta A, Mishra S, Upadhyay VD, et al. Rapunzel syndrome in a seven-year-old girl. Indian J Surg. 2022:84:236-37.
4. Frey AS, McKee M, King RA, et al. Hair apparent: Rapunzel syndrome. Am J Psychiatry. 2005;162:242-8.
5. Lyons D. Large gastric trichobezoar causing failure to thrive and iron deficiency anaemia in an adolescent girl: a case report emphasizing the imaging findings and review of the literature. BJR Case Rep. 2019;5:20180080.

Vitamin D Levels and Cardiopulmonary Status in Infants with Acute Bronchiolitis: Tip of the Iceberg?

We read with interest the recent article assessing the association of vitamin D deficiency (VDD) with cardiopulmonary status in infants with acute bronchiolitis [1]. They have concluded that low vitamin D levels were associated with clinical severity and impaired cardiopulmonary status in infants with acute bronchio-litis [1]. We have the following comment on their study [1].

VDD is common in infants with respiratory tract infections and those with respiratory syncytial virus (RSV) bronchiolitis [2]. However, there are many studies in which the association between vitamin D levels and disease severity was not detected [2]. Vo, et al. [2] have suggested that bioavailable 25-OHD deficiency is associated with length of hospital stay and admission to the pediatric intensive care unit (PICU) [2]. Contribution of comorbidities such as age, prematurity, congenital heart disease, chromosomal abnor-malities, chronic lung disease, or immunodeficiency to the severity of the infection should also be considered [3]. In particular, age was associated with non-invasive ventilation (NIV) failure, length of hospital stay, and disease severity [3]. We think it would be useful to consider other factors when commenting on the relationship between vitamin D levels and the severity of bronchiolitis.

Another issue we want to draw attention to is that the NIV was used with a high frequency of 25% in this study [1], but the details about the NIV methods are lacking. A meta-analysis of 15 studies [4] showed that high-flow nasal oxygen therapy is beneficial and safe, with similar failure rates with continuous positive airway pressure. Secondly, bi-level positive pressure ventilation has been reported to be associated with longer PICU stay and prolonged ventilation needs [5]. Although NIV is widely used for bronchiolitis, there is no consensus on what the indications are, what the correct method is, what the failure criteria are, and how the weaning should be done.

We feel that further studies are needed to determine the exact role of vitmin D level and cardiorespiratory status in infants with respiratory infection.

Zeynelabidin Ozturk,[1] Antonio M. Esquinas[2]
[1]Department of Pediatric Intensive Care, Dr. Sami Ulus Obstetrics and Gynecology, Pediatric Health and Disease Training and Research Hospital, University of Health Sciences, Ankara, Turkey.
*[2] Intensive Care Unit; Hospital Morales Meseguer, Murcia, Spain. *zeynelabidin_ozturk@hotmail.com*

REFERENCES

1. Estalella-Mendoza A, Castellano-Martínez A, Flores-González JC, et al. Vitamin D levels and cardiopulmonary status in infants with acute bronchiolitis. Indian Pediatr. 2022;59:384-87.
2. Vo P, Koppel C, Espinola JA, et al. Vitamin D status at the time of hospitalization for bronchiolitis and its association with disease severity. J Pediatr. 2018;203:416-22.e1.
3. D'Alessandro M, Vanniyasingam T, Patel A, et al. Factors associated with treatment failure of high-flow nasal cannula among children with bronchiolitis: A single-centre retrospective study. Paediatr Child Health. 2020;26:e229-35.
4. Dafydd C, Saunders BJ, Kotecha SJ, et al. Efficacy and safety of high flow nasal oxygen for children with bronchiolitis: systematic review and meta-analysis. BMJ Open Respir Res. 2021; 8:e000844.
5. Delacroix E, Millet A, Pin I, Mortamet G. Use of bilevel positive pressure ventilation in patients with bronchiolitis. Pediatr Pulmonol. 2020;55:3134-8.

AUTHORS' REPLY

We thank the readers for their thoughtful comments. We agree that the role of vitamin D as a biomarker for severity in acute bronchiolitis has been previously reported with controversial results. We think the differences in the prevalence of vitamin D deficiency (VDD), and vitamin D level cut-off points used to define VDD across studies could be the main reason for these conflicting results. Thus, the low incidence of VDD in studies showing no correlation between the severity of disease and vitamin D [1,2] is a significant limitation to consider when interpreting their results. Conversely, studies with larger sizes and higher rates of VDD [3,4] had conclusions similar to ours [1]. We also agree with the comment about the relevance of age and prematurity on the severity of acute bronchiolitis. Thus, as a significant limitation, we discussed the possible influence of age and prematurity as confounding factors on our results, which should not be fully considered until further studies determine the exact role of vitamin D in this setting.

