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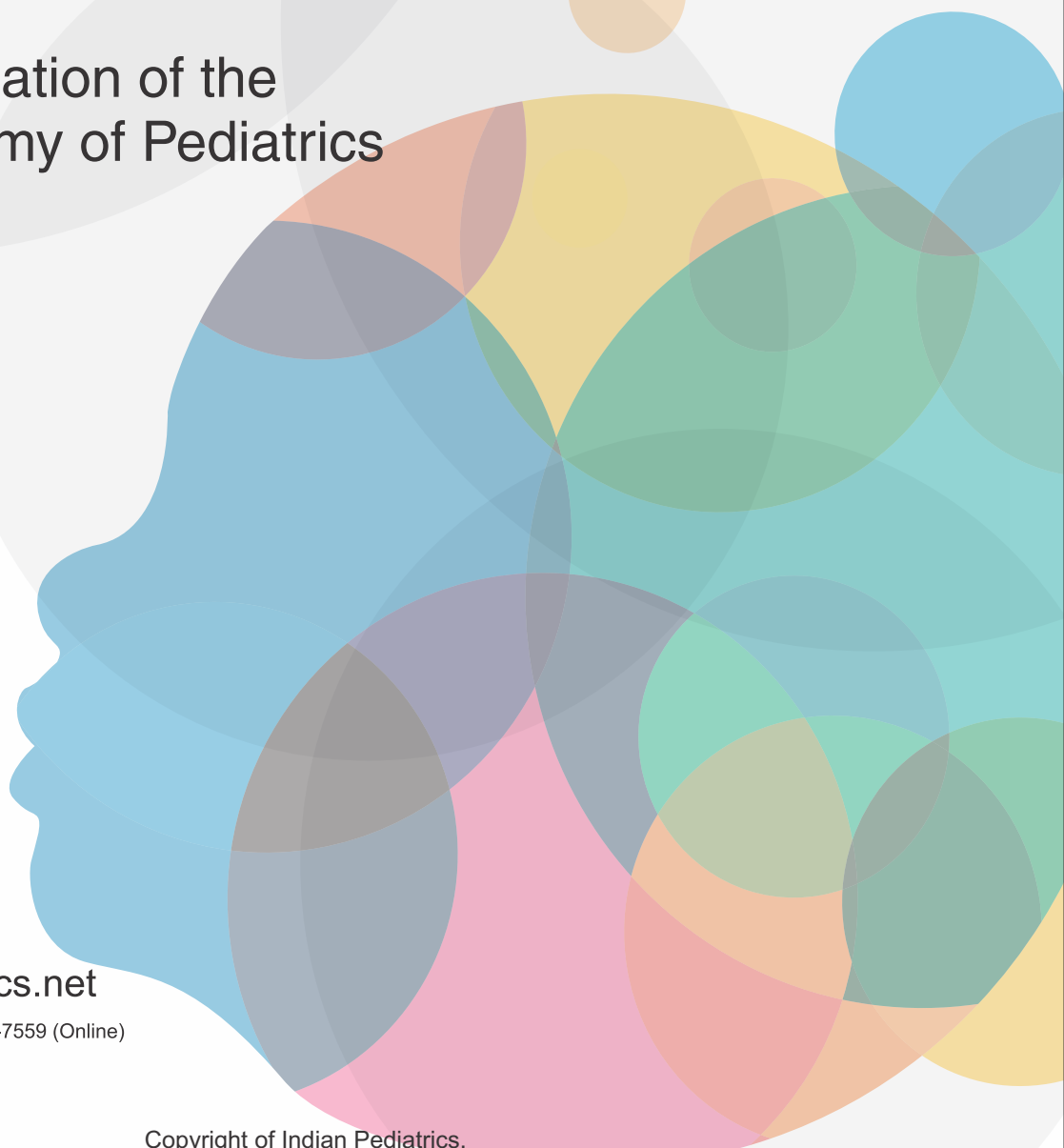

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Indian Pediatrics Case Reports (*IPCaRes*) Launching a New Journal from the Indian Academy of Pediatrics

Clinical cases are the first step of the medical student into the world of clinical medicine. Discussion on a clinical case gives the student – or for that matter, any clinician, a well-rounded view of the patient's problems, the likely causation, and the management and outcome. By extension, published case reports provide the readers exposure to the events at the frontline of clinical medicine, whether it is some rare manifestation or complication, an innovative diagnostic or therapeutic approach, or a novel association, like the one which lead to the initial identification of acquired immune deficiency syndrome. Moreover, for the practicing pediatrician, away from the esoteric concepts of epidemiology and research, it is the case-report section that provides a sense of belonging to a scientific journal. This is more relevant for journals like ours that are published by professional bodies. On the other hand, for a young doctor foraging into academic pediatrics, it is the case report section that provides both an avenue to get your feet wet in the field of publication and also an outlet for demonstrating your scientific writing skills.

Although the first written case description may be more than a few centuries old; in recent years, most journals have been reluctant to publish case reports. Even those that are publishing, continue to restrain them by limiting the word count and the number of authors. Moreover, the rejection rate for this section is one of the highest for most journals, reaching 95% for *Indian Pediatrics*. The reasons are multifold, including a gross disparity between the space we have and the number of submissions we receive.

Looking at the multi-faceted advantages of case reports and the need to make available this academic avenue to the majority of our academy members, we propose to start a new journal exclusively dedicated to publishing case reports, *Indian Pediatrics Case Reports* (*IP CaRes*). This journal will be a quarterly journal publishing case reports, case series, images, clinical videos, correspondence and related information, with e-copy mailed free to all members of the Indian Academy of Pediatrics. Print issues will be available for subscription to individuals and libraries. This high volume journal shall publish cases related to all pediatric subspecialties and allied specialties. There would not be any restriction on the number of authors, provided they meet authorship criteria, and word limits will be not too restrictive. The editorial board has already been constituted, author guidelines prepared, and the website shall be up and running within next few months. The journal will share offices with *Indian Pediatrics.*

We plan to publish the first issue in early 2021, and submissions are welcome at IPCaRes2020@gmail.com.

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The CPC Program of IAP and NCTB of IIT Madras

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As we all are hopeful for a vaccine or therapy for the coronavirus pandemic, such may not be the case for some of the other diseases that kill millions of people. Almost 10 million people die of cancer every year, and India alone accounts for about 1 million deaths. Unlike adult cancers, Pediatric cancers are treatable most of the time. However, up to 70% of children in India who succumb to the disease are not diagnosed early or treated appropriately.

World Health Organisation (WHO) has a tool on its website that predicts the future cancer incidence and mortality burden worldwide until 2040, and the figure is most disturbing, particularly in India, as we have much to do to not only effectively treat the disease but also to detect it early. Much needed solutions are missing in our system. If detected early and if the pediatrician has the advice of a Pediatric oncologist, the majority of pediatric cancers can be treated at the primary care location itself. This would not only enhance outcomes but also significantly reduce the load on our overburdened tertiary care system for cancer. It will eliminate the emotional and financial distress for thousands of families who endure the child's hospitalization.

Indian Academy of Pediatrics (IAP) has been pursuing various strategies to create a program that can empower all our pediatric clinics with tools and support to detect cancer early and to start appropriate therapy. I am glad to inform you that a promising solution has been initiated by IAP, which will go a long way towards creating a national solution for Pediatric cancer and help to save more lives than we currently do.

CATCH PEDIATRIC CANCER PROGRAM

The Catch Pediatric Cancer (CPC) Program of IAP and IIT Madras is a national program for early detection and treatment of cancer in children. CPC is an initiative of the IAP and Indian Institute of Technology Madras (IITM) and the National Cancer Tissue Biobank, a national facility financed by the Ministry of Science and Technology, Government of India.

We are working hard to launch the service in the second half of this year. The service will be available to the 130 million children who are treated annually by our member pediatricians. The CPC program will include simple detection guidelines for our doctors, tele-access to a pediatric oncologist, and locally available, comprehensive cancer diagnostics capabilities. The components of the program are:

- Training program for pediatricians on Pediatric Cancer Detection Guidelines for common cancers - delivered by IAP;
- Local sample collection and diagnostics service - delivered by IIT Madras; and
- Pediatric oncologists panel to give pediatricians pre-diagnostic counseling and post-detection therapy support, along with a legal and technology secured telemedicine and diagnostic support system - delivered by IAP.

CPC creates an inflow of tissue samples for the National Cancer Tissue Biobank, thus creating an Indian pediatric cancer genomic database. This will enable superior therapies in the future and enhance outcomes for South Asian children.

Cancer is a severe disease that requires immediate expert care. Recognizing the possible warning signs of cancer and taking quick action is the key to early diagnosis. Increased awareness of possible warning signs of cancer among doctors can have a significant impact on the disease. The CPC program is a commitment from IAP to lead and drive a movement for change.

As doctors, our *mantra* has always been "prevention is better than cure." But in the case of cancer, I would like to tweak the above mantra slightly, "Early detection is better than stage four." We aim to be the pioneers in the adoption and execution of such a program. We will do this at a national level by making it available to all our members at the earliest.

Jai Hind!
Jai IAP!

EDITORIAL

Slow and Steady Keeps Them in the Race: Metronomic Therapy in Children With Cancer

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Survival in childhood cancer has improved to approximately 80% in high-income countries (HIC) [1]. This success story is attributable to advances in diagnosis and risk stratification, and the protocolized administration of cytotoxic therapy [1,2]. However, the survival lags by 50% in low-income and low-and middle-income countries (LMIC) [1]. The road blocks faced by children with cancer in LMIC include resource constraints, a delayed presentation with advanced disease, treatment abandonment, malnutrition, and increased treatment-related toxicity. Treatment protocols that have been established and validated in HIC are not designed to address the challenges prevalent in LMICs [2]. Malignancy accounts for approximately 1% of deaths in children aged 5-14 years in India [3]. Novel Cost-effective strategies to treat children with cancer are of immense importance in LMICs such as India.

Conventionally, oncological trials have focused on the eradication of the malignancy and the reduction of relapse. With the best of infrastructure, a realistic possibility of a cure in every child with cancer is not conceivable. Five-year overall survival reported in high-risk malignancies such as acute myeloid leukamia, soft tissue/bone sarcomas and neuroblastoma; is markedly lesser when compared to malignancies such as Hodgkin lymphoma, Wilms tumor and germ cell tumors [4]. Survival is notably inferior in metastatic cancers and cancers that are refractory to therapy, or relapse following completion of therapy. Salvage treatment for such cancers is resource-intensive and toxic, and therefore often impractical in LMICs [5].

Metronomic therapy is an alternative paradigm in the management of children with cancer [5]. The approach involves the prolonged administration of chemotherapeutic agents in low, minimally toxic doses with no prolonged drug-free breaks [6]. Further, repurposed non-cytotoxic drugs, *e.g.* celecoxib, thalidomide and valproate, are incorporated into metronomic protocols [7]. In comparison, standard chemotherapy regimens utilize maximally tolerated doses (MTD) of cytotoxic drugs administered over a definite time [6]. Chemotherapy at MTD directly targets the cancer cells, which have an inherent tendency to develop mechanisms of resistance (akin to bacteria treated with antibiotics) [8]. Therapy at MTD necessitates breaks to allow recovery from toxicity, which further facilitates tumor cell proliferation [6]. Alternatively, metronomic therapy attempts to collapse the house by breaking the scaffold. That is, it targets the endothelial cells in the tumor microenvironment by anti-angiogenic mechanisms [6,8]. Additionally, metronomic therapy attempts to switch on the natural immune surveillance mechanisms against the malignant clone and induce tumor cell dormancy [6,8]. The prolonged oral maintenance therapy in acute lymphoblastic leukemia and the recent evidence favoring maintenance therapy in high-risk rhabdomyo-sarcoma stand testimony to the impact of a metronomic approach on outcome in pediatric malignancies [9,10]. ***Web Table* I** lists recent Indian studies which have demonstrated the feasibility and utility of metronomic therapy in children with high-risk cancers. Low cost, minimal toxicity, home-based intake of oral drugs, and a reduction in the need to travel to the hospital and admission comprise the self-evident benefits of metronomic therapy in LMICs [5,6].

Although metronomic therapy appears simple and attractive, there are caveats which need to be addressed. Standard chemotherapy regimens are ratified by randomized trials. Phase III trials, response criteria such as radiological remission in solid malignancies and lymphomas, minimal residual disease assessment in leukemia and outcomes such as disease-free survival may not be relevant in patients on a metronomic regimen [5,11]. Pharmacokinetic studies to optimize drug doses, identification of beneficiary disease subgroups and biological markers for response assessment constitute areas that merit research in the metronomic field [11]. This issue of *Indian Pediatrics* carries an important study in this regard. Pramanik, *et al.* [12] performed a placebo-controlled randomized trial of metronomic therapy in children with progressive extracranial solid malignancies.

The clinical aspects of the study were published previously and are briefly described in **Web Table I** [12]. The current study evaluated specific biomarkers in the same patient cohort. The authors examined the baseline and subsequent levels of vascular endothelial growth factor (VEGF) a pro-angiogenic cytokine, and thrombospondin-1 (TSP-1), an anti-angiogenic cytokine [13]. Although the study concluded that these were not reliable biomarkers for assessment of response to metronomic chemotherapy, some interesting trends were illustrated. The baseline VEGF levels were lower in responders. TSP-1 decreased in responders and increased in non-responders in the metronomic arm.A similar finding was not observed in the placebo arm. The results reaffirm the influence of metronomic treatment on angiogenesis. The study opens new vistas for research in metronomics. For instance, biomarkers such as VEGF levels may show promise as surrogates for identifying patients who would benefit with a metronomic approach. Since the effects of metronomic therapy encompass multiple pathways, the authors rightly state in their discussion that a broader spectrum of circulating biomarkers needs to be studied to yield clinically relevant indicators [13].

A survey of pediatric oncology physicians working in LMIC revealed a strong belief that the use of metronomic therapy is likely to increase with time [14]. An overwhelming majority expressed interest to participate in international studies and registries [14]. Research in metronomic therapy can fill many lacunae, if not all, in the treatment armamentarium for children with cancer.

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

REFERENCES

1. Lam CG, Howard SC, Bouffet E, Pritchard-Jones K. Science and health for all children with cancer. Science. 2019;363:1182-6.
2. Howard SC, Davidson A, Luna-Fineman S, Israels T, Chantada G, Lam CG*, et al*. A framework to develop adapted treatment regimens to manage pediatric cancer in low-and middle-income countries: The Pediatric Oncology in Developing Countries (PODC) Committee of the International Pediatric Oncology Society (SIOP). Pediatr Blood Cancer. 2017;64.
3. Causes of Death Statistics. Census of India Website. Available from: *http://censusindia.gov.in/vital_statistics/causesofdeath.html*. Accessed March 3, 2020.
4. Arora RS, Eden TOB, Kapoor G. Epidemiology of childhood cancer in India. Indian J Cancer. 2009;46:264-73.
5. André N, Banavali S, Snihur Y, Pasquier E. Has the time come for metronomics in low-income and middle-income countries? Lancet Oncol. 2013;14:e239-48.
6. André N, Banavali S, Fousseyni T, Pasquier E. Metronomic chemotherapy in pediatric oncology: A way forward for low-income countries? *In*: Oncopedia. St. Jude Cure4Kids: An International Medical Education Website and Online Collaboration Center. Available from: *https://www.cure4kids.org/*.Accessed February 23, 2020.
7. Rumore MM. Medication repurposing in pediatric patients: Teaching old drugs new tricks. J Pediatr Pharmacol Ther. 2016;21:36-53.
8. Kareva I, Waxman DJ, Lakka Klement G. Metronomic chemotherapy: An attractive alternative to maximum tolerated dose therapy that can activate anti-tumor immunity and minimize therapeutic resistance. Cancer Lett. 2015;358:100-6.
9. Andre N, Cointe S, Barlogis V, Arnaud L, Lacroix R, Pasquier E, *et al.* Maintenance chemotherapy in children with ALL exerts metronomic-like thrombospondin-1 associated anti-endothelial effect. Oncotarget. 2015;6: 23008-14.
10. Bisogno G, De Salvo GL, Bergeron C,GallegoMelcón S, Merks JH, Kelsey A,*et al.*Vinorelbine and continuous low-dose cyclophosphamide as maintenance chemotherapy in patients with high-risk rhabdomyosarcoma (RMS 2005): A multicentre, open-label, randomised, phase 3 trial. Lancet Oncol. 2019;20:1566-75.
11. Pantziarka P, Hutchinson L, André N, Benzekry S, Bertolini F, Bhattacharjee A, *et al.* Next generation metronomic chemotherapy-report from the Fifth Biennial International Metronomic and Anti-angiogenic Therapy Meeting, 6-8 May 2016, Mumbai. Ecancermedicalscience. 2016;10:689.
12. Pramanik R, Agarwala S, Gupta YK, Thulkar S, Vishnubhatla S, Batra A, *et al.* Metronomic chemotherapy *vs* best supportive care in progressive pediatric solid malignant tumors: A randomized clinical trial. JAMA Oncol. 2017;3:1222-7.
13. Pramanik R, Tyagi A, Agarwala S, Vishnubhatla S, Dhawan D, Bakhshi S. Evaluation of vascular endothelial growth factor (VEGF) and thrombospondin-1 as biomarkers of metronomic chemotherapy in progressive pediatric solid malignancies: A randomized study. Indian Pediatr. 2020;57:508-11.
14. Revon-Rivière G, Banavali S, HeississenL, Gomez Garcia W, Abdolkarimi B, Vaithilingum M, *et al*. Metronomic chemotherapy for children in low-and middle-income countries: survey of current practices and opinions of pediatric oncologists. J Glob Oncol. 2019;5:1-8.
15. Banavali S, Singh R, Prasad M, Arora B, Nahar A, Vora T, *et al.* Is there a role for a novel maintenance therapy in pediatric patients with AML? *In*: 43rd Congress of the International Society of Paediatric Oncology (SIOP) 2011 Auckland, New Zealand, 28th-30th october, 2011. Abstract PB015. Pediatr Blood Cancer. 2011;57:770.
16. Devadas SK, Banavali S. Retrospective analysis of outcomes of patients with relapsed, refractory and metastatic sarcomas who have received metronomic chemotherapy. Gulf J Oncol. 2019;1:22-8.

Web Table I Indian Studies on Metronomic Therapy in Childhood Cancer

Authors, place, year	*Subjects*	*Treatment regimen**	*Salient results*
Banavali, *et al.* [15], Mumbai, 2019	87 children with non M3-Acute myeloid leukaemia; median age 11 y	6 cycles of 6-thioguanine (40 mg/m^2) and etoposide (50 mg/m^2) days 1 to 20 given q 28 d	Overall survival of 64% at 28 mo
Pramanik, *et al.*[12], Delhi, 2017	Randomized trial comparing 52 patients on metronomic therapy and 56 patients on placebo, with extra-cranial solid malignancies failing 2 lines of treatment; age range 5-18 y	Thalidomide 3 mg/kg OD; celecoxib (100, 200 or 400 mg BD if weight <20 kg, 20-50 kg or >50 kg, respectively); etoposide 50 mg/m2/d alternating with cyclophosphamide 2.5 mg/kg (max. 100 mg) every 3 wk	Patients without bone sarcoma and those able to tolerate therapy for more than 3 cycles (9 wk) benefited. Overall, no improvement in 6-mo progression free survival
Devdas, *et al.* [16], Mumbai, 2019	49 children with relapsed, refractory or metastatic soft tissue sarcomas; age range 3-46 y	Each 28-d cycle: Tamoxifen 40 mg/m^2/d daily, cyclophosphamide and etoposide each 50 mg/m^2/d for 21 d	Clinical benefit (stable disease or response) in 79% of patients

**All the drugs mentioned were orally administered.*

EDITORIAL

Choice of Local Therapy in Children With Ewing Sarcoma

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Ewing sarcoma is the second most common bone cancer in children and adolescents and constitutes 40%-45% of malignant bone tumors in this group. Consecutive clinical trials have clearly established the essential role of multidrug chemotherapy and local control measures in curative strategies for this disease. This combined modality approach has led to improvement in long-term disease-free survival to about 70% in patients with localized or non-metastatic Ewing sarcoma. Review of published data shows that in Ewing sarcoma, local tumor control can be achieved with either surgery or radiotherapy (RT) or a combination of both. Furthermore, the trials have systematically assessed the importance of sequence and timing of local therapy on outcome. Current data recommends timing for local control to be 10-12 weeks after neo-adjuvant chemotherapy, and delay beyond 16 weeks has been shown to negatively impact survival [1].

Choice of local control modality (surgery, surgery combined with radiotherapy, or radiotherapy alone) and its impact on clinical outcomes such as survival and relapse in patients with non-metastatic Ewing sarcoma is the subject addressed by Zhu, *et al.* [2] in their article in this issue of the journal. In the absence of randomized trials that directly compare these modalities, the authors have attempted an indirect statistical approach using network meta-analysis to answer this question.

Ewing sarcoma is well known to be a radiosensitive tumor hence both surgery and RT represent effective local treatment modalities. However, recent trends for local management in Ewing sarcoma have favored the surgical approach. This trend may be attributed not only to availability of better surgical techniques, but is also based on reports of increased incidence of local failure rates with RT. These studies report three times higher local relapses in patients treated with radiation alone (failure rate of 30%) as compared to patients treated with surgery alone where the rates were less than or equal to 10% [3-6]. However, we need to interpret these results with caution, as these studies are retrospective and non-randomized and therefore susceptible to inherent selection bias. For example, it has been observed that cases where complete surgical excision was possible had better survival than those treated with RT. On the other hand, tumors that were not amenable to surgical excision and hence treated by radiotherapy alone were often associated with unfavorable features like large volume, central axis location or neurovascular involvement.

Surgery does offer certain advantages; it provides an opportunity to assess response to neo-adjuvant chemotherapy, reassess disease status and reduces the risk of second malignant neoplasms associated with RT. Therefore, the increasing use of surgery as local control modality has led to re-evaluation of the indications for radiotherapy. It is important to remember that surgery and RT are complimentary modalities in the management of Ewing sarcoma, not competitive.

Radiotherapy techniques and indications for Ewing sarcoma have evolved in the last few decades. Definitive radiotherapy is indicated for tumors that are considered unresectable (like sacral tumors crossing midline) or if the morbidity associated with surgery is deemed too high. It is generally agreed that adjuvant radiotherapy (combination of surgery and RT) should be given to patients with positive or very close margins, although various series have used differing cut-offs for amounts of viable tumor in the excised specimen. For others, there are conflicting views on integrating radiotherapy pre-operatively and post-operatively. Euro-Ewing-2012 radiotherapy guidelines recommend postoperative RT in the following clinical situations: if all tissues involved by the pre-chemotherapy tumor volume have not been surgically excised (as often seen in pelvic and sacral sarcomas) or if the histological response to pre-operative chemotherapy is poor (<90% necrosis) despite presence of negative surgical margins [7]. Other indications of postoperative RT include a displaced pathological fracture, Askin tumor presenting with pleural effusion and spinal/paraspinal disease where wide surgical excision is unlikely. There are emerging data that show benefit of pre-operative radiotherapy in certain specific clinical situations. These include Ewing sarcoma located at sites like the pelvis or rib, where it allows better delineation of tumor volume and also when the volume of treatment would be smaller than if administered post-operatively.

Newer techniques like Proton beam radiotherapy have recently been used in patients with Ewing sarcoma. Unlike the standard photon radiotherapy, with the use of proton beam one can minimize radiotherapy to adjacent normal structures while maintaining full dose to the tumor. This technique would therefore be greatly advantageous in patients where the tumor is in close proximity to critical structures like the eye or spinal cord, as well as for young children receiving curative treatment; wherein it would reduce the risk of radiation-induced second malignancy. Hence, we observe that both surgery and radiotherapy have their relative merits, but due to biases in patient selection the conclusions may be obscured.

In an ideal situation, local treatment should be individualized taking into consideration various factors based on patient characteristics, the likely benefit and harm of the treatment modality and preference of the patient. The selection strategy should be planned with the goal of optimizing local tumor control while minimizing therapy related adverse effects.

In conclusion, these decisions about local therapy are usually complex and should preferably be made by a bone sarcoma or musculoskeletal multidisciplinary team that includes surgical, radiotherapy and pediatric oncologists, together with the family. The published study [2] adds to the evidence-base for making informed decisions by such teams.

Competing interests: None*; Funding:* None.

REFERENCES

1. Lin TA, Ludmir EB, Liao KP, McAleer MF, Grosshans DR, McGovern SL, *et al*. Timing of local therapy affects survival in Ewing sarcoma. Int J Radiat Oncol Biol Phys. 2019;104:127–136.
2. Zhu H, Li Y, Xie X, Zhang S, Xue Y, Fan T. Efficacy of local control strategies for ewing sarcoma after neoadjuvant chemotherapy: A network meta-analysis. Indian Pediatr. 2020;57:527-32.
3. Bacci G, Forni C, Longhi A, Ferrari S, Donati D, Paolis M, *et al.* Long term outcome for patients with non metastatic Ewing's sarcoma treated with adjuvant and neoadjuvant chemotherapies. 402 patients treated at Rizzoli between 1972 and 1992. European J Cancer. 2004;40:73 83.
4. Donaldson SS, Torrey M, Link MP, Glicksman A, Gilula L, Laurie F, *et al.* A multidisciplinary study investigating radiotherapy in Ewing's sarcoma: End results of POG #8346. Int J Radiat Oncol Biol Phys.1998;42:125 35.
5. Krasin MJ, Rodriguez Galindo C, Billups CA, Davidoff AM, Neel MD, Merchant TE, *et al.* Definitive irradiation in multidisciplinary management of localized Ewing sarcoma family of tumors in pediatric patients: Outcome and prognostic factors. Int J Radiat Oncol Biol Phys. 2004;60: 830 8.
6. Schuck A, Ahrens S, Paulussen M, Kuhlen M, Konemann S, Rube C, *et al.* Local therapy in localized Ewing tumors: Results of 1058 patients treated in the CESS 81, CESS 86, and EICESS 92 trials. Int J Radiat Oncol Biol Phys.2003; 55:168 77.
7. Gerrand C, Athanasou N, Brennan B, Grimer R, Judson I, Morland B, *et al.* UK guidelines for the management of bone sarcomas. Clin Sarcoma Res. 2016; 6:7-27.

NOTES AND NEWS

PERSPECTIVE

Is Gradual and Controlled Approach to Herd Protection a Valid Strategy to Curb the COVID-19 Pandemic?

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Pandemics have their own natural history. They are the sum of epidemics in many countries. Most pandemics in the twentieth and twenty-first centuries have been caused by viruses – influenza, chikungunya, HIV/AIDS and now the coronavirus disease (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). When the virus is transmitted by the respiratory route, it goes around the world in a few months – pandemic flu H1N1 of 2009 was world-wide in about 3 months; COVID-19 in 4 months.

The risk to children is reported to be small [1], but in India, with 130 million children under the age of five, the numbers with severe disease and death can add up to large numbers during the epidemic period.Additionally, with many undernourished children and risk-factors like environmental and household air pollution, the epidemic can be quite worrisome for pediatricians.

Pediatric patients reportedly acquire COVID-19 either through close contact with infected family members (89%), exposure to endemic areas (33%), or both (22%); with the majority (53%) showing moderate symptoms and no severe or critical cases [2]. Though symptoms of COVID-19 are often mild in children, the prevalence of pneumonia with COVID-19 (53%) in this study is higher than with H1N1 influenza (11%), and similar to SARS (65%) [2]. This again points to the impendingchallengesin dealing with COVID-19 in children.

As pediatricians look forward to the future beyond the current extended lockdown, an important question relates to how we can navigate out of the current crisis while incurring minimal casualties, primarily among the elderly but also in the pediatric population. Globally, there are two broad approaches to taming the epidemic: by imposing lockdowns and other forced physical distancing measures to 'flatten the curve',or allowing 'herd immunity' by allowing for a graded acquisition of immunity.

We do not endorse the idea of letting the epidemic a free hand in order to create sufficient herd immunity to end the epidemic;as it would entail an enormous burden on the healthcare system – United Kingdom, at first, considered a different approach – of unrestricted spread of disease without any brakes applied, but public health experts were able to convince the government to accept the more reasonable mitigation approach. We should clarify at the outset that an approach of uncontrolled spread, one that no country is following at this time, would be a terrible mistake.

In general, a combination of mitigation and controlled herd immunity is the intervention adopted by most, if not all countries. Lockdown, cough/sneeze etiquette, hand washing (with soap and water) every time any surface potentially contaminated by droplets is touched, wearing masks in human presence, and physical distancing are the current interventions for risk-mitigation towards flattening the curve. The current approach in India appears to be based on the idea that the disease can be contained and eventually eliminated through risk mitigation plus tracing potentially infected persons through contact-tracing, testing and quarantining. In the unlikely event of this seemingly impossible task were to be accomplished, the country will have to be in a perpetual state of alert for screening and quarantining anyone who might be infected and arriving in the country. As we have observed in the past, asymptomatic infected can slip past border screenings and introduce SARS-COV-2 into the general population.

The epidemic will decline when herd immunity reaches sufficient level, determined by calculation based on the basic reproduction number, Ro. The reproductive number is the number of secondary infections produced by a single primary infection in a completely naïve population. The higher the Ro, the greater is the herd immunity that is required to prevent outbreaks or to

eliminate infection from the nation. For COVID-19, the Ro has been reported to be ~2 in early studies [3]. Recently US CDC has described Ro of 5.7 – we think that the latter pertains to coronavirus infection, the majority of which are asymptomatic. The former applies to COVID-19. The epidemiological estimate is that we need 70% herd immunity to turn the epidemic down for a Ro of 2 and 80% herd immunity if the Ro was 5.7. The proportion of the population that should be exposed to the virus for herd immunity to be effective is calculated as 1-1/Ro.

In the absence of serological studies, the true extent of spread of SARS-COV-2 in India is unknown. However, evidence from Wuhan, and more recently from France suggests that the number of asymptomatic patients may be as much as four times the number of patients who show symptoms [4,5]. At the time of writing, India has nearly 50,000 reported cases and 1700 reported deaths. Assuming an undercount by a factor of three and then adjusting for asymptomatic patients, it is likely that there are over 500,000 infections in the country.

Our view is that given the large number of asymptomatic patients, the utility of case identification and containment is fast diminishing and our best bet may lie in cluster containment. With these measures also, we can only slow down the epidemic and not stop transmission entirely. We then have only the option of holding down the epidemic through periodic shutdowns until a vaccine arrives. However, it is entirely possible that herd protection, consequent to the build-up of sufficient level of herd immunity may arrive before a vaccine does. In other words, if about 65-70% of our population is infected and acquires immunity, it becomes less likely that an infected individual comes into contact with a person susceptible to infection. At the tipping point, each infected person, on average, infects less than one other person; and here onwards, the number of cases will decline.

Furthermore, there are still many unknowns. The degree of protection afforded by antibodies among exposed is still unclear. There is evidence of immune response from the fact that patients with COVID-19 have cleared the infection. The result could either be sterilizing immunity, where re-infection can be ruled out, or weak immunity that wanes over time, and at best dampens symptoms in case of re-infection. We are not sure which end of the spectrum recovered patients from COVID-19 would fall into. It is also not known if those that have had mild or asymptomatic infections have the same degree of immunity and protection as those with symptomatic infections. A recent study showed that even asymptomatic patients had high viral loads approaching those of symptomatic patients [6].

Evidence from people who survived SARS and MERS suggests that antibodies persist for two years for SARS [7], while antibodies to MERS last nearly three years [8]. Early evidence from SAR-COV-2 from the Netherlands indicates that most PCR-confirmed SARS-CoV-2–infected persons had seroconverted by 2 weeks after disease onset [9]. All of this indicates that there is a strong likelihood of some protection through exposure to the virus and even subclinical infection.

Initial estimates from various sources prior to the lockdown were that roughly 25% of the populationwould be infected during the first wave of infection [10]. While the projected number of infections has declined significantly, because of the lockdown associated reduction in transmission, the spread of the virus will pick up speed when the lockdowns are removed. At this stage, early identification of high-risk groups, including both children and the elderly would be important to avert severe infections.

From that point on, the dual strategy of slowing spread while protecting vulnerable populations should be our main options to reach the level of herd protection that will ensure that the entire population is protected until such time that we have vaccination options. However, the likely situation post-pandemic will be endemic transmission, possibly with seasonal low and high incidences – low in summer but high during monsoons or winter.

To conclude, gradual and staged acquisition of herd protection could be a perfectly legitimate public health goal. As with all public health decisions made at a time of uncertainty, there are downside risks and there are risks and costs associated with alternative approaches as well. Available control measures, including lockdowns, should be applied selectively with the understanding that this may be inevitable and perhaps even desirable, as long as the ability of the health system to handle hospitalizations is not overwhelmed.

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REFERENCES

1. Jordan RE, Adab P, Cheng KK. Covid-19: Risk factors for severe disease and death. BMJ.2020;368:m1198. Available from:*https://www.bmj.com/content/368/bmj.m1198.long*. Accessed April 17, 2020.
2. Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: An observational cohort study.Lancet Infect Dis. 2020 Mar 25. Available from: *https://www.thelancet. com/journals/laninf/*

*article/PIIS1473-3099(20)30198-5/fulltext.*Accessed April 17, 2020.
3. Sanche S, Lin YT, Xu C, Romero-Severson E, Hengartner N, Ke R. High contagiousness and rapid spread of severe acute respiratory syndrome coronavirus 2. Emerg Infect Dis. 2020. Available from:*https://wwwnc.cdc.gov/eid/article/26/7/20-0282_article.* Accessed April 17, 2020.
4. Salje H, Kiem CT, Lefrancq N, Courtejoie N, Bosetti P, PaireauJ, *et al.* Estimating the burden of SARS-CoV-2 in France.2020. ffpasteur-02548181f. Available from:*https://hal-pasteur.archives-ouvertes.fr/pasteur-02548181/document.* Accessed April 17, 2020.
5. Li R, Pei S, Chen B, Song Y, Zhang T, Yang W, Shaman *et al*. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV2). Science. 2020:368:489-93.
6. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, *et al.* SARS-CoV-2 viral load in upper respiratory specimens of infected patients. New Engl J Med.2020;382:1177-9.
7. Mo H, Zeng G, Ren X, Li H, Ke C, Tan Y, *et al*. Longitudinal profile of antibodies against SARS-coronavirus in SARS patients and their clinical significance.Respirology. 2006;11:49-53.
8. Payne DC, Iblan I, Rha B, Alqasrawi S, Haddadin A, Al Nsour M, *et al*. Persistence of antibodies against Middle-East respiratory syndrome coronavirus. Emerg Infect Dis. 2020;22:1824-6.
9. Okba NMA, Müller MA, Li W, Wang C, vanKessel CHG, Corman VM, *et al.* Severe acute respiratory syndrome coronavirus 2 specific antibody responses in coronavirus disease 2019 patients.Emerg Infect Dis. 2020 Apr 8. Available from:*https://wwwnc.cdc.gov/eid/article/26/7/20-0841_article.* Accessed April 17, 2020.
10. Mandal S, Bhatnagar T, Arinaminpathy N, Agarwal A, Chowdhury A, Murhekar M, *et al.* Prudent public health intervention strategies to control the coronavirus disease 2019 transmission in India: A mathematical model-based approach. Indian J Med Res. 2020;151:190-9.

RESEARCH PAPER

Evaluation of Vascular Endothelial Growth Factor (VEGF) and Thrombospondin-1 as Biomarkers of Metronomic Chemotherapy in Progressive Pediatric Solid Malignancies

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Objectives: We compared Vascular Endothelial Growth factor (VEGF) and Thrombospondin-1 between patients with progressive paediatric malignancies randomized to metronomic chemotherapy *versus* placebo to determine their role as biomarker. **Methods:** In this double-blinded, placebo-controlled randomized study of 108 progressive pediatric malignancies, serum VEGF and thrombospondin-1 levels were evaluated using ELISA at baseline, A2 (week-9 or earlier if progressed) and A3 (week-18 or earlier if progressed). **Results:** Mean VEGF and thrombospondin-1 at baseline, A2 and A3 and the change from baseline to A2 were not different between two groups. In metronomic arm, responders (those completing 3 cycles) had significantly lower mean (SD) baseline VEGF levels [659.7(362.1) *vs* 1143.9 (622.0) µg/mL] (*P*=0.002) and significant decrease in thrombospondin-1 from baseline to A2 [-4.43(8.0) µg/mL *vs* 1.7(11.3) µg/mL] (*P*=0.04), as compared to non-responders. Similar changes were not observed in responders on placebo arm. No consistent trend of these biomarkers was observed. **Conclusions:** VEGF and thrombospondin-1 are not reliable biomarkers for response to metronomic chemotherapy.

Keywords: *Anti-tumor activity, Anti-angiogenic, Outcome*.

Trial registration: *Clinicaltrial.gov, NCT01858571*.

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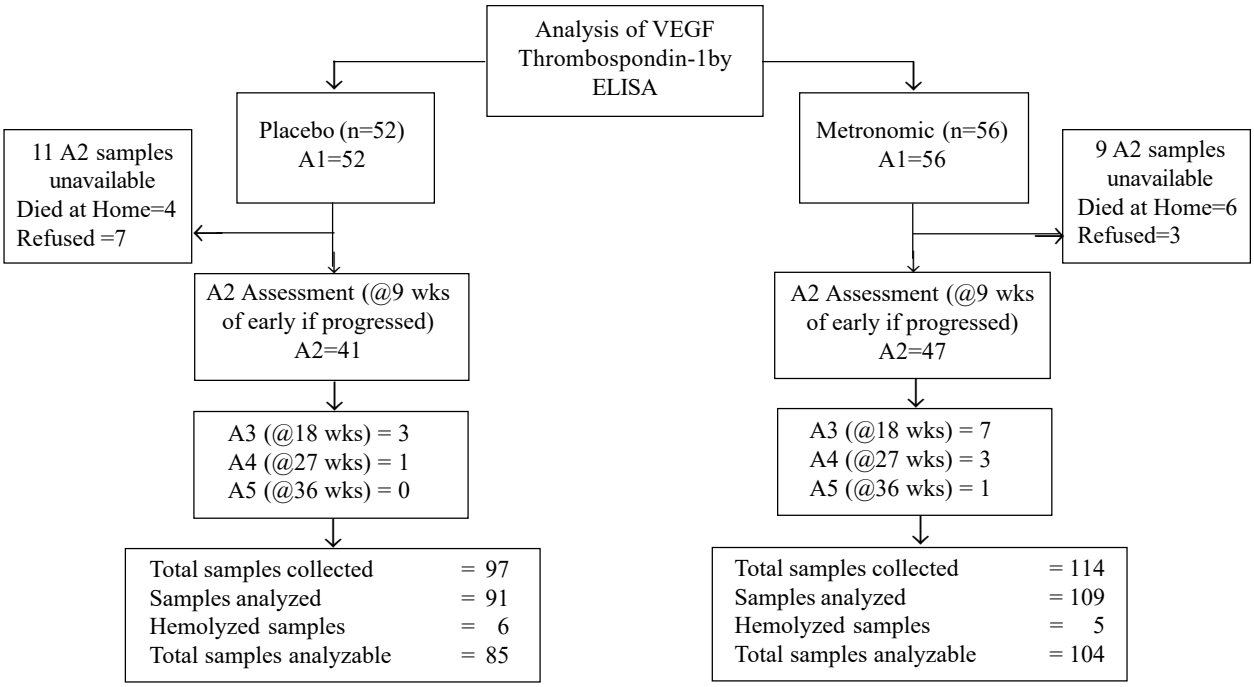
Metronomic chemotherapy is the frequent administration of chemotherapeutic drugs at doses significantly below the 'maximum tolerated dose' with no prolonged drug-free breaks; it has carved a niche in modern pediatric oncology practice, especially in the recurrent metastatic or progressive disease settings [1,2]. Powerful and reliable biomarkers are yet to be identified and validated for the selection of a metronomic regimen for a given patient, in a given clinical setting. Vascular endothelial growth factor (VEGF) is an *in vivo* proangiogenic cytokine while Thrombospondin-1 (TSP-1) is an intrinsic anti-angiogenic cytokine. Studies have shown increase in VEGF levels during successful therapy with anti-VEGF monoclonal antibodies and tyrosine kinase inhibitors (TKI) [3,4].

Even though evidence for these cytokines is contradictory [5-8], these angiogenic peptides are attractive bio-markers because of their ease of sampling and estimation in clinics. We previously published a randomized trial in pediatric metronomics comparing metronomic chemotherapy with placebo in progressive pediatric malignancies [9]. In this report, we present the planned secondary objective of the study wherein we did a comparative analysis of two angiogenic peptides between these two groups of patients at different time-points.

Accompanying Editorial: Pages 501-02.

METHODS

The design, setting, participants and methodology of the clinical study have been described elsewhere [9]. Eligible patients (*n*=108) underwent 1:1 simple centralised randomization to metronomic chemotherapy (4-drug regimen of daily celecoxib and thalidomide with alternating periods of etoposide and cyclophosphamide) and placebo groups.



A1: Baseline evaluation; A2, A3 etc: Subsequent evaluations.

Fig. 1 *Study flow diagram.*

After informed consent, blood samples were taken for biomarker evaluation at baseline (A1) and interim assessments (A2 = 9 weeks or earlier if progressed, A3 = 18 weeks or earlier if progressed) (***Fig.* 1**).

Serum was separated and centrifuged at 1000 g for 10 min within 30 min from collection. Serum was aliquoted and stored at –80°C. ELISA for VEGF and TSP-1 levels were evaluated from these samples of serum using Quantikine Human VEGF Immunoassay DVE00 and Quantikine Human Thrombospondin-1 Immunoassay DTSP10, respectively (R&D Systems, Inc, Minneapolis, MN 5541 USA).

We analyzed pattern of VEGF and TSP-1 in both study arms, comparing them at baseline, at second assessment (A2) and at third assessment (A3) as well as the change in their levels at A2. The clinical assessment during the study had shown no significant difference in Progression free survival (PFS) or Overall survival (OS) between the two arms [9]. However, in post hoc subgroup analysis, those who had completed more than 3 cycles (*i.e.* 9 weeks) and those who did not have a bone sarcoma benefitted from metronomic chemotherapy [9]. Hence, we also analyzed the patients as responders *versus* non-responders, defining responders as those who had completed 9 weeks of therapy.

RESULTS

The baseline characteristics of the 108 recruited subjects are presented in ***Table* I**. Baseline levels of VEGF greater than mean value of 1135.45 pg/mL was found to adversely affect OS with hazard ratio of 1.77 (1.18-2.65) (*P*=0.006). Baseline TSP-1 did not affect OS [HR (95% CI) =0.99 (0.99-1.00) (*P*=0.92)].

Mean level of VEGF and TSP-1 in patients at baseline, at A2 and at A3 were not different in the placebo and metronomic groups (***Web Table* I**). The difference from baseline values to second assessment (A2) for both these biomarkers in each group was also not significantly different.

In the metronomic arm, responders (*i.e.* those who completed at least 9 weeks of chemotherapy) had a significantly lower baseline VEGF levels as compared to non-responders (*P*=0.002). However, there was no difference in TSP-1 levels between them. The mean difference from baseline to the second assessment (A2-A1) for TSP-1 was significantly different (*P*=0.04); while TSP-1 decreased in the responders, it increased in the non-responders. Such a difference was not noted for VEGF (***Table* II**). There was no significant difference in the baseline levels of VEGF and TSP-1 between responders and non-responders of placebo arm. Neither

Table I Comparison of Baseline Characteristics of the Two Study Groups

Characterstics	*Placebo (n=52)*	*Metronomic (n=56)*
Age, y*	15 (5-18)	13 (5-18)
Male: female	3.3:1	3:1
ECOG-PS		
0	1 (1.9)	3 (5.3)
1	19 (36.5)	18 (32.1)
2	21 (40.3)	25 (44.6)
3	11 (21.1)	10 (17.8)
Diagnosis		
Bone Sarcoma (PNET/ Osteosarcoma)	32 (61.4)	40 (71.3)
Neuroblastoma	5 (9.6)	5 (8.9)
RMS	6 (11.5)	3 (5.3)
Esthesioneuroblastoma	1 (1.9)	1 (1.7)
STS	4 (7.6)	2 (3.8)
Others	3 (5.7)	3 (5.3)
Retinoblastoma	1 (1.9)	2 (3.8)
Previous lines		
2	48 (92.3)	53 (94.6)
3	4 (7.7)	2 (3.6)
4	0	1 (1.8)

*All P values >0.05; PNET: primitive neuroectodermal tumours; RMS: rhabdomyosarcoma; STS: soft tissue sarcoma; ANC= absolute neutrophil count; ECOG-PS: Eastern Cooperative Oncology Group- Performance Status. All values in no. (%) except *median (range).*

was there any significant difference in the mean change of both VEGF and TSP-1 from baseline to A2 (***Table* II**).

DISCUSSION

Our study showed that baseline VEGF predicted OS for the entire study population, whereas baseline TSP-1 did not predict the same. In the total study sample, there was no difference in the levels of VEGF or TSP1, neither at baseline, nor at any other time-point, between the placebo and metronomic arms. The magnitude of change from baseline to A2 was also not different significantly different between the two arms. But then, there was no difference in survival as well between the two arms.

While our findings are in contrast to studies on other solid tumors treated with anti-angiogenic agents, *eg.* metastatic colorectal cancer treated with bevacizumab [10], it corroborates with the findings in metastatic breast cancer [11]. When we focussed our analysis on the metronomic arm, we found that responders had significantly lower baseline levels of VEGF but no difference was noted in TSP-1. This is consistent with previous studies that have noted an aggressive tumor progression with injection of TSP-1 in preclinical models [12]. Although, our study demonstrated some trends, we could not provide proof of the principle that the 4-drug anti- angiogenic chemotherapy actually acts by altering the cytokine milieu of pro and anti angiogenic factors, and inhibiting angiogenesis *in vivo*. Our results are consistent with the results Stempak, *et al*. [5] and Kesari, *et al*. [8] who found that none of the four tested markers (VEGF, bFGF, endostatin, and TSP-1) were of prognostic significance.

In a previous study, baseline TSP-1 levels appeared to correlate with prolonged response; this conclusion was based on just three patients who had a baseline high TSP-1 level and did not progress for more than a year [6]. In another study of 100 patients treated with metronomic chemotherapy, 52 baseline patient samples were available and herein serum TSP-1 levels increased in patients who completed therapy than in non- completers [7]. Our study is a larger study with a placebo arm, but still we could not replicate those findings. The reason why we could not demonstrate a trend in these cytokines may probably be the fact that the small subset of proteins that we selected is unlikely to be representative of the overall effect of all of

Table II Comparison of VEGF and TSP-1 Levels Among Responders and Non-responders of Metronomic and Placebo Arms of the Study

	Metronomic (n=56)		*Placebo (n=52)*	
	Responders (n=21)	*Non-responders (n=35)*	*Responders (n=19)*	*Non-responders (n=33)*
Baseline TSP-1 (A1)	19.4 (7.0)	21.3 (10.6)	24.3 (13.4)	20.9 (12.5)
Baseline VEGF (A1)	659.7 (362.1)*	1143.9 (622.0)*	961.9 (496.3)	1238.5 (770.1)
Difference from baseline to A2 (TSP-1): (A2-A1)	–4.43 (8.0)#	1.7 (11.3)#	–4.90 (16.4)	–6.2 (12.2)
Difference from baseline to A2 (VEGF): (A2-A1)	173.1 (618.2)	90.8 (706.1)	224.9 (615.7)	–87.0 (535.9)

*All values in mean (SD); VEGF: Vascular Endothelial Growth Factor, TSP-1: Thrombospondin-1, A2= second assessment at 9 wks or earlier if progression of disease; values of VEGF are in pg/mL and the values of TSP-1 are in µg/mL respectively, P value of *0.02 and #0.04.*

WHAT THIS STUDY ADDS?

Vascular endothelial growth factor (VGEF) and thrombaspondin-I are not reliable biomarkers for metronomic chemotherapy.

the regulators of angiogenesis. Angiogenesis is a complex interacting cascade of pathways with an interplay of a large number of proteins inside and outside of the cell and we cannot gauge them by relying on only one or two of these proteins. The strengths of our study are its randomized nature and comparison with placebo.

Identifying reliable predictive and/or prognostic biomarkers for anti-angiogenic therapies has been unsuccessful to date. Looking for a biomarker for a therapy can be a realistic objective only if that therapy targets the tumor cells of interest, but when we are using metronomic chemotherapy, we are actually targeting the host endothelial cells and not directly the tumor. So, it is unlikely that universal mechanistically-driven markers will ever be unveiled for metronomic chemotherapy, especially given its varied mechanisms of action, multiple drug combinations and many clinical settings. We suggest that other biomarkers be explored for measuring the efficacy of metronomic chemotherapy like circulating cell free DNA, circulating endothelial cells, and circulating endothelial precursor cells and micro-particles.

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REFERENCES

1. Pramanik R, Bakhshi S. Metronomic therapy in pediatric oncology: A snapshot. Pediatr Blood Cancer. 2019;66: e27811.
2. Bahl A, Bakhshi S. Metronomic chemotherapy in progressive pediatric malignancies: Old drugs in new package. Indian J Pediatr. 2012;79:1617-22.
3. Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, *et al*. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol. 2006;24:16-24.
4. Rini BI, Michaelson MD, Rosenberg JE, Bukowski RM, Sosman JA, Stadler WM, *et al*. Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. J Clin Oncol. 2008;26:3743-8.
5. Stempak D, Gammon J, Halton J, Moghrabi A, Koren G, Baruchel S. A pilot pharmacokinetic and antiangiogenic biomarker study of celecoxib and low-dose metronomic vinblastine or cyclophosphamide in pediatric recurrent solid tumors. J Pediatr Hematol Oncol. 2006;28:720-8.
6. Kieran MW, Turner CD, Rubin JB, Chi SN, Zimmerman MA, Chordas C, *et al*. A feasibility trial of antiangiogenic (metronomic) chemotherapy in pediatric patients with recurrent or progressive cancer. J Pediatr Hematol Oncol. 2005;27:573-81.
7. Robison NJ, Campigotto F, Chi SN, Manley PE, Turner CD, Zimmerman MA, *et al*. A phase II trial of a multi-agent oral antiangiogenic (metronomic) regimen in children with recurrent or progressive cancer. Pediatr Blood Cancer. 2014;61:636-42.
8. Kesari S, Schiff D, Doherty L, Gigas DC, Batchelor TT, Muzikansky A, *et al*. Phase II study of metronomic chemotherapy for recurrent malignant gliomas in adults. Neuro-Oncol. 2007;9:354-63.
9. Pramanik R, Agarwala S, Gupta YK, Thulkar S, Vishnubhatla S, Batra A, *et al*. Metronomic chemotherapy vs best supportive care in progressive pediatric solid malignant tumors: A randomized clinical trial. JAMA Oncol. 2017;3:1222-7.
10. Willett CG, Boucher Y, Duda DG, di Tomaso E, Munn LL, Tong RT, *et al*. Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy: continued experience of a phase I trial in rectal cancer patients. J Clin Oncol. 2005; 23:8136-9.
11. Burstein HJ, Chen Y-H, Parker LM, Savoie J, Younger J, Kuter I, *et al*. VEGF as a marker for outcome among advanced breast cancer patients receiving anti-VEGF therapy with bevacizumab and vinorelbine chemotherapy. Clin Cancer Res 2008;14:7871-7.
12. Tuszynski GP, Gasic TB, Rothman VL, Knudsen KA, Gasic GJ. Thrombospondin, a potentiator of tumor cell metastasis. Cancer Res. 1987;47:4130-3.

