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HEMATO-ONCOLOGY

NUTRITIONAL ANEMIA - STRATEGY FOR PREVENTION AND MANAGEMENT

***Elizabeth KE**

Abstract: *This review elucidates the prevalence of anemia in various age groups in India and existing strategies for prevention and management of anemia. The inadequacies of existing strategies and their solutions are discussed. Multi-pronged approach incorporating delayed cord clamping, iron folic acid supplementation, dietary diversification and food fortification is recommended. Besides, there is a need to address iron refractory anemia, infections like malaria, worm infestations, Helicobacter pylori infection and also genetic causes like hemoglobinopathies.*

Keywords: *Anemia, Prevalence, Digital testing for hemoglobin, Anemia mukt bharat, National iron plus initiative.*

Anemia, especially nutritional, is a major public health issue in India that cuts across all age groups and all socio-economic strata. The global prevalence of anemia is also high;[1] 43% among children, 38% among pregnant mothers and 29% in women in the reproductive age group (WRA) of 15-49 years of age. As per National Family Health Survey (NFHS-4) 2015-2016, our country is having alarmingly high prevalence of anemia - 58% among 6-59 month old children, 50% among pregnant mothers and 53% among women of reproductive age (WRA).[2] Anemia prevalence of more than 40% is considered a serious and important public health problem. Despite all the existing public health measures to curb anemia, the decline in the prevalence over the last decade from NFHS-3 (2005) to NFHS-4 (2015) is only 10% among young children, 8% among pregnant and 2% among WRA.[2,3]

Anemia adversely affects oxygen carrying capacity, physical stamina, learning ability, cognition, brain development and myelination in growing children and hence increases morbidity and mortality in pregnant women besides increasing premature births, low birth weight (LBW), perinatal morbidity and mortality.[1]

Causative factors for anemia are ineffective erythropoiesis, hemolysis and blood loss. Common etiologies of anemia are nutritional deficiencies like iron, folate, vitamins B12, B6 and E, copper and protein, diseases related to bone marrow and kidney, chronic infections, and hemolytic anemias. Recently, the role of vitamin A and D deficiency in anemia has been highlighted.[4,5] The pathogenesis is through diverse biological mechanisms like differentiation of erythrocyte progenitor cells and mobilization of iron stores by vitamin A and reduction of pro-inflammatory cytokines and direct suppression of hepcidin mRNA transcription by vitamin D.[4,5] Approximately half the cases of anemia are due to iron deficiency.[6] Hence, management strategies and prevention are centered around iron and folic acid (IFA) supplementation. 'Anemia Mukt Bharat' under the 'Poshan Abhiyan Programme' aims at a 'Life cycle approach' covering all children beyond 6 months of age, including adolescents and WRA including pregnant and lactating women, in the updated version of National Iron Plus Initiative (NIPI) campaign.[7,8] Effective and universal strategies for management and prevention of anemia is expected to reduce morbidity, mortality and disability.

Iron deficiency and treatment

Iron deficiency is very rampant in developing countries and it has three stages; a stage of iron depletion, iron deficiency and iron deficiency anemia (IDA).

Table I. Iron requirement in various age groups

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	0.27 mg		-	-
7-12 months	11 mg		-	-
1-9 years	10mg		-	-
Adolescents	8-11 mg	8-15 mg	-	-
Adults	8 mg	18 mg	27 mg	9 mg

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Table II. WHO recommended hemoglobin cut off values (g/dL) among beneficiaries.[9]

Population	No anemia	Mild anemia	Moderate anemia	Severe anemia
Young Children (6-59 months)	≥11	10-10.9	7-9.9	<7
Children (5-11 yrs.)	11.5 or more	11-11.4	8-10.9	<8
Children (12-14 yrs.)	12 or more	11-11.9	8-10.9	<8
Pregnant woman	11 or more	10-10.9	7-9.9	<7
Female >15 yrs	12	11-11.9	8-10.9	<8
Male >15 yrs	13	11-12.9	8-10.9	<8
WRA	12	11-11.9	8-10.9	<8

Table III. Various laboratory tests for iron deficiency (ID) and iron deficiency anemia (IDA)

Parameter	Cutoff Value	Remarks
RBC Count - Child Adult Male Female	4.0 - 5.2 million cells/microliter 4.5 - 5.9 million/microliter 3.8 - 4.8 million cells	Decrease in IDA
Hematocrit / PCV Male Female	 40.7% to 50.3% 36.1% to 44.3%	Decrease in IDA
Red cell Indices MCV MCH MCHC	 87 ± 7 fl. 29 ± 2 picogram/cell 34 ± 2 g/dL.	MCV, MCH, MCHC decrease in IDA
RDW - Child Male Female	13 ± 1.5% 12.2-16.1% 11.8- 14.5%	Increase in ID & IDA
S. Iron- Male Female	65-177 μg/dL (11.6–31.7 μmol/L) 50-170 μg/dL (9.0–30.4 μmol/L)	Decrease in ID & IDA
S. Ferritin - Male Female	20-250 μg/mL 15-150 μg/Ml	Decrease in IDA, Increase with infalmmation
TIBC	250-370 μg/dL (45-66 μmol/L)	Increase in ID & IDA
Transferrin Saturation Male Female	 20-50% 15-50%	Increase in IDA
Trasferrin Receptor (TfR)	3 fold rise (nmol/L)	Increase in ID, Unaffected by inflammatory markers
Mentzer Index	MCV/RBC Count	> 13 in IDA, < 13 in Thalassemia
Peripheral smear	Microcytic Hypochromic Anemia	Abnormal in IDA
Retic count	0.5 - 1.5%	Decrease in IDA
Bone Marrow Stain for Iron	Prussian Blue Stain for Ferric Iron	Decrease in ID & IDA

Iron requirement varies with age and physiological status (Table I).

Dietary iron has two main forms: heme and non-heme. Bioavailability of heme iron is better than non-heme iron. Plants and iron-fortified foods contain non-heme iron only, whereas meat, seafood and poultry contain both heme and nonheme iron. Heme iron, which is formed when iron combines with protoporphyrin IX, contributes about 10% to 15% of total iron intake. A divalent metal transporter (DMT1) transports iron across the mucus membrane into the cell. From the cell, it is either stored in ferritin or exported by ferroportin 1 (FP1), located in the enterocyte. Ferrous iron is oxidized back to ferric form by ferroxidase, ceruloplasmin and hephaestin and loaded into transferrin. Hepcidin is a key regulator of the entry of iron into the circulation. Iron is incorporated into hemoglobin, myoglobin and various enzymes. 90% of circulating iron is bound to transferrin and 10% to ferritin. Ferritin reflects iron stores.

The clinical manifestations of iron deficiency and IDA are varied. WHO recommended cut off values of hemoglobin are depicted in Table II.

A variety of tests are available to detect IDA (Table III). Among these, increase in RDW, Total Iron Binding Capacity and Transferrin receptor are early markers of iron deficiency, before IDA sets in.

Various oral and parenteral iron preparations are available for treatment (Table IV). Ferrous salts and sustained release preparations are more bioavailable. A daily dose of 3 mg/kg of iron is currently recommended for treatment under the Anemia Mukt Bhart programme.[10] Iron is administered 1 hour before or 2 hours after meal for better absorption. Administering iron preparation to higher back in the tongue and oral rinsing with water are recommended to prevent staining of teeth. Revaluation is recommended after 3-4 weeks and the expected rise in hemoglobin is >1.5 g/dL. Otherwise iron refractory anemia should be considered. In those who respond, iron should be continued for 12-20 weeks after restoration of normal hemoglobin to restore stores. Hospitalization is recommended in under five children and pregnant mothers with hemoglobin <5 g/dL. Packed red cell transfusion and parenteral iron preparations are advisable in them.[10] Dose of iron for parenteral deficit therapy is calculated as follows: 4.4 X Body weight (Kg) X Hb deficit (g/dL). Various other formulae are also available for calculation. Iron therapy is contraindicated in primary hemochromatosis and hemosiderosis in children with hemolytic anemias.

Appraisal of existing management and preventive strategies

Anemia Mukt Bharat is a 6 X 6 X 6 strategy with 6 sets of beneficiaries, 6 sets of goals with 6 major interventions and 6 institutional mechanisms.[10] Currently, enteric coated tablets are getting replaced by sugar coated tablets of iron folic acid (IFA). The aim is a lifecycle approach of iron supplementation for the target group; especially females, starting biweekly for 6 months - 5 years, weekly for 5-10 years, 10-15 years and 15- 49 years old WRA. Males are included in the programme from 6 months till 15 years of age.

Table IV. Various oral and parenteral iron preparations.

Preparation	Strength	Remarks
Ferrous sulfate - hydrous	200 mg	20% elemental iron
Dried - anhydrous	200 mg	32% elemental iron
Ferrous Gluconate	300 mg	12% elemental iron
Ferrous Fumarate	200 mg	33% elemental iron
Carbonyl Iron	45 mg	97.5% for grade S and 99.5% for grade R compounds. Do not lie down for at least 10 minutes after taking
Colloidal Ferric Hydroxide	25 mg/ml	Iron (III) oxide-hydroxide or ferric oxyhydroxide
Iron Hydoxy Polymaltose		Absorption not hindered by food, less GI upset, negligible free radicals
Iron Dextran IM Iron Sorbitol IM	50 mg/ml	Maximum dose 2g, Deep IM in buttock using Z Track technique
Iron Sucrose IV	50 mg/ml	Can be given as slow infusion with NS
Ferric Carboxy Maltose (FCM)		Recommended as per Anemia Mukt Bharat Programme. Both oral and IV

Six sets of beneficiaries for anemia prophylaxis

1. 6-59 months - Biweekly 20 mg elemental iron and 100 mcg folate as IFA syrup/tablet, starting at 6 months of age plus biannual deworming starting at one year of age
2. 5-10 years - Weekly Iron and Folic acid supplementation (Junior WIFS), pink tablet containing 45 mg elemental iron and 400 mcg folic acid plus biannual deworming.
3. 10-15 years - Weekly IFA supplementation (WIFS), blue tablet containing 60 mg elemental iron and 500 mcg folic acid plus biannual deworming.
4. Pregnant mothers - Daily IFA, red tablet containing 60 mg elemental iron and 500 mcg folic acid up to 180 tablets, starting from 14 weeks of gestation till delivery.
5. Lactating mothers-red tablet containing 60 mg elemental iron and 500 mcg folic acid postnatally for six months- 180 tablets.
6. WRA- Weekly IFA red tablet containing 60 mg elemental iron and 500 mcg folic acid.

The six set of goals for 2022

Taking 2016 as base year, a 3% reduction in prevalence is targeted every year in each group, totaling 18% reduction in 6 years, i.e., by year 2022.The goals are as follows.

1. In 6-59 months old children, reduce anemia prevalence from 58% to 40%.
2. In adolescent boys, reduce anemia prevalence from 29% to 11%.
3. In adolescent girls, reduce from 54% to 36%.
4. In pregnant mothers, reduce from 50% to 32%.
5. In lactating mothers, reduce from 58% to 40%.
6. In WRA, reduce from 53% to 35%.

The six interventions

1. Prophylactic IFA supplementation.
2. Deworming.
3. Intensified year-round behavioral change communication campaign (Solid body, Smart Mind), ensuring delayed cord clamping.
4. Testing of anemia using digital methods and treatment.
5. Mandatory provision of iron folic acid fortified foods, especially in Government funded programmes.
6. Addressing non-nutritional anemia in endemic pockets with special focus on malaria, hemoglobinopathies and fluorosis.

The six institutional mechanisms

1. Intra-ministerial coordination.
2. National Anemia Mukt Bharat Unit.
3. National Centre for excellence and advanced research on anemia control.
4. Convergence with other ministries.
5. Strengthening supply chain and logistics.
6. Anemia Mukt Bharat Dashboard and digital portal - One stop shop for anemia. This is a very elaborate guide on anemia prophylaxis and treatment.

Point of Care Testing (POCT) for anemia by WHO approved methods is not yet in place.[11] The cut off values for normal hemoglobin and mild, moderate and severe anemia in various age groups are not universally disseminated.[9] For operational convenience, double the dose of prophylaxis is given for treatment, with monitoring after 2-4 weeks. If there is no improvement in hemoglobin with IFA supplementation, alternative causes like iron refractory anemia, infections like malaria, worm infestations and H. pylori infection and genetic causes like hemoglobinopathies are to be investigated. In severe anemia, judicious use of parenteral iron (Iron sucrose, ferric carboxy maltose) and packed red blood cells transfusion are recommended.

Behavioral change communication (BCC) also aims at dietary diversification including '3 Gs- grams, grains and greens', nuts, dry fruits, along with vitamin C and heme iron, along with advice for avoiding coffee and tea with major meals. Delayed cord clamping up to one to three minutes, exclusive breastfeeding till 6 months of age and cooking at least one food item in cast iron vessel by long duration simmering are the other community level interventions.

Another parallel programme is food fortification, namely iron fortified rice and other cereals for young children, iodine and iron double fortified salt and home fortification using micronutrient powders (MNPs) to supply at least 12 mg elemental iron.

Setbacks and solutions in the existing programmes

IFA supplementation is in place for several decades, but the impact on anemia reduction is not rewarding

so far. There are several operational and social reasons for this. The following are some of the issues to be analysed and apprised on a war footing:

- IFA supply is irregular and not uniform even in the governmental facilities. The private sector is often not included in the umbrella of free IFA prophylaxis programme.
- Private health facilities are not adequately sensitized or informed about the recommendations of the Anemia Mukt Bharat programme.
- Compliance to the IFA programme is only about 30% among those who receive IFA.
- WHO (2001) recommends daily iron supplementation among 6-59 months-old children, in countries with anaemia prevalence >40%, but India is following biweekly supplementation for logistic reasons.[12,13,14]
- From 1970 till recently, the elemental iron content in IFA for adolescents and adults was 100 mg, which is higher than the reported tolerable upper limit (TUL), which is estimated to be 60 mg or even less.[15,16] Currently it is recommended to give tablets containing 60 mg elemental iron.
- Enteric coated IFA has less bioavailability compared to sugar coated tablets. Currently sugar-coated tablets are recommended.
- About 90% of oral intake of iron is unabsorbed and unbound iron can lead to oxidative stress and increased morbidity from infections.[17] Hence iron has to be withheld during the initial phase of SAM management protocol (2 weeks), even in the presence of significant anemia.
- Fecal iron has an inverse relationship with fecal lactobacilli suggesting unhealthy gut microbiome.[18] Altered gut microbiota may lead to increased risk of insulin resistance and obesity.[19]
- Higher doses of iron supplementation, >60 mg of elemental iron intake during pregnancy is linked with gestational diabetes.[20]
- Ferrous Sulphate is not a gastric friendly drug and hence, compliance and adherence are low in all age groups. Development of low dose highly bioavailable iron formulations in supplementation programmes is suggested; liposomal iron, sprinkles etc. need to be piloted.
- Dietary diversity and intake of vitamin C for absorption of non-heme iron is not often practiced, instead intake of coffee and tea that hinder iron absorption is widely in practice. Bioavailability of iron has been shown to double by adding 100 g guava than with amla.[16] Amla contains the antioxidant polyphenols, which is an anti-nutrient to iron.[21] Phytate also reduces iron absorption. Recommended phytate to iron ratio is <0.4:1 and that of vitamin C to iron is >4:1 for better bioavailabilty.[22]
- Apart from supplementation and dietary diversity, food fortification is an important strategy to curb anemia. Even though, it is expensive, it can reach large majority of population. But fortification strategies like the iron and iodine fortified salt, is still in the pilot stage only.
- Use of point of care testing (POCT) in measurement of hemoglobin is not in place. Invasive methods like direct cyanmethemoglobin method (gold standard), hemoglobin color strip (HCS-HLL), digital hemoglobinometers (TrueHb), hemocue, Hemocue 301with reagent free cuvettes, Dia Spect and non-invasive devices (TouchHb), Occlusion spectroscopy-NBM 200, transcutaneous reflection spectroscopy (HemoSpect), pulse co-oximetry are recommended by WHO.[10]
- Iron refractory anemia, infections like malaria, worm infestations and H. pylori that hinder iron absorption in upper gut, prevalence of megaloblastic anemia due to B12 deficiency and genetic causes like hemoglobinopathies are to be addressed in relevant cases.
- Comparison of prevalence of anemia estimated by NFHS, District level health survey (DLHS) and annual health survey (AHS) and reporting reduction or increase in prevalence of anemia has a big fallacy due to the difference in sampling techniques and method of estimation of hemoglobin levels and cut off values.[23]

Conclusion

India has a high prevalence of anemia (>40%), which cuts across all age groups and socio-economic status. Anemia Mukt Bharat under the Poshan Abhiyan Programme which is the updated version of National Iron Plus Initiative (NIPI) campaign, is a 'Lifecycle approach' covering all vulnerable age groups with a set of interventions and different institutional mechanisms aiming at time bound targets. Multi-pronged approach of delayed cord clamping, iron folic acid supplementation, dietary diversification, cooking in cast-iron vessels and food fortification is recommended. Other issues that need to be addressed are, iron refractory anemia, infections like malaria, worm infestations and H. pylori and genetic causes like hemoglobinopathies.

Points to Remember

- ***There is high prevalence of anemia in all age groups including women of reproductive age, in our country which is a serious public health problem.***
- ***Anemia Mukt Bharat is an updated version of National Iron Plus Initiative (NIPI) campaign.***
- ***For operational convenience, double the dose of IFA recommended for prophylaxis is given for treatment of iron deficiency anemia, followed by monitoring after 2-4 weeks.***
- ***Currently the tolerable upper limit of elemental iron is estimated as 60 mg.***
- ***If there is no improvement in hemoglobin, alternate causes should be considered.***
- ***Multi-pronged approach like delayed cord clamping, iron folic acid supplementation, dietary diversification and food fortification is recommended.***
- ***Behavioral Change Communication aims at dietary diversification.***
- ***Iron fortified rice and other cereals, double fortified salt and home fortifications are recommended.***
- ***WHO 2001 recommends that children between 6 - 59 months must be prescribed daily iron if the prevalence exceeds 40%.***
- ***Dietary diversity and ideal phytate to iron ratio (< 0.4 :1) and vit C to iron ratio (4:1) are recommended for better absorption.***

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CLIPPINGS

COVID-19 anticoagulation recommendations in children.

COVID-19 caused by SARS-CoV-2infection is often associated with hypercoagulability and disseminated intravascular coagulation manifesting as progressive lung and kidney disease, pulmonary emboli, venous thrombotic events, recurrent line obstruction, and stroke in adults. Major hematology organizations like the American Society of Hematology, International Society of Thrombosis Haemostasis etc. have published recommendations for anticoagulation of hospitalized symptomatic adults with COVID-19. However similar guidelines for children with COVID-19 are lacking. The authors from Children's Hospital Colorado and University of Colorado, after reviewing published literature on adults with COVID-19 and thrombosis in childhood, developed preliminary recommendations for the hemostatic evaluation, imaging, risk assessment for thrombosis, and anticoagulation for children hospitalized with COVID-19 at their institution.

They recommend that all pediatric patients admitted for management of SARS-CoV-2 infection be evaluated upon admission, and daily thereafter for thrombotic risk and that all patients at risk for thrombosis be initiated on mechanical and/or pharmacologic prophylaxis, if appropriate. A complete blood count with platelet count, fibrinogen, prothrombin time, D dimer are obtained on admission and serially for monitoring. LMWH is suggested for prophylaxis because of extensive pediatric experience. Worsening trend in the 'DIC score' and D-dimer may dictate evaluation of new onset thrombosis and stepping up antithrombotic treatment but it is not known whether such changes have the same level of prognostication in children as in adults.

They aver that as pediatric experience with COVID-19 increases, recommendations may need modification. They suggest looking at consensus recommendations by hematology societies when applying these guidelines.

Loi M, Branchford B, Kim J, Self C, Nuss R.COVID-19 anticoagulation recommendations in children. Pediatr blood cancer 2020; 67(9):e28485.

Coping with corona: A developing country perspective on managing children with cancer during COVID-19 pandemic.

As the COVID-19 pandemic escalates and developed countries struggle to contain the virus and keep routine health-care delivery afloat, the situation in countries with already constrained health-care systems might turn into a massive tragedy. Therefore, proper planning in anticipation of the worst and making contingency plans for smooth and pragmatic delivery of care to non-COVID related illnesses, which are likely to take a backstage during this crisis, is the need of the hour to avert greater collateral damage. With the progression of the pandemic, we expect to have more data on children with cancer, and the question on how the above interventions and treatment modifications would pan out, in the end, is for the future to answer.

Saroha M, Moulik NR. Coping with corona: A developing country perspective on managing children with cancer during COVID-19 pandemic. Cancer Res Stat Treat 2020;3, Suppl S1:97-101.