The high rate of NIV in our series could be explained by the low median age (1 month) and the high rate of prematurity (20%) in our population, which are apparent risk factors for a need for ventilation in acute bronchiolitis. We used NIV in the presence of respiratory acidosis or moderate-severe respiratory distress. In the meta-analysis cited [5], HFNC was not superior to CPAP in these situations. In addition, it has not shown differences in treatment or intubation failure rates; and it does not analyze the differences between HFNC and BIPAP, which was the modality used in all the children in our series. The more extended stay in the PICU in patients with BIPAP may be due to a longer duration in days of BIPAP and the severity of bronchiolitis since they also found a significant association in the level of pH and pCO2, and prematurity as a comorbidity in the BiPAP group [6]. Although there are many studies investigating the role of vitamin D as a risk factor for severe acute bronchiolitis, addition of more data to the literature could help clarify this controversial issue, especially in the association between different cardiac variables and VDD in this setting.

Ana Castellano-Martinez
Pediatric Nephrology Division, Puerta del Mar University Hospital, Cádiz, Spain.
anacastellanomart@gmail.com

Live Leech as a Tracheal Foreign Body

A previously healthy 4-year-old girl presented with 10 days wheezing, difficulty breathing, and hemoptysis, with a 20 days history of a live leech coming out of the nasal cavity, but without cough. There was no abnormality noted physical examination. A chest computed tomography (CT) scan showed the existence of relatively high protrusions above the posterior tracheal wall at the level of sixth and seventh cervical vertebra (**Fig. 1 a,b**). She underwent tracheal foreign body removal surgery under general anesthesia with high-frequency jet ventilation. The foreign body was found sub-glottic on the posterior tracheal wall and confirmed to be a live leech (**Fig. 1c**) and no respiratory irritation related syndrome. The patient had an unremarkable postoperative course, and was discharged home on the second day after surgery.

Although, tracheal foreign bodies frequently occurs in children, an alive leech was surprising.

Funding: Doctoral Research Initiation Fund of Affiliated Hospital of Southwest Medical University (19022).

RENSHU ZHAN, YIPING BAI*
Department of Anesthesiology,
Affiliated hospital of Southwest Medical University,
Luzhou, China.
**baiyiping0608@163.com.com*

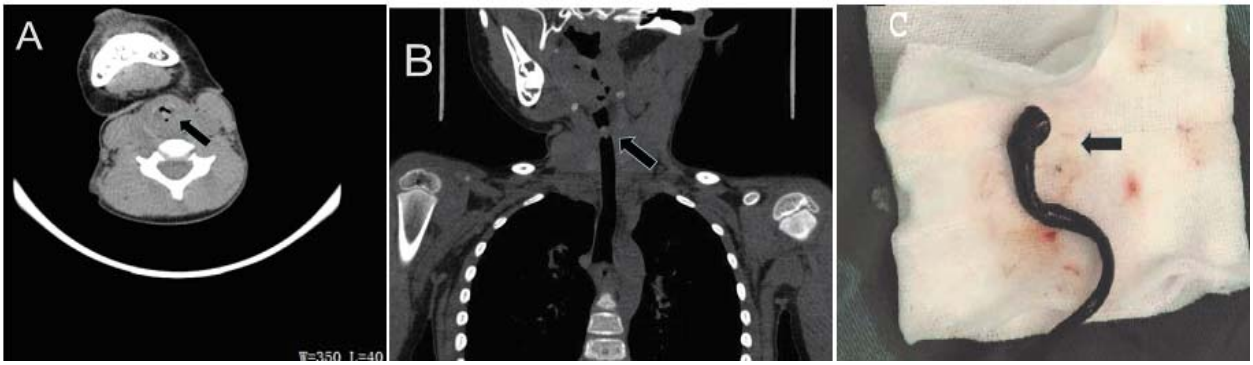


Fig. 1 *a,b*) computed tomography showing the airway blocked by a foreign body (arrow); *c*) the alive leech removed from the trachea.

Chronic Urticaria in a Child With Nephrotic Syndrome: A Double Whammy!