Web Table I VEGF and TSP-1 in Study Participants at Different Time-points

Time point	*Placebo*	*Metronomic*	*P value*
VEGF (pg/mL)			
Baseline (*n*=107)	1135.4 (688.9)	962.4 (585.5)	0.16
A2 (≤9 wks) (*n*=88)	1103.4 (712.9)	1032.9 (708.7)	0.64
A3 (≤18 wks) (*n*=7)	1471.7 (954.3)	1412.2 (687.3)	0.92
Change from baseline at A2 (*n*=88)	-57.5 (588.4)	-125.8 (664.4)	0.61
Thrombospondin-1 (µg/mL)			
Baseline (*n*=107)	22.2 (12.8)	20.6 (9.4)	0.54
A2 (≤9 wk) (*n*=87)	17.3 (9.4)	19.1 (11.2)	0.42
A3 (≤18 wk (*n*=9)	17.1(5.9)	19.2 (8.3)	0.76
Change from baseline at A2	-5.6 (14.1)	-0.9 (10.4)	0.07

VEGF: Vascular endothelial growth factor, A2: Assessment 2 (at 9 wk or earlier if disease progression); A3: Assessment 3 (at 18 wk or earlier if disease progression); all value in mean (SD).

RESEARCH PAPER

Ocular and Periocular Tumors in Asian Indian Children and Adolescents

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Objective: To describe eye tumors in Indian children (age ≤ 21 years) presenting to a multi-tier ophthalmology hospital network in India. **Methods:** Hospital-record review. **Results:** During the 3-year study period (November, 2015-October, 2018), 2911 children were diagnosed with 3003 eye tumors. The three most common benign tumors included conjunctival nevus (445, 22%), orbital dermoid cyst (206, 10%), and periocular infantile capillary hemangioma (181, 9%). The three most common malignant tumors included retinoblastoma (835, 88%), xeroderma pigmentosa related ocular surface squamous neoplasia (38, 4%), and rhabdomyosarcoma (17, 2%). Based on the age group, retinoblastoma (*n*=834, 28%) was the most common tumor in all groups, except adolescence where conjunctival nevus (*n*=194, 21%) was the most common. **Conclusion:** Overall, retinoblastoma is the most common tumor in children encountered in a referral based comprehensive ocular oncology practice in India.

Keywords: *Cancer, Conjunctival nevus, Eye, Retinoblastoma*.

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The incidence of cancer in children and adolescents is comparatively low [1,2], and the most common malignancies in children include leukemia, lymphoma, and central nervous system tumors [3].

Retinoblastoma has been reported in a population-based registry study to be the fourth most common cancer in children aged 0 to 14 years, constituting 6% cases, but it was rare in older children and adolescents (<1%) [3]. There is no big data analysis focusing on ocular tumors in children and adolescents. In this study, we describe the profile of benign and malignant ocular tumors in Indian children and adolescents.

METHODS

This was a retrospective hospital-based study of children diagnosed with eye tumors presenting between November, 2015 and October, 2018 to four referral centers spread across four states in India. For this study, a patient was considered as a child when the age was 21 years or lower [4]. They were further classified as neonate (birth to 27 days), infant (28 days to 12 months), toddler (1 to 2 years), childhood (3 to 11 years), and adolescence (12 to 21 years) [4]. Consent for electronic data privacy was obtained from the patient (if age at presentation was >18 years) or parents/guardians (if age of the patient was ≤18 years) at the time of registration. No identifiable information of the patient was used for analytical purposes. Each patient underwent a comprehensive ophthalmic examination, and data were entered into a browser-based in-house developed electronic medical records system (eyeSmart EMR). The study was approved by the Institutional Ethics Committee of the institute.

The data of all children diagnosed with eye tumors during the study period was retrieved from the EMR database. The patients with a confirmed clinical and/or histopathological diagnosis were included in this study. All patients who underwent surgical intervention had a confirmed histopathological diagnosis. Those with uncertain diagnosis or inadequate data were excluded. The data on patient demographics, ocular diagnosis, tumor status, and anatomical category were used for analysis.

Statistical analyses: Descriptive statistics was used to elucidata demographic and diagnostic data using Microsoft Excel 2013 (Microsoft Corporation, Redmond, USA).

RESULTS

Of the 728,077 new patients presenting to the four centers of the network during the period under study, 118,648 (16%) patients were at or below the age of 21 years. Of these, 3506 (2.9%) were diagnosed with eye tumors. Of

these, 595 patients who did not have a confirmed diagnosis of eye tumor were excluded from the study, and 2,911 (2.4%) patients (51% males) with a definitive diagnosis of benign or malignant tumor, either by clinical examination or confirmed by histopathology were included in the study.

The median age (range) at diagnosis was 7 years (2 days to 21 years). The age-wise distribution of patients with eye tumors included neonates (*n*=18, 0.6%), infants (*n*=242, 8.3%), toddlers (*n*=511, 17.5%), childhood (*n*=1259, 43.2%), and adolescents (*n*=655, 22.5%). Majority of patients (24%) were in the age group of 6 to 11 years.

There were 3,003 tumors (69% benign) in 2,911 patients, which were segregated into nine anatomical categories. The most common anatomical part involved was the conjunctiva with 949 (31.6%) cases and the least was lacrimal sac with 1 case ***(Table* I)**.

The three most common tumors included retinoblastoma (835, 28%), conjunctival nevus (445, 15%), and orbital dermoid cyst (206, 7%). Amongst the benign tumors, the three most common were conjunctival nevus (445, 22%), orbital dermoid cyst (206, 10%), and periocular infantile capillary hemangioma (181, 9%). Amongst the malignant tumors, the three most common were retinoblastoma (835, 88%), Xeroderma pigmentosa-related ocular surface squamous neoplasia (OSSN) (38, 4%), and rhabdomyosarcoma (17, 2%). The most common tumor in the eyelid was infantile capillary hemangioma (*n*=181, 6%); in caruncle inclusion cyst (*n*=9, <1%); in conjunctiva, conjunctival nevus (*n*=445, 15%); in iris trauma-related iris stromal cyst (*n*=15, <1%); in ciliary body, medulloepithelioma (*n*=5, <1%), in retina, retinoblastoma (*n*=835, 28%); in choroid, toxocara granuloma (*n*=15, <1%), in orbit, dermoid cyst (*n*=206, 7%); and in lacrimal sac, granuloma (*n*=1, <1%).

The most common tumor in younger children (0-2 years, 800 tumors) and older children (3 to 11 years, 1277 tumors) was retinoblastoma (50% and 31%, respectively), In adolescents (12-21 years, 926 tumors), conjunctival nevus (194, 21%) was the most common. Distribution according to the age is presented in ***Table* II**.

Table I Anatomical Distribution of Ocular and Peribocular Tumors and Tumor Status According to Age Group

Feature	*Benign (n=2058)*	*Malignant (n=945)*	*All cases (N=3003)*
Tumor location			
Conjunctiva	907 (96)	42 (4)	949 (32)
Retina	68 (7)	842 (93)	911 (30)
Orbit	483 (90)	54 (10)	536 (18)
Eyelid	469 (99)	5 (1)	474 (16)
Choroid	51 (98)	1 (2)	52 (2)
Iris	49 (98)	1 (2)	50 (2)
Caruncle	23 (100)	0	23 (<0.8)
Ciliary body	7 (100)	0	7 (<0.2)
Lacrimal sac	1 (100)	0	1 (<0.2)
Age category			
Neonate	15 (0.7)	5 (0.4)	20 (0.7)
Infant	160 (8)	94 (9.9)	254 (8)
Toddler	218 (11)	308 ( )	526 (18)
Childhood	835 (41)	442 ( )	542 (43)
Adolescence	830 (40)	96 ( )	926 (30)

All values in n (%).

Histopathological confirmation of diagnosis was available in 792 (26%) tumors, while the remaining 2211 (74%) tumors were diagnosed clinically. Accurate clinico-pathological correlation was noted in 747 (94%) tumors, while discordance between clinical and histopathological diagnosis was noted in 45 (6%) tumors. Of the cases with a discordant clinical and histopathological diagnosis, three tumors had a clinical diagnosis of benign tumor, while histopathology revealed a malignant tumor; 9 (1.1%) tumors had a clinical diagnosis of malignant tumor, while histopathology revealed a benign tumor; and 26 (3.3%) tumors had a different diagnosis in the same category of benign tumors; and 7 (0.9%) tumors had a different diagnosis in the same category of malignant tumors.

Table II Ocular and Periocular Tumor Distribution According to Age in Indian Children and Adolescents

Age group	*Commonest tumor (n=3003)*	*No. (%)*	*Commonest benign tumor (n=2058)*	*No. (%)*	*Commonest malignant tumor (n=945)*	*No. (%)*
Neonate	Retinoblastoma	4 (20)	Dermolipoma	2 (12)	Retinoblastoma	4 (80)
Infant	Retinoblastoma	95 (37)	Infantile capillary hemangioma	94 (59)	Retinoblastoma	95 (99)
Toddler	Retinoblastoma	302 (57)	Infantile capillary hemangioma	48 (22)	Retinoblastoma	302 (98)
Childhood	Retinoblastoma	394 (31)	Conjunctival nevus	240 (29)	Retinoblastoma	394 (89)
Adolescence	Conjunctival nevus	194 (21)	Conjunctival nevus	194 (24)	Retinoblastoma	39 (40)

WHAT THIS STUDY ADDS?

- Retinoblastoma was the most common ocular tumor encountered in a referral-based ocular oncology set-up.
- The most common tumor in each age group differs, with retinoblastoma being common in children and conjunctival nevus in adolescents.

DISCUSSION

Both benign and malignant ocular tumors can occur during childhood and adolescence [5-11]. We found conjunctival nevus to be most common benign tumor in children and retinoblastoma as the most common malignant tumor. Retinoblastoma was also the most common tumor in children encountered in this referral-based hospital setting.

The limitations of our study include the retrospective nature of the study and possible referral bias since it is a hospital-based study. However, the strength of the study includes larger number of patients and accurate diagnosis by a trained ocular/ophthalmic oncologist, resulting in a good clinico-pathological correlation in 94% tumors, whenever histopathological diagnosis was available.

Reddy, *et al*. [5] reviewed 75 ocular tumors in children aged 3 months to 12 years and found 52% to be benign; retinoblastoma (44%) was the most common tumor, constituting 92% of all malignant ocular tumors in children. In their study, non-specific conjunctival granuloma was the most common benign tumor constituting 33% of all benign tumors in children [5].

In a review of 806 children with conjunctival tumors, 97% were benign and 3% were malignant with the most common benign conjunctival tumor being nevus (61%) [12]. Conjunctiva was the most common tissue involved by a tumor in our study also, with nevus being the most common. OSSN was the second most common malignant tumor in our patients. All children and adolescents with OSSN had associated xeroderma pigmentosum. It is well established that patients with xeroderma pigmentosum develop ocular and periocular tumors at younger age compared to the general population [13].

In conclusion, benign tumors are more common in children and adolescents except in cases with retinal tissue involvement. Eventhough benign tumors may not be life-threatening, immediate intervention is warranted in cases which are vision-threatening. Pediatricians, who are the first point of contact in most of these cases, play an important role in diagnosis of pediatric ocular tumors and appropriate referral.

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REFERENCES

1. White MC, Holman DM, Boehm JE, Peipins LA, Grossman M, Henley SJ. Age and cancer risk: a potentially modifiable relationship. Am J Prev Med. 2014;46:S7-15.
2. Bleyer A. Young adult oncology: The patients and their survival challenges. CA Cancer J Clin. 2007;57:242-55.
3. Steliarova-Foucher E, Colombet M, Ries LAG, Moreno F, Dolya A, Bray F, *et al*; IICC-3 contributors. International incidence of childhood cancer, 2001-10: A population-based registry study. Lancet Oncol. 2017;18:719-31.
4. Williams K, Thomson D, Seto I, Contopoulos-Ioannidis DG, Ioannidis JP, Curtis S, *et al*. StaR Child Health Group. Standard 6: age groups for pediatric trials. Pediatrics. 2012;129:S153-60.
5. Reddy SC, Sarma CS, Rao VV, Banerjea S. Ocular tumours in children - A study of 75 cases. Indian J Ophthalmol. 1983;31:421-3.
6. Shields JA, Shields CL. Ocular tumors of childhood. Pediatr Clin North Am. 1993;40:805-26.
7. Shields JA, Shields CL. Pediatric ocular and periocular tumors. Pediatr Ann. 2001;30:491-501.
8. Castillo BV Jr, Kaufman L. Pediatric tumors of the eye and orbit. Pediatr Clin North Am. 2003;50:149-72.
9. Abramson DH, Frank CM, Susman M, Whalen MP, Dunkel IJ, Boyd NW 3rd. Presenting signs of retinoblastoma. J Pediatr. 1998;132:505-8.
10. Kaliki S, Patel A, Iram S, Ramappa G, Mohamed A, Palkonda VAR. Retinoblastoma in India: Clinical presentation and outcome in 1,457 patients (2,074 eyes). Retina. 2019;39:379-91.
11. Shields CL, Shields JA, Shah P. Retinoblastoma in older children. Ophthalmology. 1991;98:395-9.
12. Shields CL, Sioufi K, Alset AE, Boal NS, Casey MG, Knapp AN, *et al*. Clinical features differentiating benign from malignant conjunctival tumors in children. JAMA Ophthalmol. 2017;135:215-24.
13. Kaliki S, Jajapuram SD, Maniar A, Mishra DK. Ocular and periocular tumors in xeroderma pigmentosum: A study of 120 Asian Indian patients. Am J Ophthalmol. 2019;198: 146-53.

RESEARCH PAPER

Factors Associated With Ovarian Preservation in Children and Adolescents With Primary Tumors of Ovary

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Objective: To determine management of pediatric ovarian masses and identify the factors associated with ovarian preservation. **Methods:** From January, 2002 to January, 2019 the case records of 72 patients (median age 14 y), who underwent surgery for ovarian tumors were retrospectively reviewed. **Results:** Tumors were most common in the age group 14-17 yrs (58.3%) and most (91.7%) were benign. The most common presenting symptom was abdominal pain (80.5%) followed by vomiting, elevated body temperature, nausea and palpable abdominal mass. 25 (34.7%) patients had an ovarian torsion. Six out of 72 (8.3%) patients had malignant tumors. Of the patients with malignant tumors, 75% had abnormal alpha-fetoprotein and 50% had abnormal β-human chorionic gonadotropin levels (P<0.001). Ovarian-preserving surgery was successfully performed in 74.3% of the benign lesions and two patients (25%) with malignant tumors (P=0.004). **Conclusions:** Factors associated with ovarian-preserving surgery are benign tumor, normal tumor markers levels, and smaller size of tumor.

Keywords: *Adnexal mass, Laparoscopy, Management, Ovariectomy*.

Ovarian tumors requiring surgical intervention are uncommon in children and adolescents [1]. The incidence of ovarian tumors in pediatric population is 2.6:100 000 girls per year, with higher rates in adolescents [2]. Only about 10-20% of all ovarian tumors in children and adolescents are malignant and comprises approximately 1-2% of all childhood malignancies [1-3]. Germ-cell tumors are the most common ovarian tumors in childhood and adolescence with mature cystic teratomas accounting for 55-70% of cases [4]. The presence of an ovarian tumor in children is a diagnostic and therapeutic challenge. The signs and symptoms can mimic many abdomino-pelvic medical or surgical diseases [2]. Ovarian-conserving procedures have proven safe for children and adolescents. Over the past decade, minimally invasive surgical techniques have become the standard of care for removing benign ovarian tumors because of shorter recovery time, decreased pain, and improved cosmesis [5,6].

The aim of this study was to determine the epidemiological, demographic and clinical characteristics and the management of pediatric ovarian tumors. Secondary aim was to identify the factors that are associated with ovarian preservation.

METHODS

The case records of all children and adolescents (age 0-17 y) who underwent surgery for ovarian tumors between January, 2002 and January, 2019 in the Department of pediatric surgery, at University hospital of Split, Croatia, were retrospectively reviewed. The study was approved by the ethics committee of our hospital. Exclusion criteria were: patients with incomplete documentation and the patients where another pathological cause was found during surgical exploration. The following parameters were recorded for each patient: demographic data, presenting symptoms, lateralization of tumor, serum tumor markers, physical examination, surgical findings, tumor size, pathohistological analysis, length of hospitalization and complications. Regarding type of tumor patients were divided into two groups of benign and malignant tumors. The study population was also stratified into 4 subgroups on the basis of patient age: Group I included antenatal and newborn patients; Group II included patients age 1-8 years; Group III included patients age 8-13 years; and Group IV patients age 14-17 years.

The data were analyzed using the SPSS 24.0 (IBM Corp, Armonk, NY) software program. Differences in quantitative variables between the groups were tested

with Mann-Whitney U test. The Chi-square test was used for comparing categorical data. $P<0.05$ was considered statistically significant.

RESULTS

A total of 72 female patients (6 with bilateral tumors), with median age of 14 (IQR 8.5, 16) years and median BMI of 22.5 (IQR 18, 25) kg/m^2, underwent surgery because of ovarian tumors. Eight patients (11.1%) were diagnosed during the antenatal or newborn period, two (2.8%) at ages 1-7, 20 (27.8%) at prepubertal ages; and 42 (58.3%) patients were identified after the age of 14 years. From total number of patients, 46 (63.8%) patients underwent emergent surgery because of the suspicion of ovarian torsion. The other 26 (36.2%) patients underwent elective surgery. The most common presenting symptom was abdominal pain (80.6%) followed by vomiting (26.4%), palpable abdominal mass (9.7%), elevated body temperature (9.7%), nausea (9.7%), inappetence (6.9%), vaginal bleeding (2.8%), amenorrhea (2.8%), dysuria (2.8%) and pubertas praecox (1.4%). Ten patients were asymptomatic (13.9%). Clinical and demographic date are presented in ***Table* I.** The median tumor diameter was 9.4 cm (IQR 7.5, 14). However, there was a significant difference in tumor size between patients who had benign neoplasm and those with malignant tumors ($P<0.001$). A laparoscopic resection was performed in 43 (59.7%) and open procedure in 29 (40.3%) patients. Ovarian-preserving surgery was successfully performed in 74.3% of the benign tumors *versus* 25% with malignant tumors ($P=0.004$). Regarding the patients with malignant tumors who underwent ovary sparing surgery, one patient has been diagnosed as dermoid cyst by radiologist with no abdominal lymphadenopathy and with negative tumor markers and on patohistology immature teratoma was found. Another patient was girl with juvenile type granulosa cell tumor, which was considered as tumor with low malignant potential so we decided to perform ovarian sparing tumor resection. Both patient had uneventful recovery and at follow up after five years both were completely symptom free and without signs of tumor at magnetic resonance imagings.

Two (2.8%) patients with benign tumor (one mature teratoma and one simple ovarian cyst) had elevated level of alpha-fetoprotein (AFP). Of the patients with malignant tumors, 75% (five patients with dysgerminoma and a patient with yolk sack tumor) had abnormal AFP levels and 50% (three patients with dysgerminoma and a patient with yolk sack tumor) had abnormal b-human chorionic gonadotropin (b-HCG) levels ($P<0.001$). Outcome of treatment of all patients are presented in ***Table* II**. Regarding complications after surgery in one case of mature teratoma residual tumor was found at MR at 1 year follow up. In that case redo surgery was performed. In three cases (one granulosa cell tumor and two ovarian cysts) postoperative bleeding was recorded. In all three cases bleeding stops spontaneously and hematoma was managed conservatively. Ovarian torsion was detected in 25 patients; oophorectomy was reserved for 13 (52%) gangrenous ovaries. Of all ovarian tumors, 50 (64.1%) were non-neoplastic lesions (cysts), 20

Table I Baseline Characteristics of Children with Ovarian Tumors (*N*=72)

Parameters	*Benign*	*Malignant*
#Age, *y*	13.5 (8.5, 15.5)	14 (11, 16)
Age group, y		
0-1	7 (10.6)	1 (16.7)
2-7	2 (3)	0
8-13	18 (27.3)	2 (33.3)
14-17	39 (59.1)	3 (50)
Lateralization		
Left	20 (30.3)	2 (33.3)
Right	42 (63.6)	2 (33.3)
Bilateral	4 (6.1)	2 (33.3)
*# Tumor diameter, cm	7.5 (5.5, 10)	13.0 (10.5,17)
**Tumor markers (α-FP; β-HCG)*		
Positive	2 (2.8)	6 (75)
Negative	70 (97.2)	2 (25)

*All values in no. (%) except #median (IQR); *P<0.001.*

Table II Treatment Outcomes of Patients (0-17 y) with Ovarian Tumors (*N*=72)

Parameters	*Benign*	*Malignant*
Surgical approach		
Open surgery	25 (37.9)	4 (66.7)
Laparoscopic surgery	41 (62.1)	2 (33.3)
**Procedure*		
Ovarian-preserving surgery	52 (74.2)	2 (25)
Ovariectomy	18 (25.8)	6 (75)
Ovarian torsion		
Ovarian-preserving surgery	11 (47.8)	1 (50)
Oophorectomy	12 (52.2)	1 (50)
Complications		
Bleeding	2 (2.8)	1 (12.5)
Residual tumor mass	1 (1.4)	0
#Hospital stay	3 (2, 4)	4 (2, 6)

*All values in no. (%) except #median (IQR); *P=0.004.*

(25.6%) were benign tumors, and 8 (10.3%) were malignant tumors (***Table* III**). Regarding the patients with bilateral ovarian tumors in one case final diagnosis was bilateral dysgerminoma, two cases of bilateral matured teratoma, one case of simple ovarian cyst and matured teratoma, and two cases of bilateral simple ovarian cysts.

DISCUSSION

The results of this study showed that the risk factors for ovariectomy are a malignant pathology, elevated levels of serum tumor markers and large tumor size. Surgical management of ovarian tumors in children should be based on ovarian-preserving surgery. Most of the tumors were benign and found in prepubertal and adolescent age groups. Laparoscopy may be safe and effective method for ovarian-preserving surgery in patients with ovarian cysts and benign ovarian tumors, with abdominal pain as the most common presenting symptom. Apart from the tumor, pain may also indicate ovarian torsion, especially if accompanied by vomiting and nausea.

Retrospective character is the main limitation of this study. Also, due to low incidence of ovarian tumors in this age group, there is a relatively small number of patients included in the study, so further studies are needed to analyze the same parameters on a larger sample.

Among pediatric patients undergoing surgery for ovarian tumors, the incidence of malignancy ranges from 4 to 20% [2,3,7]. Rate of malignant tumors in this research was 10.8%, which is in accordance with previous studies [8-10]. The most common tumors in this study were germ cell tumors. Similar data were reported in other studies [10]. Dysgerminoma was the most common malignant tumor in this study similar to other published studies [11]. The most common presenting symptom of ovarian tumors is abdominal pain, which is in accordance with our study [2,4,6,8-10]. Abdominal distension and vomiting are less frequent presenting symptoms [9,10]. Malignant tumors in this study had a diameter greater than 9 cm (median 13 cm) with no difference in the age of presentation between patients who had benign tumors and those who had malignant tumors. Similar findings were confirmed in other published studies [2]. Taskinen, *et al*. [12] reported that malignant high-grade tumors were detected only in girls older than 9 years. Over the past decade, minimally invasive surgical techniques have become the standard of care for removing benign adnexal masses and many pediatric surgeons prefer laparoscopy because of shorter recovery time, decreased pain, and improved cosmesis [13-15]. Rogers, *et al*. [15] concluded that it is safe in children and adolescents to proceed with a laparoscopic approach for adnexal masses without complex features measuring less than or equal to 8 cm in maximum diameter [15]. In present study we also removed successfully cysts and teratomas larger than 8 cm. If there is a surgical indication, surgery must conform to oncologic standards and must be as conservative as possible to preserve future fertility [2,13,14]. In present study, higher oophorectomy rate was found in children with a tumor size greater than 6.5 cm. The tumor size was significantly larger in the patients who underwent oophorectomy than in those who underwent ovarian-preserving surgery. Similar findings were reported in literature [2,4]. The most common reason for oophorectomy, except malignancy, was torsion of the ovary with gangrene of the ovarian tissue. Ovarian-conserving procedures has proven safe for adolescents and over the last decade minimally invasive surgical techniques have become the gold-standard treatment. Many surgeons agree that ovary-sparing surgery should be attempted whenever possible for ovarian tumors in pediatric patients [2,4,11-15]. In all our cases when there was possibility to remove tumor, safe ovarian-sparing surgery was performed. Ovarian torsion is a true emergency that always have to be considered in the differential diagnosis of any pediatric female patient presenting with acute abdominal pain. Recently, it has been proven that the black-bluish macroscopic appearance of the ovary is not a true indication of the degree of ischemia and that there is no valid clinical method of predicting the viability of the twisted ovary [2,16]. In our study AFP and β-HCG were highly

Table III Histopathology of Ovarian Tumors in Children (*N*=78)

Histopathological type	*No. (%)*
Germ cell tumors	22 (28.2)
Mature (dermoid) teratoma	15 (19.2)
Immature teratoma	1 (1.3)
Dysgerminoma	5 (6.4)
Yolk sac tumor	1 (1.3)
*Specialized stromal ovarian tumors**	1 (1.3)
Epithelial tumors	5 (6.4)
Serous cystadenoma	3 (3.8)
Cystadenofibroma	1 (1.3)
Mucinous cystadenoma	1 (1.3)
Ovarian cysts	50 (64.1)
Simple cyst	25 (32.1)
Follicular cyst	9 (11.5)
Corpus luteum cyst	11 (14.1)
Paraovarian cyst	5 (6.4)

*All value in no. (%); 6 patients had bilateral tumors; *granulosa cell tumor (juvenile type).*

WHAT THIS STUDY ADDS?

Risk factors for ovariectomy are a large tumor, malignant pathology, and elevated levels of tumor markers.

associated with malignancy. All patients with ovarian cysts had normal levels of serum tumor markers. Two patients with benign tumor had elevated level of AFP. Papic, *et al* [17]. reported also that that AFP and β-HCG were highly associated with malignancy, and no benign tumors were positive for these markers in their study. However, other reports showed that the rate of benign lesions associated with the rise of tumor markers varies from 3% to 20% [2,16].

In conclusion, ovarian tumors in childhood are mostly benign. The most common presenting symptom of ovarian tumors in children is pain. The risk factors for oophorectomy were a malignant pathology, elevated levels of serum tumor markers and large tumor size. Surgical management of ovarian masses in children should be based on ovarian-preserving surgery whenever it is possible.

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REFERENCES

1. Heo SH, Kim JW, Shin SS, Jeong SI, Lim HS, Choi YD, *et al*. Review of ovarian tumors in children and adolescents: Radiologic-pathologic correlation. Radiographics. 2014;34:2039-55.
2. Abid I, Zouari M, Jallouli M, Sahli S, Bouden A, Ben Abdallah R, *et al*. Ovarian masses in pediatric patients: A multicenter study of 98 surgical cases in Tunisia. Gynecol Endocrinol. 2018;34:243-7.
3. Oltmann SC, Garcia N, Barber R, Huang R, Hicks B, Fischer A. Can we preoperatively risk stratify ovarian masses for malignancy? J Pediatr Surg. 2010;45:130-4.
4. Aydin BK, Saka N, Bas F, Yilmaz Y, Haliloglu B, Guran T, *et al*. Evaluation and treatment results of ovarian cysts in childhood and adolescence: A multicenter, retrospective study of 100 patients. J Pediatr Adolesc Gynecol. 2017;30: 449-55.
5. Parelkar SV, Mundada D, Sanghvi BV, Joshi PB, Oak SN, Kapadnis SP, *et al*. Should the ovary always be conserved in torsion? A tertiary care institute experience. J Pediat Surg. 2014;49:465-8.
6. Hermans, AJ, Kluivers KB, Wijen MH, Bulten J, Massuger LF, Coppus SF. Diagnosis and treatment of adnexal masses in children and adolescents. Obstet Gynecol. 2015;125: 611-5.
7. Kirkham YA, Lacy JA, Kives S, Allen L. Characteristics and management of adnexal masses in a Canadian pediatric and adolescent population. J Obstet Gynaecol Can. 2011; 33:935-43.
8. Rathore R, Sharma S, Arora D. Spectrum of childhood and adolescent ovarian tumors in India: 25 years experience at a single institution. Maced J Med Sci. 2016;15:551-5.
9. De Backer A, Madern GC, Oosterhuis JW, Hakvoort-Cammel FG, Hazebroek FW. Ovarian germ cell tumors in children: A clinical study of 66 patients. Pediatr Blood Cancer. 2006;46:459-64.
10. Rajeswari B, Nair M, Ninan A, Parukuttyamma K. Ovarian tumors in children: 10-year experience from a tertiary care center in South India. Indian J Cancer. 2016;53:292-5.
11. Mondal SK, Banyopadhyah R, Nag DR, Roychowdhury S, Mondal PK, Sinha SK. Histologic pattern, bilaterality and clinical evaluation of 957 ovarian neoplasms: A 10-year study in a tertiary hospital of eastern India. J Can Res Ther. 2011;7:433-7.
12. Taskinen S, Fagerholm R, Lohi J, Taskinen M. Pediatric ovarian neoplastic tumors: Incidence, age at presentation, tumor markers and outcome. Acta Obstet Gynecol Scand. 2015;94:425-9.
13. Berger-Chen S, Herzog TJ, Lewin SN, Burke WM, Neugut AI, Hershman DL. Access to conservative surgical therapy for adolescents with benign ovarian masses. Obstet Gynecol. 2012;119:270-5.
14. Baert T, Storme N, Van Nieuwenhuysen E, Uyttebroeck A, Van Damme N, Vergote I, *et al*. Ovarian cancer in children and adolescents: A rare disease that needs more attention. Maturitas. 2016;88:3-8.
15. Rogers, EM, Casadiego, CG, Lacy J, Gerstle JT, Kives S, Allen L. Preoperative risk stratification of adnexal masses: Can we predict the optimal surgical management? J Pediatr Adolesc Gynecol. 2014;27:125-8.
16. Spinelli C, Buti I, Pucci V, Liserre J, Alberti E, Nencini L, *et al*. Adnexal torsion in children and adolescents: New trends to conservative surgical approach – our experience and review of literature. Gynecol Endocrinol. 2013;29:54-8.
17. Papic JC, Finnell SM, Slaven JE, Billmire DF, Rescorla FJ, Leys CM. Predictors of ovarian malignancy in children: Overcoming clinical barriers of ovarian preservation. J Pediatr Surg. 2014;49:144-7.

RESEARCH PAPER

Effect of Rehydration With Normal Saline *Versus* Ringer Lactate on Serum Sodium Level of Children With Acute Diarrhea and Severe Dehydration: *A Randomized Controlled Trial*

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Objective: To demonstrate the equivalence of Normal Saline (NS) and Ringer Lactate (RL) for change in serum sodium levels during correction of severe dehydration in children with acute diarrhea based on World Health Organization (WHO) plan C.

Design: Equivalence randomized control trial.

Setting: Pediatric diarrhea unit of a tertiary care hospital from May, 2016 to April, 2017.

Participants: 72 children of 1-12 years with acute diarrhea and severe dehydration were enrolled. Children with dysentery, severe acute malnutrition, severe anemia, meningitis, and known surgical and systemic diseases were excluded.

Intervention: RL (*n*=36) or NS (*n*=36) were used as per WHO plan C. Blood samples were drawn before intravenous fluid correction and 3 h post-intervention.

Outcome Measures: Mean change in serum sodium level from the baseline between the RL and NS groups.

Results: 70 children (35 in each group) completed the study. The difference in mean serum sodium levels from baseline in RL and NS groups were 1.4 (4.5) mEq/L and 2.1(4.9) mEq/L, respectively (*P*=0.58).

Conclusions: Both RL and NS are equivalent in terms of change in serum sodium from baseline for intravenous rehydration in children with acute diarrhea and severe dehydration.

Keywords: *Acid-base balance, Hyponatremia, Intravenous fluids, pH, Serum potassium.*

Diarrhea is a leading cause of death in children accounting for 9% of all deaths among children under-5 year worldwide in 2015 [1] and an estimated 300,000 children in India each year [2]. Dehydration is associated with deaths in most cases [3] and occurs when fluid losses are not replaced adequately and a deficit of water and electrolytes develops. The total body sodium deficit in diarrheal dehydration in young children is about 70-110 millimoles per liter of water deficit. Potassium and chloride losses are in a similar range [3]. The preferred regime for treatment of children with severe dehydration is rapid intravenous rehydration using World Health Organization (WHO) Plan C. WHO recommends use of Ringer lactate or normal saline in case of non-availability of Ringer lactate, for intravenous rehydration in children under plan C [3].

METHODS

This equivalence randomized control trial was done in the Department of Pediatrics, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi during the period May, 2016 – April, 2017. The study was approved by Institute ethics committee. Children between 1 to 12 years of age with acute diarrhea and severe dehydration were enrolled after taking informed consent from their parents. Acute diarrhea was defined as ≥3 loose stools in previous 24 hour and duration of diarrhea less than 14 days. Severe dehydration was defined as per WHO guidelines [3] with two or more of the following: lethargic or unconscious, drinks poorly or not able to drink, skin pinch goes back very slowly (>2 second) and sunken eyes. Children with dysentery, severe acute malnutrition (WHO criteria), severe anemia (significant palmar pallor), meningitis, seizures, known surgical problems (*e.g.* ileostomy), known systemic disease and hypoglycemia (Blood glucose <54 mg/dL) were excluded. Eligible children were randomly assigned to receive either Ringer lactate or normal saline (***Fig.* 1**). Allocation sequence was computer generated (*www.randomization.com*) and allocation concealment was done through serially numbered opaque sealed envelopes (SNOSE).

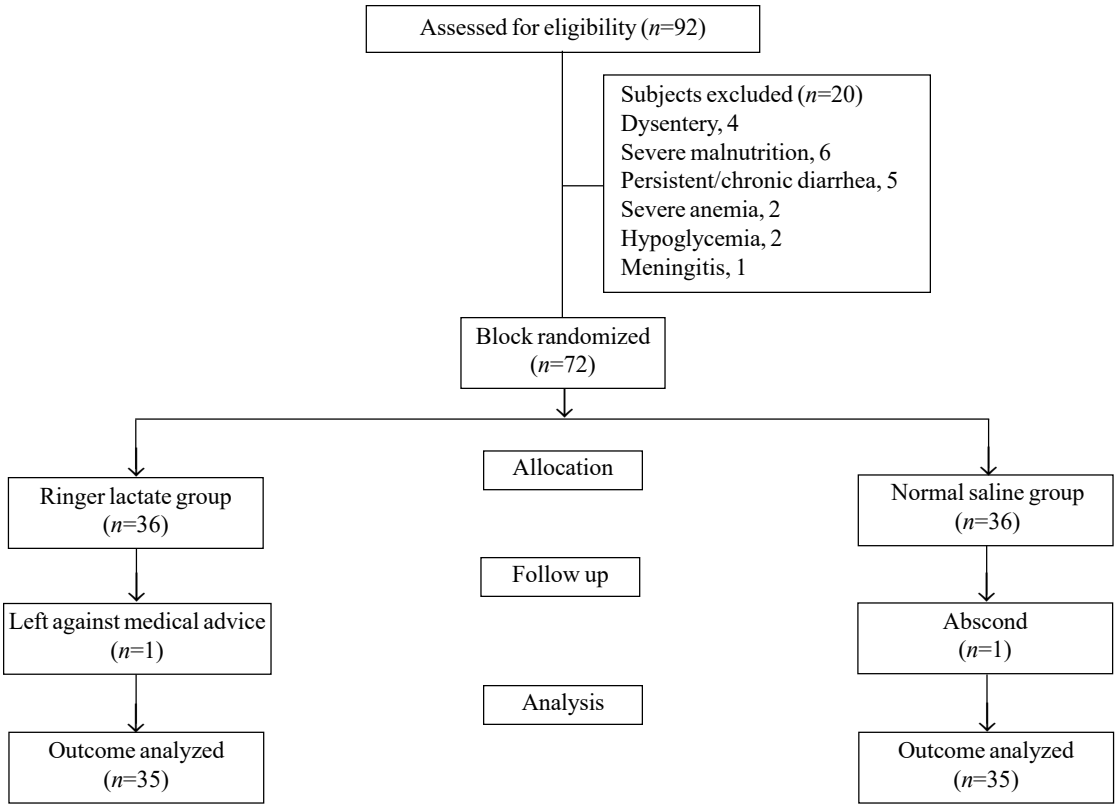

Fig. 1 *Flow diagram of patients*.

Before commencement of rehydration, blood samples were taken for blood gas analysis, kidney function tests and serum electrolytes (sodium and potassium). Hyponatremia was defined at serum sodium <135 mmol/L. Children received Ringer lactate or normal saline according to WHO guidelines in doses of 100 mL/kg over 3 hour and were monitored every 30-60 minutes for vital signs. They were reassessed at the end of 100 mL/kg infusion for clinical signs of dehydration. Caregivers were asked to mark the number of stool purges and the number of vomiting for the correction period. If any child was found in dehydration at the end of first correction, the child was treated according to standard WHO guidelines. At the end of first correction, blood samples were repeated for blood gas, renal function and serum electrolytes. In initial hours ongoing losses were replaced by intravenous fluid solution of 0.45% saline in 5% dextrose and 20 mEq/L potassium chloride at 10 mL/kg per loose stool at hourly intervals. Children also received age appropriate maintenance fluids. All children received oral elemental zinc supplementation at 20 mg/day. Completion of first fluid correction at 3 hour was taken as primary end point and disappearance of all clinical signs of dehydration was taken as endpoint for secondary outcome. Our primary objective was to determine the difference in the change of serum sodium level over baseline in the two groups. We also studied the difference in the change of serum potassium, pH, bicarbonate levels and base deficit at primary end point. The time taken and volume of fluid requirement for complete rehydration in the two groups were compared at secondary end point.

Sample size was calculated to demonstrate equivalence between the two interventions with an equivalence limit not exceeding 3 mEq in serum sodium level with SD of 3, $\alpha = 1\%$ and power of 90. A sample size of 30 children was calculated. Expecting 20% attrition, 36 subjects were enrolled in each group in an age stratified manner in 2:1 ratio for age groups 1-5 years and >5-12 years.

Statistical analyses: Analysis was conducted using IBM SPSS Statistics (version 22.0). The normality of quantitative data was checked by measures of Kolmogorov-Smirnov tests of normality. For primary outcome, the two groups were compared for change in serum sodium from baseline. Means of two groups were compared using independent t-test. Mann-Whitney U-test was carried out for statistical analysis of skewed

continuous variables. For comparison of normally time related variables paired t-test was applied. Proportions were compared using Fisher's exact test. All the statistical tests were two-sided and were performed at a significance level of 0.05.

RESULTS

Out of 72 enrolled children, 70 (35 in each group) completed the therapy. One child in each group opted out of the study before the first correction of dehydration. The baseline characteristics of patients are shown in ***Table* I**. Hyponatremia was present at baseline in 26 (74%) in Ringer lactate group and 25 (71%) in normal saline group. No child had symptomatic hyponatremia. Mean (SD) serum sodium values at baseline were comparable (131.3 (4.4) mEq/L in Ringer lactate group and 132.3 (4.8) mEq/L in normal saline group, *P*=0.29). The change in biochemical parameters at primary end point are depicted in ***Table* II**.

After first volume correction (WHO plan C), 23 (65%) children in Ringer lactate group and 17 (49%) children in normal saline group had persistent hyponatremia, one child had symptomatic hypokalemia in the latter group, which responded to standard therapy. A total of 29 (83%) children were completely rehydrated in each group while 6 (17%) had features of some dehydration and required Plan B. No child required subsequent rehydration. Time to rehydration was similar (range 3h-7h) in both groups. The mean (SD) fluid requirement for replacement of ongoing losses was similar in both the groups, 74.29 (35) mL/kg and 76.29 (34.8) mL/kg in Ringer lactate and Normal saline groups, respectively (*P*=0.81).

Table I Baseline Characteristics of Children With Severe Dehydration Receiving Ringer Lactate or Normal Saline for Rehydration

Characteristics	*Ringer Lactate (n=35)*	*Normal Saline (n=35)*
Age (y)	4.3 (2.9)	4.7 (2.9)
Male	16 (46)	17 (49)
Duration of symtoms, d	1.8 (1.6)	1.6 (1.4)
*Sodium, mEq/L	131.3 (4.4)	132.3 (4.0)
*Potassium, mEq/L	3.8 (0.6)	3.5 (0.7)
Blood urea, mg/dL	53 (35.8)	59.6 (28.6)
Creatinine, mg/dL	1.2 (0.7)	1.3 (0.7)
pH	7.26 (0.07)	7.28 (0.08)
Bicarbonate, mEq/L	12.66 (3.33)	12.16 (2.89)
Base deficit, mmol/L	12.58 (3.98)	12.89 (3.66)

**Serum values; data represented as Mean (SD); P >0.05 for all comparisons.*

DISCUSSION

In this study, high rate of hyponatremia was detected in children with acute diarrhea and severe dehydration which persisted after rehydration. The change of serum sodium was similar with use of either Ringer lactate or normal saline for rehydration.

The open label nature of the trial and the non-availability of serum chloride levels and non-utilization of oral rehydration solution for replacement of ongoing losses were the limitations of the study. The study was not powered to detect significant changes in pH, bicarbonate and base excess.

In a similar study by Mahajan, *et al.* [9], the change in serum sodium levels was similar after rapid intravenous rehydration with Ringer lactate or normal saline in children with acute diarrhea. The decline in serum potassium from baseline in both groups was comparable in the present study unlike seen only in normal saline

Table II Change in Biochemical Parameters During Correction of Severe Rehydration

Parameters	*Ringer Lactate (n=35)*			*Normal Saline (n=35)*			*P value**
	Baseline	*After correction*	*Mean (SD) difference*	*Baseline*	*After correction*	*Mean (SD) difference*	
Sodium (mEq/L)	131.3 (4.4)	132.7 (3.5)	1.4 (4.5)	132.3 (4.0)	134.5 (4.5)	2.1 (4.9)	0.58
Potassium (mEq/L)	3.8 (0.6)	3.6 (0.6)	0.2 (0.4)	3.5 (0.7)	3.3 (0.7)	0.2 (0.5)	0.60
Blood urea (mg/dL)	53 (35.8)	42.7 (28.6)	10.3 (18.2)	59.6 (28.6)	40.0 (15.8)	19.6 (21.9)	0.6
Creatinine (mg/dL)	1.2 (0.7)	0.9 (0.5)	0.3 (0.3)	1.3 (0.7)	0.8 (0.3)	0.4 (0.5)	0.42
pH	7.26 (0.07)	7.33 (0.08)	0.07 (0.05)	7.28 (0.08)	7.30 (0.09)	0.02 (0.07)	0.002
Bicarbonate (mEq/L)	12.66 (3.33)	15.92 (4.04)	3.25 (2.14)	12.16 (2.89)	13.19 (2.41)	1.03 (2.66)	<0.001
Base deficit (mmol/L)	12.58 (3.98)	8.85 (4.48)	3.73 (2.48)	12.89 (3.66)	11.67 (3.66)	1.22 (2.80)	<0.001

*All values in mean (SD); *P value for delta difference between both groups.*

WHAT THIS STUDY ADDS?

Ringer lactate and normal saline are equivalent in terms of change in serum sodium from baseline for rapid intravenous rehydration in children with acute diarrhea.

group in the earlier study [9], which was attributed to the composition of normal saline, which does not have potassium as a constituent. The present study had lesser metabolic acidosis in comparison to the previous study [9], which could explain the greater fall in the potassium levels in their study. In the present study, the significant changes in pH, bicarbonate and base deficit in Ringer lactate group as compared to normal saline group can be explained by the conversion of lactate to bicarbonate in the former group. Similar results were observed in an adult study [10], unlike Mahajan, *et al.* [9] where both groups showed comparable change, which was attributed to intravascular volume expansion.

To conclude, normal saline is equivalent to Ringer lactate solution in terms of change of serum sodium and serum potassium from baseline for initial rapid intravenous rehydration in children with acute diarrhea and severe dehydration. Rehydration with normal saline does not cause hypernatremia. Although, quicker resolution of metabolic acidosis occurs with Ringer lactate solution, its clinical significance may need to be studied further.

Ethical Clearance: Institutional Ethics Committee for Human Research, Maulana Azad Medical College; No. 11/IEC/MAMC/2015/317.
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REFERENCES

1. Diarrhoea remains a leading killer of young children, despite the availability of a simple treatment solution. UNICEF 2017. Available from: *https://data.unicef.org/topic/child-health/diarrhoeal-disease/#.* Accessed April 15, 2017.
2. Lakshminarayanan S, Jayalakshmy R. Diarrheal diseases among children in India: Current scenario and future perspectives. J Nat Sc Biol Med. 2015;6:24-8.
3. Bhan MK, Mahalanabis D, Pierce NF, Rollins N, Sack D, Santoshum M. The treatment of diarrhoea: A manual for physicians and other senior health workers. 4th rev. Geneva: World Health Organization; 2005.
4. World Health Organization. Cholera annual report 2006: Weekly epidemiological record. World Health Organ 2007;82:273-84.
5. Reid F, Lobo DN, Williams RN, Rowlands BJ, Allison SP. Normal saline and physiological Hartmann's solution: a randomized double-blind crossover study. Clin Sci (Lond). 2003;104:17-24.
6. Cho YS, Lim H, Kim SH. Comparison of lactated Ringer's solution and 0.9% saline in the treatment of rhabdomyolysis induced by doxylamine intoxication. Emerg Med J. 2007;24:276-80.
7. Hadimioglu N, Saadawy I, Saglam T, Ertug Z, Dinckan A. The effect of different crystalloid solutions on acid-base balance and early kidney function after kidney transplantation. Anesth Analg. 2008;107:264-9.
8. O'Malley CM, Frumento RJ, Hardy MA, Benvenisty AI, Brintjens TE, Mercer JS, *et al*. A randomized, double blind comparison of lactated ringer's solution and 0.9% saline during renal transplantation. Anesth Analg. 2005; 100:1518-24.
9. Mahajan V, Sajan SS, Sharma A, Kaur J. Ringer's lactate vs normal saline for children with acute diarrhea and severe dehydration: A double blind randomized controlled trial. Indian Pediatr. 2012;49:963-8.
10. Cieza JA, Hinostroza J, Huapaya JA, León CP. Sodium chloride 0.9% versus lactated Ringer in the management of severely dehydrated patients with choleriform diarrhea. J Infect Dev Ctries. 2013;7:528-32.

RESEARCH PAPER

Predictors of Outcome in Pediatric Anaplastic Large Cell Lymphoma

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Objective: To determine the event-free survival (EFS), overall survival (OS) and predictors of outcome in pediatric anaplastic large cell lymphoma treated with a uniform protocol. **Method:** This hospital record review was done between June, 2016 to March, 2019 and data was extracted from January, 2003 to May, 2016 for anaplastic large cell lymphoma (ALCL) in patients aged 1 to 18 years (*n*=27). EFS and OS were calculated by the Kaplan Meier method. Cox proportional model and the Cox regression model were used for univariate analysis and multivariate analysis respectively. **Results:** EFS and OS at three years was 70.4% (CI: 0.49-0.84) and 77.2% (CI: 0.56 -0.89), respectively. On univariate analysis stage III and IV, hemoglobin less than 10 g/dL and presence of pleural effusion predicted lower survival. On multivariate analysis, pleural effusion was a significant predictor of low EFS and OS. **Conclusion:** Pleural effusion is a potential clinical marker of poor outcome among children with anaplastic large cell lymphoma.

Keywords: *Management, Non-Hodgkin's lymphoma, Predictor, Prognosis*.

Anaplastic large cell lymphoma (ALCL) has been recognized as a unique subset of peripheral T cell lymphoma by the World Health Organisation (WHO) and is the most common subtype [1]. In children, it comprises 10% of pediatric non-Hodgkin lymphoma [2]. The clinical presentation is varied with extranodal site involvement commonly posing challenges in diagnosis. Event-free survival (EFS) of 60-75% with intensive protocols has been reported worldwide [3-5]. A characteristic anaplastic lymphoma kinase (ALK) mutation is present in the majority of pediatric ALCL [6,7]. Literature from South Asia is limited in this subset due to the rarity of the disease. Here we report EFS, overall survival (OS) and predictors of the outcome of pediatric ALCL treated uniformly at our center Berlin-Frankfurt-Munster (BFM).

METHODS

We retrieved data from medical records of our institute of pediatric ALCL managed from January, 2003 to May, 2016. The institute and ethics committee approved the study. The diagnosis of ALCL was based on clinical presentation, morphology, histopathology, and immunohistochemistry. Data of all patients of age 1 to 18 years were analyzed, except partially treated, relapsed patients and patients who did not receive treatment. The B symptoms were defined as non-infectious fever (no obvious focus of infection) more than 100.4ºF without evidence of infections, significant weight loss of more than 10%, and history of night sweats. For completion of staging workup, all patients underwent contrast-enhanced computed tomography (CECT) of neck, chest, and abdomen along with cerebrospinal fluid cytopathology, and bone marrow aspiration and biopsy examination. Symptom duration before presentation at our centre was categorized into early (≤30 days) and delayed (>30 days) presentation. The staging was done as per St Jude staging [8].

We categorized patients' performance status into 1 and 2 *vs* 3 and 4 [9], nodal and extra nodal sites, presence or absence of pleural effusion, B symptoms, and expression of a Anaplastic lymphoma kinase (ALK) and cluster of differentiation (CD) 3.

All patients of early stage were treated with chemotherapy protocol as per Link, *et al.* [10] and those with advanced disease were treated with Berlin-Frankfurt-Munster (BFM) BFM 90 NHL protocol [11]. After the end of two blocks of treatment, patients underwent CECT of neck, chest, and abdomen for response evaluation. After completion of treatment post six cycles of therapy, the response was documented with re-imaging. After completion of treatment, patients were followed up every three months for the initial two years and then every six-months till five years, and yearly thereafter. Patients who did not attend the scheduled visit were contacted telephonically. Relapse was defined as disease recurrence after the achievement of complete remission

post completion of therapy and progressive disease was defined as progression during treatment.

Statistical analyses: Baseline patient characteristics were assessed for survival. An event was defined as relapse, the progression of the disease, lost to follow up or death due to any cause. The EFS was calculated from the day of diagnosis until the date of the event. OS was calculated from the date of diagnosis until the date of death due to any cause. The data were censored on 31 March, 2019 or the date of the last follow-up for survival analysis.

STATA ver.13 (StataCorp, USA) was used for statistical analyses. Kaplan-Meiler method was used for survival analysis and the Log-rank test was used for comparison. The chi-square method was used to see the association between variables affecting outcomes. Cox proportional model was used for hazard calculation for univariate analysis, and for multivariate analysis Cox regression model was used. The significant univariate variables of value up to P=0.10, were considered for multivariate analysis.

RESULTS

A total of 27 patients (3 females) of pediatric ALCL were studied. The baseline clinicopathological characteristics and demographics are shown in ***Table* I**. Stage IV disease was observed in 7 (26%) patients. Extranodal involvement was seen in 20 (74%) patients; the most common site was bone in 9 (33%) patients and skin involvement in 5 (18%); other uncommon sites were adrenal glands, breast and lung parenchyma in one patient each. No patient had bone marrow involvement. Two patients had involvement of parenchymal central nervous system involvement; one patient had temporal lobe mass and the other had suprasellar mass. We also evaluated baseline absolute neutrophil count divided by total leukocyte count (ANC/TLC) ratio; categorized into two groups based on the median value of 0.65.

EFS at 3 years of the whole cohort was 70.4 (8.8)% (CI: 0.49-0.84) and OS was 77.2 (8.2)% (CI: 0.56-0.89), respectively. Median EFS and OS were not reached. Ten events occurred from the date of diagnosis. Three patients progressed while on therapy. There were four deaths unrelated to disease, progression or relapse; two patients died of chemotoxicity (one with gastrointestinal bleed and other with septic shock); one died of complication of complex congenital heart disease (patient was in CR at three years of follow up); cause of death in the fourth patient was viral hepatitis. There were three nodal relapses, out of which one died while two patients were treated with salvage therapy and autologous stem cell transplant. Of these, one died 3 years post-transplant due to central nervous system infection and refractory seizures, and the other patient is alive without disease.

The univariate analysis of prognostic factors for EFS and OS are depicted in ***Table* II**. On univariate analysis, stage III and IV, hemoglobin less than 10 g/dL and presence of pleural effusion had a trend towards predicting inferior EFS and OS with P<0.1. On multivariate analysis, only pleural effusion emerged as a significant predictor for EFS (P=0.011) and OS (P=0.02).