HEMATO-ONCOLOGY

MEGALOBLASTIC ANEMIA - AN UPDATE

***Sunil Gomber**
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Abstract: *Megaloblastic anemia is a multisystem disorder, which can easily be diagnosed with high index of suspicion. A complete blood count and review of blood and bone marrow films reflect the typical pathognomonic cytologic appearance of megaloblastic anemia. Assessment of metabolites like serum homocysteine and methylmalonic acid in the serum or in the urine is considered to be more sensitive and specific whereas serum cobalamin and folate levels are of limited value. It is highly amenable to therapy once the primary cause is established. Appropriate replacement therapy of deficient nutrient, cobalamin or folate or both, easily corrects the anemia.*

Keywords: *Anemia, Megaloblast, Replacement therapy, Children.*

Megaloblastic anemia is one of the important causes of anemia in children. It is a distinct type of anemia characterized by macrocytic RBCs and typical morphological changes in RBC precursors known as megaloblastosis. Megaloblastosis describes a diverse group of disorders with a common morphology of large cells with an arrest in nuclear maturation. Nuclear maturation is immature relative to cytoplasmic maturity, which is due to defective DNA synthesis and to a lesser extent, RNA and protein synthesis. This condition has protean manifestations in childhood, sometimes mimicking a hematological malignancy like leukemia. In developing countries, most cases of megaloblastic anemia result from nutritional deficiency of the micronutrients folic acid and B12. Folic acid and cobalamin are B-group vitamins that play an essential role in many cellular processes. Due to deficiency of one or both of these vitamins, megaloblastosis occurs due to asynchronous maturation between the nucleus and cytoplasm due to inhibition of DNA synthesis.[1-3] Other than megaloblastic anemia, deficiency of these hematopoietic micronutrients in children has been incriminated to cause neuro-developmental dysfunction, involuntary movements and failure to thrive. Diagnosing megaloblastic anemia assumes great clinical importance since it responds exceedingly well to treatment. The varied presentations of megaloblastic anemia with pancytopenia, vasculotoxic effects of hyperhomocysteinemia (resulting from folate and / or B12 deficiency) and the possible role of their deficiency in causing bone loss leading to osteopenia, osteoporosis and pathological fractures has brought B12 and folate deficiency and megaloblastic anemia back in focus.[4,5,6]

Background

Dr.George Whipple, a pathologist firmly established that food properties affects blood formation.[7] Hodgkin identified the structure of vitamin B12, for which she received the Nobel prize in 1964.[8] Jadhav, et al reported megaloblastic anemia in six South Indian infants for the first time in 1962.[9] Megaloblastic anemia resulting from deficiency of folate and B12 appears to be increasing over the last two decades. Because of variability in diagnostic criteria in different studies, anemia caused by folic acid or B12 deficiency is difficult to establish. There is no definite laboratory cut off values to define megaloblastic anemia especially serum B12 levels. Some studies have considered macrocytosis of red cells, while others have defined megaloblastic anemia by presence of megaloblastic changes in the bone marrow and few others have used subnormal micronutrient levels to define megaloblastic anemia. Gera, et al showed an almost four fold rise in proportion of macrocytic anemia in a study done in the past.[10] B12 deficiency is five times more common than that of folate.[11] Megaloblastic anemia accounts for a large number of cases of pancytopenia in many Indian series.[12-15]

Definition

Megaloblastic anemia is a general term used to describe a group of disorders caused by impaired DNA synthesis which leads to abnormal findings in peripheral blood smear (macro-ovalocytes) and in bone marrow samples (megaloblastic hyperplasia). Megaloblasts, the hallmark of this anemia, are formed as a result of

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asynchronous maturation between the nucleus and the cytoplasm due to DNA synthesis impairment.[1-3]

Epidemiology

The frequency of megaloblastic anemia is highest in countries in which malnutrition rate is high and routine vitamin supplementation for elderly individuals and pregnant woman is not available. Faulty cooking practices result in loss of folate which can lead to megaloblastic anemia. The prevalence of anemia as per National family health survey-4 (NFHS-4) data has been as high as 60%; however the data does not indicate the proportion of megaloblastic anemia among these anemic subjects.

Relative prevalence of B12 / Folate deficiency

A study by Gomber, et al reported that in early preschoolers aged 3 months to 3 years from Indian slums, pure iron deficiency anemia was the commonest noticed in 41.1% children followed by iron and B12 deficiency (22.2%), vitamin B 12 deficiency alone in (14.4%) of children. The folate deficiency was noticed in as low as 2.2% of cases.[16] Another study from a hospital in Delhi on children with nutritional anemia showed B12 deficiency in 19% cases and folate deficiency in 12%. In addition, nearly 35% cases had levels of B12 which could be classified as low.[17] Studies from Pakistan and Zimbabwe revealed B12 deficiency in over 50% cases while folate deficiency was seen in only 8% and 17% cases, respectively.[18,19] These studies done in adults may have a direct relevance to pediatric population as the most common cause of B12 deficiency in young children is the maternal deficiency leading to decreased stores at birth and its consequences. Stabler and Allen, in a review have highlighted B12 deficiency as a worldwide problem with breast-fed infants of B12 deficient mothers being at highest risk.[20] Most of the studies carried out in the last decade highlight that B12 deficiency appears to be a commoner cause than folate deficiency in children suffering from megaloblastic anemia.[21,22]

Metabolism of folate and cobalamin

The chemical name of folic acid is pteroylglutamic acid. Folates are essential in many biochemical reactions like synthesis of purines, thymine and deoxyribonucleic acid (DNA). The adenosylcobalamin and methylcobalamin are the biologically active forms of vit B12. Adenosylcobalamin is the tissue form of vitamin B12 while methylcobalamin circulates in blood. Hydroxocobalamin is the precursor of the above two forms. Nutritional factors of B12 and folate are compared in Table I.

Table I. Nutritional aspects of B12 and folate

	Vitamin B12	Folate
Content in foods	Vegetables-poor Meat-rich	Vegetable-rich Meat-moderate
Effect of cooking	10-30% loss	60-90 % loss
RDA*	0.2-1 mcg	25-200 mcg
Site of absorption	Ileum	Duodenum and jejunum
Body stores	2-5 mg	5-20 mg

* ICMR – 2010 RDA

Etiology and pathogenesis

The principal causes of megaloblastic anemia in clinical practice are cobalamin and folate deficiency either directly due to deficiency or indirectly due to drug induced suppression of DNA synthesis or inborn errors of metabolism. Various causes of megaloblastic anemia are shown in Box 1.

Major causes of cobalamin deficiency (Box 1)

Dietary: Strict vegetarians who avoid taking meat, eggs, and dairy products are more prone. Problems with cobalamin absorption include atrophic gastritis, achlorhydria and also autoimmune destruction of gastric parietal cells leading to failure of intrinsic factor production. This latter condition is called pernicious anemia. Rarely inborn errors of metabolism can lead to Vit B12 deficiency.

Major causes of folate deficiency (Box 1)

The main cause of loss of folate from food is poor food preparation due to dilution of food in water or by excessive heating and inactivation of folate. Folate deficiency occurs in situations where there is increased physiologic demand for folate as in chronic hemolytic states like sickle cell anemia, hereditary spherocytosis, elliptocytosis and in pregnancy.

Infections like human immunodeficiency virus (HIV) infection and myelodysplastic disorders cause megaloblastic anemia due to direct effect on DNA in hemopoietic and other rapidly dividing cells.

Box 1. Various causes of megaloblastic anemia

1. Cobalamine deficiency: Dietary deficiency, deficiency of intrinsic factor (Pernicious anemia) or gastrectomy, malabsorption, ileal resection, hypothyroidism, methyl malonic acidemia due to defects in early steps of vitamin B12 processing.
2. Folic acid deficiency: Dietary deficiency, defective food processing due to dilution or excessive heating, malabsorption, sprue, extensive bowel resection, anticonvulsants or oral contraceptives, increased physiologic demands as in chronic hemolytic states.
3. Miscellaneous:
   a) Congenital disorders of DNA synthesis: Orotic aciduria, thiamine responsive megaloblastic anemia, congenital dyserythropoietic anemia, Lesch-Nyhan syndrome.
   b) Acquired defects in DNA synthesis: Liver disorders, leukemia particularly acute myeloblastic anemia, sideroblastic anemia, aplastic anemia.
   c) Drug induced megaloblastosis: a) Purine analogues (6 Mercaptopurine, azathioprine, thioguanine) b) Pyrimidine analogues (5 flurouracil, 6 azauridine) c) inhibitors of ribonucleotide reductase (cytosine arabinoside, 5 hydoxy urea) d) Folate antagonists (aminopterin, methotrexate, pyrimethamine, trimethoprim and triamterene).

Clinical features

Children with megaloblastic anemia are commonly malnourished. Clinical features include pallor, anorexia, irritability and easy fatiguability like in other anemias. Clinical features peculiar to megaloblastic anemia include hyperpigmentation of knuckles and terminal phalanges,[23] hepatosplenomegaly (seen in upto 30-40% cases) and sometimes icterus. Petechial and other hemorrhagic manifestations like intracranial haemorrhage and gut bleeding presenting as hematological emergencies have also been reported.[24] Presence of bleeding with severe anemia makes them clinically indistinguishable from aplastic anemia. Children with megaloblastic anemia may also mimic acute leukemia due to the presence of hepatosplenomegaly.[24,25,26] Fever is one of the clinical manifestations, but often unrecognized and was noticed in 65% of the cases in one study from India.[24]

Neurologic symptoms like tremors, paresthesias, hypotonia, seizures, delayed development and developmental regression in association with severe anemia can sometimes be the presenting manifestation. These cases may show diffuse cortical atrophy in MRI of brain.[27,28] Impairment of cognitive function and persistence of neurological consequences even after treatment are major areas of concern.

Laboratory features

The laboratory features of megaloblastic anemia comprise of changes in peripheral blood and bone marrow picture, serum concentrations of methylmalonic acid (MMA) and homocysteine and serum levels of vitamin B12 and folate.

The megaloblastic macrocytic anemia in most cases results from deficiency of vitamin B12 or folate. This means peripheral smear and red cell indices will show macrocytes and bone marrow examination will reveal megaloblasts. The normoblastic macrocytic anemia occurs in association with a large number of various disorders. Macrocytosis is commonly seen in hemolytic anemia and post hemorrhagic anemia. Macrocytosis is occasional in alcoholism, leukemia, liver disease, aplastic anemia, sideroblastic anemia, myelodysplastic syndromes, anemia due to marrow infiltration, cytotoxic drug therapy, hypothyroidism, chronic obstructive lung disease, scurvy, etc. Here bone marrow examination will not show megaloblasts.

CBC and peripheral smear

All the blood cells are affected. Erythrocytes vary markedly in size and shape, some are large and oval (macro-ovalocytes). The morphologic changes are directly proportional to the severity of anemia. Circulating megaloblasts (i.e., nucleated red cells that failed to mature appropriately) are visible in circulation if hematocrit is less than 20%. Anemia is typically macrocytic with a mean corpuscular volume (MCV) of 100-110fL or more. Reticulocytopenia (a reticulocyte count of <1%) is a frequent finding. This occurs because of inordinate impairment of erythropoiesis culminating in intramedullary destruction of megaloblasts and resultant reticulocytopenia. There is also a progressive reduction in white blood cell count, but it rarely falls below 2000 cells/μL.

Neutrophil hypersegmentation (more than five lobes) in at least 5% of the neutrophil or even single cell showing 6 or more lobes is a cardinal feature of megaloblastic anemia. It is noteworthy that in nutritional megaloblastic anemias, hypersegmented neutrophils are an early sign of megaloblastosis. The complete blood count (CBC) often reveals anemia, leukopenia and at times thrombocytopenia. Pancytopenia is also a common finding in these cases. Rarely a microangiopathic haemolytic anemia like picture with burr cells and fragmented RBCs is seen in severe B12 deficiency. This is referred as pseudothrombotic microangiopathy.[29]

Serum vitamin B12 and folate

These tests have limited value because of their low sensitivity and specificity. There is wide variation of cut off values especially to diagnose vitamin B12 deficiency.

Previous studies showed that vitamin B12 levels were either normal or elevated in antecedent administration of vitamin B12, myeloproliferative disorders, liver disease and intestinal bacterial overgrowth. Although tissue stores may be normal, serum folate levels can decrease within a few days of dietary folate restriction. However cobalamin less than 200 pg/ml and folate less than 2 ng/mL are consistent with deficiency of these vitamins in most of the quoted studies in the literature.

Red blood cell (RBC) folate

RBC folate level is regarded as a more reliable source of determining tissue stores of folate. Unlike serum folate which is affected by dietary intake, RBC folate levels remain constant throughout the lifespan of the cell. However, assays for measuring RBC folate levels have also been fraught with unreliability.

Investigations for inborn errors of metabolism

When other common causes are excluded, urine for orotic acid and tandem mass spectrometry for other metabolic disorders should be done.

Bone marrow examination

The aspirated marrow is often hypercellular with striking imbalance in nuclearcytoplasmic maturation often referred to as nuclear cytoplasmic asynchrony. In view of erythroid hyperplasia, the ratio of myeloid to erythroid precursors (M/E ratio) is reversed and may fall to 1:1 or even lower. Megaloblastic anemia will usually show hyper segmented neutrophils in the blood and giant metamyelocytes and bands in the marrow.

It is noteworthy that a megaloblastic anemia may be misdiagnosed as acute leukemia when megaloblastic anemia is very severe. In this case, the typical megaloblasts are obviously absent and rather most cells available are bizarre megaloblastic pronormoblasts that dominate the marrow because of lack of maturation of the erythroid series and hence raising the possibility of erythroleukemia.

Serum concentrations of methylmalonic acid (MMA) and homocysteine

Overall, measuring serum MMA and homocysteine levels are well established ways of differentiating cobalamin deficiency from folate deficiency. In folate deficiency there is marked elevation of homocysteine levels while serum levels of MMA are not elevated whereas in cobalamin deficiency, there is marked elevation of both the metabolites.

LDH - Elevated LDH, a marker of ineffective erythropoiesis is often seen in megaloblastic anemia due to both B12 and folate deficiency.

Urine - Excessive elevation of methylmalonic acid in the urine (normal:0-3.5 mg/24hr) is a reliable and sensitive index of Vit B12 deficiency.

Treatment of megaloblastic anemia

Cobalamin deficiency

To treat a case of megaloblastic anemia, all efforts should be made to detect underlying cause. The duration of treatment is not as standardized as in case of iron deficiency anemia. Duration depends upon the underlying etiology. Life long therapy may be needed in children with inborn errors of metabolism. Children with neurologic manifestations need more aggressive therapy and may need B12 supplementation for a longer duration. The route of administration has to be parenteral in children with malabsorption and intrinsic factor deficiency. Drug related causes, myelodysplastic syndrome and others should be excluded. Megaloblastic anemia with established cobalamin deficiency should be given intramuscular/ intravenous administration of cobalamin of 1000 μg daily for 2 weeks or alternatively thrice weekly for 2 weeks (six doses) and then weekly for another six doses until hematocrit returns to normal. Intravenous route should be preferred over intramuscular injections in children presenting with thrombocytopenia to prevent local bleeding.

Other regimen for vitamin B12 is oral administration of vitamin B12 (1000 μg/day) for 8 weeks. Initially vitamin

B12 is given daily for 2 weeks followed by once a week for 6 weeks.[30]

Recently lot of stress has been given on oral administration of cobalamin due to ease of administration leading to better compliance. Recent studies show that response to oral methylcobalamin therapy is prompt and adequate.[31-33] It is administered at 1000-2000 μg but a wide range of doses and schedules have been recommended. Intramuscular/intravenous cobalamin is often preferable, since it has the potential to bypass all abnormalities of cobalamin absorption. However, oral cobalamin is less expensive, better tolerated by patients and preferable in patients with bleeding disorders like hemophilia patients in whom intramuscular injection should be avoided. To date the oral vitamin B12 treatment regimen is not yet formally approved. Further studies should include testing the efficacy of different molecules (cyano-, hydroxo-, methyl-cobalamin) and dosages.

Recent developments in conjunction with nano medicine for the co-administration of drugs with lipid compounds have been reported to enhance lymphatic transport. These technologies have been recently used to administer vitamin B12 sublingually or intranasally.[34-36] These methods have been found to be promising routes of administration.

Folate therapy

Pharmacologic doses of folate at 1 to 5 mg daily is required to achieve full hematologic response.[37] It is however noteworthy that administration of folate to individuals with cobalamin deficiency increases the risk and frequency of cobalamin deficiency induced neurological and neuropsychiatry disorders. Therefore, folate should not be instituted in patients with megaloblastic anemia unless cobalamin deficiency has been ruled out.

Hypokalemia, can occur during treatment of severe megaloblastic anemia because of ongoing rapid restoration of erythropoiesis in the bone marrow.[38] It is very important to closely monitor the serum potassium which falls with treatment and may result in death. Oral potassium supplementation should be given in case of hypokalemia. Iron deficiency can occur because of escalated erythropoiesis and this may impede the rate of response. Iron therapy is equally necessary.

Conclusion

The prevalence of anemia as per NFHS-4 data has been as high as 60 % , however the data does not indicate the magnitude of megaloblastic anemia among these anemic subjects. A strong suspicion of megaloblastic anemia should be entertained in any anemic child. The causes for increased incidence of cobalamin / folate deficiency needs further research in general population. Government of India should have a multipronged approach in the policy on control of nutritional anemia keeping in mind the wide spread prevalence of cobalamin deficiency either alone or in combination with iron and folate.

Points to Remember

- ***Vitamin B12 and folic acid deficiencies are the leading causes of megaloblastic anemia.***
- ***Vitamin B12 deficiency may present with pancytopenia, hemorrhagic manifestations and fever, thus mimicking diseases like aplastic anemia or acute leukemia.***
- ***Homocysteine is increased in both folate and vitamin B12 deficiency but serum MMA is increased in vitamin B12 deficiency only.***
- ***Apart from an anemic syndrome, patients with vitamin B12 deficiency may also present with neurologic symptoms.***
- ***Treatment of folate deficiency with folic acid supplements should be initiated after ruling out concomitant vitamin B12 deficiency as it increases the risk neurological and neuropsychiatric disorders.***
- ***Hypokalemia and iron deficiency can occur during treatment of severe megaloblastic anemia.***

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HEMATO-ONCOLOGY

AUTOIMMUNE HEMOLYTIC ANEMIA

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Abstract: *Autoimmune hemolytic anemia (AIHA) is caused by autoantibodies to red blood cells resulting in excessive destruction of erythrocytes. AIHA is either idiopathic or associated with infections, malignancies and autoimmune diseases. AIHA is classified into warm, cold and mixed types.Warm AIHA is marked by anemia, jaundice and spherocytes, due to extravascular hemolysis. Cold agglutinin disease results after infections and causes red cell agglutination at colder temperatures. Positive direct antiglobulin test (DAT) in the setting of hemolytic anemia is diagnostic of AIHA. Immunosuppression is the main basis of management.*

Keywords: *Auto antibody, Extravascular hemolysis, Intravascular hemolysis, Hemoglobinuria, Direct Coomb's test, Immunosuppression.*

Autoimmune hemolytic anemia (AIHA) was initially reported by Issit in 1985. AIHA is an uncommon disease caused by autoantibodies to the erythrocytes that result in excessive destruction of erythrocytes. Incidence of AIHA in children is estimated to be 0.8-1.25 cases per 100,000 children.[1] Boys are more commonly affected than girls. AIHA is either idiopathic or associated with infections, hematological malignancies, autoimmune disease and lymphoproliferative syndrome. The presentation of AIHA is acute in 70-80% of cases and the episode generally lasts for 3-6 months.[2] The mortality rate is lower in pediatric AIHA (4%)[3], however it may increase to 10%, when it occurs in association with immune thrombocytopenia (Evans syndrome).[4]

Classification and etiology

AIHA is classified into three categories - warm, cold (cold agglutinin disease and paroxysmal cold haemoglobinuria) and mixed (Table I), based on the temperature at which the auto antibody is active.Warm AIHA is commonly seen in children (60-90% of childhood AIHA) and is characterized by the presence of IgG autoantibodies that result in extravascular haemolysis. The clinical picture is predominantly characterized by the presence of anemia, jaundice and spherocytes in the peripheral blood smear.

Cold agglutinin disease usually results after infection with Mycoplasma pneumoniae or Epstein-Barr virus (EBV) (around 10% of AIHA). It is characterized by the presence of IgM autoantibodies at cold temperatures that are directed towards I/i antigen of erythrocytes and results in red cell agglutination seen in the peripheral blood smear. Paroxysmal cold hemoglobinuria (PCH) is rare in children and is characterized by the presence of IgG antibody against the P antigen of the erythrocytes that binds to red cells at lower temperature and results in complement-mediated intravascular hemolysis at 37°C.