Co-occurrence of nephrotic syndrome and chronic urticaria in a child is quite rare. Herein, we describe the concomitant occurrence of these two entities and possible role of dietary pseudoallergens in the causation.

An 18-months-old boy presented to pediatric inpatient department with five days history of periorbital and pedal edema, decreased urine output, fever, and recurrent urticarial rash. Based on these clinical presentations, nephrotic syndrome was suspected and specific laboratory testing was performed to establish diagnosis. Diagnostic workup by the treating pediatrician confirmed the diagnosis of nephrotic syndrome. Corticosteroid therapy (prednisolone) was started and tapered over period of 12 weeks. However, urticaria reappeared as the steroids were tapered and stopped. It did not resolved even with anti-histaminics and steroid therapy, and followed a relapsing remitting course. On dermatological review, urticarial rash had been present since 8 months of age, ever since the introduction of formula feed in the child's diet. Our differential diagnosis included hypocomplementemic urticarial vasculitis syndrome (HUVS) and pseudoallergen - induced chronic urticaria. Suggested investigations could not be carried out due to resource constraints. Complete blood count, erythrocyte sedimentation rate, C reactive protein, anti streptolysin O titre, antinuclear antibodies, autologous serum skin test, absolute eosinophil count, thyroid function test, IgE levels, CH50, C1q, C2, C3, C4 levels, stool microscopy and culture, urine analysis, dental and ENT examination were done. All investi-gations were within normal limits. Skin biopsy was performed to rule out vasculitis. Allergen test was not done to due to feasibility issues. Absence of any other symptoms, normal complement levels and painless itchy fleeting wheals with duration of 1 to 24 hours ruled out HUVS. Since, there was a temporal association between initiation of baby formula feed and onset of urticaria, we looked at its ingredients in detail, which were found to be partially hydrolyzed milk protein (casein and whey), azo dyes as coloring agent, and sodium benzoate as preservatives. All these are known pseudoallergens. One week analysis of child's diet revealed that he was not having any finished, packaged or convenience products except formula feed. After pediatric consultation, we removed this formula feed from his diet and put him on a low pseudoallergen diet for 3 weeks. This was followed by a marked improvement in the chronic urticaria, and no recurrence of the lesions.

Pseudoallergens in diet are one of the most common causes of chronic urticaria in adults [1]. However, there is still scarcity of literature on pseudoallergen-induced urticaria in children [2].

Pseudoallergies can only be diagnosed via a strict exclusion diet.

Role of allergens in pathogenesis of nephrotic syndrome have already been elucidated in the literature. Fanconi, et al. [3] suggested that allergens could be the triggering factor in the development of proteinuria. There are several case reports exemplifying the role of food allergens in minimal change disease. Laurent, et al. [4] studied the effect of allergen-free diets in steroid-dependent or steroid-resistant idiopathic nephrotic syndrome. Although there is dearth of data on role of pseudoallergens in diet as a triggering factor in nephrotic syndrome, our case suggests the possibility. Further research is needed to confirm this correlation.

AAKANKSHA ARORA,* ALPANA MOHTA
Department of Dermatology, Venereology and Leprology, Sardar Patel Medical College, Bikaner, Rajasthan.
**akarora357@gmail.com*

REFERENCES

1. Zuberbier T, Chantraine-Hess S, Hartmann K. Czarnetzki BM. Pseudoallergen-free diet in the treatment of chronic urticaria - a prospective study. Acta Derm Vencreol (Stockh) 1995:75:484-7.
2. Ghiordanescu I, Roxana SB. Pseudoallergic reactions to food and drugs in children with chronic idiopathic urticaria. Clinical Translational Allergy. 2014;4:31.
3. Fanconi G, Kousmine C, Frisch B, Knecht W. Prognosis of the nephrotic syndrome. Helv Pediatr Acta. 1951;6:219-24.
4. Laurent J Guy R, Robeva R. Is adult idiopathic nephrotic syndrome food allergy? Value of oligoantigenic diets. Nephron. 1987;47:7-11.

Subcutaneous Calcification at Honeybee Sting Site

Children very frequently become victims of honeybee sting because of their limited self-defence and curiosity. Clinical manifestations are usually acute, and chronic complications are extremely rare [1]. We report late onset, local subcutaneous calcification following an apparently uneventful honeybee sting.