Notably, there was no significant difference in pleural effusion patients with hypoalbuminemia (40%) and in those without hypoalbuminemia (12%), P=0.09. We also did not find any significant difference in pleural effusion in patients with poor performance status (36%) and good

Table I Baseline Characteristics of Children With Anaplastic Large Cell Lymphoma (N=27)

Age, y*	12 (1-17)
Male gender	24 (89)
Symptom duration, mo*	3 (0.5-12)
>30 days	23 (85)
Stage	
I	1 (4)
II	2 (7)
III	17 (63)
IV	7 (26)
Performance status (ECOG)	
1 and 2	14 (52)
3 and 4	13 (48)
Extranodal site	20 (81)
B symptom	21 (78)
Pleural effusion	6 (22)
ALK positive	18 (95)
CD 3 negative	13 (62)
Hemoglobin, (g/dL)*	10.1 (4.3-15.1)
WBC*, per cu.mm	9100 (2300-26200)
>12000 per cu.mm	11 (41)
ANC/TLC ratio*	0.65 (0.29-0.86)
Serum albumin, (g/dL)*	3.7 (2.3-5.3)
≤3.5 g/dL	10 (37)
Normal serum LDH[#]	15 (68)

LDH: *Lactate dehydrogenase; ANC*: *Absolute neutrophil count*; *TLC*: Total leucocyte count; ECOG*: Eastern co-operative group; Values in no. (%) except *median (range). ALK*: *anaplastic large kinase*[#], *ALK evaluated in 19 children, CD3 in 21 children, and LDH in 22 children*.

Table II Factor Predicting Event Free Survival in Children with Anaplastic Large Cell Lymphoma (*N*=27)

Variable	*No*	*Event free survival HR (CI)*	*P*	*Overall survival HR (CI)*	*P*
Age <12 y	13	0.52 (0.15-1.86)	0.32	0.53 (0.15-1.90)	0.33
Female sex	3	1.35 (0.17-10.69)	0.77	1.41 (0.18-11.17)	0.75
Rural residence	14	1.25 (0.36-4.36)	0.73	1.43 (0.41-5.05)	0.57
Symptom duration >30 d	23	1.78 (0.38-8.45)	0.47	1.13 (0.24-5.34)	0.88
Performance status ECOG 1 and 2	14	0.42 (0.11-1.62)	0.21	0.35 (0.09-1.37)	0.13
No B symptom	6	1.07 (0.23-5.04)	1.07	1.13 (0.24-5.34)	0.88
No Pleural effusion	21	8.51 (2.34-30.90)	0.00	1 7.77 (2.14-28.19)	0.002
[#]CD 3 positive (*n*=21)	8	4.04 (0.73-22.18)	0.11	2.58 (0.46-14.34)	0.28
Hb ≤10.0 g/dL	13	0.32 (0.08-1.24)	0.10	0.29 (0.07-1.12)	0.07
WBC ≤11 × 10^9	16	0.71 (0.20-2.51)	0.59	0.64 (0.18-2.27)	0.49
ANC/TLC ratio ≤0.65	13	1.78 (0.50-6.33)	0.37	2.41 (0.66-8.76)	0.18
Serum albumin (g/dL) ≤3.5	10	0.36 (0.10-1.29)	0.12	0.39 (0.11-1.40)	0.15
[#]Raised Serum LDH (*n*= 22)	7	1.62 (0.33-7.80)	0.55	1.57 (0.33-7.61)	0.57

ALK: Anaplastic large kinae; ANC/TLC: Absolute neutrophil count /Total leukocyte count; CD: Cluster differentiation; CI: Confidence interval; ECOG: Eastern co-operative group, Hb: hemoglobin; HR: Hazard ratio, LDH: Lactate dehydrogenase; ULN: Upper limit of normal; WBC: White blood count; [#]Data was not available for all patients; [$]As all patient in stage I and II survived, hazard ratio and confidence interval was not evaluable; Since only one patient was ALK negative, data was not evaluable and it was excluded from multivariate analysis.

performance status (8%), *P*=0.08. Out of six patients who had pleural effusion, one patient received anti-tubercular therapy. In our study, pleural effusion was not drained and on the initiation of therapy, it resolved.

DISCUSSION

In this cohort of pediatric non-Hodgkin lymphoma, we only analyzed clinical factors as predictors of EFS and OS. Minimal disseminated disease in peripheral blood and bone marrow at diagnosis, minimal residual disease, and Anaplastic large kinase titer which are novel biological predictors of outcome were not evaluated [12]. The strength of this study is that it includes a cohort of a relatively rare subtype of NHL treated with a uniform protocol. However, the fact that it is a retrospective study with a limited number of subjects is a limitation of the study.

In this study, the EFS of patients with ALCL is 10-15% lower as compared to other contemporary series from developed countries [3-5,13,14]. When the results are compared with developing countries, the outcomes are similar [9,15,16]. Our study highlights some of the challenges as almost 90% of our patients presented in an advanced stage of the disease and more than 50% of subjects had a poor performance status. Further, the median duration of symptoms was three months, and one-fifth of the patients received anti-tubercular therapy before being diagnosed as a malignancy. This is similar to the observation in pediatric Hodgkin lymphoma [17].

Prognostic factors for ALCL are not consistent across studies due to mostly retrospective series, a small number of patients and varying protocols [18]. We found pleural effusion as a predictor of poor EFS and OS. In a study of 225 patients [19], the involvement of the lymph nodes, mediastinum, skin and liver was associated with the risk of relapse; however, it is to be noted that three different protocols were used in treating these groups of patients. In NHL-BFM 90 trial, only B symptoms were associated with poor EFS [4].

Our study implies that the presence of pleural effusion is a potential clinical marker of poor outcomes in pediatric patients with ALCL. Pleural effusion resolves with treatment and that no additional intervention is usually required. There is a need to spread awareness about this subtype of NHL amongst pediatricians and primary care physicians so that they are referred early for treatment and thereby presentation of the disease in advanced stages is minimized.

Ethical clearance: Institutional Ethics Committee of AIIMS, Delhi; No. IESC/T-331, June 23, 2015.
Contributors: AP: designed the research study, analysed the data, drafted the paper; DP: analyzed the data and drafted the paper; MCS,ST: acquisition and interpretation of the data for the paper, drafted the paper; SB: conception and designed the research study, analyzed the data, drafted the paper. All authors contributed to the drafting of the work, finally approved the work and agreed to be accountable for integrity of the data.
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WHAT THIS STUDY ADDS?

Pleural effusion is a potential clinical predictor of poor outcome in pediatric anaplastic large cell lymphoma.

REFERENCES

1. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, *et al*. The 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms. Blood. 2016;127:2375-90.
2. Burkhardt B, Zimmermann M, Oschlies I, Niggli F, Mann G, Parwaresch R, *et al*. The impact of age and gender on biology, clinical features and treatment outcome of non-Hodgkin lymphoma in childhood and adolescence. Br J Haematol. 2005;131:39-49.
3. Lowe EJ, Sposto R, Perkins SL, Gross TG, Finlay J, Zwick D, *et al*. Intensive chemotherapy for systemic anaplastic large cell lymphoma in children and adolescents: Final results of Children's Cancer Group Study 5941. Pediatr Blood Cancer. 2009;52:335-9.
4. Seidemann K, Tiemann M, Schrappe M, Yakisan E, Simonitsch I, Janka-Schaub G, *et al*. Short-pulse B-non-Hodgkin lymphoma-type chemotherapy is efficacious treatment for pediatric anaplastic large cell lymphoma: A report of the Berlin-Frankfurt-Münster Group Trial NHL-BFM 90. Blood. 2001;97:3699-706.
5. Rosolen A, Pillon M, Garaventa A, Burnelli R, d'Amore ES, Giuliano M, *et al*. Anaplastic large cell lymphoma treated with a leukemia-like therapy: report of the Italian Association of Pediatric Hematology and Oncology (AIEOP) LNH-92 protocol. Cancer. 2005;104:2133-40.
6. Gambacorti-Passerini C, Messa C, Pogliani EM. Crizotinib in anaplastic large-cell lymphoma. N Engl J Med. 2011; 364:775-6.
7. Mossé YP, Lim MS, Voss SD, Wilner K, Ruffner K, Laliberte J, *et al*. Safety and activity of crizotinib for paediatric patients with refractory solid tumours or anaplastic large-cell lymphoma: A Children's Oncology Group phase 1 consortium study. Lancet Oncol. 2013;14: 472-80.
8. Murphy SB, Fairclough DL, Hutchison RE, Berard CW. Non-Hodgkin's lymphomas of childhood: An analysis of the histology, staging, and response to treatment of 338 cases at a single institution. J Clin Oncol. 1989;7:186-93.
9. Lakshmaiah KC, Guruprasad B, Shah A, Kavitha S, Abraham LJ, *et al*. Anaplastic large cell lymphoma: A single institution experience from India. J Cancer Res Ther. 2013;9:649-52.
10. Link MP, Shuster JJ, Donaldson SS, Berard CW, Murphy SB. Treatment of children and young adults with early-stage non-hodgkin's lymphoma. N Engl J Med. 1997;337: 1259-66.
11. Reiter A, Schrappe M, Tiemann M, Ludwig WD, Yakisan E, Zimmermann M, *et al*. Improved treatment results in childhood B-cell neoplasms with tailored intensification of therapy: A report of the Berlin-Frankfurt-Münster Group Trial NHL-BFM 90. Blood. 1999;94:3294-306.
12. Damm-Welk C, Pillon M, Woessmann W, Mussolin L. Prognostic factors in paediatric anaplastic large cell lymphoma: Role of ALK. Front Biosci Sch Ed. 2015;7: 205-16.
13. Pillon M, Gregucci F, Lombardi A, Santoro N, Piglione M, Sala A, *et al*. Results of AIEOP LNH-97 protocol for the treatment of anaplastic large cell lymphoma of childhood. Pediatr Blood Cancer. 2012;59:828-33.
14. Alexander S, Kraveka JM, Weitzman S, Lowe E, Smith L, Lynch JC, *et al*. Advanced stage anaplastic large cell lymphoma in children and adolescents: results of ANHL0131, a randomized phase III trial of APO versus a modified regimen with vinblastine: A report from the Children's Oncology Group. Pediatr Blood Cancer. 2014; 61:2236-42.
15. Ceppi F, Ortiz R, Antillón F, Vasquez R, Gomez W, Gamboa J, *et al*. Anaplastic large cell lymphoma in Central America: A report from the Central American Association of Pediatric Hematology Oncology (AHOPCA): Treatment of ALCL: A Report From a LMIC. Pediatr Blood Cancer. 2016;63:78-82.
16. Resham S, Khan R, Ashraf S, Rizvi A, Altaf S. Clinical features and treatment outcomes of children with anaplastic large cell lymphoma in Pakistan: A multicenter study. J Pediatr Hematol Oncol. 2019;41:5.
17. Arya LS, Dinand V, Thavaraj V, Bakhshi S, Dawar R, Rath GK, *et al*. Hodgkin's disease in Indian children: outcome with chemotherapy alone. Pediatr Blood Cancer. 2006;46: 26-34.
18. Lowe EJ, Gross TG. Anaplastic large cell lymphoma in children and adolescents. Pediatr Hematol Oncol. 2013;30: 509-19.
19. Le Deley M-C, Reiter A, Williams D, Delsol G, Oschlies I, McCarthy K, *et al*. Prognostic factors in childhood anaplastic large cell lymphoma: Results of a large European intergroup study. Blood. 2008;111:1560-6.

SYSTAMATIC REVIEW

Efficacy of Local Control Strategies for Ewing Sarcoma After Neoadjuvant Chemotherapy: *A Network Meta-analysis*

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Objective: This network meta-analysis aimed at comparing the efficacy of local control strategies after neoadjuvant chemotherapy in patients with Ewing sarcoma.

Design: Network meta-analysis was used to synthesize direct and indirect evidence in a network of trials that compare multiple interventions and has the potential to rank the competing treatments according to the studied outcome.

Setting: There are three treatment options for local Ewing's sarcoma after neoadjuvant chemotherapy, namely surgery, radiotherapy and surgery plus radiotherapy (SR).

Participants: Records of 2540 patients from 11 studies were analyzed.

Main outcome measures: Potentially relevant studies were retrieved from PubMed and Embase, and screened according to inclusion and exclusion criteria. Hazard ratios and the associated 95% confidence intervals were used to describe the efficacy of different interventions on 5-year local recurrence rate and 5-year event-free survival rate. Surface under the cumulative ranking curve (SUCRA) was calculated for ranking probabilities of different treatment.

Results: Compared with radiotherapy, surgery had better efficacy [local recurrence, OR (95% CI) 0.48 (0.33 - 0.87)] and SR had a similar effect as surgery [local recurrence, OR (95% CI) 0.50 (0.29 - 0.82)]. There were no statistically significant differences between three different local control strategies in 5-year local recurrence rate. SUCRA values suggested that surgery was better than SR for 5-year local recurrence rate (0.79 *vs* 0.70) and 5-year event free survival rate (0.67 *vs* 0.50), respectively.

Conclusions: Both surgery and SR were superior to radiotherapy in reducing 5-yer local recurrence of patients with Ewing sarcoma after neoadjuvant chemotherapy. Surgery had higher efficacy than SR on improving the prognosis of patients.

Keywords: *Management, Metastasis, Outcome, Prognosis, Relapse*.

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Ewing sarcoma is the second most common primary bone cancer affecting mainly adolescents in the second decade of their life [1]. It has a predilection for long bones (47%), pelvis (26%), chest wall (16%) and spine (6%) [2]. Pain is the most common initial symptom as with other bone sarcomas [3]. Ewing sarcoma is highly metastatic; although, it can be locally controlled by radiotherapy or surgery, historically, 85%-90% of patients die within a few months from a metastasis without systematic treatment neither before nor after local treatment [4]. After the addition of doxorubicin to vincristine, actinomycin D, and cyclophosphamide (VACD regimen), the 5-year overall survival rate of local disease increased from 28% to 65% in the 1970s [5]. Chemotherapy was initially used as systematic treatment to control metastasis, and later in a neoadjuvant setting to enhance local control with confirmed efficacy [6].

Accompanying Editorial: Pages 503-04.

Local control is an important method to improve the overall survival rate and local control rate of Ewing sarcoma patients. Local treatment is recommended after chemotherapy for all patients. Current local control strategies include isolated radiotherapy, isolated surgery, or combined surgery and radiotherapy [7]. The debate over whether surgery and radiotherapy are comparable in terms of local control continues [8]. The optimal local control strategy for Ewing sarcoma remains unclear. The French association for pediatric research suggested that surgery or surgery combined with radiotherapy is the best local treatment for pelvic tumors, while radiotherapy is only available to patients who cannot undergo surgery or patients who are resistant to chemotherapy, or surgery involves amputation [10,11]. Zogopoulos, *et al*. [12] suggested that surgery is the most

effective method for local treatment, while radiotherapy should be used sparingly. Moreover, with the neoadjuvant application of chemotherapy, we are still looking for a conclusive analysis concerning whether surgery and radiotherapy are comparable in terms of local control. This network meta-analysis aimed at comparing the efficacy of local control strategies, including surgery, radiotherapy and combined treatment with radiotherapy and surgery after neoadjuvant chemotherapy in Ewing sarcoma patients.

METHODS

PubMed and Embase database were searched from inception through July 30, 2018, using controlled vocabulary supplemented with keywords describing Ewing sarcoma and neoadjuvant chemotherapy. Possible related studies were also manually identified by screening a reference list of retrieved articles. Two reviewers independently primarily evaluated the eligibility of retrieved articles by screening their titles and abstracts. Disagreement was resolved by discussion. Subsequently, full text of eligible articles was reviewed according to inclusion criteria. The included documents fulfilling the following criteria were eligible for our analysis: (*i*) patients were diagnosed with Ewing sarcoma, and tumors were clinically diagnosed as operable and non-metastatic; (*ii*) all the patients were treated with neoadjuvant chemotherapy; (*iii*) efficacy of at least two of three investigated local control strategies, *i.e.* surgery, radiotherapy, and surgery combined with radiotherapy, should be compared in the clinical trial, and all treatments for local control were performed after neoadjuvant chemotherapy; and (*iv*) available data was sufficient for further analysis. Furthermore, trials were excluded for duplicates, articles based on the same clinical trials, and those not reported in English. We applied Cochrane collaboration's tool for assessing risk of bias [13] to evaluate the quality of enrolled randomized clinical trials, and Methodological index for non-randomized studies (MINORS) for the quality of randomized trials [14].

In our analysis, we used 5-year local recurrence rate (5-LR) and 5-year event-free survival rate (5-EFSR) as outcomes of investigated treatment. Considering that the main evaluation method of Ewing sarcoma is local recurrence rate, and the survival data is relatively lacking, we used the local recurrence rate as the main outcome index and the survival data as the secondary outcome index.

Relevant data were extracted by two authors independently and discrepancies were dealt by discussion. General information including first author, year of publication, nationality of subjects, study design, sample size and treatment were documented. Odd ratios (ORs) for OS and EFS were either extracted from original articles as the summary statistics or estimated indirectly from survival curve or using other available information.

Statistical analyses: This meta-analysis was performed according to the guidelines of PRISMA with Bayesian

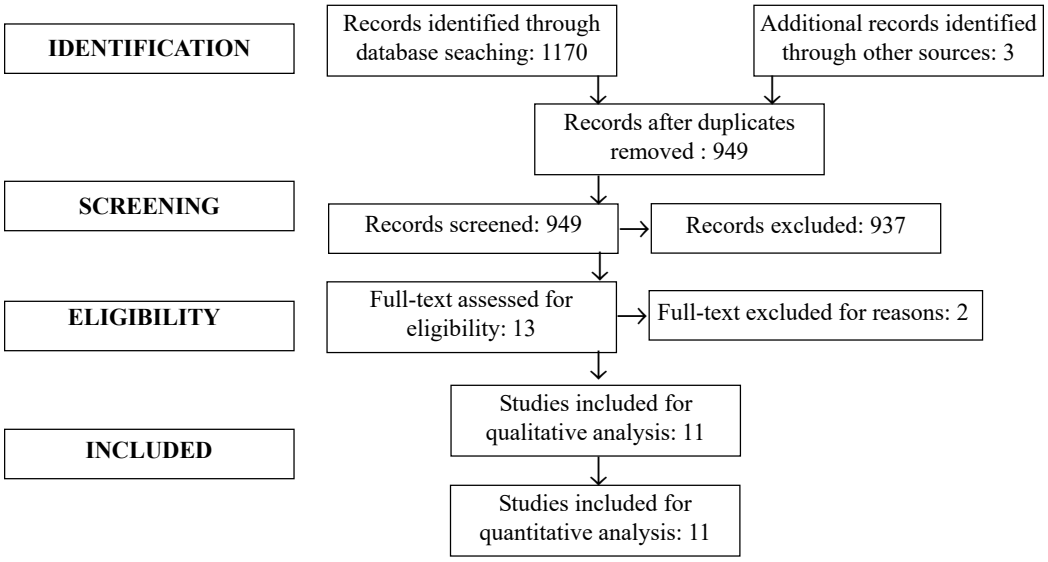

Fig. 1 *Flow diagram summarizing results of study identification and selection.*

Table I Details of Studies Included in the Meta-analysis

Author, year	*Country*	*Design*	*Follow-up (y)*	*Male*	*Age*	*N*	*Neo-CT*	*Intervention*
Reporting only LR recurrence rate								
Ahmed [9], 2017	US	Retrospective	8.3	69.0%	20 (6.6-64.9)	23	VDC/IE 41/Other 7	Surgery *vs.* RT *vs.* SR
Ahmed [15], 2017	US	Cohort	NR	55.0%	13 (0.5-45)	956	IE based	Surgery *vs.* RT *vs.* SR
Laitinen [16], 2016	UK	Retrospective	5.2	51.1%	12.4(2-16)	88	NR	Surgery *vs.* RT *vs.* SR
Shankar [21], 1999	UK	Retrospective	5.5	55.8%	12 (1-27)	190	IVAD	Surgery *vs.* RT *vs.* SR
Carrie [10], 1999	France	Retrospective	6.5	50.7%	12.9	53	EW-85/88/9	Surgery *vs.* RT *vs.* SR
Reporting only EFSR								
Grevener [17], 2016	Germany	Retrospective	10	54.0	11.5 (3-66)	43	VIDE	Surgery *vs.* RT *vs.* SR
Reporting both LR and EFSR								
Donati [8], 2007	Italy	Retrospective	7.3	47.1 %	18.4 (6-46)	66	CNR/ISG	RT *vs.* SR
Dubois [7], 2014	US	Cohort	NR	54.4%	12.4 (0.7-33)	465	VDC/IE	RT *vs.* SR
Bacci [18], 2009	Italy	Retrospective	15	62.0%	17.9 (3-40)	55	IOR	Surgery *vs.* RT *vs.* SR
Yock [19], 2006	US	Retrospective	4.4	52.0%	NR	75	VACA/VACA-IE	Surgery *vs.* RT *vs.* SR
Bacci [20], 2006	Italy	Retrospective	12	64.1%	NR	512	REN	RT *vs.* SR

CT: Chemotherapy; RT: radiotherapy; SR: Surgery combined with Radiotherapy; VDC/IE, Vincristine, Doxorubicin, Cyclophosphamide, and Ifosfamide, Etoposide; LR: Local recurrence rate; EFSR: Event-free survival rate.

model in WinBUGS (MRC Bio-statistics Unit, Cambridge, UK) for network meta-analysis and STATA 12.0 (Stata Corp, College Station, TX) for other analyses. For survival analysis, ORs and the associated 95% credible intervals (CrI) were used to describe the efficacy of different intervention on 5-LR and 5-EFSR. Surface under the cumulative ranking curve (SUCRA) was calculated in order to compare the relative ranking of different therapies. Publication bias was assessed using Begg and Egger tests. A *P* value less than 0.05 indicated the presence of publication bias. A two-side *P* value less than 0.05 was considered as significant.

RESULTS

As illustrated in the flow diagram **(*Fig.* 1)**, a total of 1170 articles were retrieved from the databases, and three more records were obtained from other sources. Finally, 11 studies [7-10, 15-21] from 1999 to 2017 were included in our analysis (***Table* I**). The quality of included studies was evaluated and they were all well-designed and reported reliable results. A total of 2540 patients were enrolled in the meta-analysis in total.

In the included studies, all patients received neoadjuvant chemotherapy prior to the investigated local control strategies. Three different strategies, surgery, radiotherapy and surgery combined with radiotherapy (SR), were evaluated in the included studies (***Table* I**). ***Web Fig.* 1** shows the net plot of the qualified comparison enrolled in our analysis. The width of the line represents the cumulative number of trials per comparison; the circled area represents the cumulative number of patients per intervention. For the outcomes 5-LR and 5-EFSR, the comparison between radiotherapy and SR was the most commonly reported one.

Local recurrence rate: The efficacy of different interventions was obtained by the use of a network meta-analysis. A total of 2474 patients from 9 clinical trials were involved in our analysis. As illustrated in ***Fig.* 2** and ***Table* II**, surgery and SR showed no statistical difference in 5-LR. However, both surgery and SR had statistically significant differences with radiotherapy. Compared with radiotherapy, surgery had better efficacy [OR (95% CI) 0.48 (0.33 -0.87)] and SR had a similar effect with surgery [OR (95% CI) 0.50 (0.29 -0.82)]. Surgery and SR could significantly reduce 5-LR of patients who received neoadjuvant chemotherapy.

The SUCRA values show the relative efficacy of different strategies (***Fig.* 3**). Surgery and SR ranked the highest for 5-LR (SUCRA value 0.79 and 0.70, respectively).

Survival analysis: 749 patients from seven clinical trials

Table II Network Meta-analysis Results for 5-LR and 5-EFSR in Ewing Sarcoma

	Trials	*OR (95% CrI)*
5 LR: No. of arms=28, Patients=2474		
*SG *vs*. RD	9	0.49 (0.30-0.82)
SG *vs*. SR	9	0.94 (0.56-1.72)
*RD *vs*. SG	9	2.05 (1.22-3.32)
*RD *vs*. SR	10	1.95 (1.17-3.32)
SR *vs*. SG	9	1.06 (0.58-1.79)
*SR *vs*. RD	10	0.51 (0.30-0.85)
SG *vs*. RD	6	1.25 (0.41-3.82)
5-EFSR: No. of arms=19, Patients=749		
SG *vs*. SR	6	1.28 (0.39-3.86)
RD *vs*. SG	6	0.8 (0.26-2.46)
RD *vs*. SR	7	1.03 (0.35-2.86)
SR *vs*. SG	6	0.78 (0.26-2.53)
SR *vs*. RD	7	0.97 (0.35-2.89)

*5-LR: 5 year local recurrence rate; 5-EFSR: 5 year event-free survival rate; OR (95% CrI) odds ratio (95% Credible interval); SG: surgery; RD, radiation therapy; SR, surgery combined with radiation; *P<0.03.*

were included in the analysis. There were no statistically significant differences between the three local control strategies in 5-EFSR (***Fig.* 2** and ***Table* 2**). As per SUCRA values, surgery ranked the highest for 5-EFSR. Radiotherapy and SR had lower SUCRA values for improving 5-EFSR (0.32 and 0.50, respectively). The publication bias of various studies for 5-LR and 5-EFSR is shown in ***Web Fig.* 2**.

DISCUSSION

In the present meta-analysis, radiotherapy was the least favorable for improving the prognosis of Ewing sarcoma patients, with significantly higher 5-LR when compared with SR and surgery. No significant difference was observed between surgery and SR, yet the SUCRA value indicated that surgery had higher ranking probability on decreasing 5-LR. Surgery, radiotherapy and SR showed no significant difference of 5-EFSR, while surgery ranked the highest as per ranking probabilities by SUCRA value.

DuBois, *et al*. [7] reported that radiation had a higher risk of local failure, when compared with that of localized ES patients treated with surgery. A study conducted by Bacci, *et al*. [20] showed that the recurrence rate after radiation therapy was high in patients with ES family tumors. In addition, the risk of second malignancies was another significant consideration for patients receiving radiation therapy [23]. Surgery was suggested to be better than radiotherapy in cases of extremity ES family tumors with achievable adequate surgical margins, and thus surgery was the optimal treatment for sites like extremities, which brought a better prognosis to patients [20].

Out results show that surgery is superior to SR. Our results are consistent with those of several previous researchers [7,18-20], which also indicated that additional radiotherapy did not show better outcomes when compared with surgery alone. However, the location of tumor may influence the efficacy of surgery. As reported previously, surgery was the best treatment for small tumors at humerus, yet surgery was only recommended for large tumors when good functional results and quality of life can be expected, and adequate surgical margins are achievable. The best treatment is uncertain for long bones that need to be rebuilt after large segmental resection (femur, tibia, and humerus) [18]. Moreover, the use of surgery for pelvic tumors in Ewing sarcoma is controversial [24,25].

Surgery combined with radiotherapy is the standard of care in the majority of high-risk extremity soft-tissue

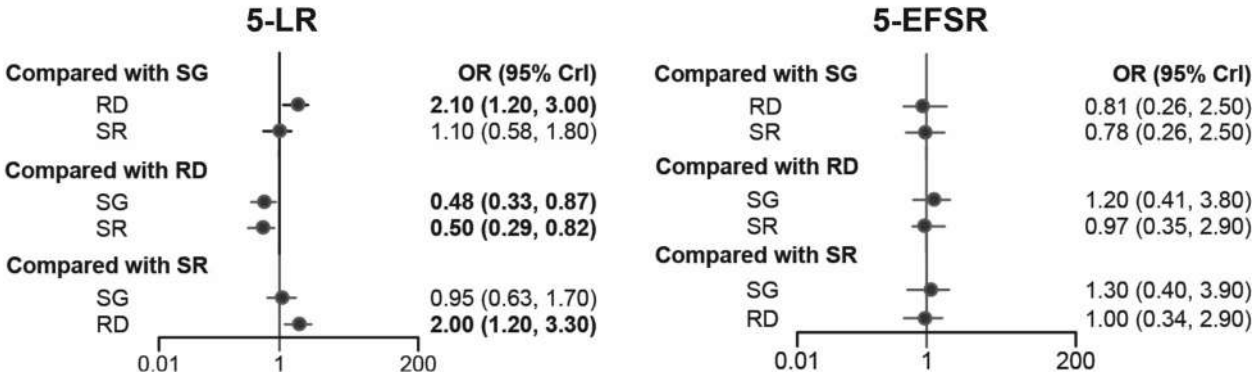

Fig. 2 *Five-year local recurrence rate and 5-year event-free survival rate in Ewing Sarcoma.*

WHAT THIS STUDY ADDS?

Surgery is the optimal option for improving 5-year local recurrence and 5-year event free survival in Ewing sarcoma patients, following neoadjuvant chemotherapy.

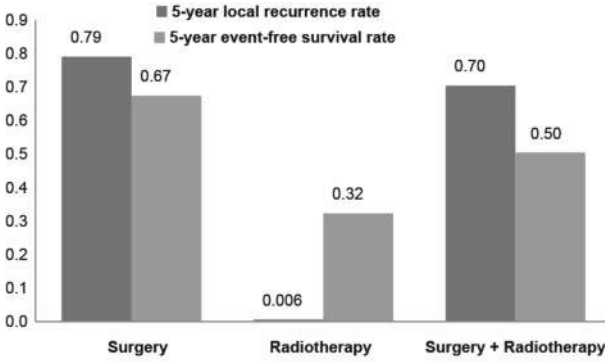

Fig. 3 *Surface under the cumulative ranking curve (SUCRA) of all treatments. Each column shows the probability of that treatment being ranked at the top.*

sarcomas [26]. Several retrospective studies reported that combined therapy had a local tumor control advantage over surgery alone, especially when tumor was larger than 200 mL at diagnosis or the removal of tissues were incomplete during surgery [23,27,28]. However, we did not find any survival benefit when combined intervention was compared with surgery alone. Moreover, combined radiotherapy after surgery resulted in increased risk of long-term treatment-associated toxicities [7]. Due to the lack of sufficient direct data, the adverse effects of SR and surgery were not compared in our network meta-analysis.

A previous meta-analysis enrolled eight retrospective clinical trials and reported inconsistent results in the efficacy of radiotherapy compared with surgery in localized Ewing sarcoma [1]. Whereas in our analysis, five newer studies were included, and one article was excluded due to lack of sufficient data [22]. Moreover, in the present analysis, we focused on the efficacy of local control strategies after neoadjuvant chemotherapy. Neoadjuvant chemotherapy helps to treat the disease early, reducing the chance of metastatic dissemination and also reduces tumor volume, making it resectable.

Some limitation of this study need to be highlighted. Firstly, the number of studies enrolled for our analysis were very limited. We were unable to investigate the effect of different local control strategies on overall survival, disease-free survival and survival rate with a shorter follow-up time due to the lack of sufficient data. Although the network meta-analysis enlarges source of evidence for different comparisons, we still need direct evidence for a robust conclusion. Secondly, since all local control strategies for Ewing sarcoma were performed after neoadjuvant chemotherapy, we did not specify different regimens and protocols for chemotherapy. This might confound the efficacy of local control strategies. Yet all the regimens and protocols used in enrolled RCTs were standard first-line treatments, the efficacy of which have been proven in previous studies. Thirdly, the used local treatment was the clinicians'choice based on patient and tumor characteristics. Radiation therapy is often used in cases of narrow or intralesional surgical margins or poor histological response to chemotherapy or when surgery would be too mutilating. Additionally, results of survival analysis were reported by odds ratios with extracted binary data from original articles. Hence, we were not able to compare the survival curves of different local control strategies. Moreover, since no reliable RCTs have been performed regarding to the efficacy of local control strategies on Ewing sarcoma patients, we enrolled only retrospective cohort studies in our analysis. The quality and reliability of involved data may thus limit the interpretation of our results.

In conclusion, this network meta-analysis suggested that surgery might be the optimal option for improving 5-LR and 5-EFSR of Ewing sarcoma patients. However, due to the lack of high-quality data, the results should be interpreted with caution. The choice of local control strategy should be decided through consideration of patient characteristics, potential adverse effects, and patient preference. Further research and well-designed randomized clinical trials are warranted to clarify the optimal local control strategy for Ewing sarcoma.

Contributors: HZ,YL,XX: substantial contribution to the conception and design of the work; HZ,SZ,YX: acquisition, analysis, and interpretation of the data; HZ: drafting of the manuscript; TF: revising the manuscript critically. All authors have read and approved the final article.
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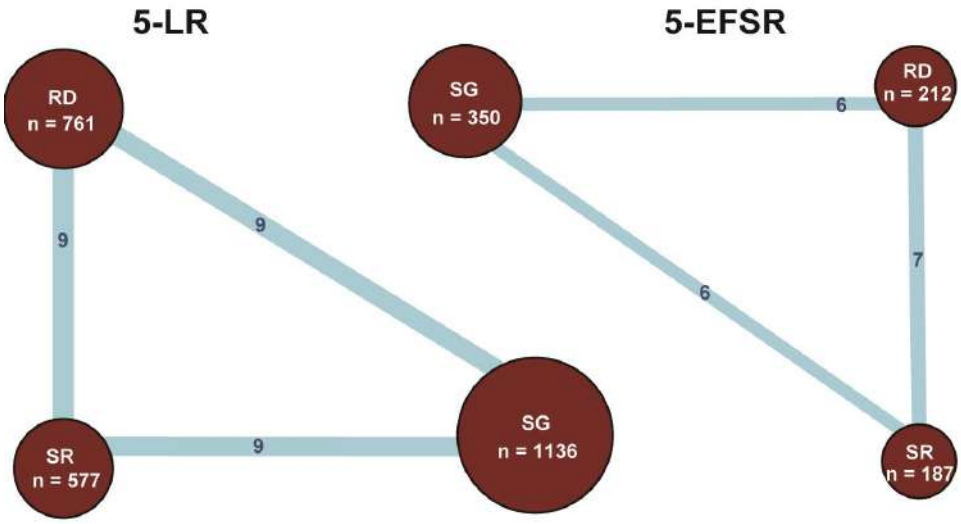
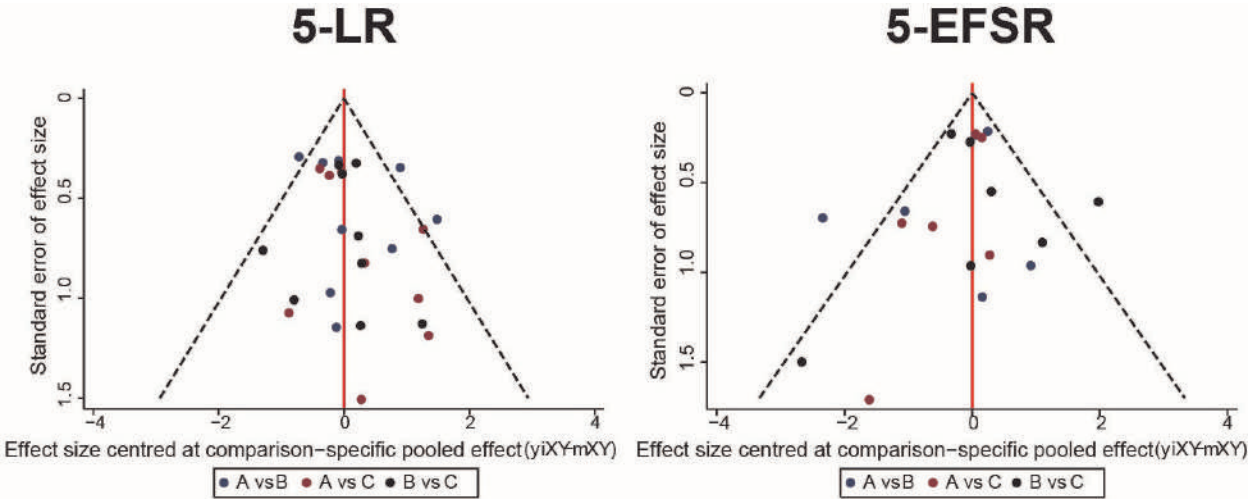
REFERENCES

1. Werier J, Yao X, Caudrelier JM, Di Primio G, Ghert M, Gupta AA, *et al*. A systematic review of optimal treatment

strategies for localized Ewing's sarcoma of bone after neo-adjuvant chemotherapy. Surg Oncol. 2016;25:16-23
2. Bolling T, Hardes J, Dirksen U. Management of bone tumours in paediatric oncology. Clin Oncol (R Coll Radiol). 2013;25:19-26.
3. Moore DD, Haydon RC. Ewing's sarcoma of bone. Cancer Treat Res. 2014;162:93-115.
4. Falk S, Alpert M. Five-year survival of patients with Ewing's sarcoma. Surg Gynecol Obstet. 1967;124:319-24.
5. Pretz JL, Barysauskas CM, George S, Hornick JL, Raut CP, Chen YE, *et al*. Localized adult ewing sarcoma: Favorable outcomes with alternating vincristine, doxorubicin, cyclophosphamide, and ifosfamide, etoposide (VDC/IE)-based multimodality therapy. Oncologist. 2017;22:1265-70.
6. Wardelmann E, Haas R, Bovée J, Terrier P, Lazar A, Messiou C, *et al*. Evaluation of Response After Neoadjuvant Treatment in Soft Tissue Sarcomas; the European Organization for Research and Treatment of Cancer–Soft Tissue and Bone Sarcoma Group (EORTC–STBSG) Recommendations for Pathological Examination and Reporting. Eur J Cancer. 2016;53:84-95.
7. DuBois SG, Krailo MD, Gebhardt MC, Donaldson SS, Marcus KJ, Dormans J, *et al*. Comparative evaluation of local control strategies in localized Ewing sarcoma of bone: A report from the Children's Oncology Group. Cancer. 2015;121:467-75.
8. Donati D, Yin J, Di Bella C, Colangeli M, Bacci G, Ferrari S, *et al*. Local and distant control in non-metastatic pelvic Ewing's sarcoma patients. J Surg Oncol. 2007;96:19-25.
9. Ahmed SK, Randall RL, DuBois SG, Harmsen WS, Krailo M, Marcus KJ, *et al*. Identification of patients with localized Ewing sarcoma at higher risk for local failure: A report from the Children's oncology group. Int J Radiat Oncol Biol Phys. 2017;99:1286-94.
10. Carrie C. Nonmetastatic Pelvic Ewing Sarcoma: Report of the French Society of Pediatric Oncology. Med Pediatr Oncol. 1999; 33:444-9;
11. De Marco S, Pollera CF, Cognetti F. Nephroblastoma in the adult. Med Pediatr Oncol. 1999;33:497-9.
12. Zogopoulos G, Teskey L, Sung L, Dix D, Grant R, Greenberg ML, *et al*. Ewing sarcoma: Favourable results with combined modality therapy and conservative use of radiotherapy. Pediatr Blood Cancer. 2004;43:35-9.
13. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, *et al*. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. British Med J. 2011;343:d5928.
14. Slim K, Nini E, Forestier D, Kwiatkowski F, Panis Y, Chipponi J. Methodological index for non-randomized studies (minors): Development and validation of a new instrument. ANZ J Surg. 2003;73:712-6.
15. Ahmed SK, Robinson SI, Arndt CAS, Petersen IA, Haddock MG, Rose PS, *et al*. Pelvis Ewing sarcoma: Local control and survival in the modern era. Pediatr Blood Cancer. 2017;64:e26504.
16. Laitinen M, Parry M, Albergo JI, Jeys L, Sumathi V, Grimer R. Outcome of pelvic bone sarcomas in children. J Pediatr Orthop. 2016;38:537-42.
17. Grevener K, Haveman LM, Ranft A, van den Berg H, Jung S, Ladenstein R, *et al*. Management and outcome of Ewing sarcoma of the head and neck. Pediatr Blood Cancer. 2016;63:604-10.
18. Bacci G, Palmerini E, Staals EL, Longhi A, Barbieri E, Alberghini M, *et al*. Ewing's sarcoma family tumors of the humerus: Outcome of patients treated with radiotherapy, surgery or surgery and adjuvant radiotherapy. Radiother Oncol. 2009;93:383-7.
19. Yock TI, Krailo M, Fryer CJ, Donaldson SS, Miser JS, Chen Z, *et al*. Local control in pelvic Ewing sarcoma: analysis from INT-0091–A report from the Children's Oncology Group. J Clin Oncol. 2006;24:3838-43.
20. Bacci G, Longhi A, Briccoli A, Bertoni F, Versari M, Picci P. The role of surgical margins in treatment of Ewing's sarcoma family tumors: Experience of a single institution with 512 patients treated with adjuvant and neoadjuvant chemotherapy. Int J Radiat Oncol Biol Phys. 2006;65: 766-72.
21. Shankar AG. Local therapy and other factors Infuencing site of relapse in patients with localised Ewing's sarcoma. Eue J Cancer. 1999;35:1698-1704.
22. Sokolov T, Stoyanova A, Mumdjiev I, Mihova A. Comparison of two treatment approaches to localized Ewing's sarcoma. Ortopediya i Travmatologiya. 2000;36: 509-15.
23. Shankar AG, Pinkerton CR, Atra A, Ashley S, Lewis I, Spooner D, *et al*. Local therapy and other factors influencing site of relapse in patients with localised Ewing's sarcoma. United Kingdom Children's Cancer Study Group (UKCCSG). Eur J Cancer. 1999;35:1698-704.
24. Kawai A, Healey JH, Boland PJ, Lin PP, Huvos AG, Meyers PA. Prognostic factors for patients with sarcomas of the pelvic bones. Cancer. 1998;82:851-9.
25. Laitinen M, Parry M, Albergo JI, Jeys L, Sumathi V, Grimer R. Outcome of pelvic bone sarcomas in children. J Pediatr Orthop. 2018;38:537-42.
26. O'Sullivan B, Davis AM, Turcotte R, Bell R, Catton C, Chabot P, *et al*. Preoperative versus postoperative radiotherapy in soft-tissue sarcoma of the limbs: A randomised trial. Lancet. 2002;359:2235-41
27. Foulon S, Brennan B, Gaspar N, Dirksen U, Jeys L, Cassoni A, *et al*. Can postoperative radiotherapy be omitted in localised standard-risk Ewing sarcoma? An observational study of the Euro-EWING group. Eur J Cancer. 2016;61:128-36.
28. Bacci G, Forni C, Longhi A, Ferrari S, Donati D, De Paolis M, *et al*. Long-term outcome for patients with non-metastatic Ewing's sarcoma treated with adjuvant and neoadjuvant chemotherapies. 402 patients treated at Rizzoli between 1972 and 1992. Eur J Cancer. 2004; 40:73-83.



Web Fig. 1 *Evidence net plots for local control strategies for Ewing sarcoma. The node size represents the sample size and the width of the lines represents the cumulative number of trials.*



Web Fig. 2 *Funnel plot for local control strategies for Ewing sarcoma.*

SPECIAL ARTICLE

Coronavirus Disease (COVID-19) and the Gastrointestinal System in Children

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), though primarily a respiratory pathogen, also involves the gastrointestinal tract. Similar to the respiratory mucosa, angiotensin converting enzyme-2 (ACE-2) receptor and transmembrane serine protease 2 (TMPRSS2) co-express in the gastrointestinal tract, which facilitates viral entry into the tissue. Less than 10% of children with infection develop diarrhea and vomiting. Prolonged RT PCR positivity in the stool has raised the possibility of feco-oral transmission. Elevated transaminases are common, especially in those with severe coronavirus disease (COVID-19). Children with inflammatory bowel disease and post liver transplant patients do not have an increased risk of disease, and should remain on medications they are already on. Children with chronic liver disease should continue their medications as usual. All elective procedures like endoscopy should be postponed.

Keywords: *Diarrhea, Fecal shedding, Inflammatory bowel disease, Liver transplant, SARSCoV-2.*

In Severe acute respiratory syndrome corona virus 2 (SARS-CoV2) infection the respiratory system is the main target organ; however, the gastrointestinal tract and the liver may also be involved, either symptomatically or with only laboratory derangements. The virus has been detected in respiratory secretions, feces and blood.

COVID-19 AND THE INTESTINE

Fecal-Oral Transmission

The virus attaches to the angiotensin converting enzyme-2 (ACE 2) receptors of the intestine. Among 73 adult patients, 39 tested positive for RNA in stool samples and 17 remained positive for the virus in stool even after becoming negative in respiratory samples [1]. Xu, *et al*. [2] reported that 8 of their 10 pediatric patients were positive for RT-PCR in rectal swabs, which remained detectable well after nasopharyngeal swabs turned negative. Thus fecal oral transmission is being proposed as another route of spread of infection and it is possible that even asymptomatic patients may be shedding the virus in the stool. However, till date there is only report of the virus cultured from a single stool specimen. At present it is unclear whether the persistence of RNA in the stool is secondary to its continued positivity in bronchoalveolar lavage, even when nasopharyngeal mucosa swabs are negative. While more data, particularly on fecal infectivity are awaited, this finding has grave implications for developing countries [1,2].

Gastrointestinal Manifestations

Some children have reported nausea, vomiting, diarrhea and abdominal pain during the course of the disease. Diarrhea most often occurred 1 to 8 days after the onset, with a median time of 3.3 days. Some patients had watery diarrhea as the first symptom. Lu,*et al.* [3] have reported that diarrhea and vomiting were observed in 15 (8.8%) and 11 (6.4%) in a cohort of 171 children. While Jin, *et al*. [4] reported that the presence of gastrointestinal symptoms in adults was associated with more severe illness, there is no similar report in children.

Intestinal involvement has many reasons. Angiotensin converting enzyme 2 (ACE2) receptor and transmembrane serine protease 2 (TMPRSS2) are key proteins in the cell entry process of the virus. Co-expression of these two proteins in the same cell is critical for viral entry. Like alveolar type II cells in the lung, gland cells of the esophagus and absorptive enterocytes in ileum and colon express them together. After viral entry, virus-specific RNA and proteins are synthesized in the cytoplasm of these cells to assemble new virions, which are released to gastrointestinal tract. This

gastrointestinal tropism explains the digestive symptoms and the viral shedding in stool. Xiao, *et al.* [5] have given more convincing evidence of primary gastrointestinal tract involvement in one patient using endoscopic biopsies. They observed that in more than 20% of their adult patients, the viral RNA remained positive in the stool even after it became negative in the respiratory tract. They have recommended that RT PCR of the stool should be performed before deciding that a hospitalized patient is not infective. The virus also seems to alter the intestinal flora, even when only the respiratory mucosa is involved, through the common mucosal immune system regulation called the 'gut-lung axis' [6].

Available evidence suggests that IBD (inflammatory bowel disease) patients do not have an increased risk of developing COVID-19 and should stay on IBD medications. Surrogate markers of inflammation (ESR, CRP, patient-reported outcomes) may be an alternative to face-to-face office visits during the epidemic, especially for those in remission. Newly diagnosed patients should be treated according to the standard protocols as before the epidemic. There is currently no evidence that any of the drugs used in IBD including immune-modulators and biologicals increases the severity of COVID and the risk of a disease flare outweighs any estimated risk of SARS-CoV2 infection. Corticosteroids can be used to treat disease relapses, but as always recommended in children, the drug should be weaned as soon as possible. The use of anti-tumor necrosis factor drugs should be continued as earlier, while making sure that infusion centers in hospitals take standard prevention measures of COVID. Switching from infliximab to adalimumab in a stable child should be discouraged unless impossible to provide intravenous infusions, considering the higher risk of disease exacerbation.

All routine endoscopic procedures must be avoided, since they are aerosol generating, more so in children where it also involves airway management. The upper gastrointestinal endoscopy carries a higher risk of aerosols than lower gastrointestinal endoscopy. Acute upper or lower gastrointestinal bleeding, esophageal obstruction, foreign body ingestion etc. may require endoscopy without delay, but should be done with full personal protection equipment including the N95 mask. Biopsies must be placed in formalin immediately. Endoscopy room and disinfection policy should be followed as per standard protocol [7,8].

COVID AND THE LIVER

A mild rise in transaminases is common with COVID-19 disease but serious liver dysfunction is uncommon. The elevated transaminases are often accompanied by high creatine kinase and lactate dehydrogenase suggesting the possibility that viral myositis may also be the cause. Elevated liver enzymes are more common in those with severe spectrum of the disease (40-60%), compared to those who are asymptomatic or have mild disease (18-25%). Bilirubin levels are also more than double in those with severe infection, when compared to those with milder disease. This is probably related to virus triggered auto reactive T cells and cytokine storm. Though hepatocytes and biliary epithelium are abundant in angiotensin-converting enzyme 2 (ACE2) receptors, which is the same receptor that the virus uses for entering the lungs, there is no evidence of active replication of the virus in hepatocytes. Hypoxic injury from respiratory distress and drug induced liver injury (remdesivir, tocilizumab) are other possible causes for the abnormal liver function tests in patients [9].

Any child with COVID-19 disease and raised transaminases should be investigated for other causes of liver disease. For patients who are asymptomatic or have only mild disease, hospital visit is unnecessary and a tele- or video-consultation is sufficient. Newly diagnosed patients with jaundice, aspartate amino transferase/ alanine amino transferase >500IU/L or recent onset hepatic decompensation should be evaluated in hospital. At present, there is no concrete evidence to show that COVID-19 co-infection causes significant worsening in of underlying chronic liver disease. However, evidence from the previous SARS CoV epidemic suggests otherwise, but more data is required. Elevated transaminases in COVID-19 disease are not a contraindication for antiviral therapy, with regular monitoring of liver function [10].

Post-liver transplant patients need particular emphasis on preventive measures like frequent hand washing, cleaning frequently touched surfaces and social distancing etc. As the cell injury in COVID-19 disease is thought to be immune-mediated, immunosuppression and mycophenolate should not be reduced or stopped in asymptomatic post-transplant patients. In an established COVID-19 infection, continue calcineurin inhibitors targeting a lower trough levels and lower the dose of mycophenolate or azathioprine. Patients on high dose steroids should have it reduced to a minimum dose based on body weight to prevent adrenal insufficiency. At present there is no recommendation for any antivirals or hydroxychloroquine prophylaxis either in post-liver transplant children or those with COVID-19 associated acute liver disease.

Children on treatment for chronic liver diseases like Wilson disease, autoimmune hepatitis, hepatitis B and C

Key Messages

- There is gastrointestinal mucosal involvement in COVID-19 and viral RNA is detected in the stool even after the nasopharyngeal swabs are negative.
- Elevated transaminases are common in severe disease, but serious liver dysfunction is uncommon.
- Routine endoscopies should be avoided during the epidemic, since it is an aerosol-generating procedure.
- Children with inflammatory bowel disease, chronic liver disease and post liver transplant patients do not seem to have an increased risk of disease and should continue their medications.

should continue their treatment protocols. All elective liver transplantations should be postponed.

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REFERENCES

1. Hindson J. COVID-19: Faecal–oral transmission? Nature Rev Gastroenterol Hepatol. 2020. Available from *https://www.nature.com/nrgastro/s41575-020-0295-7*. Accessed March 30, 2020.
2. Xu Y, Li X, Zhu B, Liang H, Fang C, Gong Y,*et al.*Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nature Med.2020. Available from:*https://www.nature.com/naturemedicine/s41591-020-08174*. Accessed March 15,2020.
3. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J,*et al.* SARS-CoV-2 infection in children. New England J Med. 2020. Available from: *https://www.nejm.org/NEJMc2005073*. Accessed March 29, 2020.
4. Jin X, Lian J-S , Hu JH, Gao J, Zheng L, Zhang YM,*et al*. Epidemiological, clinical and virological characteristics of 74 cases of corona virus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. Gut.2020. Available from:*https://gut.bmj.com/gutjnl-2020- 320926*. Accessed March 20, 2020.
5. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H.Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology. 2020. Available from: *https://www.gastrojournal.org/j.gastro.2020.02.05*. Accessed March 27, 2020.
6. Pan L, Mu M, Ren HG, Yang P, Sun Y, Wang R,*et al.*Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: A descriptive, cross-sectional, multicenter study. Am J Gastroenterol. 2020. Available from: *https://journals.lww.com/ajg/ajg.0000000000000620*. Accessed March 31, 2020.
7. Danese S, Cecconi M, Spinelli A. Management of IBD during the COVID-19 outbreak: Resetting clinical priorities. Nature Rev Gastroenterol Hepatol.2020. Available from:*https://www.nature.com/nrgastro/s41575-020-0294-8.*Accessed March 30, 2020.
8. Mao R, Liang J, Shen J, Ghosh S, Zhu LR,Yang H,*et al* on behalf of the Chinese Society of IBD, Chinese Elite IBD Union, and Chinese IBD Quality Care Evaluation Center Committee. Implications of COVID-19 for patients with pre-existing digestive diseases. Lancet Gastroenterol Hepatol. 2020. Available from: *https://www.the lancet.com/langas/S2468-1253(20)30076-5*. Accessed March 30, 2020.
9. Zhang c, Shi l, Wang F-S. Liver injury in COVID-19: Management and challenges. Lancet Gastroenterol Hepatol. 2020. Available from: https://*www.thelancet.com/gastrohep*S246812532030057-1. Accessed April 06, 2020.
10. Bangash MN, Patel J, Patrekh D. COVID-19 and the liver: Little cause for concern**.** Lancet GastroenterolHepatol. 2020. *https://www.thelancet.com/gastrohep*S2468-*1253(20)30084-4*. Accessed April 06, 2020.