AIHA can also be classified as primary or secondary depending upon the underlying aetiology; secondary AIHA is common in children. Primary AIHA is idiopathic and no evidence of underlying systemic illness is found. In the pediatric population, AIHA is primary in 37% cases, 53% are secondary to immune disorders and 10% are post-infectious.[5] Primary AIHA is usually of warm antibody type. Secondary AIHA also belongs to warm type, except in secondary causes like infection with Mycoplasma pneumoniae which results in cold agglutination. However, Mycoplasma infection has also been described on rare occasions of warm type AIHA.[6]

Immunodeficiency disorders, lymphoreticular malignancy, systemic lupus erythematosus (SLE) are common causes for secondary AIHA in children.[7,8] Other autoimmune disorders that are associated with AIHA include Sjogren syndrome, juvenile idiopathic arthritis, dermatomyositis, vitiligo, autoimmune thyroiditis and type 1 diabetes mellitus.[9]

Primary immunodeficiency diseases associated with AIHA include common variable immune deficiency, autoimmune lymphoproliferative syndrome (ALPS),

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Table I. Classification of autoimmune hemolytic anemia

Characteristics	Warm AIHA	Cold agglutinin disease	Paroxysmal cold hemoglobinuria
Aetiology	A. Primary B. Secondary 1. Autoimmune diseases (lupus) 2. Primary Immunodeficiency diseases 3. Post-HSCT 4. Infections (HIV, CMV, EBV) 5. Hematological malignancy 6. Drugs 7. Evans syndrome	A. Primary B. Secondary 1. Infections (EBV, mycoplasma) 2. Malignancy	A. Primary B. Secondary-post-infectious
Autoantibody	IgG	IgM antibodies against I/i antigen	IgG antibodies against P antigen
Coombs test	37°C- IgG ± C3 positivity	37°C- IgG negative, C3 positive 4°C- IgG negative, C3 positive	37°C- IgG negative, C3 positive 4°C- IgG and C3 positive
Therapy	First line: Corticosteroids Second line: IVIg, rituximab, sirolimus, splenectomy	First line: Avoidance of cold temperature Second line: rituximab, plasmapheresis	First line: Avoidance of cold temperature Second line: Corticosteroids

HIV- Human immunodeficiency virus; CMV- Cytomegalovirus;
EBV- Epstein-Barr virus; IVIg- Intravenous immunoglobulin; HSCT - Hematopoietic stem cell transplant

agammaglobulinemia and Wiskott Aldrich syndrome. The combination of AIHA and immune thrombocytopenia referred to as Evans syndrome is seen in 15-30% of childhood AIHA. ALPS and ALPS-like disorders are seen in around 50% of cases of childhood Evans syndrome. It is imperative that all children with Evans syndrome must be screened for ALPS. Lymphoreticular malignancies like leukemia and lymphoma can also be associated with warm AIHA.

Cold agglutinin disease in children is usually secondary to infections with M. pneumoniae or EBV. Other infections such as human immunodeficiency virus, measles, mumps, rubella and varicella can also result in AIHA. Allogenic hematopoietic stem cell transplantation (HSCT) can also trigger AIHA that typically occurs in the first 2-6 months post engraftment. AIHA, post HSCT or solid organ transplantation is usually refractory to most of the traditional therapies used. Drugs associated with childhood AIHA include ibuprofen, acetaminophen, antibiotics such as penicillin, cephalosporins, and tetracycline. Amongst the penicillin group, piperacillin is found to be commonly associated with AIHA.

Clinical presentation

The clinical picture of AIHA include symptoms of anemia and symptoms related to haemolysis, which may be intra or extravascular (Fig.1). Progression of anemia is usually gradual and well compensated. Sometimes, life threatening features such as hypovolemic shock and acute kidney injury can occur due to rapid onset of intravascular hemolysis. Patients with warm AIHA usually present with signs of extravascular haemolysis such as pallor, icterus and splenomegaly.

The hallmark of intravascular hemolysis such as dark-coloured urine due to hemoglobinuria is seen in cold agglutinin disease or PCH. Children with cold agglutinin disease can sometimes present with symptoms related to vascular occlusion secondary to red cell agglutination on exposure to cold. Acrocyanosis (blue discolouration over fingertips, nose, ears and toes) is usually seen and can sometimes result in gangrene of fingers or toes in cold agglutinin disease.

Differential diagnosis: History and clinical examination in a child with clinically suspected AIHA must be directed

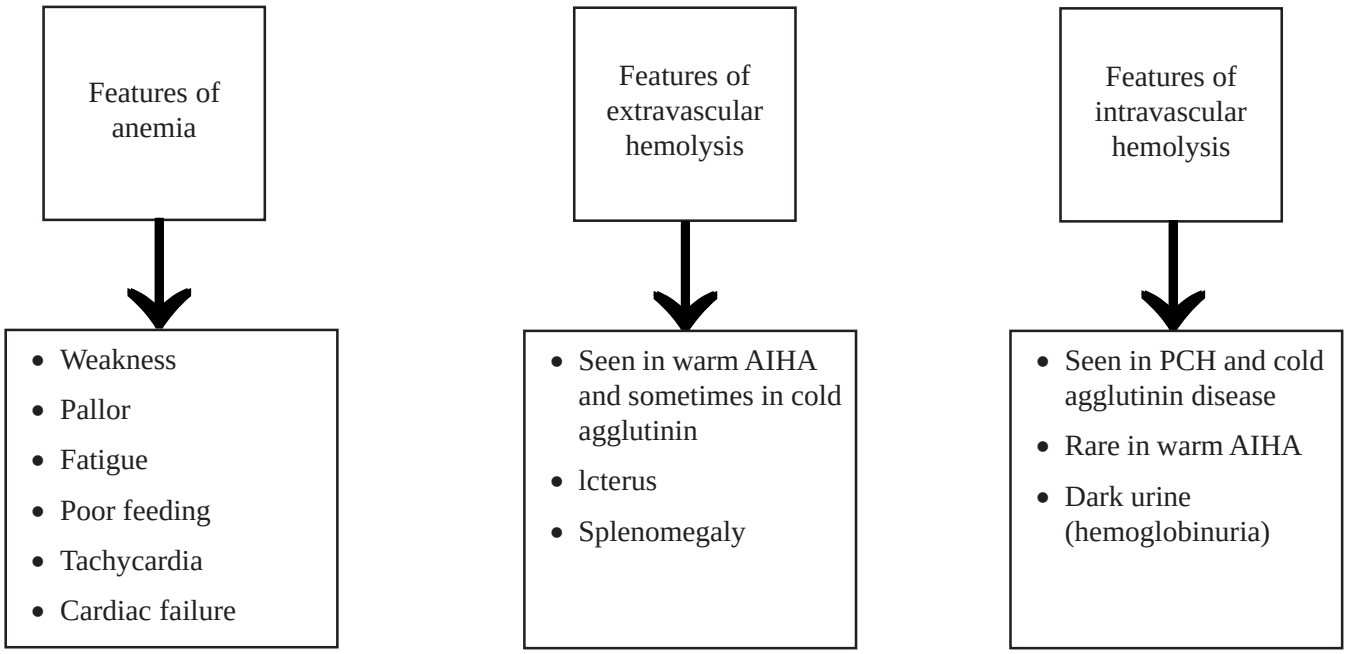

Fig.1. Clinical presentation of autoimmune hemolytic anemia

*AIHA - Autoimmune hemolytic anemia; PCH - Paroxysmal cold hemoglobinuria

Table II. Differential diagnosis and clues for underlying cause

Disease	History in favour of the disease
1. AIHA	Anemia, splenomegaly, features of intravascular hemolysis such as hemoglobinuria, difficultyin cross-matching and immediate reappearance of anemia following a blood transfusion
2. G6 PD deficiency	History of exposure to a drug, past or family history
3. Hemolytic uremic syndrome	Presence of hypertension, thrombocytopenia and uremia in a child with anemia
4. Pure red cell aplasia related to parvovirus infection	Progressive anemia usually seen after solid organ transplantation
5. Underlying malignancy, ALPS, infections like EBV or mycoplasma and systemic lupus erythematosus.	Presence of generalized adenopathy, hepatosplenomegaly in a child with clinically suspected AIHA suggests an underlying systemic disorder. In addition to this, oral ulcers, malar rash, thrombocytopenia or proteinuria suggests underlying lupus.

towards identification of underlying trigger or systemic illness and exclusion of other close differential diagnoses such as glucose 6 phosphate dehydrogenase (G6PD) deficiency, pure red cell aplasia related to parvovirus infection and hemolytic uremic syndrome. Presence of hypertension, thrombocytopenia and uraemia in a child with anemia suggests hemolytic uremic syndrome. Sudden onset of hemolysis following exposure to a drug or family history could suggest G6PD deficiency. Difficulty in cross-matching and immediate resurfacing of anemia following a blood transfusion are usually seen in AIHA. Exposure to drugs, past history of infections and family history of primary immunodeficiency disease must be sought in all cases of AIHA. Presence of significant generalized adenopathy or massive organomegaly in a child with clinically suspected AIHA suggests an underlying systemic disorder such as hematological malignancy, autoimmune lymphoproliferative syndrome, or infections

Box 1. Initial basic laboratory tests

- Complete blood count, peripheral smear and reticulocyte count
- Direct antiglobulin test (DAT) or Coombs test
- Urinalysis
- Renal function tests
- Markers for hemolysis such as indirect bilirubin
- Aspartate aminotransferase
- Lactate dehydrogenase levels

such as HIV or EBV. Presence of oral ulcers, malar rash, thrombocytopenia or proteinuria suggests underlying lupus (Table II).

Investigations (Table III)

The confirmation of diagnosis of AIHA is made in the presence of laboratory features suggestive of hemolysis and auto-antibody positivity. The basic laboratory tests for any child with suspected AIHA is shown in Box 1.

Cold agglutinin titres can be measured in children with suspected cold agglutinin disease. It represents the highest dilution of serum at which erythrocyte agglutination can be still documented. Titre higher than 1 in 256 is typically seen in cold agglutinin disease.

Once the diagnosis of AIHA is confirmed, work-up for underlying aetiology is warranted. Anti-nuclear antibody by indirect immunofluorescence and serum immunoglobulin profile (IgG, IgA, IgM) are required in most of the cases. A careful review of the history for possible drug exposure must be sought. Serology for EBV and mycoplasma must be performed for children with cold AIHA. Chest radiograph can show evidence of pneumonia in case of infection with M pneumoniae. Bone marrow examination is not routinely indicated; however, may be performed if there is a clinical suspicion of hematological malignancy or to exclude bone marrow failure syndromes. Serology for HIV, lymphocyte subsets and double-negative T cells by flow cytometry can be performed in case of suspicious clinical features of ALPS.

Alloantibodies to RBCs can develop in AIHA patients who are already exposed to erythrocyte transfusion and can result in transfusion reactions during subsequent blood transfusions. If alloantibodies are found, adsorption technique and a more detailed blood phenotyping may be required to identify the compatible blood.[10]

Treatment

Treatment of AIHA must be individualized and if there is evidence of any underlying disease, it should be treated accordingly. Presently, most of the treatment guidelines are based on small retrospective studies, case reports and expert opinion.

a) Emergency measures: Blood transfusion in AIHA is indicated in case of severe anemia or in case of congestive cardiac failure. However, low volume blood is considered for transfusion slowly over time. In case of severe anemia due to warm AIHA, intravenous pulse methylprednisolone (30 mg/kg, max: 1g) is required. Rituximab (375 mg/m^2) or plasmapheresis may be needed in case of severe anaemia in cold reactive AIHA. Identification of the compatible blood can be difficult in case of AIHA. The blood bank and the transfusion medicine specialist must be alerted to the possibility of AIHA and its type, in case of requirement of blood transfusion. Severe hemolytic reactions are common in cold-reactive AIHA that acts via complement fixation resulting in intravascular haemolysis, while, it is uncommon in warm AIHA. In cases of cold-reactive autoantibodies, the blood has to be warmed to 37°C before transfusion. The rate of transfusion must be slow to start with for early identification of transfusion reactions. Despite all the difficulties in the selection of appropriate matched blood, blood transfusion should not be withheld in decompensated AIHA as blood transfusion is the most effective method to correct hypoxia in patients with severe anemia.[11] Whole blood exchange transfusion has also been suggested as an effective modality of treatment in severely affected patients.[12] Intravenous hydration to maintain renal perfusion is needed in cases of severe intravascular haemolysis to prevent renal injury. Volume overload must be avoided and electrolyte abnormalities must also be corrected.

Box 2. Various steps of management of warm type of AIHA

- Glucocorticoids
- Rituximab (anti-CD20 agent)
- High dose intravenous immunoglobulin (IVIg)
- Other immunosuppressive drugs as steroid-sparing agents: Mycophenolate mofetil, cyclosporine A, azathioprine, sirolimus
- Plasmapheresis in refractory haemolysis.
- Splenectomy in recalcitrant or chronic cases but contraindicated in immunodeficiency and monogenic disorders of immune dysregulation.

Table III. Investigations in autoimmune hemolytic anemia

Investigation	Result	Remarks
Complete blood count	• Anemia (Hb ≤ 7 g/dL) • Leukocyte and platelet count: normal or elevated. Thrombocytopenia seen in Evans syndrome. • MCV may be high due to the presence of RBC clumps • MCHC (>36 g/dL) can be seen in cases with an excess of spherocytes	Anemia and thrombocytopenia can also be seen in hemolytic uremic syndrome. Presence of leukopenia and thrombocytopenia can suggest other causes such as bone marrow failure syndromes or marrow involvement due to active infection or infiltrative disorders.
Blood smear	Classical finding in warm AIHA is the presence of spherocytes. Polychromasia can be seen that suggests increased circulating reticulocytes. Presence of nucleated RBCs and Howell-Jolly bodies suggest accelerated erythropoiesis. Presence of RBC clumps or agglutinates suggests cold agglutinin disease.	Spherocytes can also be seen in many other conditions like hereditary spherocytosis, Wilson disease, burns and clostridial sepsis. The predominance of schistocytes and fragmented RBCs are seen in HUS.
Direct antiglobulin test (DAT) or Coombs test	• Warm AIHA- IgG ± C3 positivity at 37°C • Cold agglutinin disease- IgG negative, C3 positive at 37°C; IgG negative, C3 positive at 4°C • PCH- IgG negative, C3 positive at 37°C; IgG positive, C3 positive at 4°C	Positive DAT in the setting of haemolytic anaemia is diagnostic of AIHA. DAT is found to be positive in 0.001% of the normal population and in sick children with hypergammaglobulinemia, liver disease and HIV infection. False-positive DAT is found following IVIg administration. In contrast, DAT is negative on several occasion of AIHA due to fewer IgG molecules attached to the RBC membrane surface.
Reticulocyte count	Increased	25% of patients with AIHA will never have raised reticulocyte count due to immune-mediated destruction of RBC precursors.
Indirect bilirubin	Increased	If the conjugation rate overcomes the hemolysis, then icterus may be absent.
Lactate dehydrogenase (LDH)	Increased	This is a sensitive test but not specific for hemolysis because many conditions, like malignancy, liver disease, infections like pneumonia, encephalitis etc and bone fractures also raise the serum LDH level.
Haptoglobin	Decreased	Haptoglobin usually falls to a very low level in hemolysis. Haptoglobin levels are raised in several inflammatory conditions. In these conditions, haptoglobin may not be low in spite of hemolysis. In advanced liver disease, haptoglobin is not produced.
Urine hemosiderin	Positive iron staining of cells in urinary sediments in case of chronic haemoglobinuria	Usually a late manifestation of extravascular hemolysis

*AIHA- Autoimmune hemolytic anemia; HIV- Human immunodeficiency virus; HUS- Hemolytic uremic syndrome; PCH-paroxysmal cold hemoglobinuria

b) Specific measures- warm reactive AIHA

Immunosuppression is the main basis of management of autoimmune hemolytic anemia (Box 2).

Glucocorticoids are the cornerstone in the management of all cases of warm type AIHA. Initial treatment involves the use of oral prednisolone at a dose of 1-2mg/kg/day. With poor compliance to oral administration, intravenous methylprednisolone can be used. Routine use of high dose steroids is not recommended. Steroid tapering should always be slow, in order to extend the treatment for at least 6 months. A gradual and sustained reduction of the steroid dose correlates with a lower incidence of relapse.[13] Patients must be serially monitored for blood counts, reticulocyte count,serum markers for haemolysis and glucocorticoid toxicity. Blood pressure should be checked regularly. Glucocorticoids must be stopped only after normalization of haemoglobin and other markers of hemolysis. Direct antiglobulin test (DAT) can remain weakly positive even after therapy and normalization of haemoglobin, reticulocyte count and LDH values. If DAT is positive in high titres during follow-up, it may indicate the presence of underlying lupus or systemic autoimmune conditions. More than 90% of cases recover within 1 month of initiation of glucocorticoid therapy and the majority of children do not require therapy for more than 6 months. Around 15-40% of patients can develop relapse, 6 months to one year after the first episode. In case of relapse, glucocorticoids can be started at the lowest possible dose to maintain remission in order to avoid steroid toxicity.

Second-line therapy is indicated in children who do not respond within 1-2 months of initiation of glucocorticoids or who require a high dose of prednisolone ($\geq$1 mg/kg/day) to maintain clinical remission. Children who develop frequent relapses requiring long term steroid intake are also eligible for second-line therapy. Rituximab (anti-CD20 agent) is the preferred second-line therapy and is given at 375 mg/m^2 (given as an intravenous infusion over 3 hours) once every week for 4 weeks. Baseline serum immunoglobulins and lymphocyte subsets must be obtained before initiation of rituximab. Monitoring of B lymphocyte sub-population (CD19/ CD20) can help to assess the response to rituximab and titration of therapy. Some children can develop profound hypogammaglobulinemia after use of rituximab and may need immunoglobulin replacement therapy.

High dose intravenous immunoglobulin (IVIg) 1g/kg can also be used in cases of warm AIHA. However, the response is only transient. In a review of 73 cases of AIHA, Flores et al. concluded that treatment with IVIg (0.4-0.5gm/kg for 5 days) was effective in 39.7% of patients, with higher efficacy (54.5%) in children.[14]

Several immune-suppressive drugs have been used in AIHA with variable response. Mycophenolate mofetil (MMF) (800-1000 mg/m^2/day), cyclosporine A (1-3 mg/kg/day), azathioprine (2-2.5 mg/kg/day) and sirolimus (1 mg/m^2/day) have also been used as steroid-sparing agents with promising results.[15] In the case of lupus associated warm AIHA, azathioprine or MMF is preferred. Sirolimus is preferred over rituximab in case of ALPS and other monogenic disorders of immune dysregulation. In the case of drug-induced AIHA, the offending drug must be stopped and a short course of oral prednisolone can be considered. Humanised anti CD52 agent, alemtuzumab, has also been found effective in steroid nonresponsive AIHA in children.[16] Bortezomib, a proteasome inhibitor and ofatumumab, a monoclonal antibody to CD20 are under trial in the management of AIHA.

Plasmapheresis can be considered in cases with refractory haemolysis. Splenectomy can be effective for children with recalcitrant or chronic warm-reactive AIHA. However, a thorough work-up for underlying PID such as ALPS, CVID or other monogenic disorders of immune dysregulation must be performed before splenectomy. Splenectomy is generally avoided in those conditions. Patients must receive timely immunization against Hemophilus influenzae type B, Streptococcus pneumoniae, and Neisseria menigitidis before splenectomy. A complete response may not be found following splenectomy, but the requirement of steroids in reduced maintenance dosage following splenectomy has been documented.[17,18] Whenever possible, splenectomy should be avoided in children less than 5 years of age.

c) Specific measures- cold reactive AIHA: Pharmacologic therapy is generally not needed, as the anemia is generally mild in physiological temperatures. Primary therapy is the maintenance of warmth and avoidance of exposure to cold, especially in cases of paroxysmal cold hemoglobinuria (PCH). Antibiotic therapy should be considered in case of infection with M. pneumoniae. Azithromycin is generally used at 10 mg/kg on day 1 and 5 mg/kg for the next 4 days.

In case of severe anemia, rituximab or plasmapheresis can be considered. Eculizumab (anti-complement 5 agent) may be promising in the near future. Significant reduction in hemolysis is documented with use of eculizumab in cases of cold agglutinin disease.[19]

Prognosis

Mortality in childhood AIHA is generally 3-4% and the main causes include post-splenectomy sepsis, life-threatening hemorrhage in case of Evans syndrome, and complications of the underlying disease (lupus or malignancy). The course of illness in cold-reactive AIHA (cold agglutinin disease and PCH) is generally self-limited, though the episode can be severe at presentation. Relapses are more common in warm-reactive AIHA. Factors for poor prognosis include the presence of underlying PID and concurrent thrombocytopenia (Evans syndrome).

Points to Remember

- ***AIHA is either idiopathic or associated with infections, malignancies, autoimmune diseases, and lymphoproliferative syndrome. AIHA is classified into warm, cold and mixed types.***
- ***Warm AIHA is marked by the presence of anemia, jaundice and spherocytes, due to extravascular hemolysis.***
- ***Cold agglutinin disease results after infection with Mycoplasma pneumoniae or Epstein-Barr virus and causes red cell agglutination at colder temperatures. Features of cold antibody AIHA and PCH include features of intravascular hemolysis and microvascular occlusive episodes.***
- ***Primary immunodeficiency diseases associated with AIHA include common variable immune deficiency, autoimmune lymphoproliferative syndrome (ALPS) and Wiskott Aldrich syndrome.***
- ***Positive DAT in the setting of haemolytic anaemia is diagnostic of AIHA. Other laboratory parameters include increased reticulocyte count, indirect bilirubin, and LD, decreased haptoglobin and presence of hemosiderin in urine sediments.***
- ***Bone marrow examination is indicated, in cases of clinical suspicion of hematological malignancy or bone marrow failure syndromes.***
- ***Blood transfusion in AIHA is indicated in case of severe anemia. Immunosuppression is the main basis of management.***
- ***Splenectomy or plasmapheresis are indicated in refractory cases.***

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CLIPPINGS

Measurable residual disease after the first consolidation predicts the outcomes of patients with acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy.