An 8-year-old girl presented with a subcutaneous mass over the left suprascapular region for 7 months. There was a history of sting by honeybee at the same site 10 months back. The child had mild self-limiting pain following the incident. A slowly progressing mass was noticed by the parents three months after the sting. On examination, the mass was 25mm x7 mm in size, irregular, firm, mobile and nontender (**Fig. 1a**). The overlying skin was free, with a small visible hypopigmented swelling. Chest *X*-ray revealed subcutaneous calcification. Ultrasonography over the swelling revealed a subcutaneous, hyperechoic deposit with a linear morphology that produced an acoustic shadow which was suggestive of calcific lesion. The lesion was measuring 18mm x 8mm, without any evidence of vascularity (**Fig. 1b**). Serum calcium levels were normal. A diagnosis of subcutaneous calcification secondary to honey bee sting was made. Parents were counseled regarding the benign nature of the lesion.

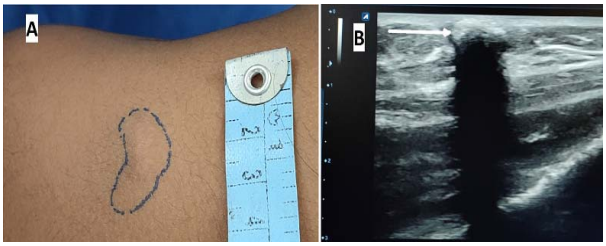


Fig. 1 *a*) Crescent shaped left suprascapular swelling measuring 2.5×0.7 cm.; *b*) High resolution ultrasonography image showing subcutaneous, hyperechoic deposit with a linear morphology (white arrow) and acoustic shadow suggestive of calcific lesion.

In honeybee sting, the stinger is detached from the body following sting which leads to death of the insect [1]. The clinical features following honeybee sting may comprise of allergic reactions, organ dysfunction and rarely, late manifestations like formation of granuloma and subcutaneous tissue calcifications [2,3].

Unlike honeybee sting, subcutaneous calcifications have been reported following sting by other insects belonging to Hymenoptera [4]. Both dystrophic and metastatic calci-fications can occur due to toxic reactions by the venom, direct inoculation of bacteria and secondary to immune reactions. Hyperphosphatemia, raised levels of TNF-α and corticosteroid use have been linked to the metastatic calcification following the sting [4].

Long term follow up is necessary in these cases in view of the possibility of granuloma formation and calcification. Clinicians should be aware of this rare and late complication in order to avoid misdiagnosis and unnecessary treatment in affected children.

DIPTIREKHA SATAPATHY, THIRUNAVUKKARASU ARUN BABU*
Department of Pediatrics,
All India Institute of Medical Sciences (AIIMS),
Mangalagiri, Andhra Pradesh, India.
** babuarun@yahoo.com*

REFERENCE

1. Vikrant S, Jaryal A, Parashar A. Mortality due to mass Hymenoptera attacks: A serious but underrecognized public health problem in a mountainous state of India. Indian J Public Health. 2019;63:154-6
2. Poddar K, Poddar SK, Singh A. Acute polyradiculoneuropathy following honey bee sting. Ann Indian Acad Neurol. 2012;15:137-8
3. Yamamoto K, Nakayama Y, Matsusue Y, et al. A foreign body granuloma of the buccal mucosa induced by honeybee sting. Case Rep Dent. 2017;7902978.
4. Liu WJ, Li W, Tang Y, et al. Soft tissue calcifications secondary to Hymenoptera stings: a potential prognostic CT imaging sign in pediatric patients. Clin Toxicol (Phila). 2018;56:886-92.

Uterus and Vaginal Prolapse in a Neonate

A girl weighting 2.5 Kg was born at term gestation, via caesarean section due to (breech presentation) was referred to us on day 1 of life as ambiguous genitalia. On examination, it was found to be uterine and vaginal prolapse through introitus, which was confirmed from an ultrasound (uterus was not present in the pelvic region). A diagnosis of the uterus and vaginal prolapse was made and prolapse was reduced completely, and did not reoccur. The differential diagnosis of mass from introitus at birth are vaginal tumor, vaginal polyp, and urethral prolapse. Treatment of uterus and vaginal prolapse is by careful digital reduction.