Perinatal-Neonatal Management of COVID-19 Infection – Guidelines of the Federation of Obstetric and Gynaecological Societies of India (FOGSI), National Neonatology Forum of India (NNF), and Indian Academy of Pediatrics (IAP)

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Justification: During the current rapidly evolving pandemic of COVID-19 infection, pregnant women with suspected or confirmed COVID-19 and their newborn infants form a special vulnerable group that needs immediate attention. Unlike other elective medical and surgical problems for which care can be deferred during the pandemic, pregnancies and childbirths continue. Perinatal period poses unique challenges and care of the mother-baby dyads requires special resources for prevention of transmission, diagnosis of infection and providing clinical care during labor, resuscitation and postnatal period. **Process:** The GRADE approach recommended by the World Health Organization was used to develop the guideline. A Guideline Development Group (GDG) comprising of obstetricians, neonatologists and pediatricians was constituted. The GDG drafted a list of questions which are likely to be faced by clinicians involved in obstetric and neonatal care. An e-survey was carried out amongst a wider group of clinicians to invite more questions and prioritize. Literature search was carried out in PubMed and websites of relevant international and national professional organizations. Existing guidelines, systematic reviews, clinical trials, narrative reviews and other descriptive reports were reviewed. For the practice questions, the evidence was extracted into evidence profiles. The context, resources required, values and preferences were considered for developing the recommendations. **Objectives:** To provide recommendations for prevention of transmission, diagnosis of infection and providing clinical care during labor, resuscitation and postnatal period. **Recommendations:** A set of twenty recommendations are provided under the following broad headings: 1) pregnant women with travel history, clinical suspicion or confirmed COVID-19 infection; 2) neonatal care; 3) prevention and infection control; 4) diagnosis; 5) general questions.

Keywords: *Covid-19, Diagnosis, Labor, Management, Newborn, Pregnancy, SARS-CoV-2, Outcome.*

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Coronaviruses are RNA viruses with glycoprotein spikes that give them a crown like appearance [1,2]. Four species have been in circulation for a long time and cause mild respiratory disease. They have a lot of genetic diversity and have jumped the species barrier leading to severe respiratory disease (the SARS virus in 2002-2003 and the MERS virus in 2012-2013). In December, 2019, a novel coronavirus emerged in Wuhan City of Hubei Province in China; this was later termed as SARS-CoV-2 or COVID-19. This virus has subsequently spread throughout the world causing more than 3.7 million cases and 2,64,000 deaths (till May 07, 2020) [3]. More than 52,000 cases and 1700 deaths have been reported from India [4].

The disease spreads by droplets generated by infected people during sneezing and coughing. These are large droplets that travel for 1-2 m and settle on surfaces on which the virus can remain alive for hours to days [5]. Infected persons can also spread the infection even before

the onset of symptoms. Infection is acquired by either inhalation of infected droplets or touching surfaces/ fomites contaminated with the infected droplets and then touching the eyes, nose or mouth. Incubation period varies from 2-14 days with a median of 5 days. The average number of people infected by one infected individual is between two to three. The clinical symptoms are variable, ranging from asymptomatic state to acute respiratory distress syndrome and multi-organ dysfunction. In adults, common symptoms include fever, cough, breathlessness, fatigue, myalgia, headache and sore throat, while vomiting, diarrhea, sneezing and conjunctivitis are uncommon. Current evidence suggests that 80-85% of cases are mild, 10-15 % are severe with lower respiratory tract involvement, and 5% are critical, needing ICU care. The fatality rate is reportedly between 2-3% but can vary from 0.5-10% depending on the number tested, the percentage of elderly people in the population and availability of critical care support in the hospitals. The severity and fatality are higher in the elderly, especially above the age of 60 (among those aged more than 80 years, fatality rate was 15%), and those with comorbidities like heart disease, hypertension, diabetes etc. There is paucity of data on COVID-19 in pregnancy and neonates. Available data suggests that in general the outcome among pregnant women and neonates is good. A large proportion of infected pregnant women are likely to be asymptomatic or have mild symptoms [6]. However, severe disease needing admission to intensive care unit has been reported among pregnant women. Emerging evidence indicates that among women infected with COVID-19 in the third trimester, the risk of vertical transmission is low. Reported clinical features of COVID-19 infection in neonates include fever, lethargy, cough, vomiting and respiratory distress, thus mimicking the presentation of bacterial sepsis [2,7,8].

METHODS

These guidelines have been developed jointly by the Federation of Obstetric and Gynaecological Societies of India, National Neonatology Forum of India, and Indian Academy of Pediatrics. The GRADE approach recommended by World Health Organization (WHO) was used to develop the guideline [9]. A Guideline development group (GDG) comprising of obstetricians, neonatologists and pediatricians was constituted. The GDG drafted a list of questions which are likely to be faced by clinicians involved in obstetric and neonatal care. An e-survey was carried out amongst a wider group of clinicians to invite more questions and prioritize. Literature search was carried out in PubMed combining the search term ("coronavirus"[MeSH Terms] OR "coronavirus" [All Fields])) AND 2019/12 [PDAT]: 2030 [PDAT]) OR 2019-nCoV [All Fields] OR 2019nCoV [All Fields] OR COVID-19 [All Fields] OR SARS-CoV-2 [All Fields]) with other key words relevant to the practice question being answered (search updated on 30 April, 2020). In addition, websites of the relevant international and national professional organizations were searched [10-20]. Guidelines, systematic reviews, trials, narrative reviews and other descriptive reports were reviewed. For PICO (participants, intervention, control and outcome) questions, the evidence was extracted into evidence profiles. The context, resources required, values and preferences were considered for developing the recommendations.

OBECTIVES

The objective of these guidelines is to provide guidance on the short-listed clinical practice questions (***Box* I**).

Pregnant Women

Of the 3323 articles on the coronavirus infection, 80 addressed the issue in pregnant women. No clinical trials have compared specific care including isolation strategies in pregnant women. A total of 13 studies (12 case series/reports and 1 retrospective cohort study) reported outcome in 113 women with pregnancy and coronavirus infection [21-26]. Due to absence of comparative group it is not possible to estimate the effect of COVID-19 infection in pregnancy. However, almost all pregnant women had mild infection. One died due to severe disease. No clinical trials have compared specific care including isolation strategies in pregnant women. Majority of women in these studies were delivered by C-section; however, in the only case-control study, all controls were also delivered by C-section. Incidence of C-section is high in China, from where all studies have originated, and it is not possible to infer that Covid-19 infection increases the probability of C-section. Literature indicates possibility of higher incidence of fetal distress in infected pregnant women. However, due to small sample size and lack of comparison group, no definite inference can be made. As severe disease and pneumonia have been reported in few reports, pregnant women with infection need to be monitored for respiratory compromise during childbirth.

The treatment of COVID-19 viral infection has been attempted by two approaches. The first approach is the use of a combination of hydroxychloroquine and azithromycin [27]. These drugs are readily available in India and are cost-effective. The other approach has been to use antiviral drugs, some of which are difficult to procure. Hydroxychloroquine in a dose of 600 mg (200 mg thrice a day with meals) and azithromycin (500 mg once a day) for 10 days has been shown to give virologic cure on

Box I Short-listed clinical Practice Questions Addressed in the Guidelines

Pregnant women with travel history, clinical suspicion or confirmed infection

1. What should be the care of pregnant women with history of travel or exposure to a confirmed/suspected case of COVID-19?
2. Which pregnant women need testing for COVID-19?
3. Where in a healthcare facility should a pregnant woman with suspected or active COVID-19 infection deliver?
4. What infection control measures should be undertaken in triage, labor and delivery of pregnant women with active or suspected COVID-19 infection?
5. What should be the method of labor induction and mode of delivery in pregnant women with active or suspected COVID-19 infection?
6. What should be the specific care of pregnant women with active COVID-19 infection?

Neonatal care

7. What precautions should the neonatal resuscitation team take when attending the delivery of a woman with suspected or confirmed COVID-19 infection?
8. What should be the feeding policy for stable neonates born to COVID-19 mothers?
9. Is it necessary to separate the mother and baby if mother is suspected or confirmed to be COVID-19 positive?
10. Should symptomatic neonates needing intensive or special care be nursed in common room NICU/SNCU or isolation facility?
11. What are the special precautions to be taken while providing respiratory support to neonates exposed to COVID-19 infection?
12. In symptomatic neonates, what is the role of specific treatment in case of perinatal exposure and in case of confirmed infection with COVID-19?

Prevention and infection control

13. What should be the specific disinfection practices in NICU /SNCU?
14. When should Personal protective equipment (donning and doffing) be used?
15. What should be the biomedical waste disposal protocol while managing a suspected or confirmed case of COVID-19?

Diagnosis

16. What should be the testing protocol for neonates born to mothers with suspected or confirmed COVID-19?

General questions

17. What should be the visitation policy and preventive measures for visitors during the COVID-19 outbreak?
18. What should be the discharge policy of neonates born to suspected or confirmed COVID-19 mothers?
19. What should be the occupational health policy specific to COVID-19 pandemic?
20. What should be the immunization policy for neonates born to suspected or COVID-19 positive women?

day 6 of treatment in 100% of treated patients in one study. The study included 20 treated patients with upper and lower respiratory symptoms. In this study, pregnancy was an exclusion criterion. However, as such, both these drugs have been used in pregnancy and during breastfeeding without significant effects on the mother or fetus. Alternative dosage regimens for hydroxychloroquine are to give 400 mg twice a day on day 1 and then 400 mg once a day for the next four days. Chloroquine can also be used as an alternative. The dose is 500 mg twice a day for 7 days. Some authorities recommend that azithromycin should be added only where there is a clinical suspicion of superadded bacterial infection. Lopinavir-ritonavir was the first antiviral combination used to treat COVID-19 infection. However, there was no difference in time to clinical improvement or mortality at 28 days in a randomized trial of 199 patients with severe COVID-19 given lopinavir-ritonavir (400/100 mg) twice daily for 14 days in addition to standard care *versus* those who received standard care alone [28]. Other agents such as remdesivir are being evaluated in randomized trials [29]. Clinicians should follow the latest updated national guidelines released by Indian Council of Medical Research/Ministry of Health and Family Welfare.

Maternal-fetal Transmission and Neonatal Cases

Among 707 neonates reported born to Covid-19 positive women, 111 (15.7%) were admitted to NICU, 20 had pneumonia and 3 died. However, in majority of neonates, the reason of admission to NICU was isolation from the infected mother or other morbidities unrelated to COVID-19 infection. Among the 707 births, vertical transmission is suspected in 17 neonates (pooled rate of 2.4%), based on virologic and serological reports [30-37]. However, two individual case series have reported higher transmission rates of 7.1% (3 of 42) and 9.1% (3 of 33) [36].

Of the 17 neonates with suspected vertical transmission, infection was confirmed by a positive RT-PCR in 11 (***Web Table* I**). In 3 neonates, infection was suspected based on elevated anti-COVID-19 IgM and IgG levels at birth [33,34]. In another 3 neonates, only IgG levels were elevated [33]. In these 6 neonates with elevated antibodies, RT-PCR was repeatedly negative indicating possibility of intrauterine infection of the fetus. Pneumonia was the most common manifestation of infection with 9 neonates of 11 with positive RT-PCR showing clinical and/or radiological evidence of pneumonia. Other clinical features included fever, lethargy and gastrointestinal symptoms. However, the disease was mild in most neonates with only one neonate needing short duration respiratory support and all being discharged alive from the hospital.

Isolation from mother was practiced in all but two of these 17 neonates [35]. Maternal infection was confirmed only in the postnatal period in mothers of these two neonates. Breastfeeding was given by these mothers without wearing masks. In one neonate RT-PCR was positive on day 1 and in second neonate it was positive on day 3.

Neonatal exposure definitions: As per the Chinese consensus guidelines, neonates are said to be exposed to COVID-19 if they are born to mothers with a history of COVID-19 infection diagnosed within 14 days before delivery or 28 days after delivery, or if the neonate is directly exposed to close contacts with COVID-19 infection (including family members, caregivers, medical staff, and visitors) [38]. They should be managed as patients under investigation (PUI) irrespective of whether they are symptomatic or not.

RECOMMENDATIONS

Pregnant Women With Travel History, Clinical Suspicion or Confirmed Infection

Recommendation 1

- Pregnant women with a history of travel or exposure to a confirmed/suspected case of COVID-19 should be isolated by using the ICMR guidelines for non-pregnant adults.
- In the absence of community spread, isolation at the designated facility and in the presence of community spread, isolation by home quarantine may be preferred. For home quarantine, the guidelines issued by ICMR/ MoHFW should be adhered to.

Recommendation 2

- Testing for pregnant women should be done as per ICMR testing strategy [39].
- In addition, ICMR recommends pregnant women residing in clusters/containment area or in large migration gatherings/evacuation centres from hotspot districts presenting in labor or likely to deliver in next five days should be tested even if asymptomatic [40].

Asymptomatic pregnant women should be tested in the health facilities where they were expected to deliver, and all arrangements should be made to collect and transfer samples to testing facilities. Women should not be referred for lack of testing facility.

Recommendation 3

- COVID care facilities should be identified in the public and private sector. These would be large multispecialty hospitals with adequate space, infrastructure and logistics. Referral pathways from non-COVID facilities should be well established.
- In such COVID care facilities, three demarcated zones, each housing all the needed equipment and services (wards, labor rooms, operation theatres, neonatal resuscitation areas and mother and neonatal ICU) are required for management of healthy, suspected and confirmed COVID-19 mothers.

The standards and facilities required for infection control in these areas should be same as that for other adults with suspected or confirmed COVID-19.

- Every pregnant woman should be triaged at entry and then allotted into one of the zones depending on the presentation.
- If a woman who delivers in a non-COVID facility turns out to be Covid-19 positive, actions should be taken as per MOHFW's 'Guidelines to be followed on detection of suspect/confirmed COVID-19 case in a non-COVID health facility'[41].

Recommendation 4

- When providing healthcare to women in labor with confirmed or suspected COVID-19 infection, follow

standard universal precautions to prevent contact with body fluids. In addition, use personal protective equipment (PPE) to prevent acquiring infection through respiratory droplets. The PPE should include masks such as the N95 and face protection by a face shield or at least goggles.

- Reception and triage should be in the same room that is to be used for admission in labor and delivery. Ideally, this should be a room with negative pressure (If not available, exhaust fans can be installed).
- Keep the room free from any unnecessary items (decorations, extra chairs, etc.) which could act as infected fomites later.
- There should be a restriction on the number of attendants and non-essential staff into the room.
- There should be facilities for health care providers to remove and safely discard PPE at the exit of the room in which the patient is being cared for.

Recommendation 5

- Mode of delivery in pregnant women infected with COVID-19 should be guided by their obstetric assessment and physiological stability (cardiorespiratory status and oxygenation). COVID-19 infection itself is not an indication for induction of labor or operative delivery.
- Continuous electronic fetal monitoring should be done during labor. If facilities for continuous electronic fetal monitoring are not available, manual monitoring by frequent auscultation of fetal heart rate should be done during the labor, as indicated for a high-risk delivery.
- Adequate equipment and trained healthcare providers should be available for intrapartum monitoring and obstetric interventions as indicated in the separate childbirth facilities for infected pregnant women.
- Oxygenation status of women during labor should be monitored by a pulse oximeter and oxygen therapy should be titrated to maintain oxygen saturation of more than 94%.

Recommendation 6

- Pregnant women with active COVID-19 infection should be managed with supportive care recommended for non-pregnant adults. Current guidelines by the Government of India do not recommend use of hydroxychloroquine, chloroquine or antiviral drugs in pregnant women.
- Currently recommended national management includes: - oxygen therapy/respiratory support for treatment of hypoxemic respiratory failure, fluid therapy, antibiotics and management of shock.

The choice of specific antiviral therapy is likely to change with rapidly emerging evidence and updated national guidance should be consulted. Updated guidance can be accessed at the website of Ministry of Health and Family Welfare: *https://www.mohfw.gov.in/*

Neonatal Care

Recommendation 7

Recommendations for neonatal resuscitation:

- If possible, resuscitation of neonate can be done in a physically separate adjacent room earmarked for this purpose. If not feasible, the resuscitation warmer should be physically separated from the mother's delivery area by a distance of at least 2 meters. A curtain can be used between the two areas to minimize opportunities for close contact.
- Minimum number of personnel should attend (one person in low risk cases and two in high risk cases where extensive resuscitation may be anticipated) and wear a full set of personal protective equipment including N95 mask.
- Mother should perform hand hygiene and wear triple layer mask.
- The umbilical cord should be clamped promptly and skin to skin contact avoided.
- Delivery team member should bring over the neonate to the resuscitation area for assessment by the neonatal team.
- Neonatal resuscitation should follow standard guidelines. If positive-pressure ventilation is needed, self-inflating bag and mask or a T-piece resuscitator with disposable tubing may be used. Disposable parts should be discarded (even if not used) and reusable equipment/parts should be disinfected after each delivery.
- Routine suction is not indicated for clear or meconium stained amniotic fluid.
- Endotracheal administration of medications should be avoided.
- Indications for intubation shall not change because of maternal COVID-19 status. Plexiglass boxes with access portholes can be used to minimize aerosol spread during intubation and suction.
- Disposables like endotracheal tubes, suction catheter,

orogastric tube, tapes for fixing ET tube, umbilical catheter, syringes placed near the resuscitation area should be discarded even if unused. Reusable equipment should be thoroughly disinfected as per hospital protocol.

- Bathing is not recommended in view of risk of hypothermia and hospital acquired infections.

Recommendation 8

A. Stable neonates exposed to COVID-19 infection from mothers or other relatives should be roomed-in with their mothers and be exclusively breastfed. For supporting lactation, nurses trained in essential newborn care and lactation management should be provided. A healthy willing family member who is not positive for COVID-19, and has not been in direct contact with suspected or confirmed COVID-19 person and is asymptomatic may be allowed in the room to provide support for breastfeeding and helping in taking care of the neonate.

B. If rooming-in is not possible because of the sickness in the neonate or the mother, the neonate should be fed expressed breast milk of the mother by a nurse or a trained family member who has not been in contact with the mother or other suspected/proven case, provided the neonate can tolerate enteral feeding.

Weak recommendation, based on consensus among experts in the absence of evidence for any beneficial effect or harm in the risk of COVID-19 following direct breastfeeding or expressed breastmilk feeding.

Conditions to be met for safe breastfeeding:

- Mothers should perform hand hygiene frequently, including before and after breastfeeding and touching the baby.
- Mothers should practice respiratory hygiene and wear a mask while breastfeeding and providing other care to the baby; they should routinely clean and disinfect the surfaces.
- Mothers can express milk after washing hands and breasts and while wearing a mask. If possible, a dedicated breast pump should be provided. If not, it should be decontaminated as per protocol. This expressed milk can be fed to the baby without pasteurization. The collection and transport of EBM to the baby should be done very carefully to avoid contamination.

C. Mothers are not eligible to donate milk in any of the following COVID-19 related situations in addition to standard criteria [42].

- COVID-19 positive donor till she is declared free of infection.
- History of having stayed or transited in a containment zone during the previous 14 days.
- History of close contact with a confirmed or probable case of COVID-19 in previous 14 days.
- Suffering from symptoms like cough, fever, sore throat, running nose till found to be COVID-19 negative on nasopharyngeal sample RT-PCR.
- Person who worked in or attended a health care facility in which a case of COVID-19 infection has been confirmed.

Recommendation 9

- Healthy neonate may be roomed-in with mother. The mother-baby dyad must be isolated from other suspected and infected cases and healthy uninfected mothers and neonates.
- Direct breastfeeding can be given. Mother should wash hands frequently including before breastfeeding and wear mask. If needed due to neonatal or maternal condition, expressed breast milk may also be fed.
- If safe, early discharge to home followed by telephonic follow-up or home visit by a designated healthcare worker may be considered.

Recommendation 10

- Neonates who are symptomatic/ sick and are born to a mother with suspected or proven COVID-19 infection should be managed in separate isolation facility.
- This area should be separate from the usual NICU/ SNCU with a transitional area in-between. This isolation facility should preferably have single closed rooms.
- In case enough single rooms are not available, closed incubators (preferred) or radiant warmers could be placed in a common isolation ward for neonates. The neonatal beds should be at a distance of at least 1 meter from one another. Suspected COVID-19 cases and confirmed COVID-19 cases should ideally be managed in separate isolations. If it is not feasible to have separate facilities and the neonates with suspected and confirmed infection are in a single isolation facility, they should be segregated by leaving enough space between the two cohorts.
- The isolation ward should have a separate double door entry with changing room and nursing station. It should be away from routine NICU/SNCU/labor room/ postnatal ward in a segregated area which is not

frequented by other personnel. The access to isolation ward should be through dedicated lift or guarded stairs.

- Negative air borne isolation rooms are preferred for patients requiring aerosolization procedures (respiratory support, suction, nebulization). If not available, negative pressure can also be created by exhaust fans driving air out of the room.
- Isolation rooms should have adequate ventilation. If room is air-conditioned, ensure 12 air changes/ hour and filtering of exhaust air. These areas should not be a part of the central air-conditioning.
- The doctors, nursing and other support staff working in these isolation rooms should be separate from the ones who are working in regular NICU/SNCU. The staff should be provided with adequate supplies of PPE. The staff also needs to be trained for safe use and disposal of PPE.

If the facilities of isolation intensive care are not available in the hospital where symptomatic or sick newborn is born or referred with COVID-19 infections, the newborn should be immediately shifted to the closest state designated COVID hospital where such facilities are available. Complete safety, PPE policies and precautions must be followed during transport.

Recommendation 11

- Respiratory support for neonates with suspected/ proven COVID-19 infection is guided by principles of lung protective strategy including use of non-invasive ventilation.
- NIPPV and high flow nasal cannulas should preferably be avoided.
- Healthcare providers should practice contact and droplet isolation and wear N95 mask while providing care in the area where neonates with suspected/ proven COVID-19 infection are being provided respiratory support.
- If intubation is needed:
  - Consider use of pre-medication for non-emergent intubation.
  - Intubation should be performed by the healthcare worker who is most experienced with airway management.
  - Consider use of aerosol box during intubation and suction.
  - Consider using in-line suction device.
  - Attach a HEPA filter in the path of exhaled gas when using a mechanical ventilator or positive pressure ventilation device.
  - The area providing respiratory support should be a negative air pressure area.

Recommendation 12

- Specific anti-COVID-19 treatment - antivirals or chloroquine/hydroxychloroquine - is NOT recommended in symptomatic neonates with confirmed or suspected COVID-19.

Weak recommendation, based on consensus among experts in the absence of evidence for any beneficial effect or harm with the use of any of the options available.

- Use of adjunctive therapy such as systemic corticosteroids, intravenous gamma globulin and convalescent plasma is NOT recommended in symptomatic neonates with confirmed or suspected COVID-19.

Weak recommendation, based on consensus among experts in the absence of evidence for any beneficial effect or harm with the use of any of the options available.

Prevention and Infection Control

Recommendation 13

Disinfection of surfaces in the childbirth/neonatal care areas for patients with suspected or confirmed Coronavirus infection are not different from those for usual Labor room/OT/NICU/SNCU areas and include the following [12]:

- Wear personal protective equipment before disinfecting
- If equipment or surface is visibly soiled first clean with soap and water solution or soaked cloth as appropriate before applying the disinfectant
- 0.5% sodium hypochlorite (equivalent to 5000 ppm) can be used to disinfect large surfaces like floors and walls at least once per shift and for cleaning after a patient is transferred out of the area.
- 70% ethyl alcohol can be used to disinfect small areas between uses, such as reusable dedicated equipment.
- Hydrogen peroxide (dilute 100 ml of H_2O_2 10% v/v solution with 900 ml of distilled water) can be used for surface cleaning of incubators, open care systems, infusion pumps, weighing scales, standby equipment-ventilators, monitors, phototherapy units, and

shelves. Use H_2O_2 only when equipment is not being used for the patient. For ensuring the efficacy of disinfection with H_2O_2 use the contact period recommended by manufacturer. Usually a contact period of 1 hour is required.

Recommendation 14

Minimal composition of a set of PPE for the management of suspected or confirmed cases of COVID-19 infection is provided in ***Box* II**.

Recommendation 15

- Follow routine biomedical waste disposal handling, segregation, transport and final disposal guidelines as prescribed by the Government of India [15].

Diagnosis

Recommendation 16

Guidelines on testing of neonates for COVID-19 are provided in ***Box* III**.

General Questions

Recommendation 17

- Parents and families of the COVID-19 exposed, suspected and infected mothers and neonates should receive informed healthcare. They should be aware of and understand the isolation, monitoring, diagnostic and treatment plans of the mothers/babies and be given a periodic update about the health condition.
- Visitors to both routine and COVID-19 specific childbirth/neonatal care areas should be screened for symptoms of COVID-19 infection.
- Persons (including parents) with suspected or confirmed COVID-19 infection should not be allowed entry in the childbirth/neonatal care area where care to parturient women/sick neonates is being provided.
- For neonates roomed in with mother having suspect/ confirmed COVID-19 infection, one healthy family member following contact and droplet precautions should be allowed to stay with her to assist in baby care activities.
- Visitation policy for COVID-19 infected mother to see her neonate admitted in NICU. Mother may be allowed to visit if
  - Resolution of fever without the use of antipyretics for at least 72 hours AND
  - Improvement (but not full resolution) in respiratory symptoms AND
  - Negative results of a molecular assay for detection of SARS-CoV-2 from at least two consecutive nasopharyngeal swab specimens collected ≥24 hours apart

Recommendation 18

- Stable neonates exposed to COVID-19 and being roomed-in with their mothers may be discharged at time of mothers' discharge.

Weak recommendation, based on consensus among experts based on the incubation period of SARS-CoV-2 infection in adults and older children.

- Stable neonates in whom rooming-in is not possible because of the sickness in the mother and are being cared by a nurse or a trained family member may be discharged from the facility by 24-48 hours of age.

Weak recommendation, based on consensus among experts in the absence of evidence for any beneficial effect or harm with early discharge following exposure to COVID-19

Remarks

- Early discharge to home may be followed by a telephonic follow-up or home visit by a designated healthcare worker.
- Mothers and family members should be counselled regarding the danger signs and advised to report back to the facility if the neonate develops any of the danger signs

Box II Desired Protection and Suggested Personal Protection Equipment for the management of Suspected/Confirmed patient of COVID-19

Respiratory protection

- Triple layered surgical mask
- N95 facemasks are needed when performing an aerosol-generating procedure or in an area where neonates are being provided respiratory support by CPAP device/ventilator.

Eye protection

Goggles (will not be usable by those using vision glasses) or face shield

Body protection

Full-sleeved water-resistant gown including head and complete shoe cover. A single piece head to toe water resistant body cover will be ideal for attending resuscitation in delivery room or OT

Hand protection

Well-fitting gloves

Box III Guidelines for Testing of Neonates for COVID-19

Which neonates?

- History of exposure to COVID-19 positive adult (Irrespective of symptoms):
  - Mother had COVID-19 infection within 14 days before birth, or
  - History of contact with COVID-19 positive persons (including mother, family members in same household or direct healthcare provider) in postnatal period
- Irrespective of history of exposure:
  - Presenting with pneumonia or SARI that requires hospitalization, with onset at more than 48-72 h of age, unless there is another underlying illness that completely explains the respiratory signs and symptoms.

Features which suggest acute respiratory illness in a neonate are respiratory distress, with or without cough, with or without fever.

When?

- If symptomatic, specimens should be collected as soon as possible
- If asymptomatic, take swab at 48 hours. If neonate's test at 48 hours is negative, repeat test should be done between 5-14 days.

What sample?

Not mechanically ventilated: Upper respiratory nasopharyngeal swab (NP). Collection of oropharyngeal swabs (OP) is a lower priority and if collected should be combined in the same tube as the NP swab.

Mechanically ventilated: Tracheal aspirate sample should be collected and tested as a lower respiratory tract specimen in addition to NP swab.

How to collect?

Upper nasopharyngeal swab

- Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing.
- Insert a swab into nostril parallel to the palate. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.
- Place swabs immediately into sterile tubes containing 2-3 mL of viral transport media. *Oropharyngeal swab (e.g., throat swab)*Swab the posterior pharynx, avoiding the tongue.*Nasopharyngeal wash/aspirate or nasal aspirate*Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.*Other samples* Currently not advised; stool, urine and blood specimens, since the isolation is less reliable than from respiratory specimens. Do not take these specimens for testing (based on current advisory recommendations)

What PPE is needed for sample collection?

Clinicians should wear appropriate personal protective equipment during sampling.

Nasopharyngeal swab

- Hand hygiene
- Disposable single use glove
- Disposable plastic apron
- Surgical facemask
- Eye protection (surgical mask with integrated visor or full-face shield or visor or goggles/safety spectacles)

For any sampling from lower respiratory tract in intubated neonates, a full set of PPE is a must.

- Hand hygiene
- disposable single use glove
- Long sleeved disposable gown
- N95 mask or another respirator mask
- Eye protection

Labelling the sample

Contd....

Box III continued

Label each specimen container with the patient's name, hospital ID number, specimen type and the date the sample was collected. Handle the sample with precautions under biosafety level 3 (BSL-3) conditions until is rendered non-infectious by laboratory.

How to store?

Samples should be collected in viral transport media procured from microbiology laboratory and transported immediately in icepacks. One can use disposable thermocol cartons or plastic bags with ice cubes for in-house transport. If sending to another laboratory, store specimens at 2-8°C for up to 72 h after collection. Storage can be done in a refrigerator dedicated for this purpose. If a delay in testing or shipping is expected, store specimens at -70°C or below. This requires deep freezers.

How to send?

If transporting by shipping, the samples need to be packed as per instructions Guidance for sample Collection, Packaging and Transportation for Novel Coronavirus.

Where to send?

Authorized laboratories (See MOHFW website for latest list)

What test?

Reverse transcriptase PCR is a rapid test for detecting COVID-19

- If the neonate develops any danger signs or becomes unwell during home isolation, he/she should be taken to a designated hospital facility for assessment as well as COVID-19 testing (if indicated)
- Mothers and family members should perform hand hygiene frequently including before and after touching and feeding the baby
- Mothers should practice respiratory hygiene and wear a mask while breastfeeding and providing other care to the baby; they should routinely clean and disinfect all the surfaces.
- If the discharged neonate is positive for COVID-19, uninfected individuals >60 years of age (*e.g.* grandparents) and those with comorbid conditions should not be assigned to provide care if possible.

Recommendation 19

- Healthcare professionals working in any childbirth or neonatal area should report to their supervisor if they have respiratory or other symptoms suggestive of COVID-19 infection. Such healthcare professional should not be put on clinical duty and should be replaced by a healthy healthcare professional to maintain appropriate patient-provider ratio.
- Healthcare professionals directly involved in the care of patients with suspect/proven COVID-19 infection may consider taking hydroxychloroquine (HCQ) prophylaxis as advised by Government of India, on medical prescription [43]. However, this advisory is based on low-quality evidence and may change in near future.

Recommendation 20

- Follow routine immunization policy in healthy neonates born to mothers with suspected/proven COVID-19 infection [44].
- In neonates with suspected/proven infection, vaccination should be completed before discharge from the hospital as per existing policy.

Conclusion

This clinical practice guideline has been jointly developed by FOGSI-IAP-NNF based on the current scientific literature, advisories issued by ICMR and MoHFW and the ground realities of Indian healthcare system. However, our understanding of SARS-Covid-2 virus is incomplete and new insights are being gained everyday. In due course, the guideline shall need to be revised. Readers should check for latest updates.

Disclaimer: The guidelines in this document are based on limited evidence, as is available now. As new evidence accumulates, some of the recommendations may change. Users should use these guidelines in accordance with the latest government regulations and ICMR advisories.

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REFERENCES

1. Singhal T. A Review of Coronavirus Disease-2019 (COVID-19). Indian J Pediatr. 2020;87:281-6.
2. Lu Q, Shi Y. Coronavirus disease (COVID-19) and neonate:

What neonatologist need to know. Journal of Medical Virology. 20 92:564-67.
3. CSSE. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). Available at *https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6; 2020.*
4. Ministry of Health and Family Welfare. COVID-19 status. Available at *https://www.mohfw.gov.in/; 2020.*
5. Tang B, Bragazzi NL, Li Q, Tang S, Xiao Y, Wu J. An updated estimation of the risk of transmission of the novel coronavirus (2019-nCov). Infect Dis Model. 2020;5:248-55.
6. Sutton D, Fuchs K, D'Alton M, Goffman D. Universal Screening for SARS-CoV-2 in Women Admitted for Delivery. N Engl J Med. 2020 /NEJMc2009316. Available at *https://www.nejm.org/doi/full/10.1056/NEJMc2009316*
7. Kamali Aghdam M, Jafari N, Eftekhari K. Novel coronavirus in a 15-day-old neonate with clinical signs of sepsis, a case report. Infect Dis (Lond). 2020;52:427-9.
8. Wang J, Qi H, Bao L, Li F, Shi Y, National Clinical Research Center for Child Health and Disorders and Pediatric Committee of Medical Association of Chinese People's Liberation Army. A contingency plan for the management of the 2019 novel coronavirus outbreak in neonatal intensive care units. Lancet Child Adolesc Health. 2020;4:258-9.
9. Alonso-Coello P, Schünemann HJ, Moberg J, Brignardello-Petersen R, Akl EA, Davoli M, et al. GRADE Evidence to Decision (EtD) frameworks: A systematic and transparent approach to making well informed healthcare choices. 1: Introduction. BMJ. 2016;353:i2016. Available at *https://www.bmj.com/content/353/bmj.i2016*
10. American College of Obstetricians and Gynecologists. Practice Advisory: Novel Coronavirus 2019 (COVID-19). Available from: *https://www.acog.org/Clinical-Guidance-andPublications/Practice-Advisories/Practice-Advisory-Novel-Coronavirus2019?* Accessed on March 25, 2020.
11. Centers for Disease Control. Interim Clinical Guidance for Management of Patients with Conûrmed Coronavirus Disease (COVID-19). Available from: *https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html*. Accessed March 20, 2020.
12. National Guidelines for Infection Prevention and Control In Healthcare Facilities, Ministry of Health and Family Welfare, Available from: *https://www.mohfw.gov.in/*. Accessed March 25, 2020.
13. Favre G, Pomar L, Qi X, Nielsen-Saines K, Musso D, Baud D. Guidelines for pregnant women with suspected SARS-CoV-2 infection. Lancet Infect Dis. 2020 Mar;S1473309920301572. Available at: *https://www.thelancet.com/pdfs/journals/laninf/PIIS1473-3099(20)30157-2.pdf.* Accessed on March 25, 2020.
14. Guidance for sample Collection, Packaging and Transportation for Novel Coronavirus. Available from: *https://www.mohfw.gov.in/pdf/5Sample%20collection_packaging%20 % 202019-nCoV.pdf.* Accessed on March 25, 2020.
15. Guidelines for handling, treatment and disposal of waste generated during treatment, diagnosis and quarantine of COVID-19 patients. March 2020 Published by Central Pollution Control Board, Parivesh Bhawan, New Delhi-110032. Available from: *https://www.imageima.org/files/New-CPCB-Guidelines-for-COVID-19-Biomedical-Waste-Management.pdf*
16. Interim Guidance for Healthcare Facilities: Preparing for Community Transmission of COVID-19 in the United States. Available from: URL:*https://www.cdc.gov/coronavirus/ 2019-ncov/healthcare-facilities/guidance-hcf.html.* Accessed March 20, 2020.
17. World Health Organization. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected. Interim Guidance. 2020. Available from:*https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected*. Accessed on March 25, 2020.
18. World Health Organization. Water, sanitation, hygiene, and waste management for the COVID-19 virus: Technical brief. 2020. Available from: *https://www.who.int/publications-detail/water-sanitation-hygiene-and-waste-management-for-covid-19.* Accessed on March 25, 2020.
19. Royal College of Obstetricians and Gynaecologists (RCOG). Coronavirus infection and pregnancy : Information for pregnant women and their families. Available from: *https://www.rcog.org.uk/globalassets/documents/guidelines/coronavirus-covid-19-infection-inpregnancy-v3-20-03-18.pdf.* Accessed on March 25, 2020.
20. RCPCH Guidelines: COVID-19 - guidance for paediatric services. Available from: *https://www.rcpch.ac.uk/resources/covid-19-guidance-paediatric-services#working-in-neonatal-settings.* Accessed on March 25, 2020.
21. Chen H, Guo J, Wang C, Fan Luo, Xuechen Yu, Wei Zhang, *et al*. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. Lancet. 2020;395:809-15.
22. Li Y, Zhao R, Zheng S, Chen X, Wang J, Sheng X, *et al.* Lack of vertical transmission of severe acute respiratory syndrome Coronavirus 2, China. Emerg Infect Dis. 2020;26(6). Available from: *https://wwwnc.cdc.gov/eid/article/26/6/20-0287_article*
23. Liu D, Li L, Wu X, Dandan Zheng Jiazheng Wang, Lian Yang,, *et al.* Pregnancy and Perinatal Outcomes of Women With Coronavirus Disease (COVID-19) Pneumonia: A Preliminary Analysis [published online ahead of print, 2020 Mar 18]. Am J Roentgenol. 2020;1-6. Available from: *https://www.ajronline.org/doi/full/10.2214/AJR.20.23072.* Accessed on March 25, 2020.
24. Liu W, Wang Q, Zhang Q, Chen L, Chen J, Zhang B, et al. Coronavirus Disease 2019 (COVID-19) During Pregnancy: A Case Series. 2020 Feb 25 [cited 2020 Mar 21]; Available from: *https://www.preprints.org/manuscript/202002. 0373 /v1.* Accessed on March 25, 2020.
25. Liu Y, Chen H, Tang K, Guo Y. Clinical manifestations and outcome of SARS-CoV-2 infection during pregnancy. J

Infect. 2020 Mar 4; Available from: *https://www.journalofinfection.com/article/S0163-4453(20)30109-2/fulltext.* Accessed on March 25, 2020.
26. Qi Lu, Yuan Shi, Coronavirus disease (COVID-19) and neonate: what neonatologist need to know; J Med Virol. 2020. Available from: *https://onlinelibrary.wiley.com/doi/full/10.1002/jmv.25740*. Accessed on March 25, 2020.
27. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, *et al*. Hydroxychloroquine and Azithromycin as a treatment of COVID-19: results of an open label non-randomized clinical trial. Int J Antimicrob Agents. 2020 Mar 20:105949. Available from: *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102549/*. Epub ahead of print Accessed on March 25, 2020.
28. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, *et al*. A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med. 2020. *Available from: https://www.nejm.org/doi/pdf/10.1056/NEJMoa2001282*. Accessed on March 25, 2020.
29. Li Z, Wang X, Cao D, Sun R, Li C, Li G. Rapid review for the anti-coronavirus effect of remdesiv. Drug Discov Ther. 2020;14(2):73-76.
30. Carosso A, Cosma S, Borella F, Marozio L, Coscia A, Ghisetti V, et al. Pre-labor anorectal swab for SARS-CoV-2 in COVID-19 pregnant patients: Is it time to think about it? European Journal of Obstetrics & Gynecology and Reproductive Biology. 2020;S0301211520302025.
31. Zamaniyan M, Ebadi A, Mir SA, Rahmani Z, Haghshenas M, Azizi S. Preterm delivery in pregnant woman with critical COVID-19 pneumonia and vertical transmission. Prenatal Diagnosis. 2020. Available from : *https://www.pubfacts.com/search/Prenatal+Diagnosis%0D%09+%0D%5BJournal%5D.* Accessed May 2, 2020
32. Alzamora MC, Paredes T, Caceres D, Webb CM, Valdez LM, Rosa ML. Severe COVID-19 during Pregnancy and Possible Vertical Transmission. Am J Perinatol.; 2020; Apr 18. Available at *https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0040-1710050.*
33. Zeng H, Xu C, Fan J, Tang Y, Deng Q, Zhang W, *et al.* Antibodies in Infants Born to Mothers With COVID-19 Pneumonia. JAMA. 2020 Available at: *https://jamanetwork.com/journals/jama/fullarticle/2763854.*
34. Dong L, Tian J, He S, Zhu C, Wang J, Liu C, et al. Possible Vertical Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn. JAMA. 2020; Available at: *https://jamanetwork.com/journals/jama/fullarticle/2763853.*
35. Zeng L, Xia S, Yuan W, Yan K, Xiao F, Shao J, *et al.* Neonatal Early-Onset Infection With SARS-CoV-2 in 33 Neonates Born to Mothers With COVID-19 in Wuhan, China. JAMA Pediatr. 2020; Available at : *https://jamanetwork.com/journals/jamapediatrics/fullarticle/2763787.*
36. Wang S, Guo L, Chen L, Liu W, Cao Y, Zhang J, *et al*. A Case Report of Neonatal 2019 Coronavirus Disease in China. Clin Infect Dis. 2020; Available at : *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7108144/*
37. Yu N, Li W, Kang Q, Xiong Z, Wang S, Lin X, *et al*. Clinical features and obstetric and neonatal outcomes of pregnant patients with COVID-19 in Wuhan, China: A retrospective, single-centre, descriptive study. The Lancet Infectious Diseases 2020;20:559-64.
38. Wang L, Shi Y, Xiao T, Fu J, Feng X, Mu D, *et al*. Chinese expert consensus on the perinatal and neonatal management for the prevention and control of the 2019 novel coronavirus infection (First edition). Ann Transl Med. 2020;8:47. *Available from: http://atm.amegroups.com/article/view/35751/html*. Accessed on March 25, 2020.
39. Indian Council of Medical Research. Department of Health Research. Strategy for COVID19 testing in India (Version 4, dated 09/04/2020). Available at: *https://www.icmr.gov.in/pdf/covid/strategy/Strategey_for_ COVID19_Test_v4_09042020.pdf* . Accessed on April 15, 2020.
40. Indian Council of Medical Research. Department of Health Research. Strategy for COVID19 testing for pregnant women in India (Version 1, dated 20/04/2020) *Available at https://www.icmr.gov.in/pdf/covid/strategy/COVID19_Testing_Strategy_for_Pregnant_Women.pdf*. Accessed on April 22, 2020
41. Ministry of Health and Family Welfare Government of India. Guidelines to be followed on detection of suspect/confirmed COVID-19 case in a non-COVID Health Facility.2020. *Available at https://www.mohfw.gov.in/pdf/GuidelinestobefollowedondetectionofsuspectorconfirmedCOVID19 case.pdf*. Accessed on May 1,2020.
42. India HMBA, Infant & Young Child Feeding Chapter of Indian Academy of Paediatrics, PATH. Guidance for use of Human Milk in India in the context of COVID-19 Pandemic. 2020. Available at : *https://www.path.org/resources/guidance-use-human-milk-india-context-covid-19/.*
43. Indian Council of Medical Research. Department of Health Research. Recommendation for empiric use of hydroxychloroquine for prophylaxis of SARS-CoV-2 infection. Available at *https://www.icmr.gov.in/pdf/covid/techdoc/HCQ_Recommendation_22March_final_MM_V2.pdf.* Accessed on March 27,2020
44. IAP Committee on Vaccines & Immunization. ACVIP Guidelines on Immunizations during COVID 19 Pandemic. 2020. Available at : *https://iapindia.org/pdf/FINAL-IAP-COVID-19-Pandemic-Immunization-Activities.pdf.*

Annexure I

Use of Personal Protective Equipment

Sequence of Donning

Before wearing the PPE for managing a suspected or confirmed COVID-19 case, proper hand hygiene should be performed. The gown should be donned first. The mask or respirator should be put on next and properly adjusted to fit; remember to fit check the respirator. The goggles or face shield should be donned next and the gloves are donned last. Keep in mind, the combination of PPE used, and therefore the sequence for donning, will be determined by the precautions that need to be taken.

Steps in Removing PPE (Doffing)

Wearing the PPE correctly will protect the healthcare worker from contamination. After the patient has been examined or desired procedure is performed, the removal of the PPE is a critical and important step that needs to be carefully carried out in order to avoid self-contamination because the PPE could by now be contaminated.

1. The gloves are removed first because they are considered a heavily contaminated item. Use of alcohol-based hand disinfectant should be considered before removing the gloves. Dispose of the gloves in a biohazard bin.
2. After the removal of gloves, hand hygiene should be performed, and a new pair of gloves should be worn to further continue the doffing procedure. Using a new pair of gloves will prevent self-contamination. Unbuttoning of the backside of the gown, performed by an assistant. Removal of gown to be performed by grabbing the back side of the gown and pulling it away from the body. Single-use gowns can now be disposed of; reusable gowns have to be placed in a bag or container for disinfection
3. After the gown, the goggles should be removed and either disposed if they are single-use, or placed in a bag or container for disinfection. In order to remove the goggles, a finger should be placed under the textile elastic strap in the back of the head and the goggles taken off. Touching the front part of the goggles, which can be contaminated, should be avoided. If goggles with temples are used, they should be removed as per manufacturer's recommendations.
4. The respirator/ mask should be removed next. In order to remove the respirator/mask, a finger or thumb should be placed under the straps in the back and the respirator taken off. The respirator (or the surgical mask) should be disposed of after removal. It is important to avoid touching the respirator/mask with the gloves (except for the straps) during its removal.
5. The last PPE items that should be removed are the new set of gloves that were worn after disposal of the contaminated gloves. Use of alcohol-based solution should be considered before removing the gloves. The gloves should be removed Dispose of the gloves in a biohazard bin.
6. After glove removal, hand hygiene should be performed.

Annexure II

Guideline Development Group (Alphabetical)

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WebTable 1: Summary of Reported Neonatal Cases With Suspected Vertical Transmission

Study	*Case number*	*Mode of delivery*	*ICU adm*	*Pneu-monia*	*Iso-lation*	*Breast-feeding*	*Mask while breastfeeding*	*Other morbidities*	*Outcome PCR*	*RT-*	*Antibodies*	*Age when first positive*
Yu N	1	C-section	No	Yes	Yes	NK	NK	No	Discharged alive	Yes	-	36 h
Zeng L	2	C-section	Yes	Yes	Yes	NK	NK	Fever, lethargy	Discharged alive	Yes	-	Day 2
Zeng L	3	C-section	Yes	Yes	Yes	NK	NK	Fever, lethargy, vomiting	Discharged alive	Yes	-	Day 2
Zeng L	4	C-section	Yes	Yes	Yes	NK	NK	RDS, Pneumonia, proven sepsis, coagulopathy	Discharged alive	Yes	-	Day 2
Wang S	5	C-section	Yes	Yes	Yes	No	NA	No	Discharged alive	Yes	-	36 h
Ferrazzi E	6	Vaginal	Yes	Yes	Yes	No	NA	GI symptoms	Discharged alive	Yes	-	Day 3
Ferrazzi E	7	C-section	Yes	Yes	No	Yes	No	NI	Discharged alive	Yes	-	Day 3
Ferrazzi E	8	Vaginal	Yes	Yes	No	Yes	No	Ni	Discharged alive	Yes	-	Day 1
Alzamora M	9	C-section	Yes	Yes	Yes	No	NA	Resp failure due to depression at birth	Discharged alive	Yes	Negative	16 h
Zamaniyan M	10	C-section	Yes	No	Yes	No	NA	Fever	Discharged alive	Yes	-	24 h
Carosso A	11	Vaginal	Yes	No	Yes	No	NA	Nil	Discharged alive	Yes	IgG positive, IgM negative	At birth
Dong L	12	C-section	Yes	No	Yes	No	NA	Nil	Discharged alive	Negative	IgG and IgM positive	2 h
Zeng H	13	C-section	Yes	No	Yes	No	NA	Nil	Discharged alive	Negative	IgG and IgM positive	At birth
Zeng H	14	C-section	Yes	No	Yes	No	NA	Nil	Discharged alive	Negative	IgG and IgM positive	At birth
Zeng H	15	C-section	Yes	No	Yes	No	NA	Nil	Discharged alive	Negative	IgG positive, IgM negative	At birth
Zeng H	16	C-section	Yes	No	Yes	No	NA	Nil	Discharged alive	Negative	IgG positive, IgM negative	At birth
Zeng H	17	C-section	Yes	No	Yes	No	NA	Nil	Discharged alive	Negative	IgG positive, IgM negative	At birth

NK: Not known, NA: Not applicable

Genomic Testing for Diagnosis of Genetic Disorders in Children: Chromosomal Microarray and Next–Generation Sequencing

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Chromosomal microarray and Next-generation sequencing are two widely used genomic tests that have improved the diagnosis of children with a genetic condition. Chromosomal microarray has become a first-tier test in evaluating children with intellectual disability, multiple malformations and autism due to its higher yield and resolution. Next generation sequencing, that includes targeted panel testing, exome sequencing and whole genome sequencing ends diagnostic odyssey in 25-30% of unselected children with rare monogenic syndromes, especially when the condition is genetically heterogeneous. This article provides a review of these genomic tests for pediatricians.

Keywords: *Chromosomal disorders, Exome sequencing, Whole genome sequencing.*

Genomic testing refers to the analysis of human DNA to detect disease-causing variations. These variations could be chromosomal abnormalities or single gene defects (monogenic or Mendelian disorders). Chromosomal abnormalities can be numerical (aneuploidy) or structural, which include loss or gain of a large part of one or more chromosomes, translocations, inversions and insertions. Loss or gain of smaller regions of a chromosome, known as copy number variations (CNV), usually involve more than one gene and are implicated in many human diseases [1]. While chromosomal aneuploidies are traditionally detected by karyotyping, chromosomal microarray analysis (CMA) is now widely used to detect chromosomal abnormalities. Next generation sequencing (NGS), which includes targeted panel testing, exome sequencing (ES) and whole genome sequencing (WGS), has emerged as the most powerful tool for diagnosis of monogenic disorders, which are mostly caused by sequence variations in the coding portion of the DNA. With technological advances, cost of these tests has decreased drastically and they have become widely available. This review discusses the techniques, clinical utility, advantages and limitations of CMA and NGS.

CHROMOSOMAL MICROARRAY

CMA, otherwise known as genomic microarray, enables the study of chromosomes at a higher resolution as compared to traditional karyotyping. It has replaced karyotyping as the first-tier investigation of children with intellectual disability, multiple malformations and autism [2,3].

Principle

CMA is based on complementary hybridization of nucleotides in the probe and target DNA. Probes are oligonucleotides, varying in length from 25 to 70 bp, which are immobilized on a glass slide or a chip (array) [4-7]. They are spread across the genome at regular intervals (form the 'backbone' and defines the resolution of CMA) and are usually enriched for regions of clinical interest. They are designed to detect CNVs or single nucleotide polymorphisms (SNPs) or both. A CNV is a segment of DNA, which is 1kb or more, and has a variable copy number (extra or less) compared to reference genome [8]. SNPs are the most common genetic variations found in a population across the human genome. Genotyping of millions of SNPs across the genome provides information on alleles and their copy numbers, in addition to mosaicism, uniparental disomy, triploidy and regions of homozygosity. The different types of oligo array platforms include comparative genomic hybridization arrays (array CGH) and SNP arrays (***Fig.*** **1a** and **1b**). Most commercially available platforms are hybrid arrays and contain oligonucleotide probes for detecting both CNVs and SNPs. Array design can be targeted (for specific regions of interest), whole genome (evaluates entire genome) or a combination of whole genome and targeted (most commercially available platforms).