Patients with newly diagnosed acute promyelocytic leukemia (APL) stratified according to a white blood cell (WBC) count of ≥3×109/L (high risk) or<3×109/L (low risk) before administering risk-adapted chemotherapy in combination with all-trans retinoic acid (ATRA). In total, 27 low-risk and 23 high-risk patients were assigned to receive induction and three courses of consolidation with ATRA and anthracycline, followed by 2-year maintenance regimen. High-risk group additionally received cytarabine during 1st consolidation and another one-shot idarubicin treatment during 3rd consolidation. Measurable residual disease (MRD) was monitored after induction and each consolidation. In the low-risk and high-risk groups, 5-year disease-free survival (DFS) rates were 86.5% and 81.2% (p=0.862) and 5-year overall survival rates were 100% and 84.8% (p=0.062), respectively. In the MRD-negative and MRD-positive groups, 5-year DFS rates were 91.7% and 78.4% (p=0.402) and 84.7% and 60.0% (p=0.102) after induction and 1st consolidation, respectively. Relapse rates were 8.3% and 13.3% (p=0.570) and 9.0% and 40.0% (p=0.076) after induction and 1st consolidation, respectively. Achieving MRD-negativity after 1st consolidation, rather than after induction, was a potential predictor of relapse and DFS in patients with APL treated with ATRA+chemotherapy.

Henzan, H, Takase, K, Kamimura, T. Mori Y, Yoshimoto G, Iwasaki H, et al. Measurable residual disease after the first consolidation predicts the outcomes of patients with acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy. Int J Hematol 112, 349-360 (2020). https://doi.org/10.1007/s12185-020-02911-z.

Effect of caregiver vaccination on the incidence of chickenpox in children with cancer: Experience from a longstay oncology facility in eastern india.

Varicella Zoster virus (VZV) causes significant morbidity and potential mortality in children with cancer. Two primary VZV outbreaks occurred at St. Jude India Cancer Care Centre (SJICCC), a charitable longstay facility for children and parents undergoing treatment at the hospital in 2014-2016. The objective was to see if VZV immunization of caregivers reduced the incidence of primary VZV in patients and prevented outbreaks. In March 2017-April 2019, screening identified 91 non-immune caregivers/staff who were vaccinated. There were 17 cases of VZV amongst children and caregivers in 2014-16 (incidence rate 2.26%). During 2017-19, only 2 children developed VZV (incidence rate 0.16%). Effective household VZV immunization programme was found to be cost - effective in reducing the incidence of primary VZV in children with cancer and their caregivers.

Bhattacharyya A, Das A, Dalvi-Mitra S, Goel G, Bhattacharya S, Saha V. Effect of caregiver vaccination on the incidence of chickenpox in children with cancer: Experience from a longstay oncology facility in eastern india. Pediatr Hematol Oncol 2019; 4(2):S60. https://doi.org/10.1016/j.phoj.2019.08.172.

HEMATO-ONCOLOGY

CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA - AN UPDATE

***Srinivasan Peyam**
****Amita Trehan**

Abstract: *Acute lymphoblastic leukemia (ALL) comprises 75% to 80% of all childhood leukemias. ALL occurs commonly between 2 and 5 years of age with 80-85% being of B-lineage, T-lineage accounting for 10-15% and around 5% being uncommon variants. An improved understanding of the biological heterogeneity of the disease has led to marked improvement in outcome, with current 5-year event-free survival (EFS) being 85% and overall survival (OS) rates being around 90%.*

A diagnosis of leukemia is confirmed by doing a bone marrow examination which ideally includes morphology, flowcytometry, cytogenetics and molecular genetics. Current day therapy is dependent on the risk assessment and the response of the disease to therapy. Precursor B ALL is stratified into standard, intermediate and high risk disease with minimal residual disease assessment at the end of Induction therapy being the most important indicator of prognosis. T-ALL is treated with a protocol similar to HR ALL. Combination chemotherapy consisting of drugs acting at different phases of the cell cycle is the cornerstone of therapy. Treatment broadly consists of 4 phases: Induction, consolidation, delayed intensification or re-induction and maintenance therapy.

A hematopoietic stem cell transplant is required in very few with contemporary treatment. Targeted therapy/ immunotherapy are the newer approaches for refractory/ relapsed leukemias. Supportive care which includes treatment and prophylaxis for infections, transfusion support, nutritional support and psychological support are vital to the management of disease.

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Keywords: *Childhood ALL, Risk, Response, Treatment, Genetics.*

Acute leukemia is the most common childhood malignancy accounting for one third of all childhood cancers; Acute lymphoblastic leukemia (ALL) comprises 75% to 80% and acute myeloid leukemia (AML) approximately 20% of all acute leukemias.[1] ALL occurs commonly between 2 and 5 years of age, with a possible rising trend in incidence over recent decades.[2] In India, cancer is the ninth common cause of death among children between 5 and 14 years and approximately 45,000 children are diagnosed with cancer annually. Preponderance of childhood leukemia cases is reported in boys as against girls.[3] On immunophenotyping, 80-85% of children with ALL are of B-lineage, with T-lineage accounting for 10-15% and around 5% being other uncommon variants. An improved understanding of the biological heterogeneity of the disease has led to marked improvement in outcome. The 5-year event-free survival (EFS) is 85% and overall survival (OS) rates are around 90% with contemporary therapies.[4] However, relapses continue to occur in 15-20% of children treated for ALL.[5] Cytogenetics and molecular genetics are important tools which help in risk stratification and prognostication. The treatment modalities change based on the risk. Genomics have become a part of routine investigation in most centres treating acute leukemias. Though the tests are expensive and not freely available they have become standard of care and are ideally required for proper management of leukemias. Hence the need for knowledge in ALL genetics. This article gives an overview of childhood ALL with an emphasis on contemporary diagnostics, especially cytogenetics/ molecular genetics. Management is discussed in brief with an outline of newer therapeutic modalities. Relapsed ALL is not discussed in this article.

Clinical features

Few common and uncommon clinical features are listed in Box 1.

Laboratory evaluation

Baseline investigations: Complete blood counts with peripheral smear to look for blasts/atypical cells, complete

Box 1. Clinical features at diagnosis in Childhood ALL[1,2]

Symptoms and physical findings

Fever

Bone pains

Limp, refusal to walk

Pallor

Fatigue

Bleeding diathesis

Petechiae/ecchymoses/epistaxis/gum bleeds

Bony tenderness

e.g. Sternal tenderness

Lymphadenopathy

painless, generalized/localized

Hepatomegaly

Splenomegaly

Parotid swelling (Mikulickz syndrome)

Subcutaneous nodules (leukemia cutis)

Central nervous system involvement*

Testicular involvement

Painless scrotal enlargement (uncommon)

Ocular involvement

Hypopyon, blurring of vision, photophobia or ocular pain (uncommon)

Joint manifestations

Arthralgia/arthritis (rare)

Superior mediastinal/superior vena caval/ hyperviscosity syndrome

Dilated veins and congestion of face/neck/ upper limbs/ upper chest, respiratory distress, orthopnea, stridor, hoarseness of voice, dysphagia, irritability, headache, vomiting, seizures, focal neurological deficits, etc

***CNS involvement:** Mostly, it is asymptomatic CSF positivity for leukemic cells. When symptomatic, features include headache, vomiting, lethargy, irritability, meningismus, papilledema, cranial nerve (CN) palsies (III/IV/VI/VII, occasionally visual disturbances with CN II involvement). Rarely, spinal cord compression, hypothalamic-obesity syndrome and intracranial leukemic cell mass.

serum biochemistry (electrolytes, liver function tests, renal functions and uric acid), chest x-ray and a coagulation profile are mandatory first line investigations. A diagnosis is confirmed by doing a bone marrow examination which includes morphology, flowcytometry, cytogenetics and molecular genetics. Occasionally, in cases with a high total leukocyte count (TLC) at presentation, peripheral blood may be sufficient for confirmation of diagnosis.

Bone marrow examination (BM): A BM aspirate and trephine biopsy are the gold standard for the diagnosis of leukemia. Demonstration of ≥20% lymphoblasts in the marrow is consistent with the diagnosis of ALL. The aspirate is subjected to morphologic evaluation and biologic studies as described, while biopsy specimens are useful in estimating marrow cellularity, fibrosis and dysplasias or when aspiration is a 'dry'tap (inconclusive with minimal cellularity) as can occasionally occur. The modern classification and risk stratification of leukemias require the integration of clinical and few laboratory features, bone marrow morphologic examination, immunophenotypic (flowcytometric) analysis, cytogenetics and molecular findings.

Flowcytometric analysis (Immunophenotyping): Assignment of the lineage (B or T) is done by flowcytometry (FCM). This is required as the treatment of ALL is dependent on the lineage. FCM aids in delineating few uncommon variants like acute leukemia of ambiguous lineage and Early T-cell precursor (ETP) acute lymphoblastic leukemia which helps in prognostication of disease and treatment strategy.

Genomics (cytogenetic/molecular) of childhood ALL: The genomics of ALL are heterogenous with multiple distinctive subtypes. Two important points are required based on cytogenetics (i) Ploidy status (Ploidy is a measure of the number of chromosomes in a cell) and (ii) molecular aberrations (recurrent chromosomal translocations/ segmental deletions/gains) (Fig.1).[6] The current WHO 2016 classification integrated the current knowledge about the ALL genetics in precursor B-ALL sub-grouping (Table I).

Ploidy status

Ploidy is determined by conventional karyotyping and/ or flowcytometry based DNA index (ratio of DNA content of test (tumour) sample / standard DNA fluorescence) measurement and/or interphase fluorescence in situ hybridization (FISH). Based on modal chromosomal number per leukemic cell/sample (ploidy), several different ploidy groups are identified which hold prognostic and therapeutic relevance (Table II).

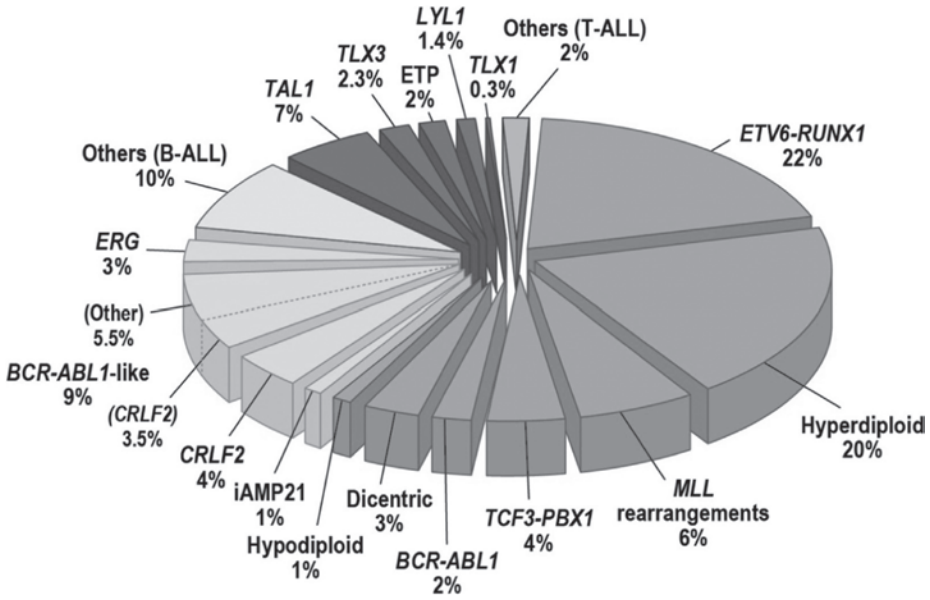

Fig.1. Genomic characterization of childhood ALL[6]

Table I. WHO classification of acute lymphoid leukemias 2016:[7]

B-Lymphoblastic leukemia/Lymphoma

• B-lymphoblastic leukemia/lymphoma, not otherwise specified (NOS).
• B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities.
  • B-lymphoblastic leukemia/lymphoma with t(9;22) (q34.1;q11.2); *BCR-ABL1*.
  • B-lymphoblastic leukemia/lymphoma with t(v;11q23.3); *KMT2A* rearranged.
  • B-lymphoblastic leukemia/lymphoma with t(12;21) (p13.2;q22.1); *ETV6-RUNX1*.
  • B-lymphoblastic leukemia/lymphoma with hyperdiploidy.
  • B-lymphoblastic leukemia/lymphoma with hypodiploidy.
  • B-lymphoblastic leukemia/lymphoma with t(5;14) (q31.1;q32.3); *IL3-IGH*.
  • B-lymphoblastic leukemia/lymphoma with t(1;19) (q23;p13.3); *TCF3-PBX1*.
• *Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1-like.*
• *Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21.*

i. T-Lymphoblastic leukemia/Lymphoma
• *Provisional entity: Early T-cell precursor lymphoblastic leukemia* • *Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma*
ii. Acute leukemias of ambiguous lineage
• Acute undifferentiated leukemia • Mixed phenotype acute leukemia (MPAL) with t(9;22) (q34.1;q11.2); BCR-ABL1 • MPAL with t(v;11q23.3); KMT2A rearranged • MPAL, B/myeloid, NOS • MPAL, T/myeloid, NOS

Table II. Ploidy grouping and its prognostic significance[1,2,8]

Ploidy group	Chromosome number (per leukemic cell)	DNA index (FCM)	Prognosis
Hypodiploidy	< 46	<0.86	Unfavourable
High hypodiploidy	40-45	0.89-0.95	
Low hypodiploidy	31-39	0.70-0.88	Poor
Near haploidy	24-30	0.55-0.69	Poor
Diploidy	46	0.96-1.05	
Pseudodiploidy	46 with numerical / structural aberrations	0.96-1.05	
Low hyperdiploidy	47-50	1.06-1.15	
High-hyperdiploidy	51-65	1.16-1.39	Good
Near triploid	66-80	1.40-1.79	Favourable
Tetraploidy	> 80	1.8-2.28	Favourable

High hyperdiploidy (51-65 chromosomes or DNA index > 1.16)

This occurs in 20%-25% of cases of B-ALL, being very rare in T-ALL. It is associated with clinically favorable prognostic factors (Age 1-10 years, initial WBC < 50,000/mm^3) and is an independent favorable prognostic factor. When hyperdiploidy occurs with chromosomal translocations, the risk stratification is based on the type of translocation.[8,9] Trisomies of chromosomes 4, 10 and 17 render a favorable outcome.

Hypodiploidy (< 46 chromosomes)

Hypodiploidy is an independent poor prognostic factor in ALL. Patients with fewer than 44 chromosomes have a worse outcome as compared to patients with 44 or 45 chromosomes.[10] Common subtypes are (i) near-haploid and (ii) low-hypodiploid groups which are associated with an increased risk of treatment failure. Patients with high post-induction minimal residual disease (MRD) (≥0.01%) have poor outcomes (5-year EFS~ 25% to 47%) compared to hypodiploid patients with low MRD (5-year EFS~ 64%-75%.[11, 12] The associated genetic alterations in near-haploid ALL involves RTK/RAS signaling pathway and IKZF3, while low-hypodiploid involves TP53, RB1 and IKZF2. Approximately 2/3 of ALL patients with germline TP53 variants have hypodiploidy.

Recurrent (genetic) translocations and gains/deletions of chromosomes

Conventional cytogenetics and/or molecular analysis (RT-PCR/FISH as balanced translocations may be missed by routine cytogenetics) can identify biologically and clinically significant disease subtypes in ALL which has prognostic relevance and aid in precise risk stratification. It is to be noted that recurrent cytogenetic/molecular abnormalities can only be identified in 60% to 80% of ALL cases with current methods and are mutually exclusive of each other.

ALL with the t(12;21) (p13;q22);ETV6/CBFA2 (or TEL/AML1) fusion transcript: The most common subtype observed in 20-25% of B-ALLs & rarely in T-ALL.[1,8] Identification of this translocation requires FISH or RT-PCR techniques. It is usually seen in the 1-10 years age group with FCM revealing the frequent expression of CD10. It is associated with a favourable prognosis, attributed to a higher sensitivity to L-asparaginase.

ALL with the t(9;22) (q34;q11.2)BCR/ABL1 fusion transcript (Philadelphia chromosome-positive ALL, Ph+ve ALL): It occurs in 3-5% childhood ALL cases. It is associated with an older age (>10years), high initial WBC counts, high CNS positivity and poor prognosis. Ph+ is an independent poor risk factor in ALL. FCM usually shows CD10 positivity. In the pre-imatinib era, outcomes were dismal and an allogeneic hematopietic stem cell transplant (Allo-HSCT) was recommended in first remission in children with Ph+ALL. Currently, addition of tyrosine kinase inhibitors (TKIs) (imatinib/dasatinib) to a high risk chemotherapy back-bone has been demonstrated to have a similar outcome as compared to HSCT (5-year EFS~70% vs 65%, p=0.6).[13,14]

ALL with t(v;11q23.3); KMT2A-rearrangements: The most common rearrangement is t(4;11) (q21;q23).ie.,

AF4/MLL fusion transcript and occurs in 2-3% of pediatric ALLs. The incidence is as high as 50- 80% in infant ALLs.[1] The characteristics of this translocation include high initial WBCs, bulky extra-medullary involvement and frequent CNS involvement. Treatment failures are higher in this group. FCM usually is negative for CD10/surface CD22/ cyto Igμ and positive for CD15 and/or CD65. Infants with t(4;11) ALLs show enhanced sensitivity to cytarabine, owing to increased expression of cytarabine metabolizing enzymes.[1] Another subtype with t(11;19) (q23;p13.3) involving KMT2A and MLLT1/ENL occurs in approximately 1% of ALL cases and in both precursor B-lineage and T-ALL. Like their t(4;11) counterpart, t(11;19) carries a poor outcome in infants but the translocation is relatively favorable in older children with precursor B-ALL/ T-ALL.[15]

ALL with t(1;19) (q23;p13) E2A/PBX1 fusion transcript: This transcript occurs in 3-6% of all childhood ALL cases. It is frequently associated with cytoplasmic Igμ+ve. The adverse prognostic impact of this transcript is largely negated by current aggressive chemotherapy regimens but with slightly higher risk of CNS relapse suggesting the need of more intensive CNS therapy.[16]

ALL with t(17;19) with TCF3-HLF fusion: This occurs in <1% of pediatric ALLs. It is associated with disseminated intravascular coagulation and hypercalcemia at diagnosis and carries very poor outcome warranting high risk chemotherapy.[8]

Intra-chromosomal amplification of chromosome 21 (iAMP21): It is characteristically detected by metaphase FISH with RUNX1 gene probe and shows 5 or more copies (signals) of gene per cell (or 3 or more on a single abnormal chromosome 21). It occurs in ~ 2% of children with ALL generally seen in those >10 years of age with low WBC counts (< 50,000/mm3), female predominance and high post-induction MRD. It is associated with an adverse prognosis requiring treatment as a high risk ALL irrespective of initial risk or MRD response.[17,18]

Philadelphia chromosome-like ALL (Ph-like ALL) or BCR-ABL1-like ALL: In genomic studies, approximately 30% of patients present with no major cytogenetic abnormalities. These are known as B-other- ALL. Among them, Ph-like ALL forms a major entity routinely missed on genetic analysis, with unfavourable and poor outcomes (Fig.2).[19] Ph-like ALL has gene expression profile similar to Ph+ve ALL but without BCR-ABL1 translocation. It accounts for approximately 10-15% of pediatric ALL which is 5-fold higher than Ph+ve ALL. This group is observed in older children (>10yrs) with initial WBC >50,000/mm.[3] It is associated with a poor outcome. It comprises a wide spectrum of genetic lesions affecting primarily cytokine receptor and/or kinase signaling pathways and includes translocations, fusions,

Risk Factors	B-ALL Standard Risk	B-ALL Intermediate Risk	B-ALL High Risk
Age (years)	1-10	>10	
Initial WBC (/mm$^{3)}$	<50,000	>50,000	
Bulky extramedullary	No	Yes	
Testicular enlargement	No	Yes	
High risk cytogenetics /molecular genetics	No	No	Yes
Day 8 absolute blast count (/mm^3)	<1000	<1000	>1000
CNS disease	Negative	Negative	Positive
End induction MRD	<10^{-4}	<10^{-4}	>/=10^{-4}

↓ ↓ ↓

Risk and MRD based chemotherapy regimen
Induction, consolidation, interim maintenance, delayed intensification, maintenance phase
Allogenic HSCT when indicated

Fig.2. ALL diagnosis and risk stratification

point mutations and deletions making the diagnosis challenging.[20] The 5-yr-EFS for Ph-like ALL is approximately 60%, 40% and 25% in children with high-risk ALL, adolescents and young adults respectively.[21] Molecular diagnostic techniques are used to identify these abnormalities. However, as this group of abnormalities is large, diagnostics for Ph-like ALL are not in routine practice at present.

Risk stratification of childhood B-ALL based on clinic-laboratory features and MRD. All T-ALL/T-NHLs are treated under high risk category.