Chiruvella Subramanyam,* Bhavana B Lakhkar
Department of Pediatrics, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi Meghe, Wardha, Maharashtra.
**subbuch26@gmail.com*

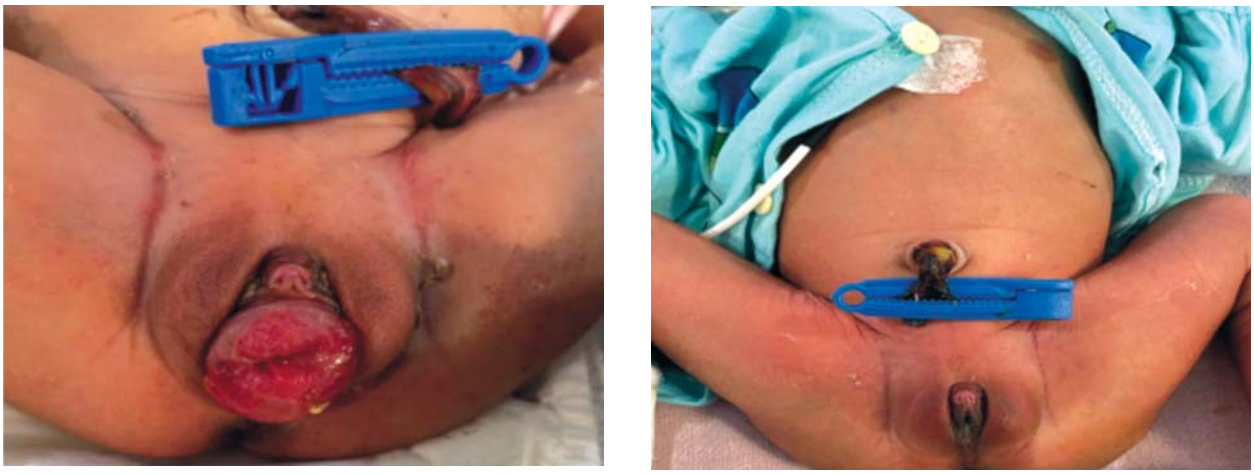


Fig. 1 (*a*) Uterus and vaginal prolapse, and (*b*) the status after reduction.

Riga-Fede Disease

A 2-month-old girl presented with ulceration of tip and ventral surface of the tongue since 3 weeks with history of failure to thrive (**Fig. 1**). The lesion had first started as a small erosion of mucosa of tongue, with progressive enlargement and was now measuring 1 cm in diameter. It was covered with white plaque in contact with the lower incisors. Baby was planned for extraction of neonatal tooth, and feeding counselling was provided.

Riga-Fede disease (RFD) is an uncommon benign mucosal ulceration of the tongue caused by tongue movements over mandibular anterior incisors resulting in repetitive traumatic injuries. Its most common site is on the tongue but lip, palate, gingiva, vestibular mucosa and floor of the mouth may also get involved. It usually presents in early infancy in association with natal or neonatal teeth or eruption of lower incisors. Failure to diagnose RFD may perpetuate tongue ulceration, poor feeding and poor weight gain and occasional dislodgement and swallowing/aspiration of teeth. Principal differential diagnosis includes ulcerative candidiasis, tuberculosis, cytomegalovirus, lymphomas and leukemias. Treatment includes dental extraction and local antiseptic pain relieving and soothing gel over area of ulceration.

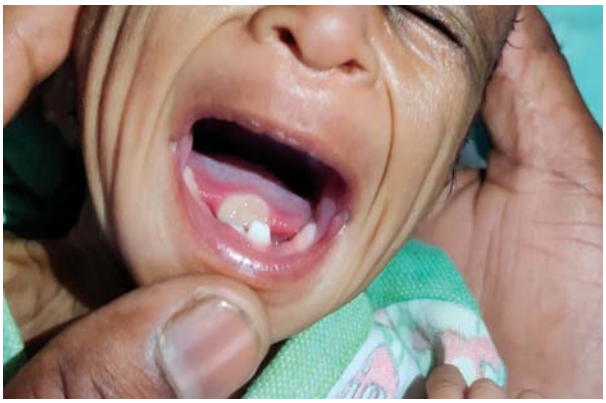
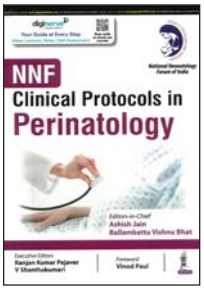


Fig. 1 Neonatal teeth causing Riga-Fede disease in a 2- month-old infant.