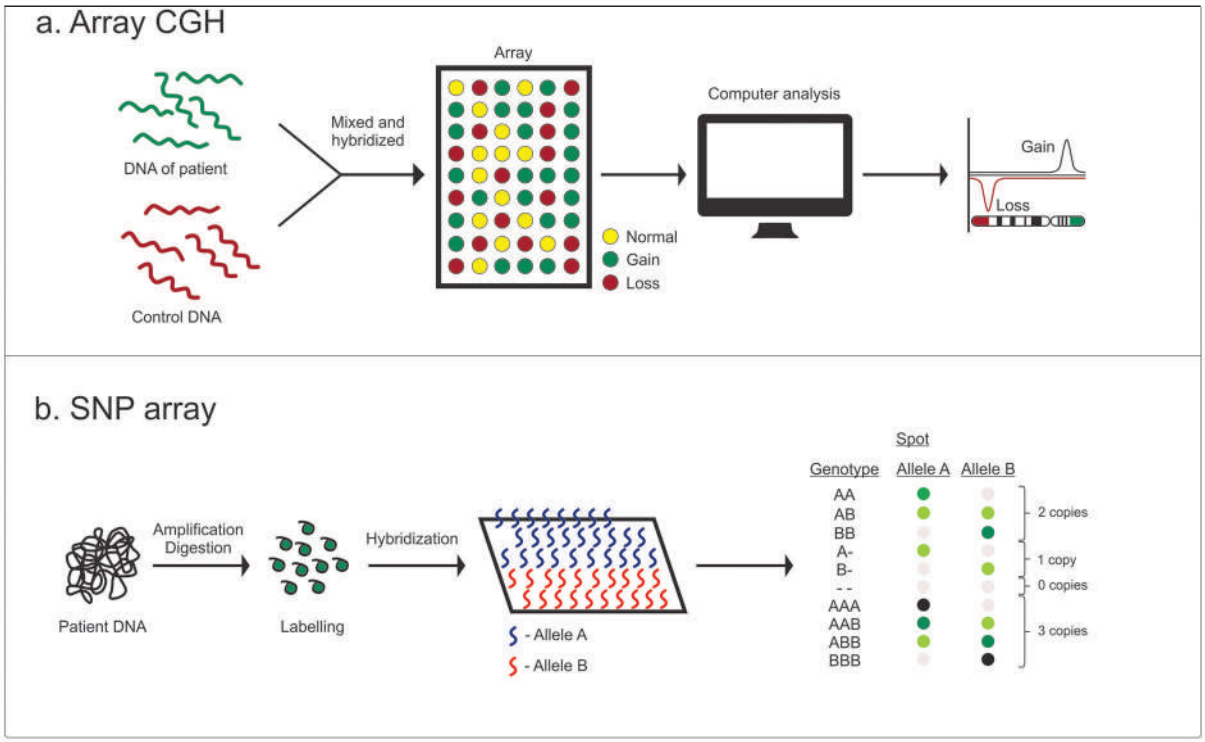

Fig. 1 *(a) Comparative genomic hybridization array, and (b) Single nucleotide polymorphism array.*

Interpretation

The variants identified are critically evaluated based on their size, gene content and published reports in literature [9,10]. Penetrance (how many of individuals with this variant have a phenotypic effect) and variable expressivity (varying severity of disease in individuals with a particular genotype) are considered. The databases used for CNV interpretation are given in ***Web Table* I**. The CNVs are classified into pathogenic, benign or variant of uncertain significance (VOUS) based on American College of Medical Genetics and Genomics (ACMG) criteria given in ***Table* I**. VOUS are variants, which are not directly linked to the patient's phenotype but have some evidence for causation. Usually laboratories using SNP arrays report variants above 50 to 100kb in size [11]. Testing of parents may be required to ascertain the significance of the variant.

CMA has the highest diagnostic yield for any single test in evaluating cognitive impairment, developmental delay, multiple malformations of unknown etiology or autistic spectrum disorder [2,12]. It is the first line investigation for antenatally detected structural abnormalities, stillbirth or intrauterine demise [13], and when a karyotype shows a marker chromosome or extra chromosome material of unknown origin. CMA can identify gain or loss of chromosomal material in up to 20% of individuals with an apparently balanced chromosome translocation [14,15]. ***Box* I** enumerates the advantages and disadvantages of CMA as compared to karyotyping.

One should know the design and resolution of the testing platform and the genomic regions covered. Most of the commercial platforms available have probes for known microdeletion/ duplication syndromes along with genome wide probes for other clinically significant CNVs. In a clinical setting, a low-resolution array, covering all well-delineated microdeletion and microduplication syndromes is usually sufficient. High-resolution arrays are more accurate in delineation of CNVs and SNPs, but result in a large number of variants, which are difficult to interpret. Its utility is limited to the research context. Both pretest counseling (for the yield, specific benefits and limitations) and post-test counseling are also essential.

NEXT-GENERATION SEQUENCING

NGS, also known as massively parallel sequencing or deep sequencing, is a high throughput sequencing technology which allows simultaneous sequencing of millions of DNA base pairs at a comparatively lower cost and higher speed. Exomes comprise only 1% of 6.2 billion base pairs in human DNA, which code for proteins [16].

Table I Classification of Copy Number Variants (CNVs) Based on American College of Medical Genetics and Genomics criteria [9]

Type of CNVs	*Criteria*
Pathogenic	
	• CNVs associated with a known microdeletion/duplication syndrome
	• CNVs reported as clinically significant in peer-reviewed journals and public databases
	• CNVs that are more than 3-5Mb size and are cytogenetically visible
Uncertain clinical significance	
Likely pathogenic	
	• CNVs reported in a single case report, but with breakpoints and phenotype correlating to the patient's features
	• CNV interval has a gene whose function is relevant to the clinical features of the patient
No sub-classification	
	• CNVs described in multiple peer-reviewed journals with no conclusive evidence regarding clinical significance.
	• CNV interval has genes but it is not known whether the genes are dosage sensitive
Likely benign	
	• CNVs are seen in small number of people in databases of variations in normal individuals
	• No gene in the CNV interval; but it is included because of the size cut off set by the laboratory
Benign	
	• CNVs reported as benign variants in multiple peer- reviewed publications or curated databases
	• CNVs whose benign nature has been characterized
	• CNVs represents a common polymorphism and has a population frequency of more than 1%

Box I Advantages and Limitations of Chromosomal Microarray over Karyotyping

Advantages

- CMA can be done from DNA isolated from any type of tissue unlike karyotyping which requires live, actively dividing cells.
- Higher resolution: CMA detects CNVs as small as 10 to 20 kb [9], unlike karyotype for which the resolution is 5 Mb.
- Objective result interpretation
- Can detect cryptic imbalances in chromosomes in apparently balanced karyotype.

Limitations

- Does not detect balanced translocations that do not alter the CNVs.
- Inability to detect point mutations, deletions or duplications at the single gene level.
- Does not detect low-level mosaicism and polyploidy.
- Missing of variations in regions that are not targeted by the probes in targeted arrays.
- Difficulty interpretation of VOUS.

CNV : *Copy number variant*; VOUS: *Variants of unknown significance*.

NGS can analyze the whole genome (whole genomic sequencing, WGS), exome (exome sequencing, ES) or a targeted region of interest in the human genome (targeted gene panel testing). The features of WGS, ES and targeted sequencing are summarized in ***Table* II.** The steps involved are illustrated in ***Web Fig.* I**. Depth of sequencing is the number of times a nucleotide is read during sequencing. A depth of 20x implies that a particular variant or nucleotide is sequenced 20 times. Coverage usually refers to the fraction of the target region of interest sequenced satisfactorily (usually at least 20 times or 20x).

Interpretation

The variants are sorted to narrow down to a single variant that is likely to explain the disease or phenotype. As monogenic diseases are rare, it is assumed that the disease-causing variant is usually not seen in genomes of healthy individuals in the population. Disease-causing variants are likely to result in a change in quantity or quality of the protein coded by the gene, thus affecting the function of the protein. They are also likely to be conserved across different species. Several computational tools are now available to predict the effect of a change in the nucleotide sequence of a gene. The sorting (also popularly called filtering) is also aided by published databases of normal variants and disease-causing variants (***Web Table* II**). If in-house databases with frequency of variants in a particular population are available, they can be very powerful tools for variant analysis as we expect unique genetic variations in different ethnicities. In 2015, ACMG published

Table II Characteristics of NGS Based Tests

NGS platform	*Regions covered*	*Advantages*	*Disadvantages*
Targeted gene panel	Genes of interest (usually associated with the same phenotype/ disease)	Can cover the regions of interest with increased depth. When the genes of interest are less in number, targeted panel testing is less expensive than exome or genome testing.	Will not be able to identify new genes responsible for a phenotype. Gene panels get outdated as new genes are discovered for the same phenotype.
Exome sequencing (also called whole exome sequencing')	Exons and flanking intronic regions of all genes	Covers entire coding region (exome) New genes responsible for a phenotype may be identified	Coverage is less compared to targeted panel. Does not cover non-coding portions of genome well, unless specific modifications are done. Secondary findings (in other genes, not relevant for the disease in question) may be identified.
Whole genome sequencing	Entire coding and non-coding regions in human genome	Coverage of coding regions is better than exome sequencing as this technique avoids 'capture' step of exome sequencing. Covers non-coding regions of the genome	Expensive currently. Secondary findings may be identified.

guidelines for interpretation of sequence variants and categorized them into five categories, i.e., pathogenic, likely pathogenic, benign, likely benign and VOUS [17]. The results are then correlated with clinical features and communicated to the patient. For efficient filtering and clinical interpretation of the variants, a patient should be referred to a trained clinical geneticist.

NGS testing generates a large number of variants in an individual's exome or genome. Clues from evaluation of pedigree, clinical examination and routine medical tests are vital to determine the effect of the variant on the phenotype. Often Human Phenotype Ontology [HPO] terms are used for this purpose. NGS should not be considered as an alternative for thorough clinical examination and ancillary laboratory tests.

Clinical Indications

- Targeted panel testing can be done when a particular phenotype is caused by variations in more than one gene (locus heterogeneity). For example, variations in about 20 different genes are implicated in osteogenesis imperfecta. A panel, which covers all the genes for osteogenesis imperfecta is more efficient than Sanger sequencing one gene after the other. Other examples are deafness, Noonan syndrome (RASopathies), congenital myopathy and pediatric epilepsy. Large genes like dystrophin can be tested by NGS either singly or in a panel for muscular dystrophy or myopathy when deletion and duplications are ruled out by multiplex ligation dependent probe amplification (MLPA) in a child with Duchenne muscular dystrophy.
- ES can be performed in patients with genetically heterogenous monogenic disorders when targeted panel testing fails.
- WGS may be considered when ES fails to identify a disease-causing variant. It detects variants in coding and non-coding regions of the genome and regions not well captured and sequenced in ES, CNVs and structural chromosomal abnormalities. It has the potential to become a single test replacing most of the current tests.
- NGS-based tests hold promise in area of carrier testing, pre-symptomatic testing, pharmacogenetic testing, and predictive testing, which are beyond the scope of this review.

Even though genome sequencing and exome sequencing are described as 'whole' genome or 'whole' exome sequencing, they do not evaluate all the genes in the human genome. The word 'whole' distinguishes these tests from panel testing and should not mislead clinicians and patients to believe that these tests would be 100%

sensitive to detect all the disease-causing variants. The coverage of known genes by these tests vary from 85%-92% [18]. 'Clinical exome' or 'focused exome' is a commercial panel test that uses a customized capture kit to interrogate only genes associated with a known clinical phenotype, usually listed in Online Mendelian Inheritance in Man (OMIM). Hence the term 'clinical exome' is better avoided. In strict sense, 'clinical' genome or exome sequencing implies sequencing of exome or genome for clinical applications [19]. Before ordering a test, it is essential to check the coverage of genes of interest. The decision whether to order a targeted panel test or ES or WGS will depend on the clinical features of a patient and the ability of a clinician to arrive at a diagnosis. An ideal targeted panel test should be able to diagnose disease-causing variants in the genes of interest of the suspected genetic disorder and should also include methods to detect deletion and duplications, which can cause a specific disease phenotype. Analyzing only selected regions or genes of interest may not qualify to be called a targeted panel, unless the laboratory fills the gaps in sequencing by alternate methods like Sanger sequencing and does a deletion/ duplication analysis. For example, in a child with leukodystrophy, before ordering a targeted panel test for leukodystrophy, it is essential to check whether all the genes of interest are covered. Krabbe disease is often caused by deletions in GALC gene and might be missed if an NGS test is ordered without asking for deletion/duplication analysis of GALC gene. If a specific genetic diagnosis cannot be made, ES or WGS may be considered. ES is cheaper and is often preferred to WGS as the first investigation for undiagnosed single gene diseases, which mostly result from variations in exons. A singleton or single exome means exome sequencing of a proband, whereas 'trio' exome means exome sequencing of the proband and parents.

Consent and Counseling in NGS Tests

Informed consent is essential before NGS based testing. Pretest counseling is essential to explain the yield, utility and implications of a 'negative' or 'positive' report for family. Limitations of science in interpreting VOUS and identification of secondary variants are specific issues in NGS testing. Secondary variants in genes are associated with diseases unrelated to the proband's condition and are common in ES and WGS. Secondary findings in genes causing cancer and sudden cardiac death may have implications for the patient and family members. A genetic diagnosis may not have any direct impact on the treatment of the patient but may aid in long-term management, genetic counseling and prenatal diagnosis. Post-test counseling by a geneticist is thus needed. Sanger sequencing is done to validate the variant in the proband and for segregation analysis. Good quality NGS often obviates the need for Sanger confirmation. Segregation analysis determines segregation of the variants in the other affected or unaffected members in the family and is crucial for causal association in the proband. If a negative test result is obtained, the family should be counseled about the need to re-evaluate the data at a later date.

At present there are no regulations governing clinicians, laboratories and counselors in India. Direct marketing of these tests may result unregulated commercialization.

Variables to Consider in NGS Report

The NGS report mentions the methodology, capture kit, depth and coverage of sequencing. Capture kits may be customized for different panel tests and ES. It is important to check for depth and coverage of sequencing before conveying the report to the patient.

Some clinical scenarios where CMA and NGS have aided in diagnosis are described in ***Web Table*** **III.**

CONCLUSIONS

Chromosomal microarray, exome sequencing and whole genome sequencing using NGS techniques are powerful methods to investigate variations in human genome. It is essential for a pediatrician to know the strengths, limitations and advantages of these testing methods over traditional medical tests to apply optimally in clinical practice of pediatrics.

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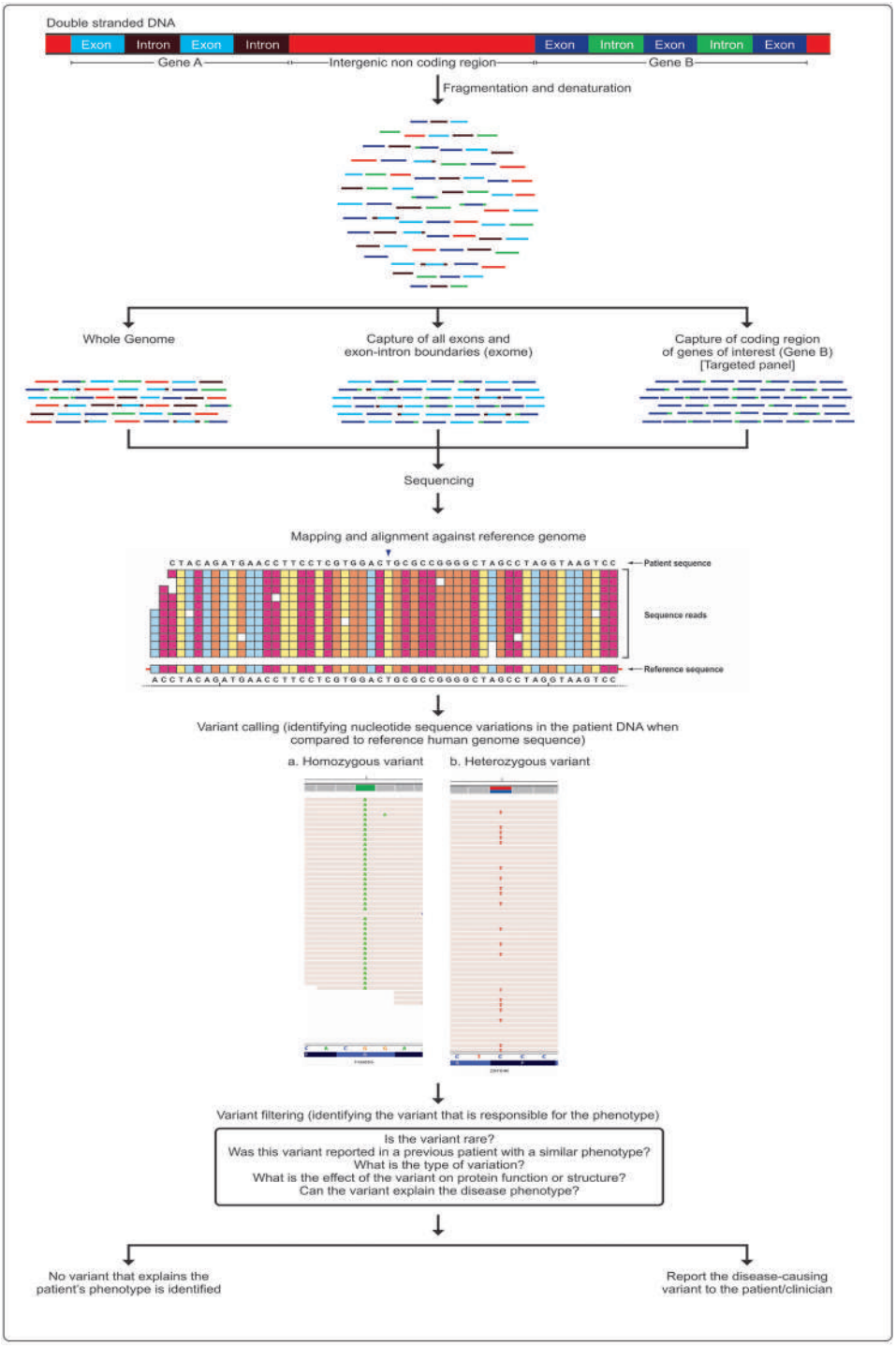
REFERENCES

1. Bernardini L, Alesi V, Loddo S, Novelli A, Bottillo I, Battaglia A, *et al.* High-resolution SNP arrays in mental retardation diagnostics: How much do we gain? Eur J Hum Genet. 2010;18:178-85.
2. Manning M, Hudgins L. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. Genet Med. 2010;12:742-5.
3. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, *et al*. Consensus Statement: Chromosomal Microarray is a First-tier Clinical diagnostic Test for Individuals With Developmental Disabilities or

Congenital Aanomalies. Am J Hum Genet. 2010;86: 749-64.
4. Snijders AM, Nowak N, Segraves R, Blackwood S, Brown N, Conroy J, *et al.* Assembly of microarrays for genome-wide measurement of DNA copy number. Nat Genet. 2001;29:263-4.
5. Beaudet AL. The utility of chromosomal microarray analysis in developmental and behavioral pediatrics. Child Dev. 2013;84:121-32.
6. Oostlander AE, Meijer GA, Ylstra B. Microarray-based comparative genomic hybridization and its applications in human genetics. Clin Genet. 2004;66:488-95.
7. LaFramboise T. Single nucleotide polymorphism arrays: A decade of biological, computational and technological advances. Nucleic Acids Res. 2009;37:4181-93.
8. Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. Nat Rev Genet. 2006;7:85-97.
9. Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST. American College of Medical Genetics Standards and Guidelines for Interpretation and Reporting of Postnatal Constitutional Copy Number Variants. Genet Med. 2011;13:680-5.
10. South ST, Lee C, Lamb AN, Higgins AW, Kearney HM. ACMG Standards and Guidelines for Constitutional Cytogenomic Microarray Analysis, Including Postnatal and Prenatal applications: Revision 2013. Genet Med. 2013;15:901-9.
11. Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. Fertil Steril. 2018;109:201-12.
12. Rauch A, Hoyer J, Guth S, Zweier C, Kraus C, Becker C, *et al.* Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. Am J Med Genet A. 2006;140:2063-74.
13. Karampetsou E, Morrogh D, Chitty L. Microarray technology for the diagnosis of fetal chromosomal aberrations: Which platform should we use? J Clin Med. 2014;3:663-78.
14. Edelmann L, Hirschhorn K. Clinical utility of array CGH for the detection of chromosomal imbalances associated with mental retardation and multiple congenital anomalies. Ann NY Acad Sci. 2009;1151:157-66.
15. Sismani C, Kitsiou-Tzeli S, Ioannides M, Christodoulou C, Anastasiadou V, Stylianidou G, *et al.* Cryptic genomic imbalances in patients with de novo or familial apparently balanced translocations and abnormal phenotype. Mol Cytogenet. 2008;1:15.
16. Thiffault I, Lantos J. The challenge of analyzing the results of next-generation sequencing in children. Pediatrics. 2016;137:S3-7.
17. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, *et al.* Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-24.
18. Biesecker LG, Biesecker BB. An approach to pediatric exome and genome sequencing. Curr Opin Pediatr. 2014;26:639-45.
19. Biesecker LG, Green RC. Diagnostic clinical genome and exome sequencing. N Engl J Med. 2014;371:1170.
20. Schwarze K, Buchanan J, Taylor JC, Wordsworth S. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genet Med. 2018;20:1122-30.
21. Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, *et al.* Clinical whole-exome sequencing for the diagnosis of mendelian disorders. N Engl J Med. 2013;369:1502-11.



Web Fig. I *Steps in NGS: Double stranded DNA is fragmented into smaller segments and denatured. In whole genome sequencing, all these fragments (exons, introns, non-coding intergenic segments) are sequenced. In exome sequencing, capture kits that selectively capture the exome (all exons and flanking introns) are used and those fragments are sequenced. In a targeted panel, capture kits that selectively capture the coding portion of the genes of interest are used (in this example, capture kit for Gene B). Once sequencing is done, mapping and alignment of reads against a reference genome is done. The next step is variant calling, which detects variants in the subject against the reference sequence. A homozygous variant is seen as a change in almost all reads whereas a heterozygous change is seen in nearly half of the total number of reads. The final step is variant filtering, interpretation and reporting. From a list of variants, pathogenic and benign variants are identified by several filtering approaches.*

Web Table I Popular Databases used in Interpretation of Copy Number Variants

Database	*Key features*
DECIPHER (DatabasE of genomiC varIation and Phenotype in Humans using Ensembl Resources) *https://decipher.sanger.ac.uk*	Interactive web based free browser where the patient's variant is displayed along with normal and pathogenic variants in that locus
DGV (Database of Genomic Variants) *http://dgv.tcag.ca/dgv/app/home*	Database of common structural variations in healthy individuals
ISCA (International Standards for Cytogenomic Arrays) *http://dbsearch.clinicalgenome.org/search/*	Database of pathogenic, likely pathogenic, uncertain, likely benign, and benign CNVs

Other databases include UCSC genome browser (University of California, Santa Cruz), ECARUCA (European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations) and OMIM (Online Mendelian Inheritance in Man).

Web Table II Databases Used in Exome or Genome Data Analysis

Name of database	*Description*	*Website*
Population database of variants		
Genome Aggregation Databases (gnomAD)	Disease specific or population specific exome and genome data from unrelated individuals	*https://gnomad.broadinstitute.org*
The International Genome Sample Resource (IGSR) (1000 Genomes Project)	Database of genetic variants with a frequency of more than 1%	*http://www.internationalgenome.org*
The Exome Aggregation Consortium (ExAC)	Exome sequencing data from disease specific and population genetic studies	*http://exac.broadinstitute.org*
Databases of disease causing variants		
The Human Gene Mutation Database (HGMD)	All known published disease-causing variants	*http://www.hgmd.cf.ac.uk/ac/index.php*
ClinVar	Clinical description and variants	*https://www.ncbi.nlm.nih.gov/clinvar/*
Leiden Open Variation Database (LOVD)	Variant database	*http://www.lovd.nl*
Online Mendelian Inheritance in Man (OMIM)	Human diseases and variants	*https://www.omim.org*

Web Table III Clinical Scenarios where Genomic Testing is Useful

Scenario 1: A non-consanguineous couple with a five-years-old girl with autism wanted to know the risk of recurrence of autism in subsequent pregnancies. Chromosomal microarray was opted as the first tier test in this child. No pathogenic copy number variant causing autism was identified. They were offered exome/genome sequencing as well, but did not opt for it in view of high cost and low yield. Only an empiric risk of recurrence of 10% was provided to the family. Since exact genetic etiology was not identified in the child, prenatal diagnosis could not be offered.

Scenario 2: A three-years-old boy born to third degree consanguineous parents had spastic diplegia and was being treated as cerebral palsy. There was no history of any adverse perinatal events. In the absence of a perinatal insult, exome sequencing done for this child, identified a biallelic pathogenic variant c.700G>C (p.Asp234His) in *ARG1* causing arginase deficiency (MIM#207800). Parents were heterozygous carriers for the same variant. The child was advised supportive care. The parents were counseled about the recurrence risk of 25% of this condition in every pregnancy and prenatal diagnosis was offered by chorionic villus sampling.

Scenario 3: A four-years-old girl had developmental delay, repetitive hand wringing movements and hyperventilation. DNA methylation analysis for Angelman syndrome and sequencing of *MECP2* gene for Rett syndrome were normal. Exome sequencing identified a de novo heterozygous disease-causing variant, c.1512insA (p.Ser505Glu*8) in TCF4 gene, causing Pitt Hopkins syndrome (MIM#610954). Since the parents did not have this variant, they were counseled about very low risk of recurrence (usually less than one percent) in subsequent pregnancies.

Scenario 4: Six-years-old girl, who was the first child of non-consanguineous parents, was evaluated for developmental delay and intellectual disability. Chromosomal microarray and fragile X mutation analysis did not reveal disease- causing variants. Exome sequencing of the child was performed. A heterozygous novel variant c.3817C>A, p.(His1273Asn) in HIVEP2 gene causing autosomal dominant mental retardation type 43(MIM#616977) was reported. The variant was interpreted as VUS (variant of unknown significance). It was noted that the parents were not tested for this variant. On testing them, the same variant was observed in heterozygous state in her father who had normal intellect. Hence this variant was re-classified as a benign variant in HIVEP2. Exome sequencing was performed in parents to complete the trio (parents-child) and a novel biallelic compound heterozygous variant in a novel gene in the proband was identified (suggesting the possibility of a hitherto unknown disease with intellectual disability and its genetic cause). Further validation of these findings by more patients with similar condition and experiments are awaited to provide definitive genetic counseling and prenatal diagnosis to the family.

Scenario 5: A 12-years-old girl with multiple fractures was diagnosed to have osteogenesis imperfecta (OI). She did not have blue sclera or dentinogenesis imperfecta. Her radiographs showed hyperplastic callus and calcified interosseus membrane in forearm. Since this pointed to a specific diagnosis of osteogenesis imperfecta type V (MIM#610967), instead of ordering a panel test covering all genes causing OI, Sanger sequencing of only the particular region of *IFITM5* was done. A de novo heterozygous pathogenic variant c.-14C>T was identified in this gene. This variant was not identified in her parents. Clinical and radiological examination is useful even in genomic era.

Multimedia Instructional Design Principles: Moving from Theoretical Rationale to Practical Applications

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Since the introduction of cognitive theory of multimedia learning more than a decade back much empirical evidence has substantiated the theoretical rationale of multimedia instructional design principles. Medical educators use multimedia mostly for delivering lectures in the form of power-point presentations. Abundant literature is available giving instructions on the appropriate use of font type and size *etc*. to be used in power-point slides, but the literature applying multimedia instructional design principles for preparing effective presentations leading to active and meaningful learning is scanty. This paper deals with theoretical aspects of multimedia instructional design principles and the ways of effectively incorporating these principles for designing meaningful power-point presentations.

Keywords: *Cognitive theory of multimedia learning, Lecture, Power-point*, *Presentation*, *Slides*.

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With the advancement in the field of technology, the use of educational technology tools in medical education are on the rise. The 2007, Effective use of educational technology in medical education report of the Association of american medical colleges institute for improving medical education (AAMC-IME) highlighted the effectiveness of educational technology in medical education and emphasized that for designing instructional presentations for medical students, medical educators must apply the principles of educational technology learning [1]. Broadly, medical educational technology can be divided in to three functional divisions – computer-aided instructions (CAIs), virtual patients (VP), and human patient simulation (HPS).

Of the three functional divisions of medical educational technologies, CAIs in the form of use of multimedia is the most frequently utilized aspect in medical education. Sensing the utility of medical educational technologies and multimedia, many regulatory bodies started faculty development programs, focusing on this aspect. The Medical council of India (MCI) Basic course workshop in medical educational technologies (BCW-MET) covers the area as 'Improving self-directed learning through technology' [2]. From 2015, MCI has made it mandatory for medical colleges to make adequate provisions for conversion of lecture theatres in to E-class and virtual class rooms [3]. As per the competency-based undergraduate medical curriculum, establishment of skill labs by December, 2019, in order to enhance students' clinical, motor, communication skills and team work in medical colleges, has also been made mandatory by MCI, and guidelines for the same were issued recently [4].

The premise that medical students learn better with the use of multimedia and CAIs is based on sound theoretical rationale and empirical evidence, some of which is discussed herein.

THEORETICAL RATIONALE: THEORY OF MULTIMEDIA LEARNING

People learn better from a combination of words and pictures, than words alone. This multimedia principle makes the basis of theoretical rationale of multimedia learning [5]. Accordingly, Mayer gave a cognitive theory of multimedia learning, stating that meaningful learning using multimedia is more likely to happen if multimedia instructional messages are designed keeping in view how the human mind works [5]. The theory is primarily based upon four scientific criteria – theoretical plausibility, testability, empirical plausibility, and applicability. This theory is based upon three fundamental assumptions [5] (***Box* I**).

BOX I THREE FUNDAMENTAL ASSUMPTIONS OF COGNITIVE THEORY OF MULTIMEDIA LEARNING

- *Dual channels*: Human information processing system has separate auditory / verbal channels and visual / pictorial channels, which then work together to make cross-channel representations.
- *Limited capacity*: Humans have limited capacity of processing the information through each channel, at a time, the assumption being consistent with the cognitive load theory
- *Active processing*: In humans active learning happens by constructing a coherent mental representation of experiences, and by integrating incoming information with the prior knowledge – making mental model.

As per the cognitive theory of multimedia learning, relevant information in the form of words and pictures is first selected, then words and images are organized, and finally words and pictures are integrated by building connections to make sense. Mayor's theory gives sound theoretical rationale for the use of multimedia and CAIs in medical education too; an area where pictures and videos can address the bulk of the cognitive load of the medical student.

EMPIRICAL EVIDENCE

Although, low effect size of 'instructional media' was previously suggested [6], recent studies provide evidence in favor of CAIs. Issa, *et al*. [7], showed that the cohort of medical students instructed using principles of multimedia design, scored better than the students instructed using traditional designs, when evaluated for immediate retention of knowledge and total scores. In a follow-up study, the authors showed that the modified condition group scored significantly better than the traditional condition group on delayed tests of transfer conducted one week and four weeks after instruction, and on delayed tests of retention conducted one week and four weeks after instruction. The modified condition group participants also performed significantly better than the traditional condition group on immediate tests of retention and transfer [8].

In another study [9], multimedia was used for teaching of gross infective pathogen with a reformed courseware. Results showed that compared with non-reform classes, the reform classes had significant improvements in results [9]. Based upon these theoretical principles and empirical evidence, it is imperative that medical educators use multimedia for learning, as medical education requires a combination of verbal and pictorial learning [10].

DOES EVERY JINGLE MINGLE?

Though people learn deeply from combination of pictures and words than anything singularly, does that mean that any combination of words and pictures will work? Not exactly. Simply adding pictures to the words does not guarantee improved learning. This affectively means that all multimedia presentations are not equally effective. Only the multimedia instructions designed on the basis of the principles of human learning are going to provide meaningful learning [5]. Accordingly, two broad aspects which one should be conversant with, while designing effective multimedia presentations are: Science of learning and Science of Instruction [10].

Science of Learning: How People Learn

Learning is the change in the behavior of the learner. Mayer called it as the "change in the learner's knowledge attributable to experience" [11]. For fostering learning, instructors must be aware of 'how learning happens'.

For any learning to happen, material is first selected, then material is organized and finally material is integrated and connections built with previous knowledge [5]. Pursuant to the Mayor's cognitive theory, five main types of cognitive processes involved in multimedia learning have been identified *viz*, selecting words, selecting images, organizing words, organizing images, and integrating [10]. One should also take into account the Knowles' principles of adult learning, which emphasize that adults are self-directed, have prior experience on which they build-up new knowledge, and they learn better in safe learning environment [12].

The role of human memory and its processing has to be collated with other processes for driving home the concept of multimedia learning. Humans have three functional parts of memory – sensory memory, working memory, and long-term memory [10]. Sensory memory holds exact copy of information provided for <0.25 seconds. Working memory stores more processed version of the information provided for <30 seconds, with limited capacity. Long-term memory stores knowledge for a longer period of time. Sensory and long-term memory have unlimited capacity. As working memory has limited capacity, so for effective multimedia learning to happen, people must be active learners, seeking meaningful learning.

The interplay between functional parts of the human memory and cognitive processes involved in the multimedia learning has been depicted in ***Fig.* 1**.

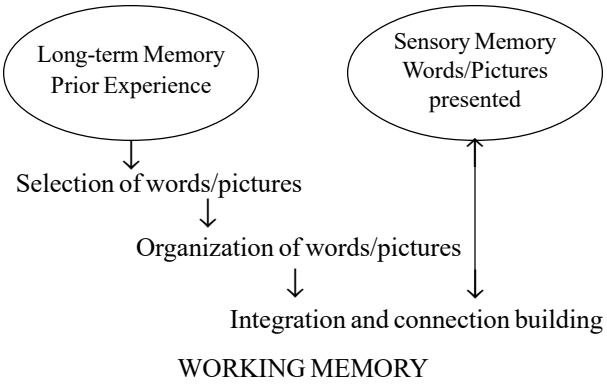

Fig. 1 *Interplay between memory and cognitive processes for multimedia learning.*

Science of Instruction: How Instructions Work

Instructional methods should be in congruence with the human learning and knowledge processing system. It is pertinent to understand how instructions work, before one can design effective instructional methods and instructions. Same applies to multimedia instructions and its application in medical education.

The first step in designing effective instructions is to specify the instructional objectives and the knowledge change in the learner [13]. An instructional objective is a statement clearly specifying – what is to be learnt, how it will be learnt and how the change in behavior will be assessed. Next in line is selecting an appropriate instructional strategy, followed by assessment. Instructional objectives, instructional strategies and assessment, all should be in alignment. Instructional objectives, strategies chosen and assessment should also be in alignment with the specific domain of learning. Well aligned instructional objectives, instructional methods and assessment, not in congruence with domain of learning will not lead to meaningful learning. Overall efforts should be made to create effective learning environments.

Authors are of the view that for designing multimedia instructions; analysis, design, development, implementation, and evaluation (ADDIE) model is best suited as it uses a behavioral approach in designing instructions [14]. As per this model, instructional designs pass through five phases of analysis, design, development, implementation, and evaluation.

In the analysis phase, the existing materials in the form of pictures, videos, exhibits, *X*-rays, ultrasounds, *etc*. will be analyzed for further use in designing multimedia instructions. In designing phase, multimedia delivery method (printed text-figures, power point presentations, computer disc, webinar, virtual classroom *etc*.) will be selected, and then instructional content will be created accordingly in next phase (***Fig.* 2**). Evaluation in multimedia instructions is largely confined to two aspects – test of retention and test of transfer [5]. Performance on these two aspects can measure learner's outcome - poor performance on both types of test indicate no learning; good performance on retention only indicates rote learning, while good performance on both types of tests indicate meaningful learning [10]. One should try to design and use multimedia assessment while using multimedia instructions, based upon cognitive theory of multimedia assessment [15].

MOVING FROM THEORY TO PRACTICE

The above mentioned theories and instructional design aspects make sound foundation for how the faculty members in medical colleges can design multimedia instructions. Mostly medical college faculty uses multimedia only for designing power point (PPT) slides-based instructions and presentations. Other multimedia contents are mostly made available by software developers as 'ready-to-use' materials. Most of the papers found in literature have restricted the discussion only about font type and size to be used in PPT slides, color combinations, number of lines *etc*. for making slides better presentable; in the next section we discuss briefly how effective PPTs can be designed based upon multimedia instructions developed through empirical evidences and practical issues.

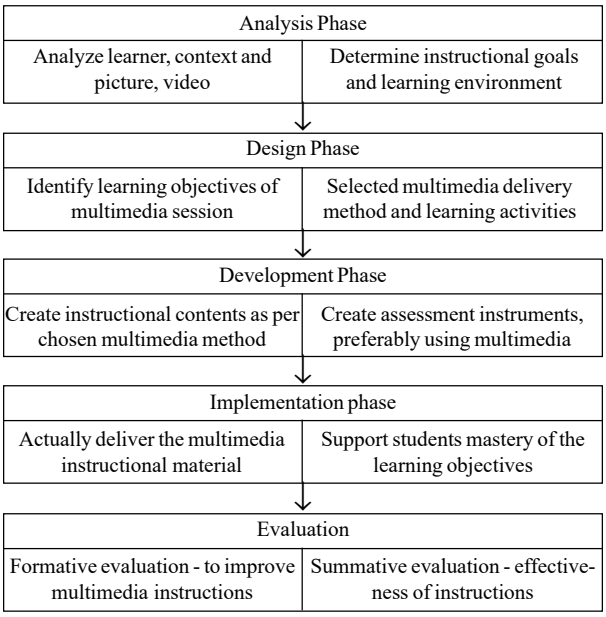

Fig. 2 *Phases and activities for designing multimedia instructions as per ADDIE model.*

After scanning almost 100 studies, Mayer identified 12 principles of developing and designing multimedia instructions [7,10,16]. These 12 principles have broadly been factorized into three generic principles in literature *viz.* curtail extraneous processing; manage essential processing, and nurture generative processing [7,10].

Extraneous processing is a type of cognitive processing that does not support the learning objective, often caused by poor instructional design, like picture on one slide and its explanation on another slide of presentation. No doubt, such processing must be reduced to have less cognitive load. Essential processing is the process of representing the essential material to the working memory, through the process of selecting and organizing. This cognitive processing must be managed. Generative processing involves the process of making sense from the presented material through integrating and organizing. This needs learners motivation and engagement too, thus generative processing needs to be nurtured. Readers interested in details of these processes may refer Mayer's write-up on the same [17].

These factors along with strategies which can be devised for effective designing of PPT based multimedia instructions have been depicted in ***Fig.* 3** [7,10,17].

Above mentioned principles can be easily incorporated to make slide presentations interesting, engaging and effective. In the next section, we have tried to present graphically the strategies to incorporate major multimedia instructional design principles for preparing effective power-point presentations.

Multimedia principle: While preparing slides, both words and pictures should be used, instead of using words alone ***(Web Fig.* 1**). This will also be in accordance with the 'dual channel' assumption of the cognitive theory of multimedia learning.

Coherence principle: Do not use unnecessary and unimportant pictures, words and animations in your presentation (***Web Fig.* 1**). These unnecessary elements are bound to increase cognitive load on the learners. Exclusion of unnecessary elements from slides will also be in accordance with the 'limited capacity' assumption of the cognitive theory of multimedia learning.

Signalling principle: The essential and important material in the slides must be highlighted (***Web Fig.* 2**). This can be done by using a text box option or by using the glow text effect.

Contiguity principle: Learning from slides is better when pictures and concerned words are presented near to each other than separated from each other on a slide (Spatial contiguity). Similarly, learning is better when pictures and related words are presented simultaneously and on the same slide rather than successively or on the next slide (Temporal contiguity) (***Web Fig.* 2**). This enhances 'active processing', another assumption of the cognitive theory of multimedia learning.

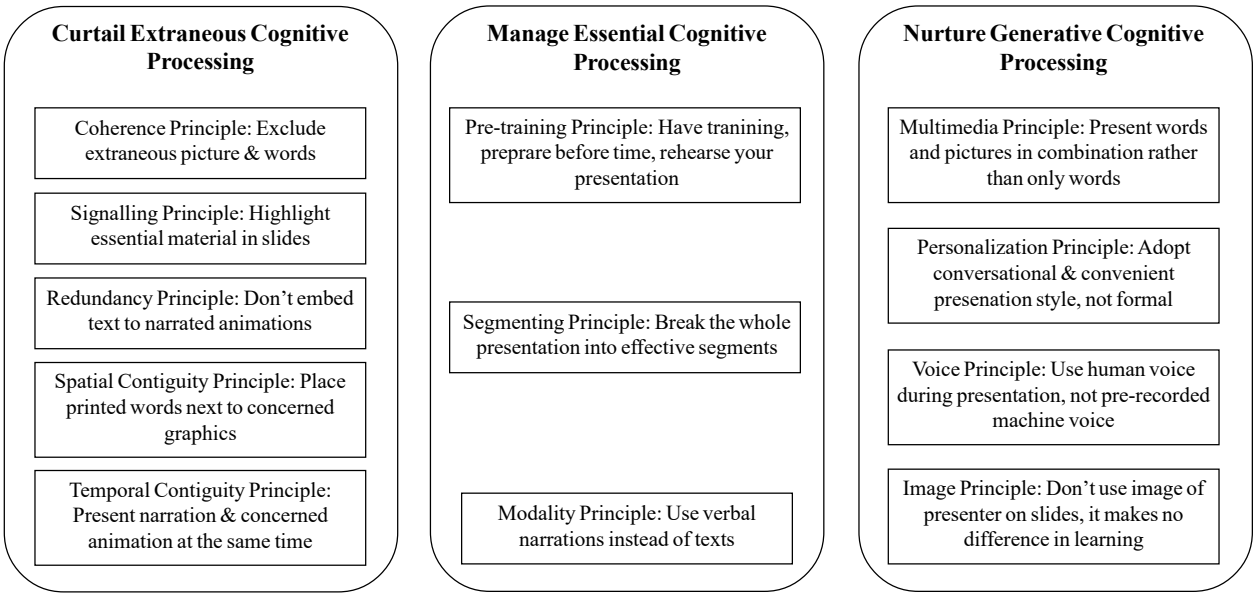

Fig. 3 *Principles of instructional design for multimedia presentations adapted for effective power-point based presentations.*

Segmenting principle: Presentation should be divided into different effective segments for better interaction with the content of the presentation and meaningful learning (***Web Fig*. 2**). Same can apply to one slide, where space must be used in different sections, if required.

Redundancy principle: While using an animation in the presentation, don't overload the slides / presentation with text also. Narration with animation makes better learning than animation, narration and text. If text is used along with animation, it will compete for the visual channel.

Multimedia presentation tools such as power point outshines in context to its applicability and feasibility at presenting knowledge in the form of visual information. The message during large group interactive sessions having embedded colored figures, charts, and graphics along with text can be better conveyed by using multimedia instructional principles while preparing power point presentations [18]. It has been proven beyond doubt that adapting powerpoint slides of lectures according to multimedia principles is likely to translate in to improved short-term retention among medical students [7].

FACULTY READINESS AND TRAINING

It has been noticed worldwide that the effective use of educational technology in medical education depends largely upon faculty readiness, which in turn depends upon faculty training. Medical faculty must be trained in at least three wider aspects of educational technology in medical education *viz*, understanding of technical operations of the technology, understanding of the ways to utilize this technology for teaching-learning, and understanding the ways to utilize the technology for students' assessment [1].

Are Faculty Members Trained and Ready?

Traditionally, medical educators are trained to use clinical settings for teaching-learning and assessment purposes. There are lot of differences between clinical setting-based teaching-learning and computer-based teaching-learning. Both will call, not only for different teaching styles but also different methods of feedback and assessment. As mentioned earlier in this paper, faculty members are most verse with theoretical aspects of the same, but application part is missing. This calls for shifting our focus from delivering 'knowledge' aspects of the training to 'competency' aspect. It is the need of the hour to structure 'competency-based faculty training programs for using advanced educational technologies' in medical education by incorporating the principles of cognitive theories of multimedia learning, and principles of multimedia design.

CONCLUSION

Cognitive principle of multimedia learning and principles of instructional designs need to be practically implemented. These principles must be used while making power-point presentations for medical education, so as to make lectures interesting, interactive and effective. These slight modifications will definitely improve the retention of the medical students.

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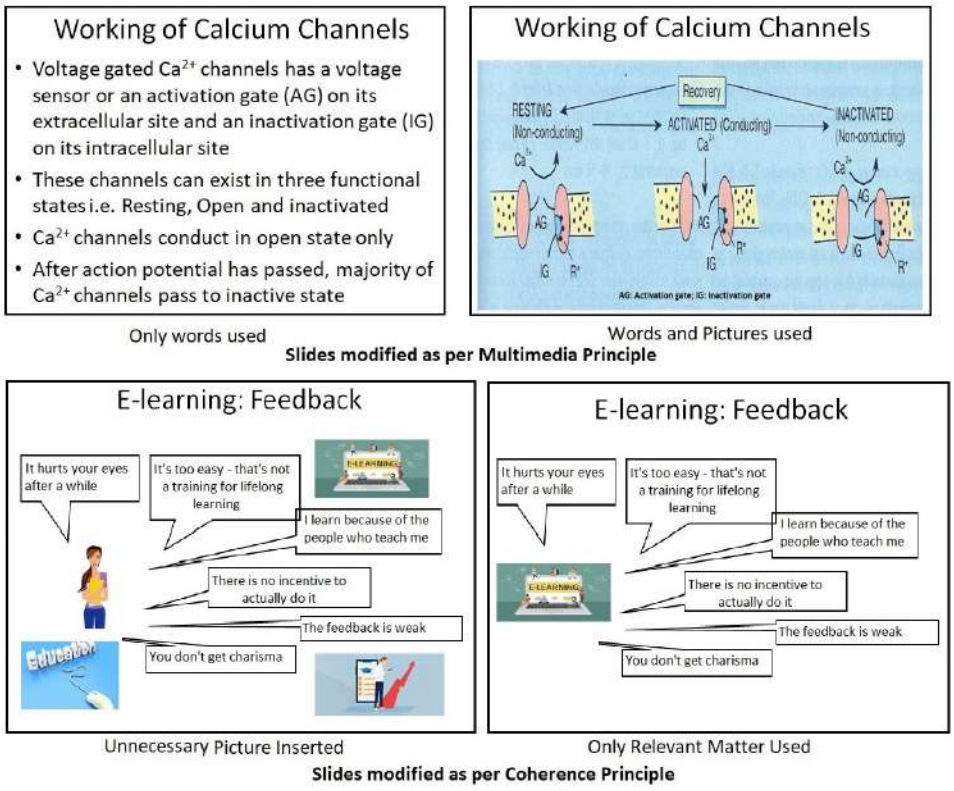
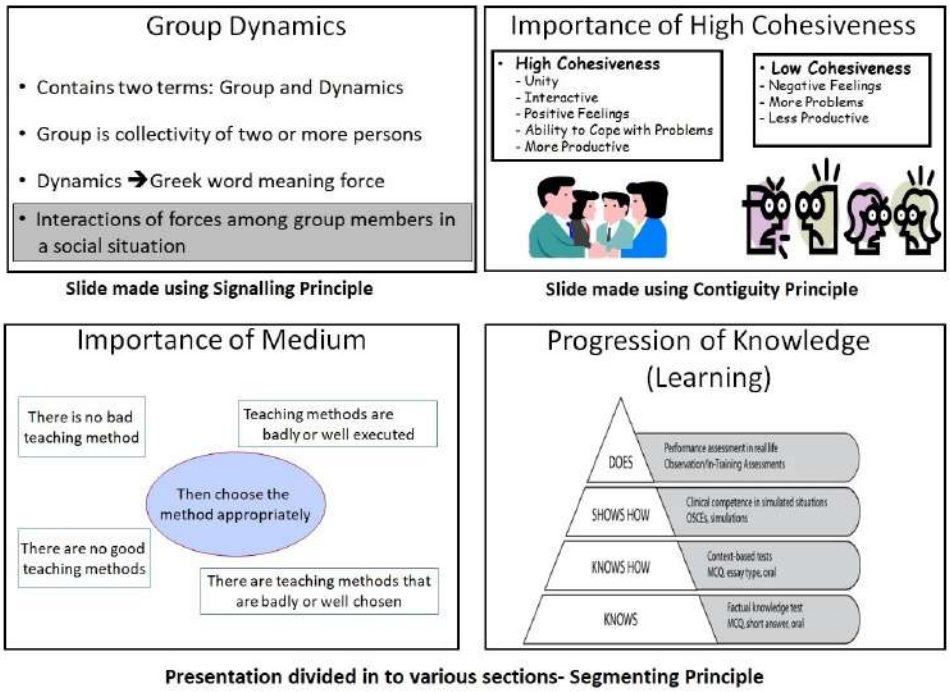
References

1. Association of American Medical Colleges. Effective Use of Educational Technology in Medical Education - Colloquium on Educational Technology: Recommendations and Guidelines for Medical Educators. Candler C, ed. AAMC Institute for Improving Medical Education. Washington, DC: AAMC; 2007.
2. Medical Council of India. Programme of the revised Basic Course Workshop w.e.f August 2015. Available from: *https://old.mciindia.org/fdp/8.%20Three%20day%20revised%20BCW%20programme.pdf*. Accessed September 6, 2019.
3. Medical Council of India. Minimum Standard Requirements for the Medical College for 150 Admissions Annually Regulations, 1999 (Amended up to January 2018). Available from: *https://old.mciindia.org/for-colleges/Minimum%20Standard%20Requirements%20for%20150%20Admissions.pdf*. Accessed September 6, 2019.
4. Medical Council of India. Guidelines for development of skills lab at medical colleges. Available from: *https://old.mciindia.org/UG-Curriculum/Guidelines_Develeopment_Skills_Labs_MedicalColleges.pdf*. Accessed September 6, 2019.
5. Mayer RE. Cognitive theory of multimedia learning. *In*: Mayer RE, *ed*. The Cambridge Handbook of Multimedia Learning. 1st ed. Cambridge, UK: Cambridge University Press; 2005. p. 31-48.
6. Hattie J. Influences on student learning. Inaugural Lecture, University of Auckland; 1999. Available from: *https://cdn.auckland.ac.nz/assets/education/ about/research/documents/influences-on-student-learning.pdf*. Accessed October 1, 2019.
7. Issa N, Schuller M, Santacaterina S, Shapiro M, Wang E, Mayer RE, *et al*. Applying multimedia design principles enhances learning in medical education. Med Educ. 2011;45:818-26.
8. Issa N, Mayer RE, Schuller M, Wang E, Shapiro MB, DaRosa DA. Teaching for understanding in medical

classrooms using multimedia design principles. Med Educ. 2013;47:388-96.
9. Zhanga C, Chen X. Use of Multimedia in Gross Infective Pathogen Experimental Teaching. Procedia Engineering. 2012;37:64-7.
10. Mayer RE. Applying the science of learning to medical education. Med Educ. 2010;44:543-9.
11. Mayer RE. Learning and Instruction, 2nd ed. Upper Saddle River, NJ: Merrill Prentice Hall Pearson; 2008.
12. Kaufman DM. ABC of learning and teaching in medicine: Applying educational theory in practice. BMJ. 2003;326:213-6.
13. Anderson LW, Krathwohl DR, Airsian PW, Cruikshank KA, Mayer RE, Pintrich P, *et al*. A Taxonomy for Learning, Teaching, and Assessing: A Revision of Bloom's Taxonomy of Educational Objectives. New York, NY: Longman 2001.
14. Khalil MK, Elkhider IA. Applying learning theories and instructional design models for effective instruction. Adv Physiol Educ. 2016;40:147-56.
15. Kirschner PA, Park B, Malone S, Jarodzka H. Toward a cognitive theory of multimedia assessment (CTMMA). *In*: Spector M, Lockee B, Childress M. *eds*. Learning, Design, and Technology. Cham, Switzerland: Springer; 2016. p. 1-23.
16. Sorden SD. The cognitive theory of multimedia learning. *In*: Irby BJ, Lara-Alecio R, *eds*. Handbook of educational theories. Charlotte: Information Age Publishing; 2012. p. 1-31.
17. Mayer RE. Multimedia Learning. 2nd ed. New York, NY: Cambridge University Press; 2009.
18. Hazari S. Applying instructional design theories to improve efficacy of technology-assisted presentations. Journal of Instruction Delivery Systems. 2004;18:24-33.