Genomics of T-ALL

Notch pathway signaling mutations with NOTCH1 (50-60%) and FBXW7 (~15%) gene are most common in pediatric T-cell ALL.[22] Few chromosomal translocations result in fusion of genes encoding transcription factors like TAL1/TAL2, LMO1/LMO2, LYL1, TLX1, TLX3, NKX2-I, HOXA and MYB to one of the T-cell receptor loci (or to other genes) and leads to dysregulation in transcription. Translocations resulting in chimeric fusion proteins include: NUP214-ABL1 fusion in 4-6% of T-ALL and other uncommon ABL1 fusion (with ETV6, BCR or EML1), SPI1 fusions (with STMN1 and TCF7), MLLT10, KMT2A, and NUP98. These are often missed by conventional karyotype, but identified using FISH or PCR. The prognostic significance of T-ALL mutations are still controversial and being studied.

Treatment

Chemotherapy

Current day chemotherapy regimens are dependent on the risk assessment and the response of disease to therapy. Risk stratification of the child is done by age, TLC count at diagnosis, presence of bulky disease, testicular involvement, immunophenotyping, CNS disease status, cytogenetics and molecular markers. Response is based on (i) Day 8 absolute blast count and (ii) end-induction MRD (Fig.2). The basic skeleton of various treatment protocols are similar and include discrete phases. At present most large centers in India are associated with the protocol "Indian Childhood Collaborative Leukemia Group (ICiCLe-ALL-14)" which has been planned for the Indian population. B-lineage ALL is stratified into standard, intermediate and high risk (SR, IR & HR) disease (Fig.2). T-ALLs/T-NHLs are treated with a protocol similar to HR ALL. Combination chemotherapy consisting of drugs acting at different phases of the cell cycle is the cornerstone of therapy. Treatment broadly consists of 4 phases: Induction, consolidation, delayed intensification or re-induction and maintenance therapy.

1. Induction therapy: This is the first phase of therapy which lasts 4-6 weeks. Induction therapy eradicates >99% leukemia cells and restores normal hematopoiesis. Children with standard risk (SR) ALL usually receive 3 drugs during induction therapy while IR and HR patients receive a 4 drugs induction. The classic combination of chemotherapy drugs in the induction phase includes a glucocorticoid, vincristine, L-asparaginase, intrathecal methotrexate and/or an anthracycline. More than 95% patients enter remission at the end of induction therapy (Box 2).
2. Consolidation therapy: The aim of this 4-6 week phase is to "consolidate remission" and to prevent the development of CNS leukemia.
3. Delayed intensification phase is given over a period of 8-12 weeks to target the remaining leukemic cells in the body and prevent development of resistance.
4. Maintenance phase is prolonged treatment over approximately 18 -24 months with low intensity anti metabolite based therapy (oral 6-Mercaptopurine and methotrexate). Some regimens include pulses of vincristine with a glucocorticoid given at monthly intervals. One of the drawbacks of prolonged maintenance therapy is poor adherence which is associated with a 4 times increased risk of relapse. In addition, host polymorphisms to 6- Mercaptopurine may influence both the efficacy and toxicity, and affect the outcomes.
5. CNS directed therapy: Regular intrathecal therapy coupled with high dose parenteral methotrexate is currently the backbone of CNS directed therapy. Cranial radiation is given only in situations where the patient has CNS disease at presentation.

Box 2. Definition of remission after induction

(i) Morphological remission

- M1 marrow is defined as less than 5% blasts in the marrow indicates morphological remission
- M2 marrow means 5-25% blasts in BM
- M3 means > 25% blasts in the BM

(ii) Molecular remission

- Minimal residual disease (MRD) assessed by FCM or FISH i.e., leukemic involvement of <0.01% of nucleated bone marrow cells at the end of remission induction therapy.

Targeted Therapy

Clinical outcomes of a subset of childhood ALL are still dismal. The advances in genetics has helped in identifying few genetic lesions that can be specifically targeted to improve the outcomes. The prototype is Ph +ve ALL with BCR-ABL1 fusion protein, targeted with tyrosine kinase inhibitors (TKIs) like imatinib combined with conventional cytotoxic chemotherapy, has resulted in improved outcomes and obviated the need for allo-HSCT.[4] Similarly, Ph-like ALL with kinase pathway lesions can probably successfully be targeted with TKIs like imatinib and Janus kinases (JAK rearrangements) with JAK inhibitors like ruxolitinib as part of precision therapy in the future (Table III).[23]

Immunotherapy

Refractory/ relapsed ALLs are often difficult to treat and treatment with ongoing trials using immunotherapy is underway. Various antigens present on B-ALL cells are targeted with engineered autologous T-cells with an anti-CD19 antibody fragment (CAR-T cells) or monoclonal antibodies with or without cytotoxic drug that enables killing of B-ALL cells (Table III). Chimeric antigen receptor (CAR) T-cell therapy involves genetic modification of patient's autologous T-cells to express a CAR specific for a tumor antigen, following by ex-vivo cell expansion and re-infusion back to the patient. Clinical trials have shown very promising results in end-stage patients with a full recovery of up to 92% in ALL.

Allogeneic hematopoietic stem cell transplant (Allo-HSCT)

With modern day chemotherapeutic regimens and risk stratification the outcome of ALL is a wonder story in malignancies and the role of HSCT is limited. The main indications are failure to achieve remission after induction therapy (M2/M3 marrow) or positive MRD after consolidation therapy. High risk cytogenetics and Ph-positive do not warrant HSCT provided patient has a negative MRD.

Though only few sub-categories are undoubtedly benefited and few show marginal benefits, in the absence

Table III. Immunotherapy in pediatric ALL[24]

Target antigen / Immunotherapy / Target therapy	Drugs	Uses
ABL/PDGFB fusion kinase inhibitors	Imatinib[$] Dasatinib[$] Ponatinib*	Ph + ve ALL Ph-like ALL
JAK2 inhibitors	Ruxolitinib*	Ph-like ALL
Proteosome inhibitors	Bortezomib[$]	Relapse/refractory ALL (IntReALL-2010 trial)
Monoclonal antibodies		
Anti-CD20 antibody	Rituximab* Ofatumumab* Obinutuzumab*	
Anti-CD22 antibody	Epratuzumab*	Relapse B-ALL (IntReALL-2010 trial)
Monoclonal antibody-drug conjugate		
Anti-CD22 antibody	Inotuzumab ozogamicin*	Relapse/refractory B-ALL
Bi-specific antibodies (BITEs) against CD19	Blinatumomab[$]	Relapse/refractory B-ALL NCT02101853) Newly diagnosed ALL (ongoing NCT03914625)
Chimeric antigen receptor (CAR) T-cell against CD19	Tisagenlecleucel[$]	Relapse/refractory B-ALL

[$]With benefits in clinical trials *Investigational drugs

Table IV. HSCT in Childhood ALL

Category	Subcategory benefited	Remarks
Induction failure (blasts > 5%)	• All T-ALLs. • Pre-B-ALL with age > 6 yrs and without high risk cytogenetics. • M3 marrow (i.e., > 25% blasts) at the end of induction.	• No additional benefit in other category especially pre-B-ALL with age 1-5 years and without high risk cytogenetics.[25]
Infant ALL with MLL-r	• KMT2A rearrangement and age < 6 months and either poor response to steroids at day 8 or WBC >3 lakhs/mm^3 benefit from HSCT.[26]	• Other KMT2A rearrangement found no benefit.
T-ALL	• Induction failure. • Persistent MRD positivity post-consolidation benefit from HSCT in CR1.	• Early T-cell precursor (ETP) ALL does not warrant HSCT universally at CR1.[27,28]
MRD positivity	• Persistent MRD positivity post-consolidation.	• End-induction MRD positivity: Chemotherapy intensification indicated, with no added benefit with HSCT.
Relapsed ALL(First relapse)	• All high risk T-ALL/ pre-B-ALL cases (very early/early relapse). • End-induction MRD positivity. • Two or more relapses.	• In isolated extra-medullary late relapses (> 6 months post completion of therapy), chemotherapy alone is sufficient.
Poor risk cytogenetics/molecular cytogenetics		
Hypodiploidy	• May be considered in CR1.	• In two recent studies, HSCT does not offer benefit over the risk directed chemotherapy irrespective of end-induction MRD.[27,29]
t(17,19)	• May be considered in CR1.[27,30]	• Very high risk with dismal outcomes.
MLL-r(in > 1 year old)	• May be considered in CR1 or in poor MRD response.	
iAMP21	• May be considered in persistent MRD positivity post-consolidation.	• Not routinely for all cases in CR1 or end induction MRD positivity.[17,18]
Ph +ve	• May be considered in persistent MRD positivity post-consolidation.	• Not routinely for all cases in CR1 or end induction MRD positivity.

of proven targeted/immune/cellular therapies, HSCT will remain a better salvage therapy in these selected cases (Table IV).

Supportive care in ALL

It is imperative to counsel the family at the start of therapy. This includes the need for staying near the treatment center during the initial intense phase of therapy (approximately 6 months), the economic impact, the help available from state governments, the need for adherence to the long treatment and the outcome of the disease. Supportive care and detailed counseling is of extra importance in India. Being a low middle income country, certain services are not easily available and once the patient completes the intensive phase of therapy and returns to his town/village there could be difficulty in access to specialized care. This specialized care may warrant appropriate instructions from the treatment center for management at his place of stay. Also, as patients belong to heterogeneous backgrounds counseling has to be tailored to the individual family. Some vital points include:

1. Maintenance of proper hand hygiene by all health care professionals to decrease the incidence of hospital acquired sepsis.

2. Restricted use of IV fluids, central lines, Foley catheter, etc.
3. Nutrition counseling and early institution of enteral nutrition when a child is undernourished or is observed to be losing weight.
4. Transfusion support: Hemoglobin <8 g/dL is generally an indication for blood transfusion in a stable patient. Indications of platelet transfusion vary with the clinical condition of the patient. In a stable patient without any co-morbidities and bleeds, prophylactic transfusions are recommended at a count below 10×10^9/ mm^3. Few studies have suggested that this threshold can be further lowered to $5\times10^9/mm^3$. Transfusion threshold of $20\times10^9/mm^3$ and $100\times10^9/mm^3$ is recommended in patients with minor (mucosal, epistaxis) and major bleeds (hemoptysis, GI or CNS bleed) and in children who are febrile and unwell. Transfusion practices may often be varied in different units, depending on physician preferences and extent of availability of blood products.
5. Growth factors: Administration of G-CSF has no role in the management of children with febrile neutropenia.
6. Patients with severe sepsis and septic shock should be managed as per the survival sepsis guidelines. Non-invasive intermittent positive pressure ventilation should be attempted in case of acute respiratory failure, before restoring to mechanical ventilation, except in those with severe hemodynamic compromise.[31]

Points to Remember

- ***Childhood ALL has a good prognosis.***
- ***B-lineage ALL constitutes around 80% and T lineage around 15% cases, 5% being mixed lineage/others.***
- ***Childhood ALL management is risk (clinical/cytogenetic/molecular analysis) and response (prednisolone response/minimal residual disease) based, indicating the need for adequate cytogenetic and molecular analysis at diagnosis.***
- ***Children who are low risk can be treated with less intensive therapy, while high risk children require intensive therapy.***
- ***Ph-like ALL is a major missed entity among B-other-ALLs, with scope for their identification by molecular diagnostics.***
- ***HSCT is needed in very few children as upfront therapy in the management of childhood ALL.***
- ***Supportive care is important and it includes infection control, transfusions and good nutrition.***
- ***Precision medicine in the future will include immunotherapy and pharmacogenomics of anti-metabolites to improve survival in the small percentage who still relapse and to decrease treatment related morbidity.***

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HEMATO-ONCOLOGY

THROMBOCYTOPENIA-CASE VIGNETTES

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Abstract: *Platelets play a vital role in coagulation and hemostasis. Thrombocytopenia is a common hematological concern in pediatric practice, the etiology of which can vary from mild viral illnesses to critical illnesses. Understanding the pathogenesis of each of these conditions is crucial as decisions such as 'to treat or not to treat' and 'how to treat' are based on this. For the same platelet count, the decision to treat varies based on the pathogenesis. In this article, we explore the common causes of thrombocytopenia in children, their pathogenesis and logic for treatment.*

Keywords: *Thrombocytopenia, Bone marrow suppression, Immune thrombocytopenia, Approach.*

Platelets play a vital role in coagulation and hemostasis. Reduction in platelet count due to inherited or acquired causes can have significant clinical consequences. Normal platelet count at any age is between 150-450x $10^3/\mu L$.[1] Thrombocytopenia was traditionally defined as platelet count $<150x10^3/\mu L$. However, in view of studies where apparently healthy individuals were found to have platelet counts between 100-150 $x10^3/\mu L$, the threshold has been reduced to $<100x10^3/\mu L$.[2] Often the trend of platelet count is more reliable than a single value as is true for many other hematological parameters. For e.g. reduction of platelet count from 440 x $10^3/\mu L$ to 160 x $10^3/\mu L$ in a child with fever should be considered ominous even though both counts are within the normal range. Thrombocytopenia in neonates and children occur usually as a result of conditions that reduce platelet production or reduce the platelet life span such as increased peripheral destruction or sequestration. Understanding the pathogenesis of each

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Box 1. Definitions of bleeding manifestations based on physical examination[4]

Skin (Dermis and epidermis)

1. **Petechiae** are small (0.5-3 mm), red, non-blanching macular lesions caused by intradermal capillary bleeding
2. **Purpura:** Larger Red (recent) or purplish (a few days old) discoloration in the skin with a diameter 4-10 mm that does not blanch with pressure and may not be palpable
3. **Ecchymosis (purpuric macule, bruises, or contusions):** Flat, rounded or irregular, red, blue, purplish, or yellowish green patch which is larger than a petechiae. It may be elevated if the underlying hematoma has spread into the superficial layers of the skin

Skin (Subcutaneous tissue)

1. **Hematoma:** Accumulation of blood in the subcutaneous or deeper tissue due to localized accumulation of blood.

Visible mucous membranes

1. **Petechiae, purpuric macules and ecchymosis:** These are same as that for skin.
2. **Bulla, vesicle and blister:** Thin-walled, raised lesions that contain blood. Vesicle is less than 0.5 cm whereas bullae and blisters are larger.
3. **Epistaxis:** Bleeding from the nose-unilateral or bilateral
4. **Gingival bleeding:** Bleeding from gum margins
5. **Subconjunctival haemorrhage:** Bleeding under the conjunctivae; colour changes with the timing of bleed.

Muscles and soft tissues

Hematoma: A localized collection of blood which is visible and palpable or revealed by imaging.

of these conditions is crucial as decisions such as to treat or not to treat and how to treat are based on this.

Platelets are tiny 2-3 microns sized, acellular fragments that are produced from megakaryocytes in the bone marrow and circulate in peripheral blood. The volume of platelets ranges from 7-9 fL which is measured as mean platelet volume in automated hematology analyzers. They are usually about 1/5th of the diameter of a normal red blood cell. In conditions where platelets are destroyed, megakaryocytes produce large platelets. In bone marrow pathology, where megakaryopoiesis is affected, usually platelets are of normal size except in certain inherited conditions.[3] After egress from the bone marrow, their life span is around 7-10 days when their major function is to promote platelet plug formation through steps of adhesion and aggregation in the primary phase of hemostasis.

Box 2. Grades of bleeding in thrombocytopenia[4]

Overall bleeding severity

0. None - Definitely no new bleeding of any kind
1. Minor - Few petechiae (≤100 total) and/or ≤5 small bruises (≤3 cm diameter); no mucosal bleeding
2. Mild - Many petechiae (>100 total) and/or >5 large bruises (>3 cm diameter); no mucosal bleeding
3. Moderate - Overt mucosal bleeding (epistaxis, gum bleeding, oropharyngeal blood blisters, menorrhagia, gastrointestinal bleeding, etc.) that does not require immediate medical attention or intervention
4. Severe - Mucosal bleeding or suspected internal haemorrhage (in the brain, lung, muscle, joint, etc.) that requires prompt medical attention or intervention
5. Life-threatening or fatal - documented intracranial haemorrhage or lifethreatening or fatal haemorrhage any site

Grades of epistaxis

0. None
1. Minor - Spotting on sheet or pillow and/or blood noted in nares, no active bleeding or need to apply pressure
2. Mild -Active bleeding on 1 or more occasions with need to apply pressure for < 15 min for stopping bleeding
3. Moderate– Active bleeding on 1 or more occasions with need to apply pressure for at least 15 min in order to stop bleeding
4. Severe- Repeated, continuous and/or profuse bleeding despite adequate pressure

Grades of oral bleeding

0. None
1. Minor -Petechiae on palate or buccal mucosa
2. Mild -One or more buccal blood blisters (haemorrhagic bullae or infiltrates) with or without petechiae, no active bleeding
3. Moderate -Intermittent active bleeding from gums, lips, buccal mucosa, or posterior oropharynx
4. Severe -Continuous bleeding from gums, lips, buccal mucosa, or posterior oropharynx

Grades of skin bleeding

0. None -No new cutaneous bleeding
1. Minor -Possibly a few new petechiae (≤100 total)
2. Mild -Definitely a few new petechiae (≤100 total) and/or ≤5 small bruises (< 3 cm diameter)
3. Moderate -Numerous new petechiae (>100 total) and/or >5 large bruises (>3 cm diameter)
4. Severe -Extensive (hundreds of) petechiae and >5 large bruises (> 3 cm diameter)

Hence, when platelet count or platelet function is reduced, primary hemostasis is impaired which manifests usually as mucocutaneous bleeding. Clinically this manifests as petechiae, purpura, easy bruisability, gum bleeding, epistaxis, menorrhagia, gastrointestinal bleeding or hematuria (Box 1). Life threatening bleeds such as central nervous system bleeds, airway bleeds and massive gastrointestinal bleeds may be encountered in thrombocytopenia depending on the etiology of the disease. Deep muscle hematomas and hemarthroses that are observed in clotting factor deficiencies are generally not observed in platelet disorders. Clinically significant "internal organ" bleeding is usually rare in thrombocytopenia; hence defining the type of bleed and grade of bleed is of utmost importance while making treatment decisions for thrombocytopenic patients (Box 2).[4]

Although platelet count less than 100 x 10^3/μL is the practical cut-off for thrombocytopenia, there is no significant risk of bleeding till it reduces to <50x10^3/μL. Clinically significant hemorrhage (mucosal bleeding) rarely occurs unless the count is <20x10^3/μL. Often there is a lack of concordance between the degree of thrombocytopenia and the risk of bleeding. The risk of bleeding is primarily related to the underlying pathology causing thrombocytopenia and is also influenced by associated fever, coagulopathy, medications and need for invasive procedures.[3] Those with platelet production defects are more likely to have serious bleeding than those with platelet destruction as in the latter, the platelets are larger and more functional. Thus, a platelet count of 15x10^3/μL in a neonate, a well child and a sick bleeding child can have very different treatment strategies.

Platelet count reported from automated hematology analyzers should ideally be manually verified in the setting of thrombocytopenia. Well calibrated automated analyzers that report mean platelet volume and provide scatter plots of platelets may obviate the need for manual verification. In the absence of these, platelet count verified manually can help identify spurious thrombocytopenia. Falsely low platelet counts are encountered due to large platelets in circulation that are under-reported by analyzer, presence of platelet clumps occurring secondary to naturally occurring EDTA dependent antibodies and entanglement of platelets in fibrin mesh due to activation of clotting inside the vacutainer following erroneous sampling. The latter often occurs during blood sampling technique of collecting from the needle or hub of the needle directly as it is followed in neonates and young children occasionally. Pseudo-thrombocytopenia due to EDTA dependent antibodies can be overcome by repeating the platelet count with sample taken in heparin or citrate vacutainer or from a blood film from a direct finger prick.

A simple format for approach to thrombocytopenia is given in Fig.1.

With this background knowledge on platelets, we will proceed to discuss few case scenarios in children, understand the clinical approach and pathogenesis of various diseases presenting with low platelet count in children. We describe the rationale for investigations and treatment in each of the scenarios.