Mukesh Vir Singh, Shahid Akhtar Siddiqui*
Department of Pediatrics, SN Children Hospital, MLN Medical College, Allahabad, Uttar Pradesh.
**sha.akht@yahoo.com*

BOOK REVIEWS



NNF- Clinical Protocols in Perinatology

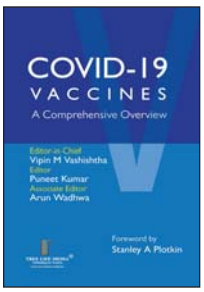
ASHISH JAIN, BALLAMBATTU VISHNU BHAT
Jaypee Brothers Medical Publishers (P) Ltd., EMCA House, Ansari Road, Daryaganj, New Delhi.
Pages: 390; Price: Rs.1995/-

This book on clinical protocols in perinatology, brought out by the National Neonatology Forum, is a step in the right direction to highlight the importance of a holistic perinatal approach to the high risk mother-newborn dyad, to improve their outcomes.

Perinatal medicine, also known as maternal-fetal medicine, has important preventive, therapeutic as well as interventional scope to reduce the burden of problems not only in the newborn baby but extending into childhood and adulthood, with the now established fetal origins of childhood and adult diseases. Attempting to prevent births with chromosomal anomalies or major structural malformations, improving outcomes of newborns of mothers with common medical problems like pre-gestation and gestational diabetes, hypertension, pregnancy related problems like pre-eclampsia, isoimmunization, multiple pregnancies, early diagnosis and management of fetal growth restriction are few of the components of perinatal medicine.

All these issues and more have been well-addressed in this book. The book has 51 topics covered under seven sections addressing fetal wellbeing, birth defects, fetal effects of maternal medical and obstetric conditions, congenital and perinatal infections, and delivery room management. There is also a chapter covering ethical and medicolegal issues in the perinatal period. Each topic is informative yet concise and has been well addressed jointly by obstetric/fetal medicine experts and neonatologists and is supported by tables, flow charts, clinical pictures and illustrations as needed. At the end of each topic is a useful section on 'chapter at a glance' as well as 'key-points' This book will serve as a ready reckoner for perinatal management for postgraduates as well as practitioners of Neonatology, Pediatrics and Obstetrics.

JAYASHREE MONDKAR
Emeritus Professor,
Department of Neonatology,
Lokmanya Tilak Municipal Medical College & General Hospital,
Sion, Mumbai, Maharashtra.
jayashreemondkar@rediffmail.com



COVID-19 VACCINES-A Comprehensive OVERVIEW

VIPIN M VASHISHTA, PUNEET KUMAR, ARUN WADHWA
Tree Life Media Publishing for Practice 2022
Pages: 425; Price: Rs.1595/-

With so much of varying opinions and claims regarding the dosage schedule, interval between doses, immediate and long-term efficacy of currently available and used COVID-19 vaccines across the globe, there was a felt need for clear information on this topic. This book, consisting of 31 sections edited by the erudite Dr. Vasishta and his team of co-editors and contributing authors, who have done an admirable job and justice in addressing, clarifying and answering most of these doubts. They have provided a clear direction, distilled from facts and data available from global studies, to care givers and beneficiaries with regard to the possibility of future outbreaks of mutants, duration of efficacy of all curently used vaccines, need for boosters at regular intervals and other issues.

After going through each of these sections, I am in total agreement with the foreword by none other than Stanley A Plotkin that this book could be serving as a blueprint for the response to a new (pandemic) – 'Disease X.'This is a strongly recommended and *a must read* book for all involved in care of infectious diseases.

S SRINIVASAN
Department of Pediatrics
Mehta Multispeciality Hospital, Chennai, Tamil Nadu.
srinivasan.renu@gmail.com

Printed and published by Dr Devendra Mishra on behalf of Indian Academy of Pediatrics and printed at Cambridge Press, Kashmere Gate, Delhi-110006 and published at 115/4, Ground Floor, Gautam Nagar, New Delhi 110 049. Editor: Dr Devendra Mishra

Mankind

Entromax

2 Billion *Bacillus clausii* Spores **Suspension**

THE RARE PROBIOTIC

For Billion Smiles

In

- Infectious Diarrhea
- Antibiotic Associated Diarrhea (AAD)

In RTIs, Typhoid Fever, Acute Otitis Media

Gudcef-100

Cefpodoxime 100mg/5ml **Dry Syrup**

Tasteful Choice for kids

Micronized Cefpodoxime for Faster Dissolution & Better Absorption