Web Fig. 1 *Examples of effective slides made using different multimedia instructional design principles.*



Web Fig. 2 *Effective slides made using multimedia instructional design principles.*

National Comprehensive Cancer Network Guidelines for Pediatric Acute Lymphoblastic Leukemia

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The National Comprehensive Cancer Network has recently published the first pediatric guidelines for the management of children, adolescent and young adults with acute lymphoblastic leukemia (ALL). The recommendations for diagnosis, work up, genetic evaluation, treatment and follow up of pediatric ALL have been provided. Genetic risk factors and newer therapeutic agents have been discussed. We highlight the major points in the guidelines.

Keywords: *Diagnosis, Management, Recommendations.*

Acute lymphoblastic leukemia (ALL) is the commonest pediatric malignancy representing 75-80% of the pediatric leukemia.The age-adjusted incidence rate in United States is 1.38 per 100,000 population per year [1]. Indian incidence varies with region and age-adjusted rates of up to 101.4 per million and 62.3 per million for boys and girls, respectively have been reported [2,3]. ALL is slightly more common in boys with peak incidence in 2-5 years. T cell ALL constitutes about 15-20% of pediatric ALL; though, in India, a higher proportion of T-ALL (20-50%) has been reported [3].

The survival rate of children with ALL has improved dramatically owing to better understanding of pathogenesis and molecular genetics, adoption of risk-stratified therapy and availability of newer therapeutic agents. Five-year overall survival (OS) rate of children has improved to 89%[4]. In India, OS has been estimated at 45-81% [5].Although the increasing survival rates give confidence, there was an unmet need for recommendations for a standard diagnostic and treatment approach in wake of the latest available evidence.

The National comprehensive cancer network (NCCN) has developed guidelines for pediatric ALLwith the goal of providing recommendations with focus on risk assessment, risk stratified therapy and supportive care [6]. These guidelines are intended to apply for the pediatric (up to 12 years of age) and adolescent and young adults (AYA) patients (up to 30 years of age). All recommendations are category 2A, unless specified.

DIAGNOSIS

Typical clinical features, bone marrow hemato-pathological examination and immunophenotyping are required to confirm the diagnosis and classify pediatric ALL. The NCCN guidelines give a cutoff of >20% blasts in bone marrow to diagnose ALL; although, most treatment guidelines require presence of >25% blasts for diagnosis. If there is significant amount of circulating disease, ≥20% lymphoblasts or 1000 circulating lymphoblasts/mm^3 in blood can establish the diagnosis. Immunophenotyping is essential, not only for diagnosis confirmation, but also to classify ALL into T cell (cCD3 or sCD3 positive) or B cell (CD19, CD22, CD79a positive), characterization of leukemic clones, and assessment of minimal residual disease (MRD) [7]. CD10 negativity correlates with presence of *KMT2A* mutations and portend poor prognosis. ETP ALL (CD1a/CD8 negative, variable CD5 positive and one or more myeloid markers positive) carries poor prognosis. Mixed phenotype acute leukemia (bi-lineage or bi-phenotypic) are defined as per WHO 2008 criteria [8].

Genetic Abnormalities and Molecular Subtypes

Genetic testing [karyotyping, fluorescent in situ hybridization *i.e*. (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR)] has become an essential component of leukemia characterization and is recommended in all patients to help in risk stratification, treatment planning and prognosis. The guidelines mention the seven recurrent genetic abnormalities for B ALL given by WHO in 2016, with two new additions in both B ALL (iAMP21, BCR-ABL like ALL) and T ALL(ETP ALL, NK cell ALL) [8]. The guideline recommends evaluation for the WHO defined recurrent genetic abnormalities, and if negative, for BCR-ABL1 and ETV-RUNX1, and

encourages evaluation for Ph like ALL as they may be responsive to tyrosine kinase inhibitor therapy.

Work-up

Clinical evaluation including testicular screening (clinical palpation, ultrasound testes only if clinically indicated) for all males and pregnancy screening for post-menarchal females; baseline hemogram, biochemical evaluation including tumor lysis syndrome panel, chest *X*-ray for mediastinal mass and appropriate imaging depending on symptomatology and signs are recommended. Both cerebrospinal fluid examination at the time of first intrathecal therapy and baseline echocardiogram are recommended. Assessment of the certain gene mutations (*e.g*. TPMT, NUDT 15) may be considered for adjusting the dose of thiopurines. Fertility counselling/preservation options should be presented to all patients. Clinical features of leukemia predisposition syndrome (*e.g*. Down syndrome), if present, should be confirmed by further tests as chemotherapy may need modification.

Risk Stratification

Risk stratification for B-ALL is done using clinical, biologic and response variables including patient age, white blood cell count (WBC), immunophenotypic/cytogenetic/ genetic subtype, presence of central nervous system (CNS) disease, and response to therapy (*i.e.* Day8/day15 peripheral smear and MRD at end of induction). This helps in tailoring therapy. T-ALL is considered high risk and none of above factors except MRD are found to be predictors of outcome. The present NCCN guideline mentions Children's oncology group (COG), St Jude Consortium approach and Dana Farber cancer institute (DFCI) ALL consortium approach for risk stratification.

TREATMENT STRATEGY

Specific treatment regimens (drugs, doses and durations) differ according to age group (pediatric, AYA and infant) and sub-type of ALL; however, basic principles of therapy remain same. Treatment consists of induction (to reduce tumor burden and eliminate maximum blasts from bone marrow), consolidation (further eradication of any residual disease), maintenance therapy (to prevent disease relapse) and extramedullary disease prophylaxis or treatment (to prevent CNS relapse and clear leukemic cells from sites which are not accessible by systemic chemotherapy due to blood brain barrier). The guideline recommends enrolling patients in clinical trials, whenever possible.

Treatment of Specific Groups

Ph negative and Ph – likeB-ALL: NCCN recommends pediatric and AYA patients with Ph negative or Ph – like should be grouped according to risk criteria and multi-agent induction be given.Patients who are MRD negative at end of induction (EOI) continue treatment on same risk arm. Patients, who are MRD positive at EOI are given intensified consolidation. In case of persistent MRD despite intensified chemotherapy, blinatumumab or tisagenlecleucel (Category 2B) can be considered. In all MRD positive cases at EOI, hematopoietic stem cell transplant (HSCT) may be considered as a part of consolidation or maintenance therapy.

Ph positive B-ALL: Pediatric and AYA patients with Ph positive disease should be treated with tyrosine kinase inhibitor containing protocols. Those who achieve MRD negative at EOI may continue the same protocol with tyrosine kinase inhibitor. For those who are MRD positive at EOI, blinatumumab or tisagenlecleucel (Category 2B) can be considered. The panel recommends HSCT for consolidation followed by post-transplant tyrosine kinase inhibitor.

T-ALL: The guideline recommends systemic chemotherapy. Those who have MRD >0.1% at end of consolidation should receive intensified chemotherapy to attain MRD negativity and thenceforth be considered for HSCT as consolidation therapy. Addition of nelrabine should be strongly considered in all patients with T cell ALL who are MRD positive or have CNS disease at diagnosis or those who fail induction.

Infant ALL: Infant ALL is to be treated with Interfant-based regimen that incorporates elements of ALL and AML therapy [9]. Assessment of baseline *KMT2A* status is essential. Infants without KMT2A rearrangement and MRD negative at EOI, continue to receive the Interfant consolidation, those who are MRD positive may receive intensified consolidation and HSCT may be considered. Those with KMT2 rearranged, are treated with intensive Interfant-based consolidation. High-risk patients (<3 months with any WBC, <6 months with WBC >3,00,000, persistent MRD after intensified consolidation) may receive maintenance therapy or may be considered for HSCT, non TBI regimen are preferable. For non-high-risk, KMT2 rearranged patients, usual maintenance therapy is recommended.

Extramedullary Disease Prophylaxis and Treatment

The guideline recommends inclusion of CNS prophylaxis or/and treatment in all regimens.Those who are CNS disease negative receive CNS-directed prophylactic therapy including intrathecal therapy and/or systemic chemotherapy that incorporates high dose methotrexate. CNS disease positive cases may receive cranial irradiation

(total recommended dose of 18 Gray) in addition as per the treatment protocol. Testicular involvement not resolved by the end of induction should be considered for bilateral testicular irradiation (total recommended dose of 24 Gray).

Hematopoietic Stem Cell Transplantation

HSCT has shown improved survival in pediatric ALL with evidence of certain high-risk features and persistent disease. In early relapse of Ph negative B-ALL, HSCT is the only known curative therapy. The benefit of allogenic HSCT is controversial in infants; only the subgroup with KMT2A rearrangement and high risk features showed survival advantage with HSCT over chemotherapy. When possible, HSCT should be done after eradication of MRD. Survival is comparable irrespective of stem cell source.

Use of Targeted Agents

Tyrosine kinase inhibitors are a standard part of treatment in Ph positive ALL. In Ph like ALL with CRLF2 or JAK mutations, Janus kinase inhibitors are being explored. Nelarabine has been approved for use in relapsed/ refractory T-ALL. Monoclonal antibodies to surface agents *e.g.* rituximab, epratuzumab, inotuzumab ozogamicin, blinatumomab; chimeric antigen receptors T cell targeting the CD19 (tisagenlecleucel) may be incorporated as part of induction, consolidation or maintenance therapy, especially in refractory/relapsed B-ALL.

Box I Criteria for Assessing Response at the End of Induction in Acute Lymphoblastic Leukemia

Complete remission (CR)

No circulating blast

No extramedullary disease

Bone marrow with trilineage hematopoiesis and <5% blasts on microscopy and <1% on flowcytometry

ANC >1000/mm^3, platelet counts >1,00,000/mm^3 and no recurrence in last 4 weeks

Complete remission with incomplete blood recovery (CRi): All criterion for CR except for ANC or/and platelet counts.

Refractory disease: Failure to achieve CR at end of induction.

Progressive disease: Increase of at least 25% in peripheral circulating or bone marrow blasts or development of extramedullary disease.

Relapsed disease: Reappearance of blasts in peripheral blood or bone marrow of >5% or >1% with molecular testing or appearance of extramedullary disease after CR.

Modified from reference 1.

Assessment of MRD

MRD has a high prognostic value in identifying patients at risk of relapse and hence needing an intensification of treatment. The optimal sample for the MRD assessment is first pull or early pull of bone marrow aspirate, done after completion of induction phase, with additional assessments at other points during therapy. MRD of greater than 0.01% in bone marrow is considered positive as assessed by flowcytometry method. Patients with MRD positivity at the EOI should receive more intensive chemotherapy and should be evaluated for HSCT.

Response Assessment

Response assessment is done during the therapy and treatment is tailored accordingly. These recommen-dations divide patients based on certain criteria into complete remission, complete remission with incomplete blood recovery, refractory and progressive diseasefor response assessment following therapy (***Box* I**).

Surveillance After Completion of Therapy

After completion of therapy, it is recommended that the patient should be periodically assessed for disease status (complete physical examination including testicular evaluation, complete blood count with differential count, liver function tests); every 1-4 monthly in first year, 3-6 monthly in second year, and 6-12 monthly in the third year. Monitoring for long term side effects of chemotherapy including weight and height monitoring, echocardiography for cardiotoxicity, neuro-psychological function and reproductive health monitoring are recommended, as previously published by COG [10].

Recommendations for Supportive Care

The recommendations for the supportive care are given in ***Box* II**.

Box II Recommendations for Supportive Care of Children and Young Adults with ALL

- Infection control
- Acute tumor lysis syndrome
- Hyperleukocytosis
- Drug toxicity management for methotrexate, vincristine, thiopurine, asparaginase and steroids
- Antiemesis
- Nutritional support
- Treatment of pain
- Transfusion of blood products/cytokine support for severe cytopenias

Modified from reference 6.

Contributors: SA: Conception, literature search and first draft; PKS: important intellectual inputs in the manuscript finalization. Both authors approved the final version.
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REFERENCES

1. National Cancer Institute. SEER cancer statistics review, 1975-2015: Leukemia, annual incidence rates (acute lymphocytic leukemia). 2018. Available at: *https://seer.cancer.gov/csr/1975_2015/.* Assessed February 15, 2020.
2. Asthana S, Labani S, Mehrana S, Bakhsi S. Incidence of childhood leukaemia and lymphoma in India. Pediatr HematolOncol J. 2018; ?:1-6.
3. Arora RS, Eden TO, Kapoor G. Epidemiology of childhood cancer in India. Ind J Cancer. 2009;46:264-73.
4. Pulte D, Gondos A, Brenner H. Improvement in survival in younger patients with acute lymphoblastic leukemia from the 1980s to the early 21st century. Blood. 2009;113: 1408-11.
5. Arora RS, Arora B. Acute leukemia in children: A review of the current Indian data. South Asian J Cancer. 2016;5: 155-60.
6. National Comprehensive Cancer Network. NCCN Guidelines Version 2.2020 Pediatric Acute Lymphoblastic Leukaemia 2019. Available from:*http.//www.nccn.org*. Accessed February 10, 2020.
7. Borowitz MJ, Bene MC, Harris NL, *et al*. Acute leukaemias of ambiguous lineage. *In*: Swerdlow SH, Campo E, Harris NL *et al*, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC; 2017:180-7.
8. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, *et al*. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391-405.
9. Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang YL, Pei D,*et al*. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371:1005-15.
10. Children's Oncology Group. COG LTFU guidelines. Version 5.0. October 2018. Long Term Follow up Guidelines for the Survivors of Childhood, Adolescent and Young Adult Cancers. Available from: http// www.survivorshipguidelines.org Accessed April 8, 2020.

2019 Update on Primary Immunodeficiency Disorders by the International Union of Immunological Societies

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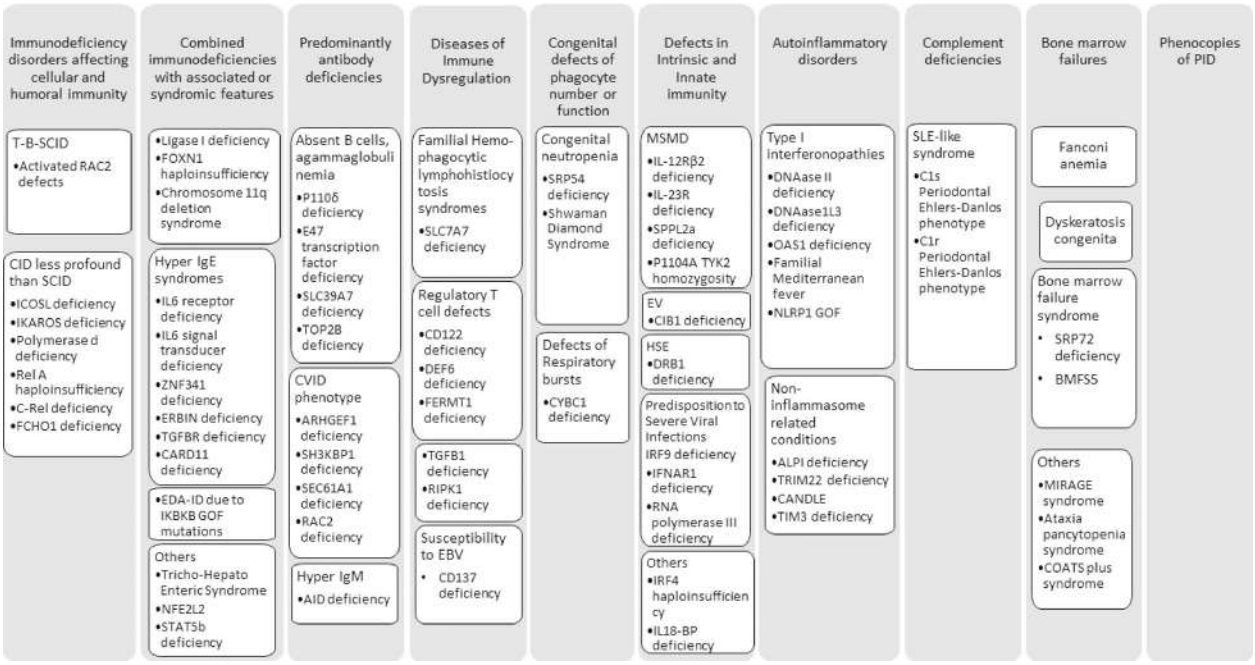
International Union of Immunological Societies working group recently updated the human inborn errors of immunity. This classification includes 65 new disorders that have been added since the last classification in 2018. This article highlights the important aspects of new classification for the benefit of general pediatricians.

Keywords: *Guidelines, Next Generation Sequencing,Inborn Errors of Immunity*

Primary immunodeficiency disorders, now known as Inborn errors of immunity, are a group of rare diseases affecting different arms of immune system. With the increased use of next generation sequencing, novel genes are being identified that have broadened our understanding of different clinical and immunological phenotypes. International Union of Immunological Societies have been updating the genetic causes for primary immunodeficiency disorders since 1970 [1]. Since 2013, the expert committee has started updating the phenotypic classification for the ease of practicing physicians [2]. In the last update, the inborn errors of immunity were classified in to nine categories, some of which were sub-classified in two categories based on severity of the disease [2]. The latest update has ten categories with a new category of bone marrow failures and 65 new disorders with total number of disorders reaching 430 (***Fig*.1**) [1,3]. Different modes of inheritance and distinct mechanisms like loss of function, gain of function, haploinsufficiency and dominant negative forms leading to different phenotypes are reported for 35 genes (***Fig*.2**).

In the category of Immunodeficiencies affecting cellular and humoral immunity, eight new genes are added. An autosomal dominant (AD) gain of function in *RAC2* gene causes recurrent bacterial and viral infections and may also be associated with neutropenia and lymphoproliferation. *ICOSL* deficiency patients have recurrent viral respiratory tract infections (RTI) with slowly progressive neutropenia and may have chronic diarrhea. *IKAROS* deficiency patients present with opportunistic infections like *P.jirovecii*, and agammaglobulinemia. These patients are at an increased risk to develop B cell ALL. Polymerase deficiency patients are of short stature and had recurrent respiratory tract and skin infections with molluscum contagiosum and viral warts. Rel A Haploinsufficiency patients have increased inflammatory cytokines signaling causing chronic mucocutaneous ulceration. c-Rel deficiency causes increased susceptibility to opportunistic organisms like Salmonella, Cryptobacterium, Cytomega-lovirus and Mycobacterium tuberculosis. FCHO1 deficient patients have failure to thrive, lympho-proliferation with predisposition to recurrent infections.

In Combined immunodeficiency with associated or syndromic features twelve new disorders have been added. LIG1 deficiency patients have growth retardation, increased sensitivity to radiation and sunlight with recurrent bacterial and viral infections. *FOXN1*-haploinsufficient patients also have recurrent bacterial and viral infections with eczema, dermatitis and nail dystrophy. Chromosome 11q23 deletion which causes Jacobsen syndrome have growth retardation, facial dysmorphism, warts and recurrent RTI. Seven disorders have been added with features like Hyper IgE syndrome due to mutations in *IL6R, IL6ST, ZNF341, ERBB2IP,TGFBR1,TGFBR2* and loss of function forms in *CARD11*. Recurrent bacterial, viral and fungal infections with ectodermal dysplasia is also seen due to gain of function mutations in *IKBKB*. Other syndromic defects are also listed (***Fig*. 1**). A dominant negative form of *STAT5b* deficiency with eczema and growth failure without immune defects like the autosomal recessive (AR) form is also added in this category.



Prepared from: SCID-Severe combined immunodeficiency disorder, CID-Combined immunodeficiency disorder, EDA ID-Anhidrotic ectodermodysplasia with immunodeficiency, CVID-Common variable immunodeficiency disorder, MSMD- Mendelian susceptibility to mycobacterial disease.

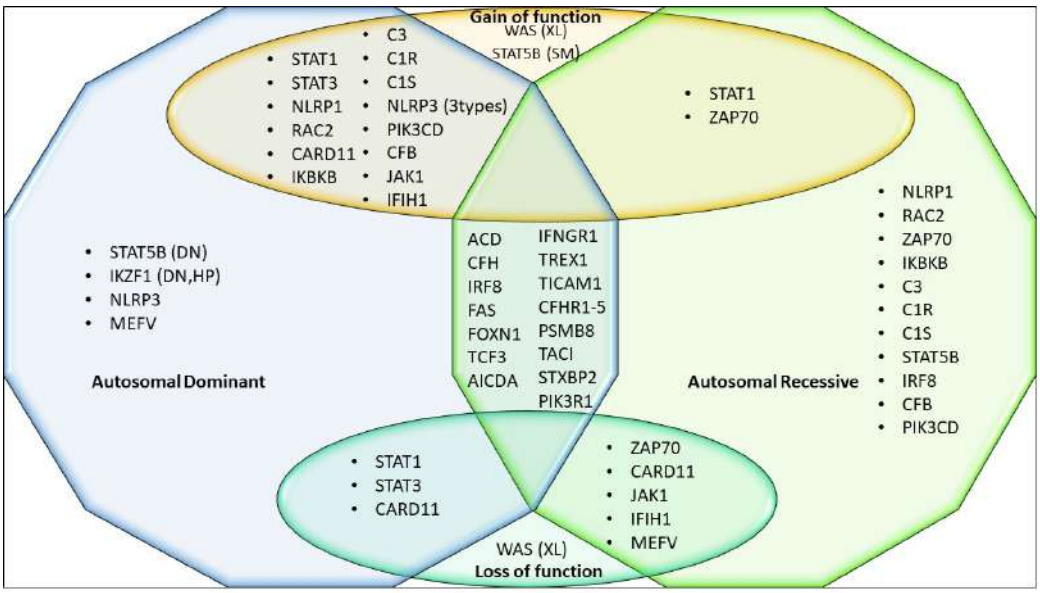
Fig. 1 *New disorders included in the 2019 update.*

In predominantly antibody deficiencies due to severe reduction in all serum immunoglobulin isotypes with profoundly decreased or absent B cells four disorders are added. p110δ deficient patient have severe bacterial infections and autoimmune complications like inflammatory bowel disease (IBD). AR forms of E47 transcription factor deficiency are more severe than the AD forms and present with recurrent bacterial infections and failure to thrive. *SLC39A7* (ZIP7) deficiency present at an early age with recurrent infections, blistering dermatosis and may have thrombocytopenia. Hoffman syndrome due to *TOP2B* deficiency present with recurrent infections with limb anomalies and facial dysmorphism. Four new disorders with Common variable immunodeficiency phenotype have been described (***Fig.* 1**). AD forms of *AID* deficiency causing Hyper IgM phenotype with lymphadenopathy and autoimmunity is also mentioned.

In diseases of immune dysregulation, one new disorder is added in familial hemophagocytic lymphohistiocytosis syndrome, *SLC7A7* defect leading to lysinuric protein intolerance which may be associated with pulmonary alveolar proteinosis and bleeding tendencies. Three new disorders with regulatory T cell (Treg) defects are CD122 deficiency with lymphoproliferation and autoimmunity, *DEF6* deficient patient had enteropathy, hepatosplenomegaly and cardiomyopathy and *FERMT1* deficient patients with severe dermatosis. *TGFB1* deficiency and *RPIK1* defects are new causes of immune dysregulation with colitis and CD137 deficiency a cause of increased susceptibility to Epstein-Barr virus and lymphoproliferation.

Two new disorders with congenital neutropenia are *SRP54* deficiency with exocrine pancreatic deficiency and neurodevelopmental delay and *ELF1* defect an additional cause for Shwachman-Diamond syndrome. A new form of functional phagocytic defect due to *CYBC1* deficiency causes inflammatory gastrointestinal symptoms with recurrent bacterial infections.

Mendelian susceptibility to mycobacterial disease involves three new genes namely *IL12RB2*, *IL23R* and *SPPL2A,* all of which predispose to mycobacterial and salmonella infections and a homozygous P1104A in *TYK2* gene which is associated with increased risk of tuberculosis. *CIB1* deficiency causes increased risk of epidermodysplasiaverruciformis (EV). *IRF9* deficient patients are at an increased risk of severe influenza infections. *IFNAR1* patients have severe disease after yellow fever and measles vaccination. Severe varicella



Prepared from: XL- X linked inheritance, SM- Somatic mutations, DN- Dominant negative, HP- Haploinsufficiency.

Fig. 2 *Genes with multiple modes of inheritance and mechanisms.*

zoster virus infections may be seen due to RNA polymerase III deficiency. *DBR1* deficiency can cause herpes simplex encephalitis (HSE) along with other viral infections of brain stem. *IRF4* haploinsufficiency is included under other inborn errors of immunity related to leukocytes presenting as Whipple disease.

Nine new disorders with Autoinflammatory phenotype are added in the update which include *DNASE2* deficiency with features likeAcardi-Goutieres Syndrome, *DNASE1L3* deficiency causing pediatric systemic lupus erythematosus, *OAS1* deficiency causing pulmonary alveolar proteinosis and skin rashes, an AD form of *MEFV* gene mostly M694del causing familial Mediterranean fever and *NLRP1* GOF variant causing juvenile onset recurrent respiratory papillomatosis, corneal scarring and palmoplantar carcinoma. Non-inflammasome related conditions included are *ALPI* and *TRIM22* deficiency causing IBD, mutations in *PSMG2* gene causing auto-immune hemolytic anemia, lipodystrophy and panniculitis with CANDLE like phenotype and T cell lymphoma subcutaneous panniculitis-like (*TIM3* deficiency).

In complement deficiency disorders GOF variants of *C1S* and *C1R* defects causing periodontal Ehlers-Danlos like phenotype causing hyperpigmentation and skin fragility are included in the new update.

To summarize, with frequent use of whole exome sequencing and whole genome sequencing the list of monogenic disorders will keep on increasing in times to come. With new genetic defects being identified,specific targeted therapies can be used increasingly like in patients with mutations in *JAK1, STAT1, STAT3, PIK3CD, DFE6, CTLA4, LRBA, IL12Râ2, IL23R AND IL18BP* genes [1].With clinical and immunophenotypic correlation of newer genes being identified, our understanding of immunology is bound to evolve.

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REFERENCES

1. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, *et al*. Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol. 2020;
2. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, *et al*. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. J Clin Immunol. 2018;38(1):96-128.
3. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, *et al*. Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. J Clin Immunol. 2020: 11; 1-16.

Point of Care Diagnosis of Anemia Using Portable Auto Analyzer

We describe a method for capillary blood sample-based point-of care testing of hemoglobin in population-based surveys using an automated analyzer system. The accuracy and precision of this method was comparable to hemoglobin estimated from venous blood sample (mean difference (SD) =0.2 (-2.77, 3.2), Pearson correlation coefficient, (0.969).

Keywords: *Accuracy, Hemoglobin, Venous blood.*

The Intensified National iron plus initiative (I-NIPI) guidelines of the *Anemia Mukt Bharat* aim at reducing anemia prevalence by 3% per annum [1]. One of the important strategies of the I-NIPI program includes screening and treatment of anemia among pregnant women (using invasive digital hemoglobinometers) and adolescent girls and boys (using non- invasive digital hemoglobinometers) [2]. The accuracy and reliability of hemoglobin estimates using these point of care devices have large variations depending on the collection technique, instrument used, and blood sampling methods (capillary *vs* venous) [3].

Automated hematology cell counters offer advantages like improved accuracy and precision, reduced subjective errors, efficiency with time, space and manpower, and safe handling of blood. However, the use of autoanalyzers is currently restricted to diagnostic laboratories due to relatively higher cost and need for use of venous blood samples [4].

Using capillary blood sample for hemoglobin estimation is advantageous for point of care diagnosis of anemia as it is less invasive, less painful and therefore more acceptable compared to venous blood samples. However, hemoglobin values using capillary samples are less accurate than venous samples [5]. Variability in sample quantity and quality based on skin thickness at puncture site and, the size and depth of incision make capillary sampling error prone. Moreover, variability of hemoglobin between consecutive blood drops has been demonstrated due to humidity, stability of reagents in cuvettes and inter-individual variation in the devices [6]. Standardized techniques that minimize inter-subject and inter-operator variation are required for both capillary blood sample collection and analytical measurements of hemoglobin. We, therefore, standardized a technique to collect capillary blood samples in microtainers for direct infusion in auto-analyzer for hemoglobin measurement. We also compared performance of different lancets for collection of capillary blood samples.

The present study was approved by the institutional ethics committee of our institution. Capillary blood samples were collected in 27 volunteers using one of the three finger-prick devices: i) Lancet A: traditional sterile lancet (Medipoint Blood lancet, NY, USA); ii) Lancet B: a pen needle device (Amkay blood lancet, Thane, India), and iii) Lancet C: contact activated lancet (Becton, Dickinson and company Ltd, Dublin, Ireland) (***Fig.* 1**). Venous sample was also collected in each participant following standard protocols [7].

Capillary blood samples were collected using protocol by Krleza, *et al*. [8]. The first drop was wiped off and 200 µL (about 4-6 drops) of free flowing sample was collected using K_2EDTA-microtainer (BD, Ltd, Dublin, Ireland) which was then mixed, and directly infused into an autoanalyzer (Horiba, Kyoto, Japan, approximate cost: Rs. 2.5 lakhs /unit) for hemoglobin measurement. Hemoglobin estimated using capillary and venous blood samples were compared using paired *t* test and Bland Altman plots.

Higher hematocrit values were observed in capillary samples compared to venous blood with all the lancets tested. The minimum difference was with lancet C (***Table* I**). The difference between capillary and venous samples in hematocrit and in hemoglobin were closely associated (r=0.9).

The touch activated lancet (lancet C) was advantageous compared to other two devices, as it reduced inter-individual variation in prick size and allowed effortless collection of 4-6 blood drops (about 200 µL in 20-25 seconds) into EDTA- microtainer tubes. This provided a capillary sample with characteristics closer to venous sample (***Table* I**). It caused less discomfort to participants, probably because the length of the incision device was not visible. The major difficulty with lancet A was related to the variable prick size which sometimes needed forced milking of the finger to collect adequate sample. The free flow of blood was achieved with lancet B; however, it took more than a minute for collecting the required volume of blood sample.

As the capillary blood sample collected with lancet C provided hemoglobin estimate closer to that using venous

Table I Comparison Between Hemoglobin Estimated Using Capillary Blood Sample and Venous Blood Sample

Lancets	*Hemoglobin (g/dL)*		
	Venous	*Capillary*	*MD (95% CI)*
Lancet A (*n*=9)	13.9 (2.7)	17.3 (2.57)	-3.5 (-12.4, 8.5)
Lancet B (*n*=9)	13.9 (1.08)	16.2 (2.94)	-2.3 (-6.5, 1.9)]
Lancet C(*n*=50)	10.7 (1.9)	10.5 (2.45)	0.2 (-2.77, 3.2)

**As the mean differences between capillary and venous sample were unacceptably high with lancets A and B, more samples were collected only with lancet C to assess the precision of the estimates; Pearson correlation coefficient (r) was –0.49, 0.84 and 0.97 for Lancet A, B and C, respectively; MD-Mean difference.*

blood sample, a large number of samples (*n*=50) were later collected in different age groups including young children in the age group 1-5 years (data not reported separately).

Capillary method of collection can overestimate hemoglobin due to higher hematocrit [4], which can be minimized by the collection method used in the present study. The (per unit) cost of lancet C was substantially higher (Rs.32) than the lancet A (Rs. 2) and lancet B (Rs. 3.50). Overall cost of analysis (about Rs 75 per sample) including, lancet C, microtainer and reagents was substantially higher than that with other commonly used PoC method like digital hemoglobinometer. However, considering the important advantages of the autoanalyzer and potential economy of scale when used in population based surveys and in screening programs for treating anemia, the benefits are likely to outweigh the cost.

Contributions: LA: analyzed and interpreted data, drafted the work; TR, RNP: designed the work, acquired data, revised the manuscript; RP, BK: conceptualized and designed the work, revised the manuscript critically for important intellectual content. All authors approved for the version to be published and agree to be accountable for all aspects of the work.

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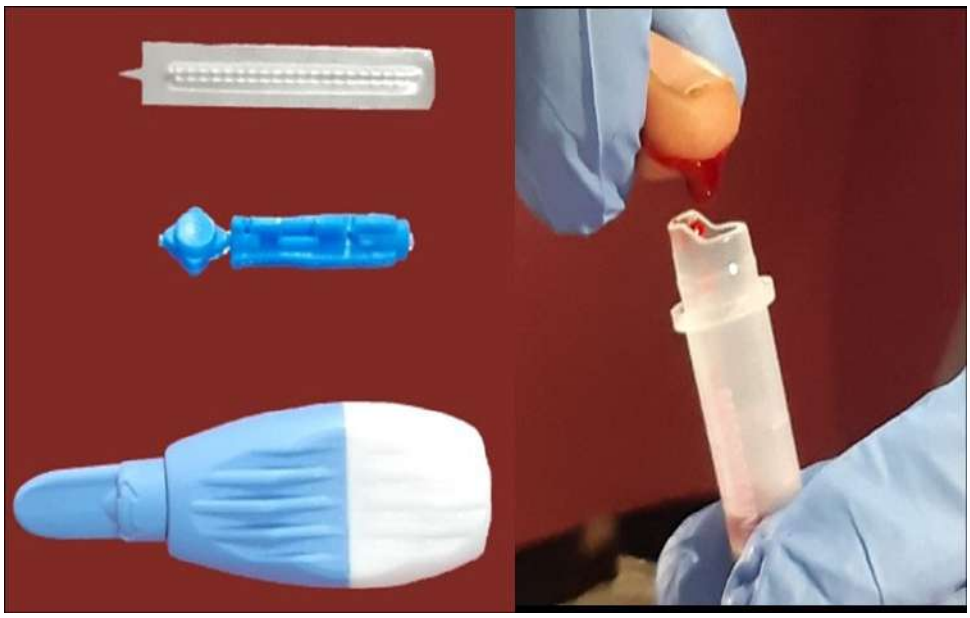
REFERENCES

1. Guidelines for control of iron deficiency anemia: National iron plus initiative. Ministry of Health and Family Welfare, Government of India 2013. Available from: *https://www.nhm.gov.in/images/pdf/programmes/child-health/guidelines/Control-of-Iron-Deficiency-Anaemia.pdf. Accessed October 15, 2019.*
2. Intensified National Iron Plus Initiative (I-NIPI): Operational guidelines for programme managers. Ministry of Health and Family Welfare, Government of India 2018. Available from: *https://www.fitterfly.com/site/pdf/anemia-mukt-bharat.pdf.* Accessed September 30, 2019.
3. Neufeld LM, Larson LM, Kurpad A, Mburu S, Martorell R, Brown KH. Hemoglobin concentration and anemia diagnosis in venous and capillary blood: biological basis and policy implications. Ann NY Acad Sci. 2019;1450: 172-89.
4. Chaudhary R, Dubey A, Sonker A. Techniques used for the screening of hemoglobin levels in blood donors: Current insights and future directions. J Blood Med. 2017; 8:75-88.
5. Tang R, Yang H, Choi JR, Gong Y, You M, Wen T, *et al*. Capillary blood for point-of-care testing. Crit Rev Cl Lab Sci. 2017;54:294-308.
6. Whitehead Jr RD, Mei Z, Mapango C, Jefferds ME. Methods and analyzers for hemoglobin measurement in clinical laboratories and field settings. Ann NY Acad Sci. 2019;1450:147-71.
7. World Health Organization. WHO guidelines on drawing blood: best practices in phlebotomy 2010. Available from: *https://www.who.int/infection-prevention/publications/drawing_blood_best/en/.* Accessed October 15, 2019.
8. Krleza LJ, Dorotic A, Grzunov A, Maradin M. Capillary blood sampling: National recommendations on behalf of the Croatian society of medical biochemistry and laboratory medicine. Biochemia Medica. 2015;25:335-58.



Web Fig. **1** *Lancet devices and microtainer; Lancet a: traditional; Lancet b: pen needle device; and lancet c: contact activated.*

GATA 2 Haploinsufficiency in Acute Myeloid Leukemia: Looking Beyond the Obvious

Infections encountered during treatment of leukemias are attributed to neutropenia and chemotherapy-induced immunodeficiency. Genetic disorders that predispose both to malignancies and infections have been recently recognized [1]. We describe a patient with GATA-2 deficiency who was evaluated after a diagnosis of acute myeloid leukemia and repeated infections.

A14-year-old boy presented with progressive anemia, macrocytosis (mean corpuscular volume 102fl), normal leucocyte and platelet count, and peripheral smear suggestive of megaloblastic anemia. He was the only child born to a non-consanguineous couple with no family history of malignancy or severe infections. Clinical examination revealed anemia and growth retardation [height 140 cm and weight 29 kg (both <5th centile)]. There was no fever, lymphadenopathy or hepatosplenomegaly. He was previously well except for episodes of diarrhea which were managed symptomatically. Bone marrow aspiration revealed 31% myeloblasts, which on immunophenotyping were positive for CD13, CD33, CD117, CD34 and negative for T and B lineages. Monosomy 7 was observed in 76% blasts by fluorescent in-situ hybridization. With a diagnosis of high-risk acute myeloid leukemia (AML), he was started on induction therapy (cytarabine, daunorubicin and etoposide). He achieved remission after first cycle following which he received another induction and 3 cycles of cytarabine-based consolidation. He tolerated chemotherapy without any unusual side-effects. Bone marrow transplantation was advised considering high risk AML; however, parents deferred the same.

At initial evaluation for AML, he was noted to have black warts along the hairline of forehead. According to the family the flat warts had been present for the last few years and would shed off occasionally. 6 months after completion of chemotherapy, he started developing episodes of protracted diarrhea. Stool was positive for *Cryptosporidium parvum* which was managed with nitazoxanide. These episodes of diarrhea persisted with weight loss to 25.2 kg. Progressive lymphopenia was noted during this period. Bone marrow and CSF were repeated and continued remission of AML was confirmed. HIV was ruled out. Serum immunoglobulins were normal. Recurrent infections with monosomy 7 associated AML was clinically suggestive of GATA 2 deficiency. Absolute lymphocyte count was done which was 735 cells/μL with 99.8% CD3 cells (733 cells/μL, normal 684-2170 cells/μL).

Next generation sequencing for cancer predisposition genes was performed; a frameshift deletion (chr3: 128205727: TG>T; c.147del) resulting in amino acid change and subsequent termination of the protein, 31 amino acids downstream to codon 49 (p. Phe49LeufsTer31) was detected in the *GATA 2* gene. This is a loss of function mutation which is not reported in ExAC and 1000 genomes databases. The nature of disease and prognosis was explained to parents and bone marrow transplantation was again advised. Upper and lower gastrointestinal endoscopy was done which was normal; thus ruling out mycobacterial infections and colitis which are reported to cause diarrhea. Fourteen months after AML treatment, he developed bilateral lobar consolidation with fatal pulmonary hemorrhage despite aggressive antimicrobial support. CT scan and bronchoalveolar lavage to distinguish between infection and pulmonary alveolar proteinosis although planned, was not performed as the child died within 12 hours of hospitalization.

The GATA (Guanine-Adenine-Thymine-Adenine) family is comprised of six zinc finger transcription factors of which *GATA 2* is vital for the proliferation of hematopoietic stem cells [2]. The gene is located on chromosome 3q21 and >100 heterozygous germline mutations are now known to result in *GATA 2* deficiency or haploinsufficiency. Frameshift or null mutations that abolish *GATA 2* activity tend to present early in life as in our case compared to missense mutations that reduce transcriptional activity [2]. This disorder has protean disease manifestations such as cytopenias (neutropenia, monocytopenia, B and NK lymphopenia, aplastic anemia), myelodysplastic syndrome, acute myeloid leukemia, infections including human papilloma virus, atypical mycobacterial infections and pulmonary alveolar proteinosis [1]. The syndrome complex has been described independently as MonoMAC (monocytopenia

with Mycobacterium Avium Complex), DCML (dendritic cell monocyte and lymphoid deficiency) and Emberger syndrome (sensorineural deafness, congenital lymphedema and viral warts). This is a sporadic entity with autosomal dominant inheritance. Germline *GATA 2* mutations are the most common defect predisposing to pediatric myelodysplastic syndrome with a high prevalence of monosomy 7 thus mandating its evaluation in every case of monosomy 7 associated MDS [3]. Allogeneic hematopoietic stem cell transplantation is the only curative option for both immunodeficiency as well as MDS/AML [4-6].

Evaluating the cause of repeated infections without dismissing them as related to malignancy/chemotherapy has helped us in reaching a diagnosis. Identification of such genetic predisposition not only helps us manage our patients better, but also has implications for donor search for bone marrow transplantation and genetic counseling for the family.

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REFERENCES

1. Mir MA, Kochuparambil ST, Abraham RS, Rodriguez V, Howard M, Hsu AP, *et al*. Spectrum of myeloid neoplasms and immune deficiency associated with germline GATA2 mutations. Cancer Med. 2015;4:490-9.
2. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, *et al*. GATA2 deficiency: A protean disorder of hematopoiesis, lymphatics, and immunity. Blood. 2014; 123:809-21.
3. Wlodarski MW, Hirabayashi S, Pastor V, Starý J, Hasle H, Masetti R, *et al*. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. Blood. 2016;127:1387-97.
4. Collin M, Dickinson R, Bigley V. Haematopoietic and immune defects associated with GATA2 mutation. Br J Haematol. 2015;169:173-87.
5. Rastogi N, Abraham RS, Chadha R, Thakkar D, Kohli S, Nivargi S, *et al*. Successful nonmyeloablative allogeneic stem cell transplant in a child with emberger syndrome and GATA2 mutation. J Pediatr Hematol Oncol. 2018;40: e383-8.
6. Vinh DC, Patel SY, Uzel G, Anderson VL, Freeman AF, Olivier KN. Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. Blood. 2010;115: 1519-29.

Acquired Recto-Vaginal Fistula as a Presenting Feature in an Infant with Severe Combined Immunodeficiency

Acquired recto-vaginal fistula in infancy is a rare entity. We report a child with Severe Combined Immunodeficiency (SCID) who presented to us with an acquired recto-vaginal fistula.

A 5½-month-old girl, firstborn of a non-consanguineous marriage presented with constipation for one month and passing stools per vaginum for three days. She was born at full term at our centre, with a birthweight of 3100 grams, to HIV negative parents. Complete physical examination, including the perineum was noted to be normal at birth. She had been hospitalised for culture-negative sepsis without meningitis at day 21 of life and uncomplicated dengue fever at three months of age. She was on exclusive breastfeeding and had received BCG, OPV, DPT, and Hepatitis B vaccines with no reactions. There was no family history of immunodeficiency disorders or unexplained infant deaths. There was no history of trauma, surgery or suspicion of abuse. Her weight was 6.4 kg (0 z score), length was 59 cm (0 to -2 z score). She had a typical BCG scar. Examination of the perineum revealed a fistulous opening next to the vaginal orifice at the nine o'clock position, suggestive of a recto-vaginal fistula and there was no obvious evidence of local infection. Systemic examination was otherwise unremarkable.

Hemogram showed a normal absolute lymphocyte count (ALC) of 7385 cells/cu.mm. She underwent a diversion colostomy, and per-operatively was found to have a fistulous tract extending from the vagina to the rectum by gentle probing. There was no gross evidence of

inflammation or infection. On post-surgery day 3, she developed a new-onset fever along with multiple nodular lesions all over the body and received intravenous piperacillin-tazobactam and vancomycin for presumed bacterial sepsis. On day 10, she developed a burst abdomen and underwent a surgical closure. She was suspected to have an underlying predisposing condition like primary immunodeficiency or inflammatory bowel disease. Biopsy of the skin nodule showed panniculitis suggestive of erythema nodosum. Xpert MTB/RIF assay of the tissue was positive for *Mycobacterium tuberculosis* (MTB) complex with no rifampicin resistance. HIV ELISA and NBT (Nitro blue tetrazolium) test were negative. Flow cytometry was suggestive of T-B+NK- type of SCID [$CD4^+$ T cell count - 43 cells × 10^9/l (9700 - 2200 cells × 10^9/l), $CD19^+$ B cell count- 2062 cells × 10^9/l (390-1400 cells × 10^9/l) and $CD3^-/CD16^+$ $CD56^+$ NK cell count-41 cells × 10^9/l (130-720 cells × 10^9/l)]. Her serum immunoglobulin levels were deficient [IgG -2348 (4669-10673) µmol/l, IgM-77 (41-237) µmol/l, IgA-176 (438-2500) µmol/l, IgE- 10.9 (0.96-842)µg/l]. Whole exome sequencing revealed a homozygous *JAK 3 kinase* mutation. Ophthalmology examination and echocardiography were normal.

She developed severe progressive pneumonia requiring ventilator support and succumbed despite treatment with anti-tuberculous drugs, antifungals, antibiotics, and co-trimoxazole for a presumed *Pneumocystis* pneumonia. Bacterial, fungal, and mycobacterial cultures of the biopsy tissue, quantitative CMV DNA PCR in blood and *Pneumocystis jirovecii* staining of the endotracheal aspirate were negative. Parents were counselled to undergo genetic screening and prenatal testing for subsequent pregnancies.

Recto-vaginal fistula, a rare type of anorectal malformation with abnormal epithelial lined connections between the rectum and vagina, has an incidence of less than 1% in children [1]. They can be congenital or acquired. Acquired causes include trauma or infections in the recto-vaginal septum such as anorectal abscesses and Bartholin gland infections, tuberculosis and lymphogranuloma venereum [2]. Rarer causes include malignancies, inflammatory bowel disease, post-radiation therapy, and operative traumas. Acquired recto-vaginal fistulas have been reported to occur in infants with HIV from Africa, representing an early manifestation of the disease [3-5]. The postulated pathogenesis is probably, secondary to a low grade and indolent perianal infection [3]. In the previous case series in HIV-positive girls, the fistulae were small and extending anteriorly from the rectum to distal vagina [3]. Most of these children had evidence of tuberculosis or lymphocytic interstitial pneumonia in the lung [3]. However, rectal fistulae in boys with HIV have only been reported rarely [6]. Surgical closure is usually unsuccessful because of poor wound healing [4,6].

SCID is a medical emergency as hematopoietic stem cell transplantation can prevent death in children before infections occur. Unusual presentations like acquired recto-vaginal fistula should be kept in mind to expedite diagnosis and management.

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REFERENCES

1. Tiwari C, Shah H, Bothra J, Sandlas G. Congenital rectovaginal fistula with anorectal agenesis: A rare anorectal malformation. Int J Pediatr Adolesc Med. 2017;4:138-40.
2. Debeche-Adams TH, Bohl JL. Rectovaginal fistulas. Clin Colon Rectal Surg. 2010;23:99-103.
3. Borgstein ES, Broadhead RL. Acquired rectovaginal fistula. Arch Dis Child. 1994;71:165-6.
4. Hyde GA Jr, Sarbah S. Acquired rectovaginal fistula in human immunodeficiency virus-positive children. Pediatrics. 1994;94:940-1.
5. Osifo OD, Egwaikhide EA. Acquired rectovaginal fistula in babies of unknown and asymptomatic retroviral positive mothers. J Pediatr Adolesc Gynaecol . 2011;24:e79-82.
6. Wiersma R. HIV positive African children with rectal fistulae. J Pediatr Surg. 2003;38:62-4.

Acrodermatitis Enteropathica as a Presentation of Cystic Fibrosis in an Infant

Cystic Fibrosis (CF) in an autosomal recessive disease due to mutation of *CFTR* gene on chromosome 7 which is involved in ionic transport across membranes. Children with CF commonly present with recurrent or persistent respiratory infections and exocrine pancreatic insufficiency, however, the clinical presentation can be varied.

A seven-month-old girl was referred with non-healing skin lesions and failure to thrive. She was born to third degree consanguineous parents with uneventful perinatal period. She was asymptomatic with normal weight gain till 4 months of age when she developed perianal maculo-papular rashes which failed to improve with topical steroids. Her weight started to plateau. There were no respiratory infections. Acrodermatitis enteropathica was suspected at 7 months of age. Serum zinc levels were low (46 µg/dL). Her length and weight were below the 3rd centile for age as per WHO growth charts. She was irritable and had sparse hair. There were no dysmorphic features or congenital anomalies. She had extensive maculo-papular erythematous rashes, especially in the perianal region with pitting pedal edema. During examination, the baby passed stools which were oily. A detailed family history revealed two siblings deaths in early infancy with recurrent respiratory symptoms. Investigations indicated hemoglobin 8.3 g/dL, aspartate aminotransferase 156 U/L, alanine aminotransferase 52 U/L, and serum albumin 2.1 g/dL. She was started on oral zinc supplementation and protein rich diet. CF was suspected as the child had oily stools, transaminitis and sibling deaths with recurrent respiratory symptoms. She was started on fat soluble vitamin supplementation with pancreatic lipase. Genetic analysis showed homozygous mutation on exon 14 of the *CFTR* gene (p.Arg709Ter), which was deemed pathogenic for cystic fibrosis. After three months, her rashes had disappeared and weight was between the 3rd-10th centiles.

Acrodermatits enteropathica is a rare autosomal recessive disorder of zinc absorption. Affected infants usually present with erythematous maculo-papular rash typically in the perianal region, diarrhoea and alopecia. The presentation usually coincides with initiation of complementary feeding. Other symptoms include irritability, conjunctivitis and nail changes. Acrodermatitis enteropathica has been described as a presenting feature of CF secondary to zinc malabsorption with pancreatic insufficiency. Hypoalbuminemia is likely to further exacerbate zinc deficiency. Most children with CF present with recurrent respiratory infections and steatorrhea. The index infant presented with FTT, oily stools as well as a family history suggestive of CF. The diagnosis of CF is more frequently considered in Indian children with increasing access to genetic testing. Delta 508, which is the commonest mutation associated with CF in the Caucasian population, is seldom found in Indian children. The index patient had a homozygous nonsense variant at exome 14 which results in a stop codon and premature truncation of protein at codon 709.

In conclusion, CF is associated with a wide range of presenting features which includes acrodermatitis enteropathica. A high index of suspicion, detailed history (including family history) and a thorough clinical examination will aid in directing investigations towards confirmation of the diagnosis. Genetic testing is often required for confirming diagnosis in infants with unusual presentation

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REFERENCE

1. Zedek D, Morrel DS, Graham M, Goodman D, Groben P. Acrodermatits enteropathica like eruptionsand failure to thrive as presenting signs of cystic fibrosis. J Am Acad Dermatol. 2008;58;S5-8.
2. Mei-Zahav M, Solomon M, Kawamura A, Coates A, Durie P. Cystic Fibrosis presenting as kwashiokor in a Sri Lankan infant. Arch Dis Child. 2003;88:724-5.
3. Estivill X, Bancells C, Ramos C, Biomed CF. Mutation analysis consortium geographic distribution and regional origin of 272 cystic fibrosis mutations in European Populations. Hum Mutat. 1997;10:135-54.

A Novel Mutation in *G6PC3* Gene Associated Non-syndromic Severe Congenital Neutropenia

Severe congenital neutropenia (SCN) characterized by congenital neutropenia and maturation arrest in myeloid series is an orphan disorder with estimated prevalence of 6 per million [1]. Homozygous or compound heterozygous mutations in *G6PC3* gene, which encodes glucose-6-phosphatase enzyme, constitute around 2% of cases of SCN (MIM 612541) [2,3]. Till date, around 60 patients of this deficiency have been described in literature [4]. Majority of G6PC3 deficiency are syndromic and associated with prominent superficial veins, cardiac, genitourinary and renal disorders. Dursun syndrome has additional features involving non-myeloid hematopoietic cell lines, extra-hematologic features and pulmonary hypertension [1]. Recently, a non-syndromic phenotype has been described [4,5]. We describe a novel mutation associated with non-syndromic G6PC3 deficiency.