STEP 1 → History, examination
Complete blood count and peripheral smear: Other cell lines, manual platelet count

STEP 2 →

Sick child	Well child
1. Infections	1. Immune thrombocytopenia
2. Malignancy	2. Inherited thrombocytopenias
3. Hemophagocytic lymphohistiocytosis	3. Drug induced
4. Hemolytic uremic syndrome	4. Sequestration
5. Thrombotic thrombocytopenic purpura	

STEP 3 → Is bone marrow evaluation and Hemato-Oncology consult needed at present?
Mandatory 1. Bone marrow failure 2. Leukemia / Lymphoma 3. Hemophagocytic lymphohistiocytosis
In the rest, based on clinical condition.
Bone marrow preferred always prior to starting steroids for any condition causing thrombocytopenia

Fig.1. Approach to thrombocytopenia

Case 1: Immune thrombocytopenia

A 4-year-old girl presents with history of bruises noted over legs and arms since the past one week, associated with bleeding from nose. She has been suffering from an episode of upper respiratory infection and mother noted blood stained nasal discharge on few occasions. As per advice from her pediatrician, a complete blood count was done, which was reported as follows. Hemoglobin: 12 gm/dL, WBC Count: 7700 cells/μL with 36% neutrophils, 54% lymphocytes, 8% monocytes and platelet count of 8000/μL. Mean platelet volume was 10.8 fL with marked thrombocytopenia and few large platelets

Table I. Etiology of thrombocytopenia in children and neonates

1. Increased destruction	
Immune causes	**Immune thrombocytopenia**
	Associated with other autoimmune diseases like **ALPS, SLE, Evans syndrome**, Antiphospholipid antibody (APLA) syndrome
	Drug Induced (Including heparin induced thrombocytopenia)
	Post transfusion purpura
Non immune thrombocytopenia	**Thrombocytopenia of infections** (Table III) (**Viral/ bacteremia**/ Fungemia / **protozoal**)
	Thrombotic thrombocytopenic purpura / Hemolytic uremic syndrome
	Disseminated intravascular coagulation
	Hemophagocytic lymphohistiocytosis
	Drug induced (Table II)
	Congenital heart disease
	Type 2b Von Willebrand disease (VWD) or platelet type VWD
	Kasabach- Merritt syndrome
	BMT- associated microangiopathy
2.Impaired production	
Hereditary disorders	Congenital amegakaryocytic thrombocytopenia
	Amegakaryocytic thrombocytopenia with radioulnar synostosis
	Thrombocytopenia with absent radii
	Wiskott Aldrich syndrome
	Bernard Soulier syndrome
	MYH 9 related thrombocytopenias
Acquired disorders	**Aplastic anemia**
	Myelodysplastic syndrome
	Marrow infiltrative process (leukemia/ lymphoma/ solid tumours)
	Nutritional deficiency (folate, vitamin B12)
3. Sequestration	
	Hypersplenism
	Hypothermia
	Burns

ALPS - Autoimmune lymphoproliferative syndrome, SLE - Systemic lupus erythematosis. Common etiologies are given in bold letters.

Table II. Drug induced thrombocytopenia

Drug category	Drugs
Heparins	Unfractionated heparin, LMWH
Antimalarials	Quinine, quinidine
Anti-rheumatic agents	Gold salts, D penicillamine
Anticonvulsant agents	Carbamazepine, phenytoin, valproic acid
Antimicrobial agents	Linezolid, rifampin, sulfonamides, vancomycin
Histamine receptor antagonists	Cimetidine
Analgesic agents	Acetaminophen, diclofenac, naproxen
Diuretic agents	Chlorthiazide

seen in circulation. She was admitted at another center, evaluated, transfused platelets and IV Immunoglobulin. She received 2 grams/kg of IVIG over the next 2 days which was tolerated well. She was discharged on the 5th day of admission with a platelet count of 95,000/μL. There were no further episodes of mucosal bleeding and the previous bruises and petechiae faded during hospital stay. CBC done one week later however showed a platelet count of 35,000/μL. Parents were anxious about the 'recurrence of low platelets'.

Thrombocytopenia in children can be classified pathologically into those due to increased destruction and reduced production (Table I). The usual mechanisms of destruction are immune mediated as seen in immune thrombocytopenia (primary or secondary) or non-immune causes. During evaluation, the duration of symptoms, abruptness of presentation, recent history of viral illness, ongoing fever, associated rash, history of drug intake (e.g. steroids may mask leukemia, non-steroidal anti-inflammatory drugs can result in thrombocytopenia or mask fever that can suggest a more sinister pathology), intake of indigenous medicines and transfusions received are to be noted (Table II). In immune thrombocytopenias, usually children are well with no other clinical manifestations such as pallor, lymphadenopathy or fever. Those with reduced platelet production have features of other cell line involvement and extra medullary disease such as lymphadenopathy, hepatosplenomegaly, gingival hyperplasia, etc.

Platelet size also can give valuable clues to the etiology of thrombocytopenia. Platelets in ITP are larger (MPV of 10-15 fL) and approach half the diameter of a red blood cell on peripheral smear. A close evaluation of complete blood count to look for other cell lines and clues towards bone marrow failure/ infiltration is vital before labeling as immune thrombocytopenia. Often iron deficiency with microcytic hypochromic anemia may be co-existent with ITP in children. The presence of normo or macrocytic anemias in association with thrombocytopenia needs further evaluation to rule out bone marrow pathology such as subclinical leukemia, aplastic anemia, myelodysplasia, etc. Children with substantial hemorrhage (gastrointestinal/ hematuria/massive epistaxis), etc. may also present with associated normocytic anemia. Evidence of fragmented red cells, hemophagocytosis, red cell agglutination, activated lymphocytes, presence of blasts, etc. are other findings in peripheral blood smear that may alert us to an alternative diagnosis.

Childhood ITP is a self-limiting condition that often requires no treatment despite the dramatic presentation. Acute or newly diagnosed ITP typically occurs in children younger than 10 years and often follows a brief viral illness 1-2 weeks prior. The disease occurs as a result of auto antibodies produced in response to a viral illness or a vaccination which can interact with the glycoproteins on the surface of platelets and megakaryocytes. Although the bleeding tendency is much less than expected for the degree of thrombocytopenia, at times, there is additional disturbance in platelet function due to autoantibodies directed against platelet receptors responsible for agglutination and aggregation. In response to peripheral destruction, megakaryocytes are increased in number; at times, there may be antibodies directed against megakaryocytes or additional mechanisms causing reduced platelet production as well. The autoantibodies produced as a post-viral phenomenon are short lasting. Hence, the disease usually is short lived and platelet count improves even without any treatment in the next 6-12 weeks as the titer of antibody falls. So in most cases, the treatment plan would be just to wait and watch and ensure a regular follow up.

Treatment is no longer given for those with no, minor or mild bleeding manifestations (Box 2). The usual agents for treatment i.e., glucocorticoids, IV immunoglobulin G or IV antiD improve the platelet count by countering the effect of the autoantibodies in circulation. All three drugs used in treatment of acute ITP are short acting and do not have any role in modifying the natural history of ITP. Around 20% of children with ITP would proceed into

chronicity (duration of symptoms more than 12 months). This occurs when the auto antibody levels do not reduce or continue to be produced by dysregulated T cells which promote expansion of autoantibody producing B cell clones. A search for underlying etiology is to be done (Table I) in some cases periodically as underlying disease can evolve with the passage of time. Even in chronic ITP, pharmacological treatment would be given only to those children with repeated mucosal bleeding to keep them safe from life threatening bleeds and not to 'normalize' platelet count. Parents are counseled to reduce risk of bleeding by avoiding nose picking, constipation, non-steroidal anti-inflammatory drugs, head trauma and intramuscular (IM) injections. Parents' understanding of the natural history and treatment plan is vital in immune thrombocytopenia to avoid panic, multiple doctor visits and use of treatments that often have no benefit.

Case 2: Megaloblastic anemia

A 12-year-old girl was admitted with history of low grade fever of 3 weeks duration associated with generalized weakness, loss of appetite and yellowish discolouration of eyes. Her tiredness progressed to difficulty in walking and climbing stairs and an episode of giddiness at home. On admission, she was noted to have pallor, icterus with petechiae and bruises on arms. CBC showed pancytopenia, haemoglobin 3.6 grams/dL, MCV of 114fL, WBC count of 4200 cells/μL with neutropenia (absolute neutrophil count: 820 cells/μL), relative lymphocytosis and platelet count of 24000/μL. Peripheral blood smear showed marked anisocytosis with macrocytosis, tear drop cells, polychromasia, with 30 nucleated RBCs/100 WBCs and hyper segmented neutrophils. Bone marrow evaluation was suggestive of cellular marrow with megaloblastic erythroid series and megakaryocytic hyperplasia. Serum B12 level was low (102pg/ml; normal 211-911pg/ml). She was started on parenteral vitamin B12 and oral folic acid. She responded with improvement in general sense of well-being and rise in haemoglobin over the next 4 weeks. Platelet count improved by D10 and remained normal thereafter.

Anemia with thrombocytopenia is encountered in a wide variety of conditions in pediatric practice.[5] The common scenarios encountered are:

1. Infections - Malaria, enteric fever, chronic infections, gram negative sepsis
2. Nutritional anemia - Megaloblastic anemia
3. Bone marrow hypoplasia / aplasia - Aplasticanemia (idiopathic / constitutional), drug induced or infection induced hypoplasia
4. Hemophagocytic lymphohistiocytosis (Genetic/ Secondary)
5. Bone marrow infiltration - Acute leukaemia (lymphoblastic/ myeloid), infiltration from lymphomas/ solid tumours such as neuroblastoma, Ewing sarcoma
6. Consumptive coagulopathies
    a. Disseminated intravascular coagulation
    b. Hemolytic uremic syndrome/ Thrombotic thrombocytopenic purpura/ other thrombotic microangiopathies
    c. Kasabach Merritt syndrome
7. Immune thrombocytopenia
    a. Autoimmune haemolytic anemias with thrombocytopenia (Evan's syndrome)
    b. Systemic lupus erythematosus
    c. Antiphospholipid antibody syndrome
8. Hypersplenism

In addition to the above scenarios, any condition causing thrombocytopenia may have associated anemia secondary to iron deficiency, acute or chronic blood loss. History and evaluation is directed at identifying an underlying cause with judicious use of investigations. In the case described above, the presence of severe anemia with macrocytosis, directs the evaluation to bicytopenias / pancytopenias with raised MCV. Reticulocyte count and bone marrow evaluation helps us to further narrow down the possibilities. Hypocellular/ aplastic bone marrow with decreased absolute reticulocyte count is seen in patients with either bone marrow hypoplasia or aplastic anemia. The mean corpuscular volume of red cells will be normal to slightly high in idiopathic aplastic anemia and raised in constitutional causes of bone marrow failure such as Fanconi anemia and Dyskeratosis congenita. Prompt identification of these conditions can avoid unnecessary treatment including platelet transfusions for non-bleeding patients.

Cellular to hyper-cellular bone marrow is observed in all the other conditions mentioned above causing anemia with thrombocytopenia. The presence of blasts, megaloblastic erythropoiesis, dyspoietic erythropoiesis, hemophagocytosis, etc. helps differentiate between the causes.

Megaloblastic anemia is a prototype for ineffective erythropoiesis where precursor cells generated in the bone marrow are destroyed there itself. Although anemia is the

earliest cytopenia, as the deficiency of B12 or folate progresses, the megaloblastic process affects myeloid and megakaryocytic lineages also, thus presenting as pancytopenia. The degree of thrombocytopenia in megaloblastic anemia is usually mild to moderate. Severe thrombocytopenia and mucosal bleeds are rare in megaloblastic anemia. In most cases, diagnosis can be made on peripheral smear itself without the need for B12 and folate assays or bone marrow evaluation. This is because, B12 levels may be normal in patients with frank megaloblastic anemia and low B12 levels may be seen in normal individuals. Inter laboratory differences in normal values have also been observed. In patients presenting with pancytopenia, bone marrow evaluation is prudent to rule out acute leukemia and myelodysplasia which can present in a similar manner. Reticulocyte count and lactate dehydrogenase (LDH) can also help to differentiate megaloblastic anemia from aplastic anemia. With appropriate treatment (B12 or folic acid as deemed appropriate), rise in platelet count is observed within 48 hours and normalization is seen within 7-14 days.[6]

Case 3: Infection associated thrombocytopenia

A 7-year-old boy presented with history of fever of 5 days' duration associated with complaints of pain in both knees, difficulty in walking and a bruise below the left eye. On examination, he had multiple petechiae over the trunk, oral cavity and conjunctiva, flushed skin and splenomegaly. CBC done at admission showed a haemoglobin level of 14 grams/dL, haematocrit of 45%, WBC of 2600/μL with 33% neutrophils and 60% lymphocytes and platelet count of 11000/μL. There was evidence of mild transaminitis and raised blood urea at admission. Dengue serology (NS1 and IgM) was positive. He was managed conservatively with adequate hydration and intensive monitoring. On second day of admission, he had one episode of hematemesis. He was managed with platelet transfusion and tranexamic acid in addition to IV fluids. He remained afebrile after admission and over the next 48 hours there was improvement in platelet count.

Thrombocytopenia is a common hematological manifestation in infections (Table III) especially viral.[7] Mild to moderate thrombocytopenia is observed in most viral infections (including the recent pandemic due to SARS-COV-2) where platelet production is hampered either by direct infection and apoptosis of megakaryocytes, decreased maturation of megakaryocytes or by suppression of hematopoietic precursors. Enhanced platelet destruction occurs during viremic states by immune or non-immune destruction. Additionally, platelets may be destroyed in the spleen or by hemophagocytosis secondary to the infection. In viral hepatitis, low platelet count can occur following platelet specific antibodies directed against glycoproteins or by immune complexes that bind to platelet surface. Hepatitis C infection related liver dysfunction in addition to portal hypertension and hypersplenism can also cause reduction in thrombopoietin production, which results in platelet production defects.

Table III. Infection associated thrombocytopenia

Viral infections	Dengue, Epstein Barr virus, hepatitis C, HIV, parvo virus B19, CMV, rubella, COVID-19, mumps, measles
Bacterial infections	Gram negative sepsis, enteric fever, rickettsial infections, brucellosis, disseminated Tuberculosis
Parasitic infections	Malaria, leishmaniasis, toxoplasmosis

In addition to the above mechanisms, dengue virus can also induce complement mediated lysis of platelets and endothelial activation with enhanced consumption, both of which can enhance the degree of thrombocytopenia.[8] The onset of thrombocytopenia usually occurs with the start of the febrile illness. It worsens during the critical phase where capillary leak and raised hematocrit is observed and persists during the initial recovery phase before it starts to recover. Mucosal bleeding is expected in most patients of dengue fever who are admitted to intensive care units and hence should be prevented as far as possible by measures such as avoiding NSAIDs, gastro-protection, lubrication of nasogastric tube and urinary catheter to prevent trauma, correction of co-existent coagulopathy and improvement of tissue perfusion. There is no role for prophylactic platelet transfusions in dengue infection.[9] Monitoring of platelet count in dengue infection is in fact to predict plasma leakage rather than to decide about transfusion. In those patients with mucosal bleeding or platelet count $<20 \times 10^3/\mu L$, single donor platelet transfusions are preferred.[9]

Failure of recovery of platelet count as expected with the natural history of dengue viral infection should prompt search for hemophagocytosis, super-added nosocomial infections with Gram negative bacilli (enteric fever or leptospirosis) or even additional folate/B12 deficiency. The presence of large platelets in the circulation indicate the presence of recovering bone marrow and heralds the

normalization of platelet count. Immature platelet fraction (IPF) is a measure of reticulated platelets in the circulation and reflect the degree of thrombopoiesis.[10] Reticulated platelets, the "reticulocyte" equivalent of platelet series, are immature platelets that are recently released from bone marrow, have more RNA and are physiologically more active. Mean platelet volume is also a surrogate marker of bone marrow activity. IPF >8% predicts platelet recovery within the next 24 to 48 hours in dengue patients and can reduce anxiety as well as platelet transfusions in these patients.[10]

Case 4: Hemophagocytic lymphohistiocytosis

A 5 year old girl presented with complaints of high grade fever of 1-week duration and yellowish discolouration of eyes of 3 days duration. At admission, she had pallor, icterus and hepatosplenomegaly. Following evaluation, she was diagnosed to have acute viral hepatitis as she was positive for IgM hepatitis A. At admission, CBC showed haemoglobin of 8 grams/dL, WBC count of 5500/μL with 25% neutrophils and 66% lymphocytes and platelet count of 75,000/μL. Liver function tests showed serum bilirubin of 5.6mg/dL with 50% direct fraction, SGPT of 1500units/dL and SGOT of 2450 units/dL. She was managed symptomatically. Fever persisted and repeat evaluation showed declining trend for all hematological cell lines. Repeat CBC showed hemoglobin of 6.5 grams/dL, WBC count of 3200/μL with 10% neutrophils and 76% lymphocytes and platelet count of 22,000/μL. Evaluation for dengue, enteric fever, malaria and leptospirosis was negative. Peripheral smear showed lymphomonocytoid cells with no evidence of malignancy. In view of persisting fever, splenomegaly and pancytopenia, HLH was considered. Serum ferritin was 5800ng/ml, triglycerides were 380mg/dL and fibrinogen was 120 mg/dL. Bone marrow done showed reactive marrow with prominent macrophages. In view of these findings, she was managed with dexamethasone and etoposide for 8 weeks to which she responded well. Fever subsided and platelet count improved within 2 days of starting dexamethasone. She completed treatment 2 years back and is currently doing well.

Thrombocytopenia is commonly encountered in critically ill children and neonates. The timing of thrombocytopenia, rapidity of fall, presence of mucosal bleeding can have significant implications towards diagnosis and treatment. Endothelial activation causes platelets to adhere to the vascular endothelium and activate coagulation cascade.[11] In addition to these, complement mediated platelet destruction, thrombotic microangiopathy, drug induced platelet destruction and hemophagocytosis are other reasons for thrombocytopenia in sick children.

Hemophagocytic lymphohistiocytosis (HLH) is being increasingly recognized as a complication in sick patients which results from inflammation triggered by infections, malignancies and rheumatological illnesses. It is characterized by uncontrolled proliferation of lymphocytes and macrophages that release cytokines into the circulation.[12] This cytokine storm results in a constellation of clinical manifestations such as persistent fever, splenomegaly, pancytopenia or serial reduction in absolute values of all three hematological cell lines, hypofibrinogenemia, hypertriglyceridemia, hyperferritinemia and evidence of hemophagocytosis in bone marrow, lymph nodes or spleen. In some families with a genetic predisposition for this inflammation, HLH can present in an autosomal recessive or X linked manner with early clinical presentation and poor outcome in the absence of bone marrow transplantation.[13] In the vast majority, it is secondary and requires early identification, prompt search for an underlying genetic cause and treatment to reduce the inflammatory drive.

The appearance and persistence of thrombocytopenia in a sick, febrile child is often the first indication of HLH. Treatment is directed at suppressing the activated lymphomonocytoid cells with dexamethasone, etoposide or cyclosporine as per the co-operative group protocols from North America.[12,14] During treatment, patients are closely monitored for signs of improvement which include defervescence, recovery of platelet and neutrophil count and improvement of liver function. HLH has a highly dynamic clinical course.[14] Hence customization of therapy is warranted and treatment should be given in centers capable of handling sick and immunocompromised patients.

Thrombotic microangiopathies viz. hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are another group of conditions that present with thrombocytopenia in critically ill patients.[1,15] Both these conditions occur following consumption of platelets resulting from endothelial cell injury. In HUS, the toxin released from strains of Escherichia coli or other bacteria causing colitis results in vascular injury that primarily involves the kidneys resulting in oliguria, hypertension and thrombocytopenia. Thrombotic thrombocytopenic purpura is another dramatic condition characterized by fever, thrombocytopenia, microangiopathic hemolytic anemia, neurological and renal abnormalities. Early identification of the dyad of

thrombocytopenia and microangiopathic hemolytic anemia and initiation of therapeutic plasma exchange is vital for these life threatening diseases.[15]

Platelet refractoriness is yet another difficult scenario encountered in critically ill children. Drugs (amphotericin B, vancomycin), infections, hemophagocytosis, immune causes such as HLA sensitization are to be carefully excluded in this situation.

Case 5: Aplastic anemia

A 15 year old boy presented with complaints of fever on and off for the past 8 months. On evaluation at another centre, he was found to have anemia and thrombocytopenia, for which he was transfused packed red cells and random donor platelets on multiple occasions. At the last centre, a bone marrow aspiration was done which was reported as hypoplastic anemia. Since the past month, he had multiple episodes of bleeding in the form of gum bleeding and melena. He had tiredness, difficulty in breathing and needed help to walk. On examination at our centre, he was noted to have severe pallor, active mucosal bleeding with no hepatosplenomegaly. He did not have short stature, skeletal anomalies or hyperpigmentation. CBC showed hemoglobin of 5.5 grams/dL, WBC count of 1400/μL with 12% neutrophils and 80% lymphocytes and platelet count of 4,000/μL. Bone marrow aspiration and biopsy were done; cellularity was <20%. There was no HLA matched donor for bone marrow transplantation and hence he received immune suppressive therapy with anti-thymocyte globulin and cyclosporine to which he has responded partially. He has completed one year of follow up and is transfusion independent.

Bone marrow suppression secondary to infections or drugs have been mentioned in the previous cases. Bone marrow failure or aplastic anemia results from inability of bone marrow to produce blood cells either due to genetic causes, post infectious causes, post toxin/drug exposure or yet unknown reasons. At least 1/4th of childhood aplastic anemia occurs in children with underlying genetic predisposition to bone marrow failure.[15] Bone marrow failure is suspected in children who present with pancytopenia with no evidence of extramedullary disease (hepatosplenomegaly or lymphadenopathy). A careful history of recent exposure to drugs/ toxins and infections is part of evaluation. Complete blood count shows evidence of anemia, reticulocytopenia, neutropenia and thrombocytopenia with the absence of blasts of any kind and dysplastic neutrophils. Platelets are reduced severely and are small in size unlike what is seen in destructive states.

The diagnosis of aplastic anemia requires evidence of bone marrow hypocellularity (defined as cellularity <25% or between 25-50% where <30% cells are residual hematopoietic cells) with peripheral blood cytopenias in the absence of marrow infiltration or fibrosis. As per the approved Camitta criteria, at least 2 out of 3 criteria (reticulocyte count <20,000/μL, platelet count<20,000/μL and absolute neutrophil count <500/μL) should be satisfied. The importance of an adequate trephine biopsy of atleast 1.5-2 cms cannot be over emphasized in this regard. As is described in the case, aspiration without biopsy is hardly useful and just adds to the cost and pain suffered by the patient.

During clinical evaluation, the possibility of an underlying genetic etiology for bone marrow failure is always to be looked for. The presence of short stature, hyperpigmentation, café au lait macules, skeletal anomalies such as dysplastic thumbs, absent radius, triphalangeal thumbs, poly/syndactyly, renal anomalies, reticulate pigmentation of skin on neck, nail dystrophy, leukoplakia, warts, molluscum, history of repeated infections, family history of liver and lung fibrosis, early greying or malignancies point towards Fanconi anemia, dyskeratosis congenita, thrombocytopenia with absent radius and more recently identified conditions such as GATA2 haploinsufficiency, SAMD9/SAMDL mutations etc.[16] All patients under the age of 35 years with bone marrow failure are to be evaluated for genetic causes for the same.[17]

Early bone marrow transplantation is probably the only intervention that saves the lives of these children. In case of idiopathic aplastic anemia, in the absence of a HLA matched bone marrow donor, immunosuppressive therapy with anti thymocyte globulin and cyclosporine may be given. A very restrictive transfusion policy is followed as far as platelet transfusions are concerned in these patients prior to initiation of definitive treatment.[18] Platelet transfusions from multiple donors can induce immunization against either donor HLA or human platelet antigens. The number of pre-transplant transfusions received have a negative impact on the outcome after transplantation. Irradiated and leuco-depleted blood products are to be used in these children to reduce the risk of allosensitization. Repeated platelet transfusions can lead to platelet refractoriness which will further impair the quality of life of these children. As in most other thrombocytopenic conditions in children, there is no role for prophylactic platelet transfusions, unless platelet count is <$10x10^3$/μL. The threshold is reduced to $5x10^3$/μL in many centers in stable children without bleeding signs. However, such a low transfusion trigger requires regular

evaluation of the patient and provision for immediate transfusion in case of clinically significant bleeding.[17,18]

Case 6: Inherited thrombocytopenia

A 1.5 year old boy presented with history of fever since 5 days, redness and induration of skin on the nape of neck with bleeding and petechial skin rash. He was the first born child to non-consanguineous couple with no significant family history. He was evaluated earlier at a centre, diagnosed as immune thrombocytopenia and started on oral prednisolone. Bone marrow done showed increased megakaryocytes which was compatible with the diagnosis of immune thrombocytopenia. On examination, in addition to the petechiae, child had evidence of seborrhoea on the scalp and atopic rash. Mother recounted that this rash had been present since early infancy for which she had tried multiple emollients. CBC done showed haemoglobin: 12.6 gm/dL, WBC count: 9790 cells/μL with 63% neutrophils, 21% lymphocytes, 7% eosinophils and platelet count of 9000/μL. Peripheral smear showed the presence of occasional giant platelets among uniform small platelets. Even though MPV was reported high, based on a clinical suspicion of Wiskott Aldrich syndrome (young boy with thrombocytopenia with atopy), a genetic evaluation was performed, which was confirmatory.

Although immune thrombocytopenia and other acquired causes are the most frequent reasons for low platelets in children, a suspicion for inherited genetic mutations should be kept in mind while evaluating this condition in children of any age especially in a male child. Congenital thrombocytopenia could be classified based on the platelet size, the pattern of inheritance or by associated clinical features. The presence of consanguinity, family history of thrombocytopenia or malignancies, repeated infections often point towards a genetic cause. Wiskott Aldrich syndrome and X linked thrombocytopenias are most commonly associated with platelets that are very small in size with MPV< 7fL. Inherited macrothromboctyopenias (MPV>11 fL) are seen in May Hegglin anomaly, Epstein syndrome, Fechtner syndrome, Sebastian syndrome, Bernard Soulier syndrome etc. Thrombocytopenia absent radii and congenital amegakaryocytic thrombocytopenias present with normal platelet size (MPV 7-11 fL).[19]

Wiskott Aldrich syndrome is an X-linked primary immunodeficiency disorder that is characterized by the classic triad of severe immunodeficiency, micro-thrombocytopenia, and eczema. The WAS gene encodes the WAS protein (WASp), which is a 501-amino acid protein expressed within the cytoplasm of nonerythroid hematopoietic cells. WAS is an X-linked disorder that is manifested in males, with an absence of clinical symptomatology in obligate female carriers.[20] WAS presents with susceptibility to pyogenic, viral and opportunistic infections, eczema, autoimmune phenomena and increased incidence of lymphoproliferative disease. X linked thrombocytopenia results from mutations in the same gene, but present only with thrombocytopenia and small platelets without skin manifestations or infections.

The platelet counts in patients with WAS and XLT ranges form 3000-70,000/μL. Since there may be few platelets that are large, MPV often cannot be relied on. Also, in the presence of profound thrombocytopenia, MPV may give unreliable results. WAS should be considered in all males with immune thrombocytopenia like features, especially in those who present very early in life, those with skin features of allergy/ atopy in infancy, small sized platelets, reduced MPV, higher eosinophil counts and repeated infections.[20] Identification will help in judicious treatment for immunodeficiency and early referral for bone marrow transplantation.

Case 7: Hypersplenism

A 13 year old girl from Bengal, diagnosed at the age of 5 years with hemoglobin E-Beta Thalassemia following pallor and abdominal distention had been on transfusion support episodically. She received 2-3 transfusions in a year. She was brought currently with complaints of extreme tiredness and episodes of gum bleeding. Mother felt that her growth was poor. On examination, she weighed 25 kg and was 135 cms tall, with pallor and splenomegaly of 12 cms along the splenic axis. CBC done showed haemoglobin: 4.4gm/dL, WBC Count: 4390 cells/μL with 33% neutrophils, 61% lymphocytes and platelet count of 35,000/μL. Peripheral smear was suggestive of thalassemic hemoglobinopathy. Manual platelet count was 45,000/μL.

About 1/3rd of the body's total platelets are retained in the spleen which is exchanged constantly with the circulating pool. Thrombocytopenia due to sequestration occurs in conditions with splenomegaly where the proportion of platelets sequestered increase. In children this is observed secondary to chronic liver disease with portal hypertension, hemolytic anemias such as hereditary spherocytosis, hemoglobinopathies such as HbE beta thalassemia, beta thalassemia intermedia and storage diseases such as Gaucher disease.[1] Thrombocytopenia due to hypersplenism is usually mild to moderate and generally does not fall <50x10^3/μL.[3] No treatment is required for this mild-moderate thrombocytopenia. Hypersplenism is defined as bicytopenia or pancytopenia that occurs usually in the presence of an enlarged spleen.

In hemoglobinopathies, the treatment of hypersplenism would be the initiation of a regular transfusion regimen to keep pre transfusion hemoglobin above 10gm/dL. This usually reduces the extramedullary hematopoiesis and results in regression of splenomegaly with improvement in platelet count.

Conclusion

Thrombocytopenia is a common, yet important marker of illness in children. In the above few case scenarios, we have observed how thrombocytopenia can present in apparently normal and sick children. Understanding the pathogenesis is the key to appropriate management.

Points to Remember

- ***Thrombocytopenia is a vital clue to the diagnosis of many acute and chronic illnesses.***
- ***Management of thrombocytopenia is decided based on the underlying etiology.***
- ***It is important to focus on the clinical condition of the child than on platelet counts.***
- ***Mean platelet volume ranges from 7-9 fL which is expressed in automated hematology analyzers. In conditions where platelets are destroyed, megakaryocytes produce large platelets. In bone marrow pathology, where megakaryopoiesis is affected, usually platelets are of normal size except in certain inherited conditions.***
- ***Immature platelet fraction is a measure of reticulated platelets or "reticulocyte" equivalent of platelet series.They are physiologically more active. IPF >8% predicts platelet recovery within the next 24 to 48 hours in dengue infection.***
- ***Bone marrow failure syndromes and leukemias should not be missed while evaluating thrombocytopenia.***
- ***Inherited causes of thrombocytopenia like Fanconi's syndrome, thrombocytopenia absent radius syndrome, dyskeratois congenita, Wiskott Aldrich syndrome should be suspected when there are suggestive features on physical examination.***

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CLIPPINGS

Effect of High-dose Vitamin A Supplementation in Children With Sickle Cell Disease: A Randomized, Double-blind, Dose-finding Pilot Study.

Suboptimal vitamin A status (serum retinol <30μg/dL) is associated with poor clinical outcomes in children with the hemoglobin-SS disease (HbSS), and supplementation with the recommended daily allowance of retinol is ineffective in improving vitamin A status. In a single-center randomized blinded dose-finding pilot study, the authors compared vitamin A and nutritional status in children with HbSS to healthy children and explored the impact of high-dose supplementation on the primary outcome serum vitamin A status.

In summary, neither 3000 nor 6000 IU/d of vitamin A is sufficient to increase serum vitamin A concentrations in children with HbSS. Minor improvements in exploratory nutritional, hematologic and muscle outcomes were noted with supplementation, and deserve exploration with a larger-sample longitudinal placebo-controlled study that controls for HU use. A longer term trial will contribute to understanding the potential clinical impact of modest changes in erythrocyte quality and muscle function. Given that there were few differences in outcomes by dose and no adverse events attributable to the study supplement, both doses were considered safe in this study. Future studies designed to evaluate vitamin A impact on these outcomes may require a higher dose vitamin A and a larger sample to fully evaluate these potentially clinically-beneficial relationships.

Modeling of total body vitamin A stores using stable isotope dilution is becoming more informative and could be used in combination with serum retinol as an outcome measure in such a study. A more accurate estimation of total body vitamin A stores will likely shed light on the mechanism altering vitamin A metabolism and thus serum retinol concentrations in this unique patient population.

Brownell JN, Schall JI, Mcanlis CR, Smith-Whitley K, Norris CF, Stallings VA. Effect of High-dose Vitamin A Supplementation in Children With Sickle Cell Disease: A Randomized, Double-blind, Dose-finding Pilot Study. J Pediatr Hematol Oncol 2020; 42(2):83-91.

Use of Probiotics for the Management of Acute Gastroenteritis in Children: An Update.

Since the publication of the 2014 European Society for Pediatric Gastroenterology, Hepatology and Nutrition Working Group (WG) on Probiotics and Prebiotics guidelines for the management of acute gastroenteritis (AGE), new evidence concerning the efficacy of probiotics has become available. The recommendations were formulated if at least 2 randomized controlled trials that used a given probiotic were available. The WG made weak recommendations for (in descending order in terms of the number of trials evaluating any given strain): Saccharomyces boulardii (low to very low certainty of evidence); Lactobacillus rhamnosus GG (very low certainty of evidence); L reuteri DSM 17938 (low to very low certainty of evidence) and L rhamnosus 19070-2 and L reuteri DSM 12246 (very low certainty of evidence). The WG made a strong recommendation against L helveticus R0052 and L rhamnosus R0011 (moderate certainty of evidence) and a weak recommendation against Bacillus clausii strains O/C, SIN, N/R, and T (very low certainty of evidence).

Szajewska H, Guarino A, Hojsak I, Indrio F, Kolacek S, Orel R, et al. Use of Probiotics for the Management of Acute Gastroenteritis in Children: An Update. J Pediatr Gastroenterol Nutr 2020; 71(2):261-269.

HEMATO-ONCOLOGY

AUTOMATED ANALYZER BASED APPROACH TO ANEMIA

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Abstract: *Anemia represents an extremely common clinical problem among children in India. Automated hematology analyzers yield a wealth of data that can aid etiological diagnosis and follow-up of anemic children. Conventional approaches include the use of parameters indicating cell volume and size variability in conjunction with the reticulocyte count to classify anemias. Recent advances range from reliable enumeration of schistocytes, enhanced precision in nucleated RBC counts, multiple approaches for detection of spherocytes, improved parameters for identification of anemias due to iron deficiency and iron restriction to hematopoiesis and improved prediction of hematopoietic recovery by identifying immature reticulocyte populations. This review discusses interpretation of the analyser data and their relevance to practicing paediatricians managing anemia.*

Keywords: *Anemia, Automated analysers, Automation, Erythrocytes, Laboratory test.*

Anemia represents an extremely common clinical problem in children, especially in developing countries. It is encountered virtually in all pediatric sub-specialities and has innumerable causes, ranging from the obvious to the obscure. The automated blood count is usually the first laboratory investigation to be ordered in a child who is pale, lethargic, exercise intolerant, or otherwise suspected to be at risk for anemia. The easily available and time tested parameters for the evaluation of anemia are hemoglobin (Hb) and the red blood cell (RBC) indices including the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW), often supplemented by a peripheral smear and the reticulocyte count. With the introduction of new and technologically-advanced automated analyzers, many novel parameters have also become available (Table I). These are often generated at no or minimal additional cost during the hemogram, and can accurately reflect various disease processes. However, they are not standardized across manufacturers, reference ranges are not easily available and interpretation is often not straightforward outside of controlled studies. Hence, for various reasons including availability and comfort levels, the application of the newer parameters in real-world clinical practice has often lagged their anticipated virtues extolled in published literature.

This review begins with a brief overview of existing approaches to diagnosis of anemia using the universally available parameters and examines what is new or improvable in their assessment. Subsequently, the more advanced and/or novel automated parameters are discussed with a focus on their applicability to pediatric practice.

Defining anemia in children - Reference ranges for Hb, hematocrit (Hct) and RBC count vary substantially with age in children, and to a lesser degree with race / ethnicity. Divergence between the sexes becomes apparent with increasing age, usually by 10-12 years. Hence, it is essential to assess hemoglobin based on the appropriate normal range and these are available internationally.[1] This is becoming easier for clinicians as more laboratories adopt hospital information systems. These systems can electronically select the correct age and gender-specific reference ranges to be provided in reports being generated. Although there is discussion in literature about the cut-off percentile for Hb (2.0, 2.5 or even 5th centiles) to diagnose anemia, most studies recommend less than 2.5th centile for age, ethnicity and gender.[2]

Conventional automated analyzer-based approaches to anemia

Once anemia has been diagnosed clinically and/or confirmed by Hb estimation, the automated hematology analyser data can yield rapid insights into its cause. One of the earliest considerations is the number of cell lines involved, with the differential diagnosis for

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Table I. Parameters identified by automated hematology analyzer-based techniques

Conventional automated analyzer-based approaches to anemia	Advanced parameters for anemia and red cell analysis
a) Quasi-morphological approach based on MCV and RDW	a) Diagnosis of iron status by automated hematology analyzers. Reticulocyte hemoglobin content (CHr) and reticulocyte hemoglobin equivalent (Ret-He.)
b) Red cell kinetics estimation by the reticulocyte count	b) Newer factors related to microcytosis and hypochromia. Low Hemoglobin Density (LHD %). Red blood cell size factor (RSf)
c) RBC volume histogram analysis shows abnormalities in several disease settings	c) Reticulocyte subclassification based parameters: Immature reticulocyte fraction (IRF) - the ratio of immature reticulocytes to the total number
	d) Automated detection of spherocytes - to detect multiple inherited and acquired conditions with spherocytes
	e) Nucleated RBC enumeration - The detection of nucleated RBCs is important for several reasons in pediatric practice.
	f) Fragmented RBC enumeration - important to diagnose thrombotic microangiopathy
	g) Miscellaneous novel red blood cell parameters: e.g. recognition of typical shapes like a “comma” in thalassemia trait

Table II. RDW and MCV-based classification of anemia by a quasi-morphological approach

	Normal RDW	High RDW
Low MCV	• Thalassemia traits (α, β, δβ thalassemia), Hb Lepore and HbE traits • Anemia of chronic disease (ACD), inflammation, or malignancy (MCV declines in long-standing ACD)	• Iron deficiency • Thalassemia major or intermedia, HbH disease, double heterozygous HbE/β-thalassemia • Sideroblastic anemia • Microangiopathic hemolysis including hereditary pyropoikilocytosis • Deficiency of vitamins C and E, copper deficiency, aceruloplasminemia
Normal MCV	• Normal state • Post-chemotherapy • Acute blood loss • G6PD deficiency • Anemia of chronic disease, inflammation, or malignancy (shorter duration)	• Early/latent iron deficiency • Multiple-deficiency anemia • Hemolytic anemia due to other red cell enzymopathies or membranopathies • Sickle cell anemia • Immune hemolysis • Recently transfused patients
High MCV	• Aplastic anemia and inherited marrow failure syndromes • Hypothyroidism • Liver disease • Myelodysplastic syndromes (MDS)	• Megaloblastic anemia • RBC agglutination • Hemolytic anemia with marked reticulocytosis • Some forms of MDS

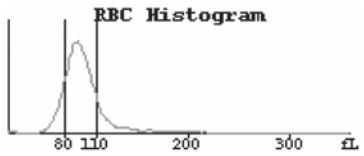
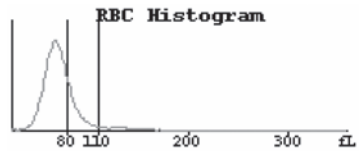
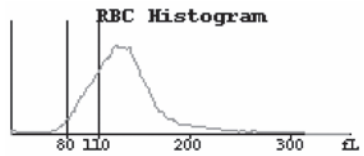
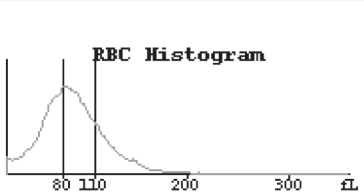
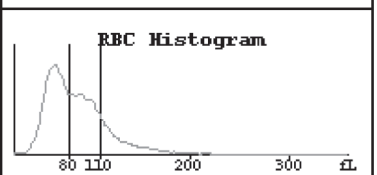
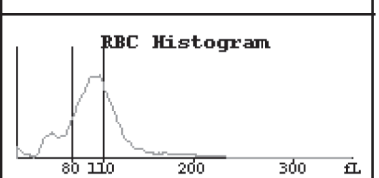
NOTE: *Spurious macrocytosis may occur in cases with reticulocytosis, marked red cell agglutination, and marked leukocytosis. Spurious microcytosis may occur in cases with schistocytes, microspherocytes, giant platelets.*

pancytopenia or bicytopenia being wider than that for isolated anemia. In the appropriate clinical setting, a rapid exclusion of hematological malignancy takes precedence over further work-up of anemia, necessitating a blood film or bone marrow examination. However, once this initial triage is done, isolated anemia is usually best dealt with by applying two commonly used approaches to the hemogram data. Mostly, both these approaches are applied simultaneously, keeping in view the history and examination findings:

1. Quasi-morphological approach: This approach involves evaluation of the mean corpuscular volume (MCV) and the degree of red cell anisocytosis as indicated by the [red cell distribution width (RDW)] to classify anemias analogous to what is done during blood film evaluation. Hence, the patient may have microcytic, normocytic, or macrocytic anemia, with or without anisocytosis. The assessment of mean corpuscular hemoglobin (MCH) as an indicator of hypochromia can further refine the differential diagnoses.[3] The various causes of different combinations of MCV and RDW are outlined in Table II.

2. Red cell kinetics estimation by the reticulocyte count: The reticulocyte count is easily available from most of the 5-part and more advanced analyzers. The percentage of red cells comprising of reticulocytes correlates with the erythropoietic activity in the bone marrow. Based on the reticulocyte count, the cases of anemia can be divided into those with (1) appropriate reticulocyte response to anemia (including anemia caused by hemolysis, acute or chronic

Table III. Red cell histogram appearances and abnormalities in various states

S. No.	Abnormality	Cause	Illustrative examples
1.	Nil (normal Gaussian curve)	-	
2.	Peak with normal width shifted to the left	Uniform microcytosis due to β- or α-thalassemia trait	
3.	Peak with increased width shifted to the right	Megaloblastic anemia, marked reticulocytosis, mild RBC agglutination	
4.	Left "shoulder" extension or a failure of the curve to touch the baseline on the left side	RBCs with smaller volumes merging into platelets indicating the presence of very small RBCs (e.g., microspherocytic or fragmented RBCs), or macrothrombocytes or platelet clumps	
5.	Right "shoulder" or trailing of erythrocyte population to the extreme right	Extremely large RBCs (macrocytes), marked red cell agglutination or marked increase in reticulocytes	
6.	Presence of two populations of red cells	Heterozygotes for X-linked sideroblastic anemia, or persons transfused with blood donated by carriers of β-thalassemia trait or HbE	

blood loss, transient marrow suppression followed by an almost immediate recovery etc.) and (2) those with inappropriately low reticulocyte responses (very low counts in marrow aplasia, pure red cell aplasia and drug/radiation induced suppression and moderately low to near-normal counts in micronutrient deficiencies and dyserythropoietic anemias).[4-10] A special situation occurs in patients with normal Hb and marked reticulocytosis, who are considered to have compensated hemolysis, most typically exemplified by the milder forms of hereditary spherocytosis and some rare enzymopathies. Patients with myelophthisis (for e.g. myelofibrosis, metastases) also have elevated reticulocyte counts.