A five-month-old girl of southern Indian ethnicity, second born of second degree consanguineous marriage (birth weight 2600 gm) presented with multiple episodes of septicemia, pneumonia, acute gastroenteritis, and otitis media. She had a BCG scar and was developmentally normal. On examination, anthropometry revealed severe wasting (weight 4 kg, length 63 cms). There was no dysmorphism and hepato-splenomegaly.

Her hemoglobin records ranged from 8.5 to 10 g/dL with normal mean corpuscular volume, persistent neutropenia with absolute neutrophil count (ANC) between 100-1500/cmm and intermittent thrombocytopenia (nadir platelet count 1,10,000/cmm). Bone marrow examination revealed cellular marrow with maturation arrest at myelocyte stage; erythrocytes and megakaryocytes were normal. In view of recurrent infections with pancytopenia, differential diagnoses considered were primary immunodeficiency, inherited bone marrow failure syndrome and autoimmune neutropenia. Immunoglobulin profile was normal [IgG 889 (176-581) mg/dL, IgM 95 (24-102) mg/dL, IgA 99 (4-58) mg/dL]. HIV-ELISA and direct Coombs test were negative. Chromosome 22qdel by FISH was negative. T and NK cell subsets, CH50 screening test for complement pathway defect, dihydrorhodamine test were normal. Immunophenotyping of thymic function and anti-neutrophilic antibodies for auto-immune neutropenia were unavailable. Anti-tissue transglutaminase IgA and TORCH titres were normal. Chromosomal breakage studies and stool for fat globules were negative. Ultrasound was not suggestive of pancreatic fibrosis or renal anomaly. Targeted clinical exome sequencing by next generation sequencing was sent for congenital neutropenia. A homozygous single base pair deletion in exon 3 of the *G6PC3* gene variant c.372delC that results in a frameshift and premature truncation of the protein at codon 125 was detected. Echocardiography did not reveal any structural heart disease or pulmonary hypertension. Brainstem evoked response audiometry suggested mild hearing loss.

She was managed with antibiotic, antiviral and antifungal prophylaxis with granulocyte-colony stimulating factor (G-CSF) at 5µg/kg/day. After G-CSF, ANC improved to >1500/cmm without recurrence of any severe infection in next 6 months. Parents were counseled regarding the disease and were advised to avoid live vaccines. There was no donor available for bone marrow transplant. A trial off G-CSF was attempted at one year age. Parents were advised to use G-CSF if ANC fell below 500/cmm. At two years of age, ANC was between 1000-2000/cmm with no reported severe bacterial infections. She had normal development and growth, with weight and height presently between 10-25th centile. She did not require any further courses of G-CSF.

G6PC3 gene maps to 17q21.31, consists of six exons and encodes the G6PC3 protein. Homozygous missense mutations leading to frameshifts are described in four out of six reported non- syndromic cases. The largest number of missense mutations have been described in exon 6 [5]. Index child had deletion mutation in exon 3 which is a novel mutation in *G6PC3* responsible for SCN. *G6PC3* mutation causes G6PC3 deficiency affecting Glucose-6-phoshatase enzyme activity resulting in increased apoptosis of neutrophils leading to maturation arrest, subsequent neutropenia and diminished respiratory burst thereby accounting for both quantitative and qualitative defect in neutrophils [4]. A phenotypic heterogeneity and founder effect in Pakistani ethnicity has recently been described [4,5].

The typical hematological features described are severe neutropenia starting in early infancy and maturation arrest in myeloid lineage or myelokathexis. Dysfunctional neutrophilic activity, anemia, lymphopenia and intermittent thrombocytopenia are additional hematologic findings.

So far only six non-syndromic cases have been described in literature [1,4,5]. Index child did not have any of the above syndromic features. It remains unclear whether growth failure was primary or secondary to

recurrent infections. Similarly, sensorineural hearing loss is described in just three cases till date and has not been shown to be syndromically associated [4]. In the index child it may be attributed to recurrent ear infections. A prominent superficial venous pattern described in around two-third of the patient may not be present in infancy and becomes prominent with age. Non-syndromic presentation of G6PC3 deficiency should thus always be thought of in a child with isolated congenital neutropenia [1].

The genetic diagnosis is important in SCN as phenotypic variability exists; wherein most severe cases may require early bone marrow transplant (BMT), while rest may require G-CSF and infection prophylaxis awaiting a definitive BMT. Chronic G-CSF therapy has been inconsistently reported to be associated with myelodysplastic syndrome/myeloid leukemia and may not correct functional deficiency of neutrophil despite reversing neutropenia to a safer limit [6]. The use of G-CSF without clinical benefit should be avoided.

To conclude, SCN-4 should be considered in differential diagnosis of early onset severe neutropenia and indiscriminate use of G-CSF should be curtailed unless clinically indicated.

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REFERENCES

1. Donadieu J, Fenneteau O, Beaupain B, Mahlaoui N, Chantelot CB. Congenital neutropenia: Diagnosis, molecular bases and patient management. Orphanet J Rare Dis. 2011;6:26.
2. Boztug K, Appaswamy G, Ashikov A, Schäffer AA, Salzer U, Diestelhorst J, *et al*. A syndrome with congenital neutropenia and mutations in G6PC3. N Engl J Med. 2009;360:32-43.
3. Xia J, Bolyard AA, Rodger E, Stein S, Aprikyan AA, Dale DC, *et al*. Prevalence of mutations in ELANE, GFI1, HAX1, SBDS, WAS and G6PC3 in patients with severe congenital neutropenia. Br J Haematol. 2009;147:535-42.
4. Banka S, Wynn R, Byers H, Arkwright PD, Newman WG. G6PC3 mutations cause non-syndromic severe congenital neutropenia. Mol Genet Metab. 2013;108:138-41.
5. Banka S, Chervinsky E, Newman WG, Crow YJ, Yeganeh S, Yacobovich J, *et al*. Further delineation of the phenotype of severe congenital neutropenia type 4 due to mutations in G6PC3. Eur J Hum Genet. 2011;19:18-22.
6. Rosenberg PS, Alter BP, Bolyard AA, Bonilla MA, Boxer LA, Cham B, *et al*. The incidence of leukemia and mortality from sepsis in patients with severe congenital neutropenia receiving long-term G-CSF therapy. Blood. 2006; 107:4628-35.

An Untold Tale of Iron Deficiency Anemia

In India the prevalence of Iron deficiency anemia in children (6-59 months) is reported as 56% [1], most common by due to nutritional iron deficiency. Iron refractory iron deficiency anemia (IRIDA) is a rare genetic condition with autosomal recessive inheritance caused by mutation in *TMPRSS6* gene, located on chromosome 22 [2]. Suspicion of IRIDA usually occurs during pediatric age group when a child with iron deficiency anemia does not respond to standard therapy and has some suggestive markers of IRIDA.

The proband, a 9-year-old boy born of a consanguineous marriage, was diagnosed to have iron deficiency anemia at one year of age and treated with various iron preparation with inadequate response. Growth and development was adequate for the age. There was no icterus, bleeding manifestations, lymphadenopathy or hepatosplenomegaly. Blood examination revealed microcytic hypochromic anemia suggestive of iron deficiency anemia. His hemoglobin was 7g/dL; RBC count 5.4 × 10^9 L; mean corpuscular volume (MCV) 52 L, mean corpuscular hemoglobin (MCH), 15 pg/mL; mean corpuscular hemoglobin concentration (MCHC) 25.8%; red cell differential width (RDW) 19.6; serum iron, 1.2 µg/dL; Serum ferritin, 32 ng/mL; total iron binding capacity (TIBC) 352 µg/dL; and transfeerin saturation, 2.7%. Iron deficiency could not be explained from his diet, gastrointestinal losses or other symptoms. He was prescribed standard therapeutic doses of oral iron (6 mg/kg); however, response was not satisfactory (increase in hemoglobin Hb <0.5g in initial 4 weeks) despite adequate compliance. Further investigations revealed normal hemoglobin electro-phoresis and stool occult blood was tested negative. His younger brother was also detected to have anemia at 9 months of age and was on iron preparation with inadequate response.

Presently he is four years and clinical features and evaluation is suggestive of iron deficiency anemia. He was also started on oral iron preparation (6 mg/kg) but response was unsatisfactory despite adequate compliance (increase in hemoglobin <0.4g initial 4 weeks). In view of third degree consanguinity, similarly affected sibling, poor response to oral iron and no other causes of iron deficiency, we considered the possibility of IRIDA for both siblings.

Sequencing of the *TMPRSS6* gene in elder sibling showed a homozygous missense variation in exon 9 (chr22:g.37480810G>A) that resulted in the amino acid substitution of Leucine for Proline at codon 357 (p.Pro357Leu; ENST00000346753.3). However, hepcidin assay could not be done because of lack of availability of standard hepcidin assay.

IRIDA is characterized by microcytic hypochromic anemia with a very low mean corpuscular volume, low transferrin saturation (<5%), no or inadequate response to oral iron, and only a partial response to parenteral iron. In contrast to classic iron deficiency anemia, serum ferritin levels are usually low to normal, RBC count will be normal to high and serum or urinary hepcidin levels are inappropriately high for the degree of anemia [2]. In our case, the phenotype had all this features and laboratory evaluation was suggestive with. The family history of consanguinity and similar phenotype in other siblings were other features pointing to the diagnosis.

IRIDA should be differentiated from acquired iron deficiency anemia and other genetic microcytic anemias. Family history of consanguinity and affected sibling are pointers to hereditary condition. Acquired iron deficiency and IRIDA are rare in neonatal period and microcytosis at birth is suggestive of other genetic conditions like DNMT1 mutations or atransferrinemia. IRIDA can be differentiated from acquired iron deficiency anemia with increase RBC Count and normal/increased serum ferritin.Beta thalassemia carriers have similar picture, but can be differentiated by normal RDW, High RBC Count, slightly elevated iron parameters and elevated Hb A2 more than 3.5%. IRIDA will have a normal to increased hepcidin in contrast to acquired iron deficiency anemia where hepcidin is low. Sideroblastic anemia can also occur in childhood, but usually associated with markers of iron overload.

IRIDA results from germline mutations in *TMPRSS6*, encoding MT-2 [3]. Different mutations have been identified in *TMPRSS6* gene, with mutation analysis in the Indian population also showing mutational heterogeneity with both intronic and exonic mutations [4]. MT-2 is a type II transmembrane serine protease which plays an essential role in downregulating hepcidin expression in liver cells. In iron deficiency hepcidin levels are reduced, whereas in IRIDA, the levels are normal or high [5].

In Indian population,38% of children with refractory anaemia had a mutation in the TMPSS6 gene suggestive of IRIDA, which shows that this condition is grossly under-disgnosed [4]. Most patients of IRIDA will be refractory to oral iron therapy and intravenous iron will be needed for the correction. Initial trial of oral ferrous sulphate along with Vitamin C (30 mg/kg) for 6-8 weeks may l be beneficial in some patients, before intravenous iron treatment [6].

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REFERENCES

1. Bharati S, Pal M, Bharati P. Prevalence of anaemia among 6- to 59-month-old children in India: The latest picture through the NFHS-4. J Biosoc Sci. 2020;52:97-107.
2. Finberg KE. Iron-refractory iron deficiency anemia. Semin Hematol. 2009;46:378-86.
3. Finberg KE, Heeney MM, Campagna DR, Aydinok Y, Pearson HA, Hartman KR. *et al*. Mutations in *TMPRSS6* cause iron-refractory iron deficiency anemia (IRIDA) Nat Genet. 2008;40:569-71.
4. Bhatia P, Singh A, Hegde A, Jain R, Bansal D. Systematic evaluation of paediatric cohort with iron refractory iron deficiency anaemia (IRIDA) phenotype reveals multiple *TMPRSS6* gene variations. Br J Haematol. 2017;177: 311-8.
5. Melis MA, Cau M, Congiu R, Sole G, Barella S, Cao A, *et al*. A mutation in the *TMPRSS6* gene, encoding a transmembrane serine protease that suppresses hepcidin production, in familial iron deficiency anemia refractory to oral iron. Haematologica. 2008;93:1473-9.
6. Cau M, Galanello R, Giagu N, Melis MA. Responsiveness to oral iron and ascorbic acid in a patient with IRIDA. Blood Cells Mol Dis. 2012;48:121-3.

COVID-19 in a Young Girl with Restrictive Cardiomyopathy and Chronic Lung Disease

Coronavirus disease (COVID-19) pandemic has resulted in severe infection with thousands of deaths among the elderly; however, very few pediatric cases need hospitalization and/or intensive care.

A 7-year-old female (weight 17 kg), who was under our follow-up for the last one year with a diagnosis of cardiomyopathy and chronic lung disease (CLD), was admitted to the emergency unit with the complaints of chest pain, dyspnea and fatigue. She had no fever, cough, vomiting, myalgia or gastrointestinal symptoms. There was no exposure to a person infected with COVID-19 in her family. The body temperature was 36.5 °C, heart rate was 110 beats/minute, respiration rate was 24/minute and blood pressure was 90/60 mm/Hg on admission. She had bilateral rales, more prominent on the right side, and grade II systolic murmur on auscultation. The liver was 3-4 cm palpable below the right lower costal margin. She had no peripheric edema and her cutaneous oxygen saturation was 85%. The chest *X*-ray showed infiltrations on the right middle and lower pulmonary zones and massive cardiomegaly (***Fig.* 1**). Electrocardiogram showed sinus tachycardia and tall and wide P waves, suggesting bi-atrial dilatation. Laboratory results were normal except for raised white blood cell count (15500/mm^3 with 80% neutrophils) and blood urea of 55 mg/dL. D-dimer and troponin levels were slightly increased (0.85 mg/L and 0.3 ng/mL, respectively). The erytrocyte sedimantation rate and C-reactive protein levels were normal.

Echocardiography demonstrated restrictive cardiomyopathy, mitral and tricuspid insufficiency and left ventricular dysfunction (ejection fraction of 40%). She had a history of using nasal continuous positive airway pressure (CPAP) and oxygen concentrator due to the CLD and oxygen desaturations (SpO_2:80-85%). Her computed tomography (CT) of the chest, performed two months ago, was compatible with the pulmonary infiltration and CLD in the right lower and middle lobes. The patient was hospitalized and treatment started with inotropic support (milrinone, dopamine, furosemide infusion) and nasal CPAP. Her fever spikes increased further and CRP and neutrophil counts were also increased on the second day of admission. After the blood, sputum and urine cultures were obtained and the respiratory pathogen panel (RPP) was sent to the laboratory, antibiotic therapy was initiated with vancomycin and meropenem.

As COVID-19 infection was suspected due to her progressive deterioration, a nasopharyngeal swab test was obtained. The RPP and culture results were negative. The patient showed rapid deterioration within a few hours on the third day. Due to the acidosis, high lactate levels and hypercarbia on her blood gase analysis, she was intubated and ventilated, but she had a cardiac arrest. There was no response to the cardiopulmonary resuscitation. The swab test result was positive for COVID-19.

The common symptoms of COVID-19 reported in pediatric patients can easily be misdiagnosed as the upper/lower respiratory tract infections of pediatric age group [1,2]. In a series of 2135 children from China, 728 of which were positive for COVID-19, 55% were mild or asymptomatic, 40% were moderate (clinical or radiographic evidence of pneumonia without hypoxemia), and 5% were severe (dyspnea, central cyanosis) and <1% were critical [3]. Diagnosis of COVID-19 have been based on the presence of at least two symptoms (fever, cough, respiratory or gastrointestinal findings or fatigue), combined with laboratory tests (normal or low wbc count, low absolute lymphocyte count and increased CRP) and an abnormal chest *X*-ray [4]. The most commonly reported

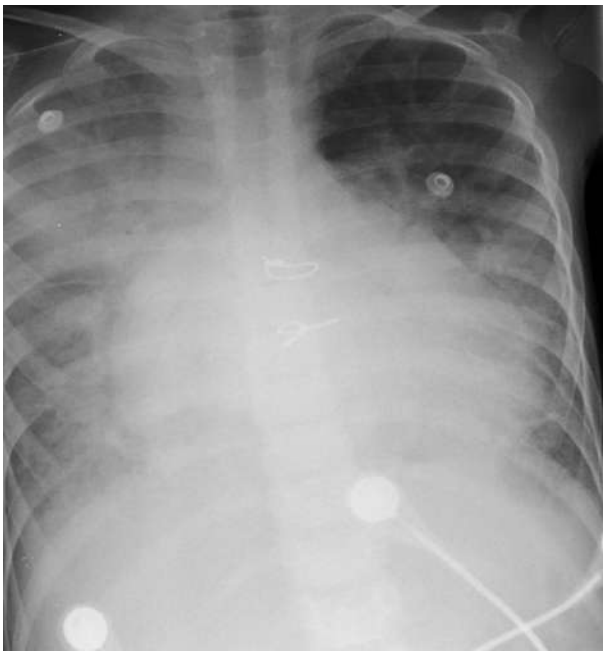

***Fig.* 1** *Chest X-ray findings in a girl with COVID-19 infection and pre-existing restrictive cardiomyopathy and chronic lung disease.*

radiologic finding is bilateral ground-glass opacity (32.7%) [5]. However, unlike adults, there are no typical radiological findings for definitive diagnosis of COVID-19 in children.

In one series, 23% of 345 children with laboratory-confirmed COVID-19 one or more comorbidity. The most commonly reported comorbid conditions were chronic pulmonary disease (including moderate to severe asthma), cardiovascular disease and immunosuppression [6]. Lu, *et al*. [5] reported that 3 out of 171 pediatric patients with COVID-19 required intensive care support and invasive mechanical ventilation; all with some underlying conditions, and only a 10-month-old child with intussuception died after 4 weeks of hospitalization [5].

Our case had no fever, no exposure to an infected person, but had restrictive cardiomyopathy and chronic lung disease that would suggest an exacerbation of her disease on admission. COVID-19 infection was suspected due to her progressive deterioration. We want to draw attention to such sub-acute clinical presentations of COVID-19 in the pediatric population with underlying comorbidities.

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REFERENCES

1. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al*. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus infected pneumonia in Wuhan, China. JAMA. 2020; 323:1061-9.
2. Qiu H, Wu J, Liang H, Yunling L, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: An observational cohort study. Lancet Infect Dis. 2020. Available from: *https://doi.org/10.1016/S1473-3099 (20)30198-5*. Accessed April 22, 2020.
3. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, *et al*. Epidemiology of COVID-19 among children in China. Pediatrics. 2020;145:e20200702.
4. Ludvigsson JF. Systematic review of COVID-19 in children shows milder cases and a beter prognosis than adults. Acta Paediatrica. 2020. Available from: *https://doi.org/10.1111/apa.15270*. Accessed April 22, 2020.
5. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, *et al*. SARS-CoV-2 infection in children. N Engl J Med. 2020;382:1663-5.
6. Bialek S, Gierke R, Hughes M, McNamara LA, Pilishvili T, Skoff T. Coronavirus disease 2019 in children- United States, February 12–April 2, 2020. MMWR Morb Mortal Wkly Rep. 2020;69:422-6.

Clinical Profile of Scrub Typhus in Newborns

Scrub typhus can affect all age groups but has been rarely reported among neonates. We retrieved records of neonates admitted with scrub typhus from our hospital database, and analyzed their clinical and laboratory features. Only patients who were confirmed as having scrub typhus according to WHO criteria were included [1].

During the period from January, 2013 to December, 2018, a total of 525 patients were admitted with scrub typhus, out of which seven (1.3%) were newborns. All the seven patients were from neighbouring districts of Chennai and had postnatal scrub typhus with fever, hepatosplenomegaly, thrombocytopenia and elevated C-reactive protein (CRP). Two cases had eschar (28%). Affected babies had complications such as shock (*n*=4), respiratory failure (*n*=3), disseminated intra-vascular coagulation (*n*=2) and multi organ dysfunction syndrome (*n*=2). Other causes of fever were ruled out as dengue IgM, peripheral smear for malaria, TORCH (toxoplasmosis, rubella, cytomegalovirus, herpes, and others) screen and blood culture were negative.

Pediatric scrub typhus in a previous study [2] presented as prolonged fever (100%), gastrointestinal symptoms (76%), lymphadenopathy (96%) and hepato-splenomegaly (61%). Eschar was seen in 50 (60%) patients and only six patients had severe illnesses (7%) [2]. In our series, six babies who were treated with doxycycline (4.5 mg/kg/day for 5 days) improved and there were no adverse reactions. One baby, treated with azithromycin 10 mg/kg/day died. Long-term follow-up data of the babies who survived was not available.

On review of English literature from 1992 to 2018 using Pub Med, 12 newborns with scrub typhus from seven publications [3-9] were identified, and nine (75%) had postnatal scrub typhus (three were due to vertical transmission). Fever, hepatosplenomegaly and thrombo-cytopenia were present in all of them and only one had an eschar (8%). Most common complications encountered were shock (*n*=8), respiratory failure (*n*=5), DIC (*n*=4) and MODS (*n*=3). Four babies who were treated with doxycycline and two out of the four babies treated with azithromycin improved. The mortality rate was 25%.

Scrub typhus should be considered in the differential diagnosis of newborns with fever, hepatosplenomegaly, thrombocytopenia and elevated CRP, especially in endemic regions. We found doxycycline to be useful in neonates without any side-effects.

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REFERENCES

1. World Health Organization. WHO Recommended Surveillance Standards. Second edition. 2006. Available from: *http://www.who.int/emc.* Accessed September 15, 2019.
2. Lakshmanan S, Sagayaraj M, Sujatha B, Vasudevan *LD*. Clinical and laboratory profile of pediatric scrub typhus in a tertiary care teaching hospital in Southern India. Indian J Pediatr. 2018;5:2092-7.
3. Wang CL, Yang KD, Cheng SN, Chu ML. Neonatal scrub typhus: a case report. Pediatrics. 1992;89:965-8.
4. Suntharasaj T, Janjindamai W, Krisanapad S. Pregnancy with scrub typhus and vertical transmission: A case report. J Obstet Gynaecol. 1997;23:75-8.
5. Aarthi P, Bagyalakshmi R, Mohan KR, Krishna M, Nitin M, Madhavan HN, *et al*. First case series of emerging rickettsial neonatal sepsis identified by polymerase chain reaction-based deoxyribonucleic acid sequencing. Indian JMed Microbiol. 2013;31:343-348.
6. Gothwal S, Dhruw SS, Choudhry R, Sitaraman S. Neonatal scrub typhus. Pediatr Em Care Med Open Access. 2016;1:2.
7. Vajpayee S, Gupta RK, Gupta ML. Scrub typhus causing neonatal hepatitis with acute liver failure-A case series. Indian J Gastroenterol. 2017; 26:61-5.
8. Jajoo M, Kumar D, Manchanda S. Scrub typhus in a new born. J Clini Diag Res. 2017;11:05-06.
9. Paramanantham P, Sathyamoorthy P, Sekar P. Neonatal scrub typhus. World J Pharmaceutical Res. 2018;7; 836-40.

Clinical Features of Patients With 7p22.1 Microdeletion

With the advent of comparative genomic hybridization (CGH) technology, more patients with microduplications or micro deletions are being reported. Herein, we report an 11-year-old girl with 7p22.1 microdeletion who presented with short stature and intellectual disability. She was the second born child to non-consanguineous parents, born at full term with a birthweight of 2 kg. Ventricular septal defect and patent foramen ovale were detected after birth and cardiac surgery was undertaken at one year of age (weight 7 kg). At four years of age, she was detected with intellectual disability and short stature (height of 87 cm) with a normal karyotype (46,XX).

At 11 years, her weight was 31.3 kg (about -1 SD) height 134.5 cm (<2 SD), with distinctive facial features including long face, high forehead, arched eyebrows, long eyelashes, flat nose bridge, broad nose, upturned lip, downturned corners of the mouth and pointed chin. Small brown pigmentation, snaggle teeth, sagging shoulders and shoulder girdle muscle bulk, scar on the chest wall, flexion of the left thumb (contracture), clinodactyly and abnormal dermatogly-phics were also noted with Tanner stage 4 breast development. Wechsler intelligence scale showed intellectual disability with a score of 56.

Laboratory data showed hypertriglyceridemia (3.64 mmol/L). The levels of follicle stimulating hormone, luteinizing hormone, prolactin, progesterone and estradiol were consistent with sexual development. The level of insulin-like growth factor 1, insulin like growth factor binding protein 3, corticotrophin, cortisol, liver function, renal function and thyroid function were in normal range. An abdominal ultrasound was normal. Copy number variation (CNV) analysis (MyGenostics Gene Technology Co., Ltd. China) was performed and a 1.673 Mb microdeletion (chr7: 5 147 686-6 820 998) at chromosome 7p22.1 was found. No similar deletion was noted from her parents by CNV analysis and her parents refused for FISH analysis.

About 30 cases with 7p22.1 microdeletion have been earlier reported [1,2]. The common clinical features in these patients included intellectual disability (58.3%), distinctive facial features (44%), short stature (33.3%), microcephaly (22.2%), hernia (14.8%), hypotonia (14.8%), VSD (13%) and abnormal extremities (7.4%). Joint laxity, bilateral radial abnormalities, PFO, pulmonary artery dilatation and high palate were also reported in one case.

The specific relationship between genotype and phenotype in 7p22.1 deletion is still unknown. The deleted region in index case contained 36 genes, including *RNF216*, *AIMP2*, *RAC1*, *PMS2* and *ACTB*, which are known pathogenic genes. Mutations in *RNF216, AIMP2, RAC1* and *ACTB* are associated with intellectual disability [1-5]. The expression levels of *ACTB* are linearly correlated with the *ACTB* gene copy number and influence the amount of β-actin, which is involved in cell motility and expressed in all eukaryotic cells [1,2]. Thus, the haploinsufficiency of *ACTB* may be accountable for intellectual disability and other clinical features in 7q22.1 microdeletion [2]. Postnatal growth retardation, high forehead, hypertelorism, arched eyebrows, broad nose with large tip, hypoplastic scapulas, and congenital heart defects in index case were consistent with the features of *ACTB* gene deficiency. However, *ACTB* gene was not deleted in few cases who showed intellectual disability, implying that other genes (*e.g., RAC1* gene) or even non-coding RNA coded in this region may contribute to the clinical features of this rare condition.

In summary, 7p22.1 microdeletion syndrome should be considered in patients with intellectual disability, distinctive facial features, microcephaly, short stature, inguinal hernia, hypotonia, and congenital heart disease and confirmed with genetic testing.

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REFERENCES

1. Palumbo O, Accadia M, Palumbo P, Leone MP, Scorrano A, Palladino T, *et al*. Refinement of the critical 7p22.1 deletion region: haploin sufficiency of ACTB is the cause of the 7p22.1 microdeletion-related developmental disorders. Eur J Med Genet. 2018;61:248-52.
2. Shimojima K, Narai S, Togawa M, Doumoto T, Sangu N, Vanakker OM, *et al*. 7p22.1 microdeletions involving ACTB associated with developmental delay, short stature, and microcephaly. Eur J Med Genet. 2016;59:502-6.
3. Husain N, Yuan Q, Yen YC, Pletnikova O, Sally DQ, Worley P, *et al*. TRIAD3/RNF216 mutations associated

with Gordon Holmes syndrome lead to synaptic and cognitive impairments via Arc misregulation. Aging Cell. 2017;16:281-92.
4. Shukla A, Das Bhowmik A, Hebbar M, Rajagopal KV, Girisha KM, Gupta N, *et al*. Homozygosity for a nonsense variant in AIMP2 is associated with a progressive neurodevelopmental disorder with microcephaly, seizures, and spastic quadriparesis. J Hum Genet. 2018;63:19-25.
5. Reijnders MRF, Ansor NM, Kousi M, Yue WW, Tan PL, Clarkson K, *et al*. RAC1 missense mutations in developmental disorders with diverse phenotypes. Am J Hum Genet. 2017;101:466-77.

Vinblastine-induced Acral Hyperpigmentation

A 5-year-old girl, diagnosed with multi-system langerhans cell histiocytosis (bone, liver, bone marrow and pituitary involvement), was started on induction chemotherapy (weekly cycles of vinblastine/ prednisolone for 12 weeks). Two months later, she developed progressive bluish-black discolouration over the nose and fingertips along with darkening of the nail beds without any itching, pain, redness, numbness, trauma or sun exposure.

Several differentials were considered, Chikungunya fever is well reported for causing an acute, brownish-black, centrofacial hyperpigmentation [1]. It can persist for three to six months after resolution of infection [1]. However, our child had no fever, arthralgia or cytopenias to suggest an infectious aetiology. Addisonian hyperpigmentation, which commonly involves mucous membranes, flexures, palmar and plantar creases, the areola, genitalia and pressure points (elbows and knees), was ruled out clinically, as well as by the absence of hyponatremia, hyperkalemia and acidosis [1]. Hyperpigmentation in thyroid disorders also has a similar distribution as in Addison disease [2]; however, the thyroid function test was normal. Exogenous ochronosis, secondary to topical hydroquinone use, responsible for bluish-black hyperpigmentation of the sun-exposed areas of the face, was also ruled out as there was no history of such application; neither was henna applied locally [1,2]. She had no preceding redness, scaling, pain, injury or cutaneous eruptions to suggest post-inflammatory hyperpigmentation [3]. Acanthosis nigricans, although classically noted over the nape of the neck, axilla and groin, can also develop over the face [4]. However, our child had neither hyperglycemia nor obesity and the lesion in question lacked the characteristic velvety thickening of acanthosis nigricans [3]. The involvement of distal phalanges, interphalangeal joints and oral mucosa, characteristic of Vitamin B_{12} deficiency, was absent in our child [2]. Moreover, her red blood cell indices were normocytic and normochromic, ruling out this possibility. Drug-induced acral hyperpigmentation was considered after ruling out other differentials and vinblastine was discontinued, following which the hyperpigmentation faded over a period of 3 weeks, but did not disappear completely.

Vinca alkaloids are notorious for causing extravasation injury [4]. Supravenous hyperpigmentation has been reported with the ABVD regimen which includes vinblastine (however, the causative drug was not implicated) [5]. Vinorelbine, a vinca alkaloid, in high doses is known to cause acral erythema [6]. Although drug-induced hyperpigmentation is responsible for 10-20% cases of acquired hyperpigmentation [2], it has not been reported with either vinblastine or prednisolone. Possible mechanisms of drug induced acral hyperpigmentation

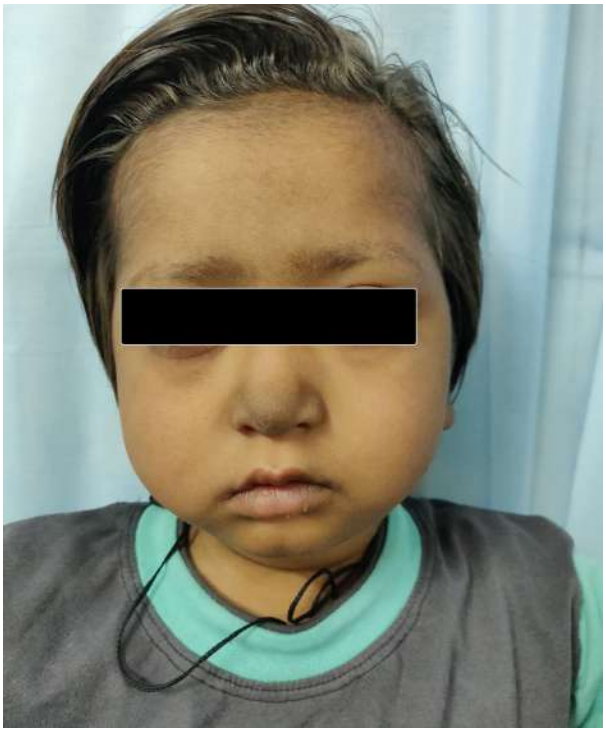

Fig.1

include increased melanin synthesis (secondary to cytotoxic effect on melanocytes), cutaneous drug accumulation or iron deposits following dermal vascular damage, and increased blood flow to acral areas causes drug deposition [4,5].

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REFERENCES

1. Khanna N, Rasool S. Facial melanoses: Indian perspective. Indian J Dermatol Venereol Leprol. 2011;77:552-64.
2. Bhalla M, Garg S. Acral melanosis. Pigment Int. 2018;5:14-27.
3. Vashi AN, Kundu RV. Facial hyperpigmentation: Causes and treatment. Br J Dermatol. 2013;169:41-56.
4. Payne AS, James WD, Weiss RB. Dermatologic toxicity of chemotherapeutic agents. Sem Oncol. 2006;33:86-97.
5. Pavey RA, Kambil SM, Bhat RM. Dermatological adverse reactions to cancer chemotherapy. Indian J Dermatol Venereol Leprol. 2015;81:434.
6. Heidary N, Naik H, Burgin S. Chemotherapeutic agents and the skin: An update. J Am Acad Dermatol. 2008;58:545-70.

Coronavirus Disease (COVID-19) With Relevance to Pediatrics

We read with interest the recent article on coronavirus disease (COVID-19) in children [1], which has, to some extent, as far as present knowledge is concerned, explained why children are less affected than the elderly. However, the possible reason why the illness is less, thus far, in our country needs to be highlighted.

We need to first understand the role of 'trained immunity'. Trained immunity represents an innate immune memory and it is formed by innate immunity cells that become memory cells after antigen exposure. The increased neutrophilic and low lymphocyte counts in COVID-19 patients during the cytokine storm, that occurs with severe deterioration of some patients, supports the hypothesis that innate immune response is both a protective and destructive phenomenon [2].

The WHO statement emphasizing that there is no evidence that BCG protects against SARS-CoV-2 virus infection was made primarily to prevent BCG being used as a prophylactic. The fact that the three studies referred to compared the incidence of COVID-19 cases in countries where the BCG vaccine is used with countries where it is not used and inferred that countries that routinely used the vaccine in neonates had less reported cases of COVID-19 to date [3] may suggest the protective effect of BCG at birth in countries where almost universal BCG vaccination is practiced.

It is known that seroconversion to oral polio vaccine (OPV) and rota virus vaccine has been poor in India compared to the developed world [4]. The frequent viral infections that probably prevent a new virus from getting a 'foothold', early immunizations like BCG, measles vaccine, 0-dose OPV, hepatitis B vaccine, maternal Tdap (or Tdvac) and influenza vaccine and exposure to atypical and typical bacterial and fungal infections expose our children to many antigens, which could contribute to effective defense against various infections – indicating the so-called trained immunity.

Telemedicine during the COVID-19 pandemic: The roles of teleconsultation, during a pandemic in outpatient and acute care settings, including virtual intensive care unit (ICU) are diverse [5] and need to be encouraged. Virtual care utilizing video and audio provider-initiated services is a well-established modality to provide direct care to patients. Hospitalized COVID positive cases could be managed by interviewing the parent and/or the adolescent and examining the child using video conferencing. It would be ideal, if possible, to provide high definition camera and digital peripherals, including stethoscopes, otoscopes, ophthalmoscopes, and dermatoscopes for this purpose. In-person visits should remain part of patients' care to ensure provider patient relationship [6]; however, telemedicine could still be deployed to provide direct care and monitoring of these patients. Nursing staff could use the facilities to conduct hourly rounds and limit unnecessary in-room visits. It definitely goes a long way in minimizing exposure of healthcare personnel and, in addition, helps conserve personal protection equipments (PPE). Triaging patients online or telephonically is useful in preventing high-risk patients from exposing others to infection. Prescription generation for in-patient and

outpatient care is time tested, however, automated dispensary systems, or pharmacy robots would reduce patient contact at the pharmacy [7]. Establishment of a telemedicine system; however, requires a robust information technology infrastructure, training of healthcare staff, receptionists, attenders, cleaning staff and security personnel; and introduction of the modifications to integrate hospital workflow. This will entail expenditure and increased cost to the patient. Development of telehealth services have in most parts of the world been hampered by the lack of insurance reimbursement for such services.

Hospitals could also consider the possibility of introducing self-administered nasal swabs for older children and adolescents; this has comparable efficacy to staff-administered swabs [8].

For children with special needs, disruptions in the schedule can be chaotic. They could be accessed through online platforms to develop home-based care programs. Teletherapy also allows the provider to document the care, train parents to maintain a regular schedule, capture sessions on video, chart progress and amend the care plan as needed.

Parents unexposed to this modality of care may be unaware of the usefulness of this form of consultation. They might feel it is impersonal and may not be satisfied with the experience. Patient acceptance can only be achieved with introduction of the often overlooked, but extremely essential operational requirement of patient education and public awareness. With these processes in place and an efficient and coordinated implementation, parents and their children would find contentment with teleconsulations like with other virtual care experiences [9].

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REFERENCES

1. Balasubramanian S, Rao NM, Goenka A, Roderick M, Ramanan AV. Coronavirus disease (covid-19) in children – what we know so far and what we do not. Indian Pediatr. 2020 Apr 9. Available from: *https://www.indianpediatrics.net/CONVID29. 03.2020/SA-00159.pdf.* Accessed April 24, 2020.
2. Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol. 2020;38:1-9.
3. World Health Organization. Bacille Calmette-Guérin (BCG) vaccination and COVID-19. Available from: *who.int/newsroom/commentaries/detail/bacille-calmette-gu%C3%A9rin-(bcg)-vaccinatio-and-covid-19*. Accessed April 24, 2020.
4. Kirkpatrick BD, Colgate ER, Mychaleckyj JC, Haque R, Dickson DA, *et al*. Designed to explore complex biologic problems the "Performance of Rotavirus and Oral Polio Vaccines in Developing Countries" (PROVIDE) Study: Description of methods of an interventional study. Am J Trop Med Hyg. 2015;92:744-51.
5. Lurie N, Carr BG. The role of telehealth in the medical response to disasters. JAMA Intern Med. 2018;178: 745-74.
6. Daniel H, Sulmasy LS. Policy Recommendations to Guide the Use of Telemedicine in Primary Care Settings: An American College of Physicians Position Paper. Ann Intern Med. 2015;163:787-9.
7. Rodriguez-Gonzalez CG, Herranz-Alonso A, Escudero-Vilaplana V, Ais-Larisgoitia MA, Iglesias-Peinado I, Sanjurjo-Saez M. Robotic dispensing improves patient safety, inventory management, and staff satisfaction in an outpatient hospital pharmacy. Eval Clin Pract. 2019;25:28-35.
8. Akmatov MK, Gatzemeier A, Schughart, K, Pessler F. Equivalence of self- and staff collected nasal swabs for the detection of viral respiratory pathogens. PLoS One. 2012:7;e48508.
9. Martinez KA, Rood M, Jhangiani N, Kou L, Rose S, Boissy A, *et al*. Patterns of use and correlates of patient satisfaction with a large nationwide direct to consumer telemedicine service. J Gen Intern Med. 2018;33: 1768-73.

Do Not Neglect the Children: Considerations for COVID-19 Pandemic

The crisis of coronavirus disease (COVID-19) pandemic is the greatest challenge we have faced since World War II [1]. Although children are less commonly infected, and severity of disease in children is usually mild, no one is completely immune to this disease [2]. Most of the reported cases of COVID-19 infection in children show a complete recovery in one to two weeks; however, those with serious underlying medical conditions are at a higher risk [3].

The reason behind the lower prevalence of COVID-19

among children compared to adults is still unclear. Latest studies suggest that less exposure, less vigorous immune response, and incomplete functionality of Angiotensin-Converting Enzyme II (ACE2) in children – as the potential receptor for SARS-CoV-2 – are responsible for the significantly fewer severe cases in children [4]. Generally, children with mild symptoms and no risk factors should be managed at home [5]. Obstructed or absent breathing, central cyanosis, and cold skin are emergency signs that need immediate airway management and oxygen therapy to the target SpO2 ≥ 94% [6]. The children with moderate and severe symptoms, along with the children with mild symptoms but with underlying risk factors, are recommended to seek hospital admission, and emergency treatment should be started according to the disease severity [5,7].

Due to a possible transmission from asymptomatic patients, children should avoid playgrounds and use a face mask in public settings. Current evidence suggests that SARS-CoV-2 is not transmittable by breast milk [8]. However, in the case of suspected or confirmed COVID-19 positive mother, feeding the expressed breast milk by a healthy caregiver is more favorable than direct breastfeeding [9]. The mother should use a mask and maintain strict hand hygiene to prevent transmission during the breast milk expressing by using alcohol-based hand rubs or soap and water. Proper disinfection should also be applied to the equipment by a healthy person.

Lockdown, along with school and playground closures, and more time spent in front of screens like video games and television, can affect children's mental health, and the lack of physical activity could result in overweight. To overcome this issue, children should be encouraged to achieve the 60-minute daily goal of physical activity, recommended by WHO, by sticking to in-door exercises [10]. Also, the quality and quantity of food should be considered seriously by the families, as the children eat unhealthier foods and the underprivileged children are undernourished due to the discontinuation of mid-day meal facility. Therefore, close supervision on physical health, nutrition, and mental health should be performed. Compared with the other groups of society, children are more vulnerable and are in need of great support [11]. It is our duty to provide support to children and families to assist them in overcoming the current situation.

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REFERENCES

1. United Nations Development Program. COVID-19 pandemic: Humanity needs leadership and solidarity to defeat the coronavirus 2020. Available from: *https://www.undp.org/content/undp/en/home/coronavirus.html.* Accessed April 13, 2020.
2. Balasubramanian S, Rao NM, Goenka A, Roderick M, Ramanan AV. Coronavirus disease (covid-19) in children – what we know so far and what we do not. Indian Pediatr. 2020 Apr 9. Available from: *https://www. indianpediatrics.net/CONVID29. 03.2020/SA-00159.pdf.* Accessed April 24, 2020.
3. Wei M, Yuan J, Liu Y, Fu T, Yu X, Zhang Z-J. Novel coronavirus infection in hospitalized infants under 1 year of age in China. JAMA. 2020. Available from: *https://jamanetwork.com/journals/jama/article-abstract/2761659.* Accessed April 13, 2020.
4. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, et al. Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in China. Pediatrics. 2020. Available from: *https://pediatrics.aappublications.org/content/pediatrics/early/2020/03/16/peds.2020-0702.full.pdf.* Accessed April 13, 2020.
5. World Health Organization (WHO). Home care for patients with COVID-19 presenting with mild symptoms and management of their contacts. Available from: *https://www.who.int/publications-detail/home-care-for-patients-with-suspected-novel-coronavirus-(ncov)-infection-presenting-with-mild-symptoms-and-management-of-contacts.* Accessed April 21, 2020.
6. World Health Organization (WHO). Clinical management of severe acute respiratory infection when COVID-19 is suspected. Available from: *https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected.* Accessed April 21, 2020.
7. Meena J, Kumar J. Fecal shedding of SARS CoV-2: Implications for disease spread and quarantine. Indian Pediatr. 2020. Available from: *https://www.indian pediatrics.net/CONVID29.03.2020/CORR-00160.pdf.* Accessed April 24, 2020.
8. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, *et al.* Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: A retrospective review of medical records. Lancet. 2020;395:809-15.
9. Centers for Disease Control and Prevention (CDC). Considerations for inpatient obstetric healthcare settings 2020. Available from: *https://www.cdc.gov/ coronavirus/2019-ncov/hcp/inpatient-obstetric-healthcare-guidance.html*. Accessed April 21, 2020.
10. World Health Organization (WHO). Global strategy on diet, physical activity and health: Activity and young people. Available from: *https://www.who.int/ dietphysicalactivity/factsheet_young_people/en/.* Accessed April 21, 2020.
11. World Health Organization (WHO). Environmental health in emergencies: Vulnerable groups. Available from: *https://www.who.int/environmental_health_emergencies/vulnerable_groups/en/.* Accessed April 21, 2020.

Coronavirus Disease (COVID-19) in Children: Indian Perspectives

Balasubramanian, *et al*. [1] have concisely described various aspects related to coronavirus disease (COVID-19) in children in the Indian setting. We would like to address additional issues related to epidemiology of COVID-19, reasons for uneventful clinical course in children, and the contributions of Indian judiciary to the health of children during the pandemic.

From the point of epidemiology, reporting and testing of children for COVID-19 are less, which lead to under-sampling and under-reporting of the disease [2]. Decreased illness severity and an overall resilience to this disease in children facilitate transmission of the organism by rendering children as carriers. Moreover, children can shed COVID-19 virus through stools for a longer time. This is attributed to increased viral load through the act of swallowing the virus containing sputum or saliva [3] and the expression of angiotensin converting enzyme 2 (ACE2) in the intestine.

From a physiological perspective, a higher frequency of beating lung cilia [4] in children hinders the virus entry into lung pneumocytes. Apart from that, they have low risk for COVID-19 associated acute respiratory distress syndrome (ARDS) due to decreased generation of thrombin, and fibrin formation [5,6]. The other reasons for the protection of lungs and airways are lack of co-morbidities and less exposure to particulate matter and pollutants [7], as also mentioned by Balasubramanian, *et al.* [1]. In addition, increased expression of ACE2 in pediatric lungs and other tissues gives additional protection and contributes to uneventful clinical course [4]. Moreover, they escape from cytokine storm [6,8] and hence, fatal complications are rarely observed [8,9]. In addition, exposure of pediatric population to various vaccines [10] carried out as per the Universal immunization program in India enhance the activation of the immune system [11,12], and contribute to uneventful clinical course. Further, we think that the relative lack of physical and mental stress in children likely gives additional protection *via* psychoneuroimmunology.

Understanding the gravity of the current and emerging situations of COVID-19, the Supreme Court of India has given directions [13] and measures to Child Welfare Committee, Juvenile Justice Boards, Children's courts, Child Care Institutions and State Governments across India towards the care and attention of children in conflict with law and those kept in various types of homes including those under foster and kinship care. Thus, Indian judiciary is the first in the globe to look into the needs of marginalized children and give specific directions for their care in this pandemic.

Over all, we believe that Indian children will withstand the outbreak of the novel coronavirus pandemic, but may be a link in transmission due to possibility of under-reporting of cases, sub-clinical syndrome and longer shedding period of virus. We have to urgently address these through effective public health approach, including possible vaccination against COVID-19, as and when available [14].

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REFERENCES

1. Balasubramanian S, Rao NM, Goenka A, Roderick M, Ramanan AV. Coronavirus disease (covid-19) in children – what we know so far and what we do not. Indian Pediatr. 2020 Apr 9. Available from: *https://www. indian pediatrics.net/CONVID29.03.2020/SA-00159.pdf*. Accessed April 22, 2020.
2. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, *et al*. Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in China. Pediatrics. 2020. Available from: *https://pediatrics.aappublications.org/ content/ pediatrics/early/2020/ 03/16/peds.2020-0702.full.pdf*. Accessed April 19, 2020.
3. Ma X, Su L, Zhang Y, Zhang X, Gai Z, Zhang Z. Do children need a longer time to shed SARS-CoV-2 in stool than adults? J Microbiol Immunol Infect. 2020. Available from: *https://www.sciencedirect.com/science/article/pii/ S1684118220300700.* Accessed April 18, 2020.
4. Chilvers MA, Rutman A, O'Callaghan C. Functional analysis of cilia and ciliated epithelial ultrastructure in healthy children and young adults. Thorax. 2003;58:333-8.
5. Moore HB, Moore EE, McIntyre RC, Moore PK, Talmor DS, Moore FA, *et al*. Is there a role for tissue plasminogen activator (tPA) as a novel treatment for refractory COVID-19 associated acute respiratory distress syndrome (ARDS)? J Trauma Acute Care Surg. 2020. Available from: *https://journals.lww.com/jtrauma/Citation/9000/Is_There_ a_Role_for_Tissue_ Plasminogen_Activator.97967.aspx*. Accessed April 18, 2020.

6. Ignjatovic V, Pelkmans L, Kelchtermans H, Al Dieri R, Hemker C, Kremers R, *et al*. Differences in the mechanism of blood clot formation and nanostructure in infants and children compared with adults. Thromb Res. 2015;136:1303-9.
7. Yang J, Chen Y, Yu Z, Ding H, Ma Z. The influence of PM2.5 on lung injury and cytokines in mice. Exp Ther Med. 2019;18:2503-11.
8. Ng PC, Lam CW, Li AM, Wong CK, Cheng FW, Leung TF, *et al*. Inflammatory cytokine profile in children with severe acute respiratory syndrome. Pediatrics. 2004;113:e7-14.
9. Liniger M, Zuniga A, Tamin A, Azzouz-Morin TN, Knuchel M, Marty RR, *et al*. Induction of neutralising antibodies and cellular immune responses against SARS coronavirus by recombinant measles viruses. Vaccine. 2008;26:2164-74.
10. Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol. 2020;38:1-9.
11. Ruf BR, Knuf M. The burden of seasonal and pandemic influenza in infants and children. Eur J Pediatr. 2014;173:265-76.
12. Benn CS, Netea MG, Selin LK, Aaby P. A small jab - A big effect: Nonspecific immunomodulation by vaccines. Trends Immunol. 2013;34:431-9.
13. Bureau IL. India Legal Bureau. Supreme court issues directions for prevention of children from Covid-19. 2020. Available from: *https://www.indialegallive.com/constitutional-law-news/supreme-court-news/supreme-court-issues-directions-prevention-children-covid-19-94696.* Accessed April 18, 2020.
14. Ella KM, Krishna Mohan V. Coronavirus vaccines: Light at the end of the tunnel. Available from: *https://www.indianpediatrics.net/CONVID29.03.2020/PERS-00163. pdf.* Accessed April 24, 2020.

Does a Crying Child Enhance the Risk for COVID-19 Transmission?

The pandemic of coronavirus disease (COVID-19) has led all of us to recalibrate both our personal and professional life [1]. In our routine pediatric outpatient practice for non-COVID cases *i.e.* well baby visits and kids presenting with afebrile, non-respiratory symptoms, a surgical face mask with proper hand hygiene and gloves has been recommended for health care professionals [2]. However, for those handling aerosol-generating procedures (AGP), respirators and additional personal protection equipment (PPE) are recommended [3]. Aerosol is defined as suspension of fine solid particles or liquid droplets in air or another gas. Aerosols of varying severity are generated on sneezing, coughing, talking and also during normal breathing [4]. AGPs are believed to produce aerosols and droplets as source of respiratory pathogens that exposes the health care workers to pathogens causing acute respiratory infections including Severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) [5]. AGPs are generated on performing certain medical procedures like intubation, manual ventilation, non-invasive ventilation, tracheostomy insertion etc. on infected cases. However, it is not clear if the risk is due to direct airborne transmission or secondary exposure to respiratory droplets.

It is established that even loud speaking results in increased aerosol generation *i.e.* aerosol super-emission [6]. Extrapolating the same logic even a crying and screaming child should produce aerosol super-emission. Although an operational definition for AGP is in place, the relation to crying and its possible effects of increased aerosol generation has so far not been stressed.

In a pandemic situation, we need to ponder on some points: even infants and toddlers who come for routine vaccinations or non-respiratory complaints can be asymptomatic carriers or in pre-symptomatic period of transmission; implementing source control measures like face mask and social distancing in this age group practically difficult; crying, a common occurrence in this age group, also increases the risk of aerosol generation and transmission; and, proximity of these kids to caregivers and their attenders along with sustained crying either due to anxiety or fear might further increase the risk and load of aerosol.

In view of the yet unknown increased risks posed by expected or unexpected crying of asymptomatic children in the transmission of COVID-19, it may be prudent to make every effort to avoid examining a crying child without adequate precautions.

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REFERENCES

1. Balasubramanian S, Rao NM, Goenka A, Roderick M, Ramanan AV. Coronavirus disease (covid-19) in children – What we know so far and what we do not. Indian Pediatr. 2020 Apr 9. Available from: *https://www.indianpediatrics.net/CONVID29. 03.2020/SA-00159.pdf.* Accessed April 23, 2020.
2. Clinical Infectious Diseases Society, India. Infection control in stand-alone out patients and small nursing homes in the setting of COVID. Available from: *http://www.cidsindia.org/pdf/covid-19/infection-control-in-standalone-OPs.pdf.* Accessed April 25, 2020.
3. Ministry of Health and Family Welfare. Government of India. Novel Coronavirus Disease 2019 (COVID-19): Guidelines on rational use of Personal Protective Equipment. Available from: *https://www.mohfw.gov.in/pdf/GuidelinesonrationaluseofPersonalProtective Equipment.pdf.* Assessed April 25, 2020
4. National Research Council. 2020. Rapid Expert Consultation on the Possibility of Bioaerosol Spread of SARS-CoV-2 for the COVID-19 Pandemic (April 1, 2020). Washington, DC: The National Academies Press. Available from: *https:// www. nap.edu/read/25769/chapter/1*. Accessed April 18, 2020.
5. Tran K., Cimon K., Severn M, Pessoa-Silva CL, Conly J. Aerosol generating procedures and risk of transmission of acute respiratory infections to healthcare workers: A systematic review. PLoS One. 2012; 7:e35797.
6. Asadi S, Wexler AS, Cappa CD, Barreda S, Bouvier NM, Ristenpart WD. Aerosol emission and superemission during human speech increase with voice loudness. Sci Rep. 2019; 9: 2348. Available from: *https://www.nature.com/articles/s41598-019-38808-z*. Accessed April 18, 2020.

The Imperative of Early Treatment for Children With COVID-19 Infection

Acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is uncommon in children [1], with greater morbidity and mortality in adults and elderly. A number of hypotheses may explain the low susceptibility of children to COVID-19 virus [2] *viz,* (*i*) immaturity and limited function of angiotensin-converting enzyme 2 (ACE2) receptors in children, as undifferentiated cells that express low levels of ACE2 are not readily infected by SARS-CoV; (*ii*) the immature innate immune system in young children results in less inflammation and consequently fewer symptoms; and, (*iii*) possible cross-reactivity of antibodies against other viruses (influenza, adenovirus, respiratory syncytial virus *etc*.) with the SARS-CoV-2, which could provide partial protection.

As COVID-19 infection is not universally mild in children [3], it is important that they are protected as a vulnerable population, as still there is limited data on the risk factors for severe infection in children.

The long-term effects on the lungs of COVID-19 in children are not known, even for those with moderate symptoms. In patients hospitalized in French pediatric units in recent weeks, the chest computed tomography (CT) scans have often been pathological, even in children with limited respiratory sign with associated decline in lung function (unpublished data). In light of this, should not all children with moderate to severe respiratory symptoms be treated, irrespective of their comorbidity? Why do pediatricians appear to be unwilling to consider employing the COVID-19 treatments that are available, e.g., hydroxychloroquine and azithromycin [4]? These drugs (which are already widely used in pediatrics in other indications) certainly have side effects that are of concern, but their use in a hospital environment shall allow these side effects to be monitored and ensure greater safety for the patient [5].

In the absence of specific antiviral treatments, pediatricians need more virological, epidemiological, and clinical data to better treat and manage COVID-19 infections. It should be kept in mind that children, even when asymptomatic, may be a potential cause of spread and transmission of the disease in their communities [6]. In light of this, barrier precaution needs to be rigorously applied within families in order to protect the elderly.

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REFERENCES

1 Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, *et al*. Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in China. Pediatrics. 2020. Available from: *https://pediatrics.aappublications.org/content/pediatrics/early/2020/03/16/peds.2020-0702.full.pdf.* Accessed April 24, 2020.
2. Li Y, Guo F, Cao Y, Li L, Guo Y. Insight into COVID 2019

for pediatricians. Pediatric Pulmonol. 2020;55:E1-E4.
3. Sun D, Li H, Lu X, Xiao H, Ren J, Zhang FR, *et al.* Clinical features of severe pediatric patients with coronavirus disease 2019 in Wuhan: A single center's observational study. World J Pediatr. 2020 Mar 19: 1-9. Available from: *https:// www.ncbi.nlm.nih.gov/pmc/articles/PMC7091225.* Accessed April 26, 2020.
4. Colson P, Rolain JM, Raoult D. Chloroquine for the 2019 novel coronavirus SARS-CoV-2. Int J Antimicrob Agents. 2020;55:105923.
5. Ponticelli C, Moroni G. Hydroxychloroquine in systemic lupus erythematosus (SLE). Expert Opin Drug Saf. 2017;16:411-9.
6. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, *et al*. Presumed asymptomatic carrier transmission of COVID-19. JAMA. 2020;323:1406-7.

Demystifying BCG Vaccine and COVID-19 Relationship

Efforts for developing vaccines for novel coronavirus disease (COVID-19) are ongoing, but it is unlikely to be available in the immediate future [1]. In the absence of specific therapy, the researchers are exploring other potential preventive and therapeutic options. Recently, there has been a buzz about the protective effect of Bacille Calmette-Guérin (BCG) vaccine in COVID-19. Based on epidemiological correlations, many unpublished preprints hypothesized that the BCG vaccine may offer protection against COVID-19. It gained so much popularity that within 20 days three randomized controlled trials (RCTs) got registered, and many more are in the pipeline [2]. To make an informed decision, we must understand the mechanism of action of BCG, and appraise the robustness of the evidence.

The basis of the possible use of the BCG vaccine against COVID-19 lies in its non-specific effects (NSEs) over the immune system [3]. The NSEs of BCG are mainly mediated by potentiating innate immune response through epigenetic mechanisms.These epigenetic changes within the innate cells act as *de novo* enhancers to boost the immune response against a secondary challenge [3-5]. This enhancing response is popularly known as 'trained immunity' and is very characteristic of BCG. This trained immunity also offers protection against a variety of pathogens (Salmonella, Shigella, malaria, respiratory viruses, *etc*.) other than *Mycobacterium tuberculosis,* and forms the basis of its use in bladder cancer, melanoma *etc.* However, this non-specific effect is mostly short-lived and wanes soon after the primary BCG stimulus is cleared from the body. By virtue of the NSEs, BCG vaccine has shown to decrease all-cause mortality in children. Though a few observational studies suggest that the NSEs may last till adulthood, but the overall evidence is still inadequate and is of low quality [3,6,7].

On critical appraisal of the non-peer reviewed pre-print evidence, at the relationship between BCG and COVID-19 is being proven by looking at correlation/ association among two data set (BCG vaccine coverage and COVID-19), without acknowledging the confounders. The variables like the difference in testing strategies, reporting bias, demographics, nation's ability to respond to the pandemic, prevalence of co-morbidities, and different stages of the pandemic across various countries might have a significant impact on these associations/correlations and must be interpreted carefully. Therefore, at this stage, this association should be considered as a hypothesis only and should be tested through appropriately designed studies.

Though the epidemiological association between BCG and COVID-19 is striking, it does not prove causal relationship unless tested in well-designed clinical trials. Also, we should not forget that the NSEs of the BCG vaccine has not been well-studied in human beings and their clinical relevance is unknown [2,3]. Therefore, in the absence of evidence, the BCG vaccination for the prevention of COVID-19 cannot be recommended. The results of the ongoing RCTs shall guide us further.

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REFERENCES

1. Ella KM, Mohan KV. Coronavirus vaccines: Light at the end of the tunnel. Indian Pediatr. 2020 Apr 15 [Epub] Available from: *https://www.indianpediatrics.net/ COVID29.03.2020/ PERS-00163.pdf.* Accessed April 26, 2020.
2. World Health Organization. Bacille Calmette-Guérin (BCG) vaccination and COVID-19. Available from: *https:/ /www.who.int/news-room/commentaries/detail/ bacille-calmette-guérin-(bcg)-vaccination-and-covid-19.*

3. SAGE Working Group on BCG Vaccines and WHO Secretariat. Report on BCG vaccine use for protection against mycobacterial infections including tuberculosis, leprosy, and other nontuberculous mycobacteria (NTM) infections. World Health Organization; 2017. Available from: *https://www.who.int/immunization/sage/ meetings/ 2017/october/1_BCG_report_revised_version_online.pdf*. Accessed April 14, 2020.
4. Moorlag SJCFM, Arts RJW, van Crevel R, Netea MG. Non-specific effects of BCG vaccine on viral infections. Clin Microbiol Infect. 2019;25:1473-8.
5. Arts RJW, Moorlag SJCFM, Novakovic B, Li Y, Wang SY, Oosting M, *et al*. BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. Cell Host Microbe. 2018;23:89-100.e5.
6. Usher NT, Chang S, Howard RS, Martinez A, Harrison LH, Santosham M, *et al*. Association of BCG vaccination in childhood with subsequent cancer diagnoses: A 60-Year Follow-up of a clinical trial. JAMA Network Open. 2019;2:e1912014.
7. Abbott S, Christensen H, Lalor MK, Zenner D, Campbell C, Ramsay ME, *et al*. Exploring the effects of BCG vaccination in patients diagnosed with tuberculosis: Observational study using the Enhanced Tuberculosis Surveillance system. Vaccine. 2019;37:5067-72.

COVID -19 Pandemic: The Challenges for Pediatric Oncology

The global pandemic of the novel coronavirus disease (COVID-19) has had a significant impact on adult and pediatric patients with many acute and chronic diseases including cancer. The purpose of this correspondence is to project the challenges faced by both, the children undergoing treatment and the treating pediatric oncologist [1].

Providing medical care to children with cancer during this pandemic is challenging given the risks of death from cancer *versus* death or serious complications in the immunocompromised hosts [2,3]. Hospitals are delaying chemotherapy, radiotherapy and surgery after being overwhelmed by COVID-19 infection. Patients with cancer also fear coming to hospital fearing the risk of infection. There are also limited supplies of personal protective equipment (PPE) for doctors and other personnel, limited beds and ICU facility, limited blood bank facilities and also strained diagnostic facilities. Limited data, though from adults, also suggests that cancer patients with COVID will fare worse [4]. In a study from China, there was a higher risk of severe events in COVID-19 patients with cancer compared with those without cancer. Theoretically, immune therapy can result in cytokine release and worsen the viral injury, which is also believed to be due to a cytokine storm, but this is a theoretical consideration and not proven due to very limited data.

Some general guidelines have been provided for children with cancer [5], which include, in addition to social distancing, mask usage outside home and hygiene, *viz.* clinic visits that can be postponed without risk to the patient should be postponed and telemedicine to be used to screen and evaluate patients. Certain other issues are briefly elucidated herein.

Difficulty in hospital consultation: Many hospitals have been designated as centers for management of COVID-19, leading to temporary stoppage of outpatient services. Children with malignancies are unable to come for outpatient consultation for clinical follow-up and chemotherapy planning. In addition, it is now difficult for outstation patients to visit referral centers, despite prior appointments.

New cases needing evaluation are also not able to reach some of the major hospitals,which have temporarily closed OPD registrations to focus on COVID patients. There is also risk of new patients being asymptomatic carriers, but the current guidelines do not allow COVID testing for all new hospital admissions even if they are immune-compromised cancer patients.

Increased risk of coronavirus infection: The pandemic poses a risk of coronavirus infection for all individuals including children. Children with cancer are assumed to be more susceptible to the coronavirus due to inherent suppressed immune function associated with cancer treatment and repeated attendance in health care facilities [2].

Treatment delay: Children with cancer, already on treatment, were advised to stay indoors and practice social distancing resulting in delay in their treatment. The nationwide lockdown further delayed the treatment due to restricted mobility even within the city or travel from outstation. The bed strength available for in-house admission has also reduced due to diversion to COVID-19 wards, leading to delayed/deferred intensive chemo-

therapies requiring in-house management [5]. Most hospitals have split the staff into two or more teams so as to reduce chances of infection and keep a reserve pool of medical staff, should one team be exposed to a COVID case. A drawback has been the increased workload for treating teams, and the possibility of delay in getting appointment for investigations/procedures**.**

Shortage of blood component: The lockdown has drastically reduced the number of voluntary blood donations, thereby creating a shortage at blood banks. In addition, relatives of patients have also been unable to come for donations due to travel restrictions. This problem has also been highlighted in centers treating thalassemia patients [6].

Social impact: The COVID -19 pandemic has caused stress to families resulting from the infection itself, delayed treatment, need of prolonged unexpected stay due to lockdown, lack of availability of accommodation and financial implications. Patients often need travel passes and travel support from treating physician and NGOs to be able to come to hospitals for treatment.Some are not willing to come for treatment for fear of contacting corona infection.

In a recent online publication [7], it was stated that there is no reason to discontinue daily activities in pediatric hematology/oncology units or to turn away children with suspected cancer during this pandemic. It seems desirable to postpone high intensity treatments, where feasible, and to prepare to triage according to prognosis. Similar treatment advice has also been released online recently by Tata Memorial Hospital, Mumbai. It may be highlighted that the rarity of cases reported precludes the development of clear chemotherapy guidelines for children being treated for cancer.

In summary, delivering cancer care during the pandemic is challenging given the risks of death from cancer *versus* death from infection. The likelihood of a severe illness from COVID-19 is higher among patients with cancer. As more information becomes available evidence-based consensus recommendations may emerge. Individual centers treating childhood cancers may come up with strategies to tackle the situation. A balance needs to be created keeping in mind risks associated with COVID-19 and the timely management of a child with cancer [8].

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REFERENCES

1. Yang C, Li C, Wang S, National Clinical Research Center for Child H, Disorders, Children's Oncology Committee of Chinese Research Hospital A. Clinical strategies for treating pediatric cancer during the outbreak of 2019 novel coronavirus infection. Pediatr Blood Cancer. 2020;67: e28248.
2. Yu J, Ouyang W, Chua MLK, Xie C. SARS-CoV-2 transmission in patients with cancer at a tertiary care hospital in Wuhan, China. JAMA Oncol. 2020 Mar 25. [Epub ahead of print]. Available from: *https://jamanetwork.com/journals/jamaoncology/fullarticle/2763673.*Accessed May 2, 2020.
3. Lewis MA. Between Scylla and Charybdis - Oncologic decision making in the time of Covid-19. N Engl J Med. 2020 Apr 7. [Epub ahead of print]. Available from:*https://www.nejm.org/doi/full/10.1056/NEJMp2006588.* Accessed May 2, 2020.
4. Xia Y, Jin R, Zhao J, Li W, Shen H. Risk of COVID-19 for patients with cancer. Lancet Oncol.2020;21:e180. Available from: *https://www.thelancet.com/journals/lanonc/article/PIIS1470-2045(20)30149-2/fulltext.* Accessed May 2, 2020.
5. Cinar P, Kubal T, Freifeld A, Mishra A, Shulman L, Bachman J, *et al*. Safety at the time of the COVID-19 Pandemic: How to keep our oncology patients and healthcare workers safe. J Natl ComprCanc Netw. 2020 Apr 15:1-6. Available from: *https://jnccn.org/view/journals/jnccn/aop/article-10.6004-jnccn.2020.7572/article-10.6004-jnccn.2020.7572.xml.* Accessed May 2, 2020.
6. Yadav U, Pal R. Challenging Times for Children With Transfusion-dependent Thalassemia Amid the COVID-19 Pandemic. Indian Pediatrics. April 07, 2020. [Epub ahead of print]. Available from: *https://indianpediatrics.net/COVID29.03.2020/CORR-00155.pdf.*Accessed May 2, 2020.
7. Bouffet E, Challinor J, Sullivan M, Biondi A, Rodriguez Galindo C, Pritchard Jones K. Early advice on managing children with cancer during the COVID 19 pandemic and a call for sharing experiences. Pediatr Blood Cancer. 2020 Apr 2:e28327. Available from: *https://onlinelibrary.wiley.com/doi/full/10.1002/pbc.28327.* Accessed May 2, 2020.
8. Chen Z, Xiong H, Li JX, Li H, Tao F, Yang YT, *et al*. [COVID-19 with post-chemotherapy agranulocytosis in childhood acute leukemia: a case report] [Chinese]. ZhonghuaXue Ye XueZaZhi. 2020 Mar 9;41:E004. Available from: *http://journal.yiigle. com/LinkIn.do?linkin_type=pubmed&DOI=10.3760/cma.j.issn.0253-2727.2020.0004.*Accessed May 2, 2020.

Management Concern for Non-COVID Children During the COVID-19 Pandemic

Indian Council of Medical Research (ICMR) has released guidelines for coronavirus disease (COVID-19) testing in India and has been updating it frequently. The current version (April 9, 2020) [1] focusses on symptoms of COVID-19 infection and any epidemiological link (either foreign travel history, direct contact of COVID-19 patient, health worker or if patient coming from hot spot area) to maximize the sensitivity of the screening criteria.

Severe acute respiratory infection (SARI) is one of the criteria in the ICMR COVID-19 screening guidelines and it includes the presence of fever and cough and/ or respiratory distress [1], though certain additional symptoms have subsequently been added [2]. Experience from other countries has shown that SARI is not much common in pediatric COVID-19 [3-6]. Moreover, respiratory problems are common in children, and many non-COVID-19 conditions could manifest as respiratory distress even in this COVID-19 pandemic. The current ICMR COVID-19 screening criteria might be appropriate for children coming from the hot spot areas but it does not appear appropriate for children from other area, where it may lead to over-testing and undue stress on the already over-burdened healthcare system.

Moreover, barring few centers in India, both children and adult are being screened, tested and made to wait in the same isolation area till the report is available, which may lead to unnecessary exposure of children and their caregivers to COVID-19 [7]. It may also lead to delay in management of non-COVID conditions, since many facilities are not available for children in common isolation area.

There is, thus, an urgent need to reconsider screening criteria for COVID-19 in children taking in account the available evidence from our country. Furthermore, we are observing lesser number of pediatric admissions for common pediatric emergencies, and this requires vigilance as children might not be able to reach hospitals and we may encounter more deaths in children without COVID-19 [8]. We should not forget that non-COVID pediatric emergencies are still more common even in the era of COVID-19 pandemic.

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REFERENCES

1. Revised Strategy for COVID19 testing in India (Version4). Available from: *https://www.icmr.gov.in/pdf/covid/strategy/Strategey_for_COVID19_Test_v4_09042020.pdf.* Accessed on April 24, 2020.
2. Symptoms of Coronavirus. Available from: *https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html.* Accessed on April 30, 2020.
3. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, Tong S. Epidemiology of COVID-19 Among Children in China. Pediatrics. 2020 Mar 16 [Epub ahead of print]. Available from: *https://pediatrics.aappublications.org/content/early/2020/03/16/peds.2020-0702.1.long.* Accessed on April 30, 2020
4. Lu X, Zhang L, Du H, Zhang J, Li Y, Qu J, *et al.* SARS-CoV-2 infection in children. N Engl J Med. 2020; 23; 382:1663-5.
5. Livingston E, Bucher K. Coronavirus Disease 2019 (COVID-19) in Italy. JAMA. 2020 Mar 17 [Epub ahead of print]. Available from: *https://jamanetwork.com/journals/jama/fullarticle/2763401*. Accessed on April 30, 2020.
6. CDC COVID-19 Response Team. Severe outcomes among patients with coronavirus disease 2019 (COVID-19) - United States, February 12-March 16, 2020. MMWR Morb Mortal Wkly Rep. 2020;69:343-6.
7. Kelvin AA, Halperin S. COVID-19 in children: the link in the transmission chain. Lancet Infect Dis. 2020 Mar 25 [Epub ahead of print]. Available from: *https://www.thelancet.com/action/showPdf?pii=S1473-3099%2820%2930236-X.* Accessed on April 30, 2020.
8. Balasubramanian S, Rao NM, Goenka A, Roderick M, Ramanan AV. Coronavirus disease (COVID-19) in children - What we know so far and what we do not. Indian Pediatr. 2020; April 9 [Epub ahead of print]. Accessed from: *https://indianpediatrics.net/COVID29.03.2020/SA-00159.pdf.* Accessed on April 24, 2020.

Prepare for Post-COVID-Pandemic Polio Problems

The current crisis of the COVID-19 pandemic will, most likely, abate in due course as herd immunity builds up in all affected countries [1]. Among all public health projects that are stalled now, global polio eradication is a critical one and it ought to be put back on rail. However, we foresee formidable obstacles and propose remedial actions.

At a global level, on 24 March, 2020, the leadership of global polio eradication recommended suspending all OPV campaigns until at least second half of the year to prevent spread of SARS-CoV-2 [2]. Globally, vaccine derived polio virus (VDPV) type 2 outbreaks were not getting contained by OPV campaigns [3], and they may expand and spread widely as lockdowns are lifted in the affected countries. We may see resurgence of wild-virus polio in Afghanistan and Pakistan.

In India, all activities under the Universal immunisation programme (UIP) were stalled at the start of the lockdown, and later resumed in different parts of the country amidst patchy availability of public transport. There are consequences, affecting all vaccine-preventable diseases, but here we focus on the problems of polio as it is under eradication mode. The major risk in India and in all countries still using bivalent OPV with types 1 and 3, is the likelihood of emergence of VDPVs type 1 and type 3 [4], on account of long periods of no immunization following its widespread use until January-February 2020, including national pulse immunization in January, 2020. It is to prevent the emergence of VDPVs that India's UIP had been keeping up high herd immunity in children through OPV under UIP plus annual pulse campaigns twice annually, in spite of eradication of wild polioviruses in 2014. As polio immunization is suspended, India must anticipate the emergence and circulation of VDPVs. We will know the full picture only after the lockdown is lifted and surveillance for acute flaccid paralysis (AFP) and for polioviruses is resumed.

We feel that the safest strategy to mitigate this impending risk of VDPVs is to prepare for intensified immunization with IPV, aiming to not reintroduce OPV in children in India after achieving high IPV coverage. Full IPV immunization requires priming and boosting. If a high proportion of children are protected with full immunizing schedule of IPV adding booster dose(s), the chances of VDPV emergence will be minimized and any emerged VDPV transmission can be intercepted.

It is time for us to make these policy decisions after dialogue with stake-holders, supported by science and evidence accumulated in the country over the past 6 decades – and choose the path which is in the best interests of our children.

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REFERENCES

1. John TJ. Will Coronavirus pandemic eventually evolve as Pan-endemic? Curr Sci. 2020;118:855-6.
2. Global Polio Eradication Initiative. Call to action to support COVID-19 response. Available from: *http://polioeradication.org/news-post/call-to-action-to-support-covid-19-response*. Accessed April 25, 2020.
3. Centers for Disease Control and Prevention. Update on Vaccine-Derived Poliovirus Outbreaks — Worldwide, July 2019–February 2020; MMWR. 2020;69:489-95.
4. World Health Organization. Polio outbreak in Papua New Guinea. Geneva: WHO; 2018 [cited 2019 Oct 7]. Available from: *https://www.who.int/westernpacific/emergencies/papua-new-guinea-poliovirus-outbreak.* Accessed May 8, 2020.

KAWASAKI DISEASE AND CORONAVIRUS

Nearly half a century ago, Dr Kawasaki described the 50 cases of the eponymous disease whose etiology continues to flummox us. It has again reared its head in the alpine city of Bergamo in Lombardy, Italy. Pediatricians in the busy Hospital Papa Giovanni XXIII noticed a 30 times increase in the monthly incidence of Kawasaki disease between February, 2020 and April, 2020 compared to the previous 5 years. They analyzed the clinical data of these children diagnosed during the COVID19 pandemic and compared them to those diagnosed with Kawasaki disease between January, 2015 and February, 2020.

They found that the children with Kawasaki diagnosed during this pandemic had higher incidence of Kawasaki shock syndrome (50%), meningeal signs (40%), macrophage activation syndrome (50%) and abnormal echocardiography (60%). Seventy percent met criteria of an abnormal Kobayashi score, which predicts IVIG resistance, and needed adjunctive therapy with steroids.

Testing for SARS CoV-2 revealed positive IgG antibodies in 80% but a nasopharyngeal PCR for virus was positive in 20% only, suggesting an immune-mediated phenomenon. Coronaviruses have been implicated earlier also as the etiological basis of Kawasaki disease but had not been proven due to low PCR positivity.

The study has put all pediatricians on high alert and may shed light on the obscure origins of Kawasaki disease.
(*Lancet Online May 13 2020*)

A VACCINE AGAINST SARS-COV-2

The COVID-19 pandemic continues its relentless march across the globe. It is clear we are in for the long haul. Consequently the race for the vaccine is heating up. As of 28 April, 2020, there are 90 contenders. At least six have started safety trials in humans.

What is the modus operandi of the various vaccines? The commonest approach is the protein subunit vaccine. There are 28 teams targeting the spike protein or its receptor binding domain. The technique has been evaluated for SARS-CoV-1 in monkeys. The downside of these vaccines is the need for adjuvants and multiple doses. Twenty five groups are working on the viral vector based vaccines. Here, attenuated viruses like measles or adeno are genetically engineered to produce coronaviral proteins. This approach has been successful in the recently approved Ebola vaccine. However, sometimes previous immunity to the viral vector may interfere with a robust immune response.

Johnson and Johnson is experimenting with a killed viral vector vaccine. And Codagenix, New York and Serum Institute, India are working together to develop an attenuated live viral vaccine. Human safety trials have already begun in Beijing using an inactivated whole viral vaccine; some other groups are attempting to make a nucleic acid based vaccine.

Unanswered questions include how rapidly these can be developed, tested and clear safety trials. Who needs it most? What will the costing be? Economics and politics will soon overshadow medicine and ethics.
(*Nature News 28 April 2020*)

WHY IS SARS-COV-2 KINDER TO CHILDREN?

Overall experience shows that children may be less severely affected by SAR-CoV-2. This means understanding the disease in children may be the key to mitigating the disease in adults.

The first hypothesis involves the differential expression of the ACE- 2 receptors in children, through which the virus enters the cell. However, ACE-2 also plays a crucial role in converting angiotensin II to Angiotensin (1-7) and thereby attenuating its damaging effects on the lungs and cardiovascular system. Patients with chronic diseases have been noted to express less ACE-2 receptors. Evaluation of ACE- 2 levels in children may shed further light on the issue. The second possibility is that the innate immune system in children may be more battle ready to handle new organisms. Children have been shown to have natural antibodies of the IgM isotype with a broad reactivity and affinity independent of previous encounter with a particular organism. Recurrent infections that children have and their ongoing regular immunization may induce an enhanced state of activation, which would result in more effective defense against novel pathogens. There are data to show that children who were immunized against measles, and also after influenza vaccinations, had an overall reduction of other infections besides measles or influenza.
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CLIPPINGS

Theme: Immunization

Vero-cell derived inactivated vaccine candidate for SARS-Cov-2 *(Science. 2020; eabc1932)*

Researchers from Sinovac Biotech, China developed a pilot-scale production of a purified inactivated SARS-CoV-2 virus vaccine candidate (PiCoVacc), which induced SARS-CoV-2-specific neutralizing antibodies in mice, rats, and non-human primates. These antibodies neutralized 10 representative SARS-CoV-2 strains, suggesting a possible broader neutralizing ability. Three immunizations provided protection in macaques against SARS-CoV-2 challenge, without observable antibody-dependent enhancement of infection. The vaccinated monkeys tolerated well a SARS-CoV-2 virus challenge after 3 weeks of vaccination and none developed a full-blown infection. The monkeys given the highest dose of vaccine had the best response. In contrast, four control animals developed high levels of viral RNA in several body parts and severe pneumonia.

The old-fashioned inactivation methodology used can be developed easily by many vaccine developers in low- and middle-income countries, and the absence of lung damage in vaccinated animals with relatively low levels of antibodies lessens the concern about vaccine enhancement. However, the number of animals was too small and the fact that monkeys do not develop the most severe symptoms that SARS-CoV-2 causes in humans.

Oxford Group's vaccine prevents severe Covid-19 pneumonia in rhesus macaques (*bioRxiv. 2020.05.13.093195*)

The Oxford group researchers show that the adenovirus-vectored vaccine ChAdOx1 nCoV-19, encoding the spike protein of SARS-CoV-2, is immunogenic in mice. A single vaccination with ChAdOx1 nCoV-19 induced a humoral and cellular immune response in rhesus macaques. They observed a significantly reduced viral load in bronchoalveolar lavage fluid and respiratory tract tissue of vaccinated animals, and no pneumonia was observed in vaccinated rhesus macaques. At 7 days post inoculation, all animals were euthanized, and tissues were collected. Viral genomic (gRNA) was detected in nose swabs from all animals and no difference in viral load in nose swabs was found on any days between vaccinated and control animals. None of the vaccinated monkeys developed pulmonary pathology after inoculation with SARS-CoV-2. Importantly, no evidence of immune-enhanced disease following viral challenge in vaccinated animals was observed.

These observations are marked contrast to the results reported from Sinovac trial, as the vaccine did not protect the animals from infection; though, it prevented severe disease. Thus, the vaccine did not provide sterilizing immunity to the virus challenge, the gold standard for any vaccine, but it may provide partial protection. The moot question is that will partial protection be enough to control the COVID-19 pandemic?

SARS-Cov-2 infection protects against re-challenge in rhesus macaques *(Science 2020; science.abc4776)*

An understanding of protective immunity to SARS-CoV-2 is critical for strategies aimed at ending the pandemic. A key unanswered question is whether infection with SARS-CoV-2 results in protective immunity against re-exposure. Chandrashekar, *et al.,* developed a rhesus macaque model of SARS-CoV-2 infection and observed that macaques had high viral loads in the respiratory tract, humoral and cellular immune responses, and pathologic evidence of viral pneumonia. Following initial viral clearance, animals were re-challenged with SARS-CoV-2 and showed 5 log10 reductions in median viral loads in bronchoalveolar lavage and nasal mucosa compared with primary infection. Anamnestic immune responses following re-challenge suggested that protection was mediated by immunologic control. These data show that SARS-CoV-2 infection induced protective immunity against re-exposure in nonhuman primates. However, it should be emphasized that there are important differences between SARS-CoV-2 infection in monkeys and humans, with many parameters still yet to be defined in both species.

DNA vaccine protection against SARS-CoV-2 in rhesus macaques *(Science.2020; eabc6284)*

In this study, researchers developed a series of DNA vaccine candidates expressing different forms of the SARS-CoV-2 Spike (S) protein and evaluated them in 35 rhesus macaques. Vaccinated animals developed humoral and cellular immune responses, including neutralizing antibody titers comparable to those found in convalescent humans and macaques infected with SARS-CoV-2. Following vaccination, all animals were challenged with SARS-CoV-2, and the vaccine encoding the full-length S protein resulted in >3.1 and >3.7 log10 reductions in median viral loads in bronchoalveolar lavage and nasal mucosa, respectively, as compared with sham controls. Vaccine-elicited neutralizing antibody titers correlated with protective efficacy, suggesting an immune correlate of protection. These data demonstrate vaccine protection against SARS-CoV-2 in nonhuman primates.

Truly little is known about immune correlates of protection and protective efficacy of candidate SARS-CoV-2 vaccines in animal models. In this study, the researchers demonstrate vaccine protection with substantial reductions in median viral loads in BAL and nasal swabs, in immunized animals compared with controls.

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Pederus Dermatitis

A 4-year-old boy presented with erythematous vesicular linear streaks over left cheek and erythematous papular eruption of lobule of left ear since 3 days (***Fig.* 1**). It was of sudden onset noticed first in the morning with burning sensation over lesions. Except for that, rest of the physical examination was within normal limits. He did not remember any contact with insects. Oral antihistamines and topical antibiotics steroid cream was advised on an out-patient basis.

Pederus dermatitis (*Bhiter* beetle dermatitis or insect-bite reaction) is a contact irritant dermatitis. It is characterized by sudden onset of erythematobullous lesions on exposed areas of the body caused by accidental contact of a beetle of genus *Paederus.* It contains a toxin paederin present in beetle coelomic fluid. Brushing or crushing of the beetle causes contact of paederin with skin resulting in peculiar skin lesions. Principal differential diagnosis includes phytophoto-dermatitis, thermal and chemical burns. If patient presents early, washing with soap and water is helpful. Treatment includes soothing agents, oral antihistamines and topical antibiotics-steroid cream for local application.

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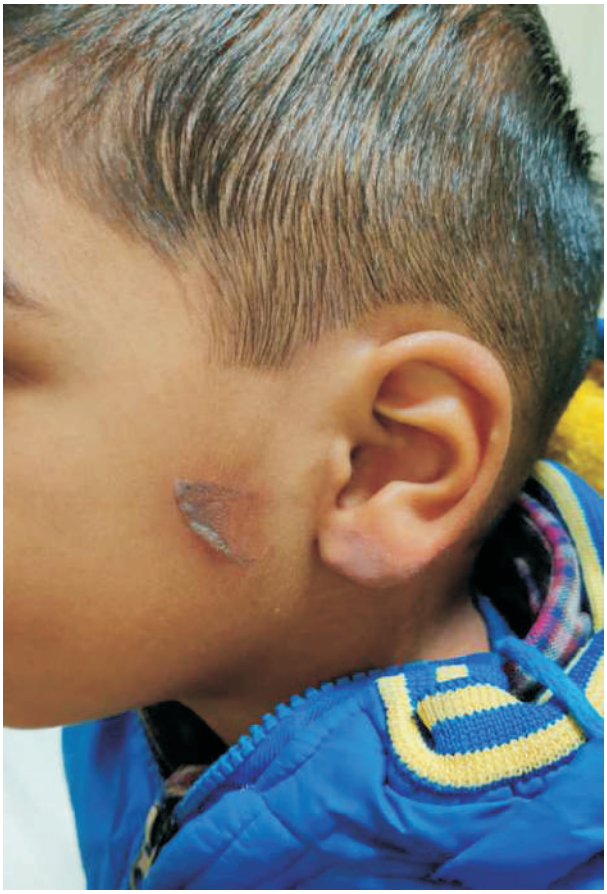

Fig. 1 *Paederus dermatitis on left check and lobule of ear.*

Cherubism

A 10-year-old girl presented with bilateral jaw swelling noticed by her parents when she was 3 years old. Her father had a similar history with his jaw swelling spontaneously regressing by the 25 birthday. Examination revealed fullness of cheeks and jaws with upward tilting of eyes exposing the sclera below inferior limbus, giving, the so-called 'cherubic' appearance (***Fig.* 1a**). Radiograph revealed multiple thin-walled cystic lesions involving rami and body of mandible (***Fig.* 1b**), reconfirmed on computed tomography images (***Fig.* 1c**). Biochemical investigations revealed mildly elevated total alkaline phosphatase. She was diagnosed as familial cherubism and reassured about the self-limiting disease course.

Cherubism is a fibro-osseous disorder characterized by the appearance of multilocular, expansile radiolucent lesions involving mandible or maxilla that usually appear at 2-7 years of age. Gain-of-function mutations in *SH3BP2* gene have been implicated. Differential diagnoses include brown tumor of hyperparathyroidism, Noonan/multiple giant cell lesion syndrome, fibrous dysplasia (as part of McCune-Albright syndrome), and ossifying fibromas seen in hyperparathyroidism-jaw tumor syndrome. Cherubism is self-limiting and begins to regress spontaneously around the age of puberty.

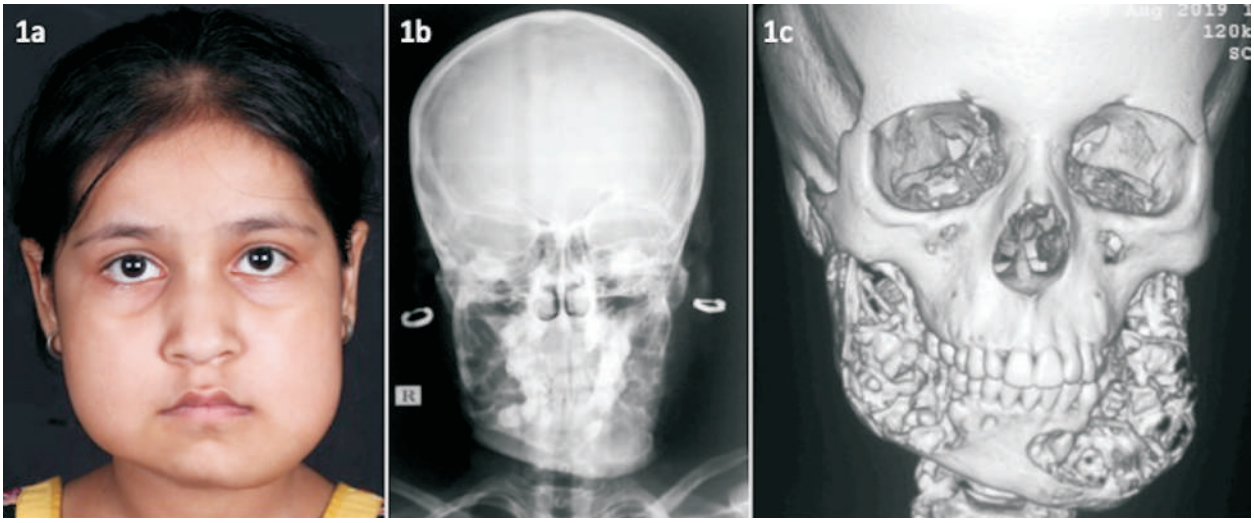

Fig. 1 *Cherubism characterized by (a) Bilateral fullness of the cheeks and jaws with slight upward tilting of the eyes, giving a 'cherubic' appearance; (b) Radiograph of the face showing multiple thin-walled cystic lesions involving rami and body of mandible; (c) Computed tomography of the face with 3D reconstructed images showing multiple expansile soft-tissue lesions involving both halves of the mandible causing areas of thinning and destruction of the overlying bony cortex.*

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Guttate Psoriasis

A 6-year-old boy presented with rash over whole body for a week, and past history of upper respiratory tract infection two weeks ago. Examination revealed extensive guttate erythema with overlying tiny scales; individual lesions measured about 2-10 mm in diameter, and predominantly involved the extremities and trunks (***Fig.* 1**). The rest of the physical examination was normal except tonsillar enlargement. Anti-streptolysin O(ASO) antibody was positive. After receiving the narrowband UVB phototherapy and oral penicillin, the skin lesions gradually faded within 8 weeks, and did not recur over a one-year follow-up.

Guttate psoriasis is a subtype of psoriasis characterized by acute eruption of numerous small, erythematous papules and plaques. It usually occurs in children and adolescents, but it can also occur in other age groups. Streptococcus infection is an important risk factor which can usually precedes its development by 2-3 weeks; although, the relationship between streptococcal infection and guttate psoriasis is not fully understood. Although diagnosis is based on clinical, but in the difficult cases, skin biopsy is needed. Differential diagnosis includes

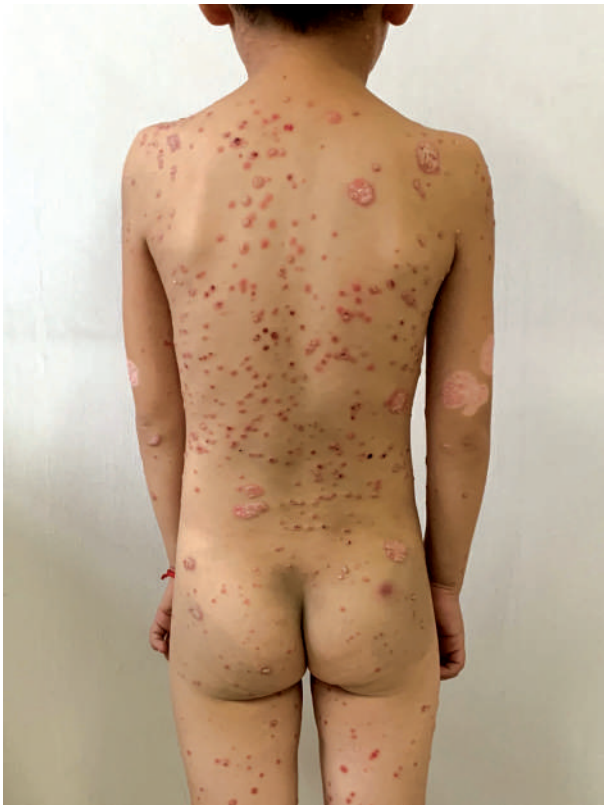

Fig. 1 *Extensive guttate erythematous lesions.*

pityriasis rosea, tinea corporis, nummular dermatitis and prurigo nodularis. Guttate psoriasis can spontaneously fade within several weeks or several months, phototherapy as a first-line treatment has a good effect, and antibiotics may be used if persisting infection is suspected. Overall, most patients have a good prognosis, just a few patients have a chronic course.

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Traumatic Anserine Folliculosis

A 10-year-old boy presented with asymptomatic roughness over the left cheek since 6 months. He acknowledged resting in a particular position, which led to prolonged localized pressure and friction, while watching television or studying. Examination revealed multiple tiny skin-coloured, discrete but grouped, follicular papules having a sandpaper-like feel (***Fig.* 1**). Considering the site of affection and characteristic history, a diagnosis of traumatic anserine folliculosis was established. He was treated with topical tretinoin cream, and advised to avoid trauma and friction to the area.

Traumatic anserine folliculosis is an under-recognized condition characterized by multiple, closely set grouped follicular papules affecting the chin, jaws, and neck. This entity should be differentiated from keratosis pilaris (keratinous follicular plugs, usually surrounded by erythema), lichen spinulosus (pruritic symmetric plaques having thorny grouped follicular papules), trichostasis spinulosa (hair tufts through follicle, resembling comedones), and trichodysplasia spinulosa (viral infection in immunocompromised). Treatment includes topical keratolytics and removal of etiological factor.

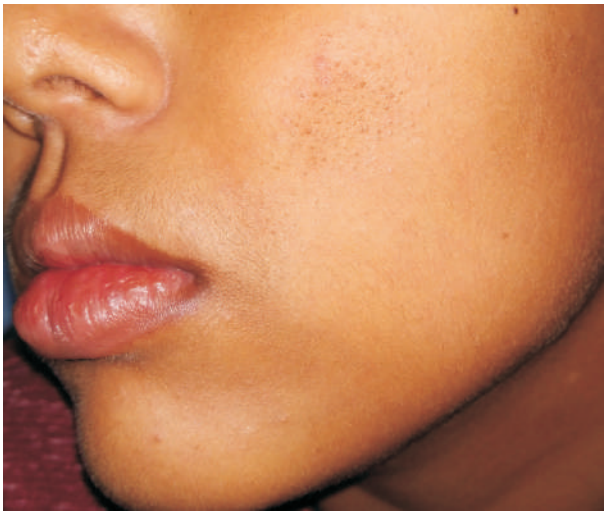

FIG. 1 *Skin-colored, discrete but grouped, follicular papules over left cheek.*

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Becker Melanosis

A 12-year-old boy presented with a gradually progressive asymptomatic area of discoloration over right forearm since last 2 years. Examination revealed a unilateral, well-circumscribed 6cm x 8cm tan-brown patch on the right forearm, and having irregular border and blotchy pigmentation at the periphery (***Fig.* 1**). Localized coarse hair and acneiform eruptions were observed, restricted to the patch. Darier sign was negative. No skeletal, soft tissue or neurological abnormalities were

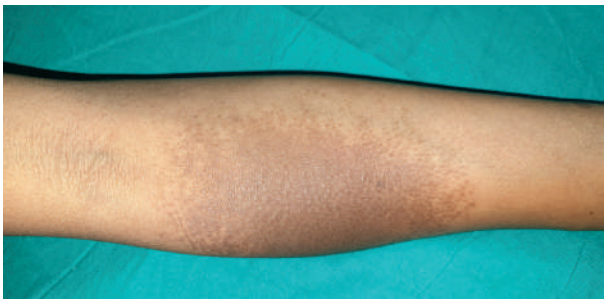

Fig. 1 *Well-circumscribed tan-brown patch on the forearm, having irregular border and blotchy pigmentation at the periphery with localized coarse hair and acneiform eruption.*

found on further examination. A diagnosis of Becker melanosis was made and the benign nature of the condition explained to the family.

Becker melanosis is typically characterized by unilateral circumscribed hyperpigmentation that usually begins at puberty and displays features of androgen sensitivity like hypertrichosis, and acneiform eruptions. Several skeletal or soft tissue anomalies can be associated with Becker melanosis (Becker nevus syndrome). The common mimickers of the condition are melanocytic nevus (usually congenital, lacks acneiform eruption), café au lait macules (present since birth, no hypertrichosis), and plexiform neurofibroma ('bag of worms' sign, presence of other features of neurofibromatosis). Topical flutamide or Q-switched ruby or Er: YAG laser can be used to treat the cosmetic concerns regarding the condition.

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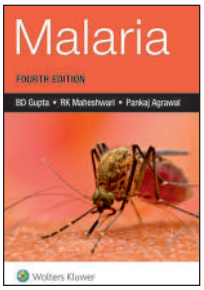
BOOK REVIEWS



Malaria: Fourth Edition

BD GUPTA AND RK MAHESHWARI
Wolters Kluwer (India) Pvt. Ltd.
Pages:374; Price: Not mentioned.

This concise book is a good ready reckoner for medical personnel dealing with malaria. Most of the recent advancements in the preventive and therapeutic strategies to control malaria have been incorporated into the book. The clinical features highlighted through case scenarios is a welcome addition, assisting easy grasp of the subject.

There are certain points that need to be taken care of in the next edition *e.g.,* the dated information on geographical distribution of malaria incidence in India, lack of alignment between tables on modes of administration of anti-malarials and on chemotherapy. There is a need to expand the section on chemoprophylaxis to include more drugs like chloroquine, proguanil and atovaquone, and provide information on the total duration of treatment.

On the whole, this book is a valuable addition to the armamentarium of the treating physician.

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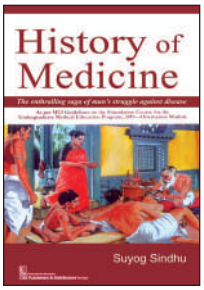

History of Medicine

DR SUYOG SINDHU
CBS Publishers & Distributors Pvt Ltd.
Pages: 86; Price: 175/-

Since the adoption of foundation course for MBBS curriculum last year, there has been a wild search for resource materials for various new topics introduced, including History of Medicine. This compact and informative book comes as a ready reckoner – composite and interesting information on history of medicine delineated on four time lines- Prehistory, Middle ages, Period of renaissance and Modern medicine. The multitude of authentic photographs is surely the icing on the cake and along with the two-color printing makes the material visually appealing. The book is written in an easy narrative style that facilitates understanding. There is a chapter dedicated to famous Indian doctors (chapter 8) that should serve as an inspiration to our students, although the selection is not exhaustive and a bit idiosyncratic. Definitely recommended!

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PRESENTS

MENVEO
Meningococcal Group A,C,W135 & Y
Conjugate Vaccine Ph. Eur.

High efficacy*
with a clinically acceptable safety profile[1]

Robust immune response
across age groups (>2 years) against all four serogroups[1,2]

~10 years
of global real world experience[3]

Approved in US & EU
only MCV licensed in both regions[4,5]

Safety information:
Most common adverse events in clinical trials:
Injection site pain, erythema, induration, sleepiness, headache, irritability, malaise, eating disorder, nausea, vomiting, diarrhea, rash, myalgia, arthralgia, fever and chills.

* The efficacy of MENVEO has been inferred by measuring the production of serogroup-specific anti-capsular antibodies with bactericidal activity[6]

^ Meningococcal Vxs global IMS data

Reference : 1. S.A. Halperin et al., Vaccine 28 (2010), 7865–7872 2. Lalwani S, et al. Int J Infect Dis. 2015;38:36-42 3. GSK data on file- Sales summary- May 2019 4. ECDC factsheet about meningococcal disease, available at https://www.ecdc.europa.eu/en/meningococcal-disease/factsheet (Accessed Nov, 2019) 5. Meningococcal Vaccine Safety, available at https://www.cdc.gov/vaccinesafety/vaccines/meningococcal-vaccine.html (Accessed Nov, 2019) 6. Menveo India Prescribing information Version MNV/PI/IN/2019/03 dated 21 August 2019

For the use only of Registered Medical Practitioners or a Hospital or a Laboratory
Abbreviated Prescribing information of MENVEO [Meningococcal Group A, C, W-135 and Y Conjugate Vaccine Ph. Eur.]
ACTIVE INGREDIENT: Each reconstituted dose (0.5ml) contains: 10 µg Meningococcal group A oligosaccharide conjugated to 16.7-33.3 µg CRM197, 5 µg Meningococcal group C oligosaccharide conjugated to 7.1-12.5 µg CRM197, 5 µg Meningococcal group W-135 oligosaccharide conjugated to 3.3-8.3 µg CRM197, 5 µg Meningococcal group Y oligosaccharide conjugated to 5.6-10 µg CRM197. Therapeutic Indication: Active immunization of children (from 2 years of age), adolescents and adults to prevent invasive meningococcal disease caused by Neisseria Meningitidis groups A, C, W-135 and Y. Posology: Children (> 2 years of age), adolescents and adults: administered as a single dose (0.5 ml). Older people: Limited data in aged 56-65. No data in aged >65 years. Booster vaccination: May be given in subjects vaccinated with same, other conjugated or unconjugated polysaccharide meningococcal vaccine. Need and timing of booster dose to be based on national recommendations. Children under 2 years of age: Safety and efficacy not established. Method of Administration: Intramuscular injection, preferably in deltoid muscle. Contraindications: Hypersensitivity to any active substance, excipient or diphtheria toxoid (CRM197), or life-threatening reaction after administration of vaccine with similar components. Postpone in acute severe febrile illness. Minor infection not a contraindication. Special Warnings and Precautions: Take all precautions for prevention of allergic or other reaction. Appropriate medical treatment and supervision should be readily available in case of rare anaphylactic reaction. Anxiety-related reactions (vasovagal reactions, syncope, hyperventilation or stress-related reactions) may occur; ensure procedure to avoid injury from fainting. Should not be administered intravascularly. Not protect against serogroups of N. meningitidis not included in vaccine. May not elicit protective immune response in all vaccinees. Waning of serum bactericidal antibody titers against serogroup A seen in studies; clinical relevance unknown. If individual at risk of exposure to Men A: consider booster dose. No data on use as post-exposure prophylaxis. Vaccination in immunocompromised individuals may not result appropriate protective antibody response. Increased risk of invasive disease in individuals with familial complement deficiencies and treatments that inhibit terminal complement activation (for example, eculizumab). Evaluate risk-benefit in persons at risk of haematoma following intramuscular injection. Interaction with Other Medicinal Products and Other Forms of Interaction: Can be given concomitantly with hepatitis A and/or B, yellow fever, typhoid fever (Vi polysaccharide), Japanese encephalitis and rabies. Administer concomitant vaccines at separate injection sites (preferably contralateral) Adolescents (11-18 years of age): Can be co-administered with Tetanus, Reduced Diphtheria and Acellular Pertussis Vaccine, Adsorbed (Tdap) alone/or Human Papillomavirus Quadrivalent (Types 6, 11, 16 and 18) Vaccine, Recombinant (HPV). Lower W-135 seroresponse on administration of MENVEO one month after Tdap; clinical relevance unknown. Children (2-10 years of age): No data on concomitantly administration with other childhood vaccines. Co-administration with other vaccines not studied. Administer concomitant vaccines at separate injection sites, preferably contralateral. Effects on Ability to Drive and Use Machines: Dizziness very rarely reported, may temporarily affect ability to drive or use machines. Undesirable Effects: Clinical Trial Data: A) Subjects aged 2 - 10 years: Very common (> 1/10): sleepiness, headache, irritability, malaise, injection site pain, erythema (≤50 mm), induration (≤50 mm) Common (≥ 1/100 to <1/10): eating disorder, nausea, vomiting, diarrhea, rash, myalgia, arthralgia, injection site erythema (>50mm), induration (>50mm), chills, fever ≥38°C Uncommon (≥ 1/1,000 to <1/100): injection site pruritus B) Subjects aged 11 to 65 years: Very common (> 1/10): headache, nausea, myalgia, injection site pain, erythema (≤50 mm), induration (≤50 mm), malaise Common (≥1/100 to <1/10): rash, arthralgia, injection site erythema (>50 mm), induration (>50 mm), fever ≥38°C, chills Uncommon (≥ 1/1,000 to <1/100): dizziness, injection site pruritis Post-marketing experience (all age groups) hypersensitivity including anaphylaxis, tonic convulsion, febrile convulsion, syncope, vertigo, injection site cellulitis, injection site swelling, including extensive swelling of the injected limb.
Version: MEN/API/IN updated 6-Sep-2019.
Registered medical practitioners can refer company website http://india-pharma.gsk.com/en-in/products/prescribing-information/ for full Product Information.
Please report adverse events with any GSK product to the company at india.pharmacovigilance@gsk.com.
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PN-IN-MNV-ADVT-190001 Date of Preparation: Dec 2019

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