A reasonable estimate of the cause of anemia is usually possible based on data derived from the above two approaches, in conjunction with the clinical background. In addition, the analysis of red cell histograms can occasionally be of help.

3. Red blood cell volume histogram analysis: The RBC size frequency distribution histogram typically displays a symmetrical or Gaussian shape. MCV and RDW are directly derived from this curve. This histogram shows abnormalities in several disease settings. These are shown in Table III. An interesting special situation among the entities listed is that of uniform microcytosis with absolute or relative erythrocytosis. The constellation of findings of reduced MCV, normal RDW and elevated RBC count (absolute or relative to the Hb) usually accompanied by reduced MCH are well known to be indicative of α- or β-thalassemia trait.[11,12] Similar results may however also be found in δβ thalassemia trait, HbE-trait as well as homozygosity and Hb Lepore trait.[13] More or less similar results also occur in iron-deficient polycythemia vera, iron deficiency on therapy and cyanotic congenital heart disease.[12]

A key feature in both the quasi-morphological approach as well as red cell histogram analysis is the RDW. Several analyzers nowadays provide the RDW both as coefficient of variation (CV) and as standard deviation (SD).

Choosing between the RDW-CV versus RDW-SD

The RDW-CV and RDW-SD are both measures of the dispersion of RBC volumes around their mean. The wider the spread of the red cell volumes, the higher the SD. Although both the indices use dispersion to estimate the degree of anisocytosis, they approach it differently (Fig.1).

The RDW-CV measures dispersion as a ratio of 1 SD to the MCV. Hence, changes in the SD (width) or MCV both influence the results. Microcytosis therefore tends to elevate the RDW-CV by lowering the ratio's denominator and macrocytosis tends to reduce it by increasing the MCV (i.e. the denominator). Thus, the RDW-CV can be spuriously high in cases with extreme microcytosis and falsely normal in macrocytic anemia.[12,14]

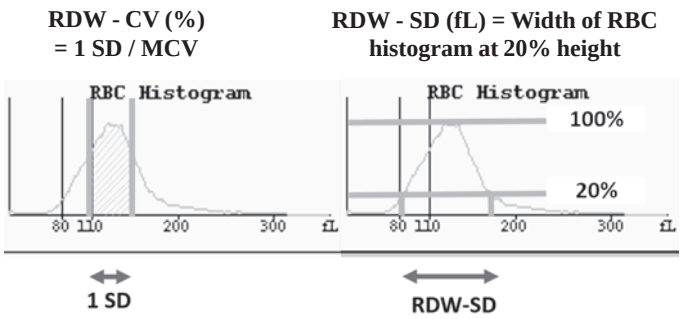


Fig.1. A comparison of calculation strategies for RDW-CV versus RDW-SD. The RDW-CV (left panel) is calculated by dividing the width of the RBC histogram at 1 SD across its centre by the MCV. The RDW-SD, on the other hand, is simply the width of the RBC histogram at 20% of its height. The latter is therefore independent of the MCV.

The RDW-SD, on the other hand, is a direct measure of the RBC histogram width taken at its 20% height level. Corpuscles below the 20% height of the curve are excluded. These include aperture artifacts, cell coincidence errors (doublets, triplets, etc.) and agglutinates on the right extreme of the histogram and platelet clumps, electrical interference and very large platelets on the left. Since the RDW-SD is not dependent on the MCV it represents the absolute measure of dispersion. Overall, it is more reliable than the RDW-CV, especially in patients with abnormal MCVs.[12,14]

Advanced parameters for anemia and red cell analysis

This section deals with selected recently developed important parameters. The focus is mostly on clinical applications of the analyzer data and less on its technological derivation and instrumentation.

1. Diagnosis of iron status (iron deficiency and iron-restricted erythropoiesis) by automated hematology analyzers

Assessment of iron status is important in not only the child with suspected iron deficiency, but also in those in whom iron deficiency may coexist with acute or chronic inflammatory states or chronic diseases, like chronic kidney disease (CKD), inflammatory bowel disease (IBD), hemophagocytic lymphohistiocytosis (HLH), pediatric

rheumatological illnesses, etc. The conventional red cell indices (MCV, MCH, MCHC, RDW) are not sensitive enough in the early or latent stages of iron deficiency and they do not alter rapidly enough to predict response to iron therapy in deficient patients. Moreover, they are not useful in distinguishing coexisting iron deficiency in inflammatory states.[15]

Reticulocyte hemoglobin content (CHr) and Reticulocyte hemoglobin equivalent (Ret-He): Automated hematology analyzers nowadays incorporate several parameters to measure the iron content of circulating erythrocytes. The most effective of these parameters reflect the availability of iron to erythroid precursors. CHr, measured by Siemens and Abbott analyzers, quantifies hemoglobin mass in reticulocytes, which is directly dependent on iron bioavailablity to these cells. Given the short maturation time of reticulocytes into erythrocytes (typically 1-2 days), hemoglobin content of these newly-released immature erythrocytes reflects the short-term dynamics of iron availability for erythropoiesis in the bone marrow. RET-He, (Sysmex analyzers) is a related biomarker serving essentially the same purpose.[12,15,16]

Both CHr and Ret-He represent physiologically appropriate iron status indicators in pediatric CKD as the synthesis of iron-replete reticulocytes is highly dependent on erythropoietic iron bioavailability. Additional advantages include lower cost vis-à-vis conventional tests, and the requirement for a lower blood sample volume as analysis is typically performed on the same EDTA specimen used for the hemogram.[12,15,16]An alternative approach to diagnosis of iron deficiency or iron-restriction is to assess the response to therapy of the immature reticulocyte fraction, which is discussed in the next section.

Newer factors related to microcytosis and hypochromia: The percentages of hypochromic and microcytic RBC (Siemens, Abbott and Sysmex) and related parameters like low hemoglobin density (LHD%), red cell size factor (RSF) (on Beckman Coulter instruments) at various thresholds can be sensitive indicators of both iron deficiency and iron restricted erythropoiesis. LHD % is a potential marker of iron availability which is provided by Beckman-Coulter analyser and is derived from the mean cell hemoglobin concentration, using the mathematical sigmoid transformation. Red blood cell size factor (RSf) is a new parameter provided by Beckman-Coulter which combines the volume of erythrocytes and the volume of reticulocytes. RSf is a potential screening parameter in evaluating patients with hypochromic microcytosis in identifying possible cases of α-thalassemia trait regardless of iron status. There may however be interference in cases with inherited forms of hypochromic microcytosis, since the frequencies of β- and α-thalassemia as well as HbE trait in India are not inconsiderable.[11,17] Hence, the interpretation of the complete blood count is recommended, preferably with complete knowledge of the specific clinical setting. These factors are also dependent on the time of sample processing. For instance, the 2016 update to the National Institutes of Clinical Excellence (NICE) guidelines for assessment of iron status in CKD patients states that the percentage of hypochromic red cells (%HRC), CHr or Ret-He are superior to serum ferritin alone in predicting therapeutic response to intravenous iron. % HRC should be used if testing is possible within 6 hours, otherwise CHr or Ret-He should be used.[18]

Although the Ret-He is reduced in both iron deficiency and in thalassemia trait, formulae incorporating novel RBC indices have been designed to distinguish these conditions. For instance, an "Urrechaga Index" has been described that uses a cut-off of >minus 7.6 on the formula "% MicroR-%Hypo-He - RDW" to recognize heterozygous thalassemia with 100% sensitivity and 92.6% specificity.[19,20] Values greater than minus 7.6 are highly suggestive of heterozygous β-thalassemia which must then be confirmed by estimating the HbA2 percentage.

2. Reticulocyte subclassification based parameters

Immature reticulocyte fraction (IRF): The IRF is the ratio of young, immature reticulocytes to the total number of reticulocytes. Analyzers use fluorescent or non-fluorescent dyes (for e.g. thiazole orange, Oxazine 750, new methylene blue, etc.) to stain reticulocyte RNA. A combination of fluorescence and narrow angle laser light scatter is used to detect the red cells and measure the reticulocyte count and the IRF. Most of the above dyes also stain DNA, but as the concentration of DNA in leukocytes is far in excess of that of RNA in reticulocytes, the cells are easily separated by their differing fluorescent intensities.[4,5,12,16]

Based on fluorescence emission intensity, reticulocytes are divided into three subsets, the most immature, the moderately immature and finally, the mature reticulocytes. Of these, the most immature and moderately immature reticulocytes constitute the IRF with the reference range being usually around 2.0% - 16.2%.[12]

Clinically, the IRF is low with a low reticulocyte count in severe aplastic anemia or renal failure and high with high reticulocyte count in hemolysis, hematinic response and erythropoietin-doping athletes. If it is paradoxically high with a low reticulocyte count, this suggests

dyserythropoiesis (myelodysplastic syndromes, congenital dyserythropoietic anemia). The IRF is high with a normal reticulocyte count in engrafting marrow and hemopoietic regeneration after myeloablative chemotherapy. Hence, it can be used to monitor marrow regeneration (engraftment) post-transplant or chemotherapy as well as check for hematinic response, together with the Ret-He. The first erythropoietic response after marrow ablation by therapy is a rise in the IRF, which precedes the increase in the reticulocyte count and absolute neutrophil count (ANC) by several days.[4,5,12,16]

3. Automated detection of spherocytes

Spherocytic red cells can be seen in multiple inherited and acquired conditions and can also be difficult to detect morphologically when present in small numbers.[1,12] Different hematology analyzers have adopted different approaches to their detection and in some cases, distinction between various causes. The earliest approaches used high-normal to raised MCHC, coupled with increased RDW and hemoglobin distribution width, with modest success rates.[3,12,21]

More recently, Nair, et al used Beckman Coulter instruments to conclude that an algorithm of MCV minus MSCV (mean sphered cell volume from the reticulocyte channel) >10 and (mean reticulocyte volume) MRV minus MSCV <25 yielded 84.2% sensitivity and 94.7% specificity in patients with hereditary spherocytosis (HS).[22] Another study done on Abbott instruments evaluated 740 pediatric patients and found that "hyperchromic erythrocytes" were significantly higher in HS patients than autoimmune hemolytic anemia (sensitivity of 96.4% and specificity of 99.1% for HS at a threshold of 4.9%). The Abbott CELL-DYN Sapphire instrument has the capability of performing the eosin-5'-maleimide (EMA) dye binding test as an investigational method.[23] Investigators using Sysmex instruments have devised a two-step algorithm using the microR (% of microcytic red cells), % of red cells with low Hb (% Hypo-He) and the immature reticulocyte fraction to yield 90.9% sensitivity and 96.3% specificity for identification of HS.[24]

4. Nucleated RBC enumeration

The detection of nucleated RBCs is important for several reasons in pediatric practice. At the most basic level, blood samples with high nRBC counts require correction of the total WBC count. In addition, nRBCs are raised in disorders characterized by increased erythropoiesis, for instance, acute hemolysis, severe hypoxia and thalassemias and hemoglobinopathies. They are also frequently seen as part of leucoerythroblastosis in hematological and metastatic malignancies and in extramedullary hematopoiesis. nRBCs have been associated with poorer outcomes in hematological and non-hematological conditions and in ICU patients. These cells can be physiologically present in newborns and young infants (up to 100 nRBCs/100 leukocytes). In this age group, their number goes up in conditions of prematurity, hypoxia, intraventricular hemorrhage and in babies with Down's syndrome.[1,12]

Automated analyzers are faster and more reliable than manual assessments in providing highly precise nRBC and corrected WBC counts. In Sysmex instruments, they are measured in a WNR (white-and-nucleated-reds) channel using a polymethine nucleic acid binding dye and cell-specific lysing agents. Due to the higher sensitivity of automated counts, nRBC <1.5% may be found in persons without enhanced erythropoiesis or an ongoing pathologic process in the bone marrow.[25]

Table IV. Commonly encountered sources of erroneous results in automated red cell analysis

Error phase	Common causes
Pre-analytical errors	Transcription errors (usually of labelling or accompanying information) including mix-up of mother/baby's samples Prior recent blood transfusion Wrong anticoagulant, incorrect sample volume Hemoconcentration due to prolonged tourniquet application Hemodilution due to sampling from catheter that is in use for an infusion Hemolysis, partial clotting, inadvertent freezing/heating, delayed despatch
Analytical errors	Inadequate mixing Probe blockage (often from microclots in the prior specimen) Carryover from previous highly abnormal specimen Presence of interfering substances
Post-analytical errors	Results issued late or never Results entered in incorrect patient's record Results interpreted incorrectly, or against incorrect reference range

5. Fragmented RBC enumeration

Detection of fragmented RBCs (FRC) is important to diagnose thrombotic microangiopathy, and typically are viewed as a medical emergency. Manual assessment suffers from interobserver bias and a few cells indistinguishable from fragmented RBCs can also be seen in membrane / hemoglobin /other hemolytic disorders and in patients on dialysis therapy, impacting specificity. Automated enumeration of fragmented red cells offers advantages of rapid availability, high reproducibility and reasonably good concordance with microscopy.[12,26]

Based on the principle of fluorescent flow cytometry, fragmented RBCs in most modern analyzers (Siemens, Sysmex) are measured in the reticulocyte channel by proprietary algorithms. They lie in an area beneath the RBC population in the scattergram and display extremely low side fluorescence signal, due to the absence of ribonucleic acids in mature RBCs and a high-angle forward scatter (FSC). In general, the automated fragmented RBC counts have very high negative predictive values (i.e. cases with less than 1% schistocytes on automated counts are highly unlikely to reveal the cells on smear evaluation), but poorer specificities in cases with higher counts. This poor specificity has been particularly common in cases with iron deficiency and megaloblastic anemias. Hence, correlation with the clinical background and the platelet and reticulocyte counts, and ultimately a peripheral smear is necessary in cases with elevated FRC%.[5,12]

Table V. Commonly encountered pitfalls and causes of spurious/unreliable results in automated red cell analysis.

Pitfall / artifact / spurious result	Cause(s)
Spuriously elevated Hb	Inadequate mixing of specimen Marked leukocytosis Hyperlipidemia (esp. in patients on total parenteral nutrition) Paraproteinemias and cryoglobulinemia
Spuriously elevated RBC count	Hyperleukocytosis Numerous large platelets Cryoglobulinemia and cryofibrinogenemia
Spuriously low RBC count	Cold agglutinating autoantibodies EDTA-induced panagglutination Extreme red cell fragmentation and microcytosis Post-sample-draw lysis due to inadvertent heating/freezing
Spuriously elevated MCV	Red cell agglutination Marked leukocytosis Plasma hyperosmolarity as seen in hypernatremia, hyperglycemia, etc. Excess di-potassium EDTA
Spuriously low MCV	Marked hypochromia of RBCs Storage at warm temperatures Plasma hypo-osmolarity as seen in hyponatremia
Spurious high reticulocyte counts	Neonatal and post-splenectomy samples (high autofluorescence) Heinz bodies in oxidatively damaged red cells Abnormal dye binding to non-RNA targets (malaria, Babesiosis, Howell Jolly bodies, irreversibly sickled RBCs, leukemic blasts, porphyrin complexes, drugs, nucleated RBCs)
Spuriously low reticulocyte counts	Automated reticulocyte counts usually require generation of their specific individual reference ranges due to variations in dye used, duration of exposure of cells to dye, temperature, and threshold settings (upper threshold to exclude bright WBCs and lower threshold to exclude background autofluorescence).

6. Miscellaneous novel red blood cell parameters

Lyse-resistant erythrocytes in the WBC channel may represent several entities including sickled RBCs and target cells. The latter have also been studied as unghosted cells (which indicate presence of RBC abnormalities like inclusions, target cells and abnormal haemoglobin) in Beckman Coulter instruments.[27] The red cell cytograms of Advia series instrument (Bayer/Siemens) can help classify red cells based on size and hemoglobinization, aiding recognition of typical shapes like a "comma" in thalassemia trait and localizations like "hyperchromic" spherocytes.[28]

Spurious results and pitfalls in automated analyzers

Automated red cell analysis and the diagnosis of anemia requires familiarity and experience with the analyser in use, as well as determination of normal patterns and ranges for that instrument and the patient population.[8,29-32] From the laboratory's viewpoint, they are best summarized as pre-analytical, post-analytical and analytical phase errors and examples are given in Table IV. These are followed in Table V by a list of some commonly encountered pitfalls and causes of spurious / unreliable results in automated cell counters.

Future directions: Automated approaches to anemia diagnosis have seen major advances in the last decade or so. The likely future advances are expected to be in the fields of digital image analysis and artificial intelligence. The commercially available CellaVision™ software scans and "reads" Romanowsky-stained slides to provide standardized reporting of RBC morphology with minimal to no manual supervision being required for standard cases. It has shown extremely encouraging results in screening for schistocytes, intra-erythrocytic parasites and inclusions, and as a screening modality for identification of inherited hemolytic anemias.[33] Computer-aided artificial intelligence systems have been developed for RBC classification in smear images. These models evaluate vastly larger cell numbers than typical manual analyses, detect subtle abnormalities invisible to the human eye and are trainable over time.[34-36]

Conclusion

Automated hematology analyzers can provide several additional clues and inputs into the diagnostic work-up of anemia in children. Recent advances in cell counting and classification technologies need to be rapidly translated into actual clinical use to realize the full potential of these powerful new parameters, and this responsibility rests on both the laboratories and their pediatrician clients.

Points to Remember

- ***Automated hematology analyzers yield a wealth of data that can aid etiological diagnosis and follow-up of anemic children.***
- ***Conventional approaches include the use of parameters indicating cell volume and size variability in conjunction with the reticulocyte count to classify anemias.***
- ***Recent advances include precise schistocyte and nucleated RBC enumeration, improved parameters for iron deficiency and iron-restricted hematopoiesis, increasing utility of immature reticulocyte populations and detection of spherocytes and other poikilocytes.***
- ***Future advances in the field are likely to include digital image analysis and artificial intelligence to analyse patterns indiscernible to the human mind and eye.***

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CLIPPINGS

COVID 19 disease in New York City pediatric hematology and oncology patients.

Viral respiratory infections in chronically ill and immunocompromised children are associated with increased morbidity and mortality compared to the general population. Little is known about the effect of COVID-19 disease on pediatric hematology, oncology, and hematopoietic stem cell transplant (HCT) patients.

In this retrospective study, all patients 21 years old or younger with COVID-19 testing done were included. For COVID-19 positive patients, data on demographics, presence of COVID-19 symptoms, complete blood counts, inflammatory markers, imaging, hospital course, and impact on cancer directed therapy were extracted from the electronic medical record. All laboratory and radiologic assessments were performed at the discretion of the treating physicians.

Most COVID-19+ patients had relatively mild disease, with almost half treated as outpatient or without the need for respiratory support. Hospitalized COVID-19+ cancer patients were generally admitted for expected complications of cancer therapy rather than complications of COVID-19 disease. Nine of 14 had their cancer directed treatments delayed for COVID-19+ infection. Five tested positive for COVID-19 during or immediately postmyelosuppressive chemotherapy; all did well without any significant complications from COVID-19. Even the four cancer patients requiring PICU level care and demonstrating clear evidence of lung involvement were recovering well. No COVID-19+ patients who were initially managed as outpatients subsequently required admission.

These data suggest that in patients without underlying comorbidities beyond their cancer diagnosis, COVID-19 may not pose a significantly greater threat than other intercurrent viral infections and that asymptomatic patients whose anticancer therapy cannot be delayed may be able to safely receive myelosuppressive chemotherapy with close monitoring and follow up. Nearly two thirds of the patients with cancer in our cohort experienced treatment delays due to COVID-19; the majority of these delays were due to decisions to defer planned treatment rather than directly due to complications of COVID-19 infection. The decision to delay critical, time sensitive anticancer therapy in these children is one of the biggest challenges being faced by pediatric oncologists. Our data suggest that in patients without underlying comorbidities beyond their cancer diagnosis, COVID-19 may not pose a significantly greater threat than other intercurrent viral infections and that asymptomatic patients whose anticancer therapy cannot be delayed may be able to safely receive myelosuppressive chemotherapy with close monitoring and follow up.

However because of the small numbers in their series, there remains an urgent need for prospective longitudinal study of the effects of COVID-19 on the pediatric hematology, oncology, and HCT population.

Gampel B, Troullioud Lucas AG, Broglie L, Gartrell Corrado RD, Lee MT, Levine J, et al. COVID-19 disease in New York City pediatric hematology and oncology patients. Pediatr blood cancer 2020; 67(9): e28420.

HEMATO-ONCOLOGY

CLOTTING FACTOR REPLACEMENT THERAPY

*Shanthi S

Abstract: *Inherited disorders of clotting factor deficiency are known to occur with all coagulation factors. Of these, Von Willebrand disease, hemophilia A and B are the commoner conditions. Fresh frozen plasma contains all coagulation factors and hence in the past it was used as the major therapy for all inherited clotting factor deficiencies presenting with bleeds. Later cryoprecipitate was discovered and used for deficiency of fibrinogen, factorVIII, factor XIII and Von Willebrand disease. Both these blood products have to be administered in large volumes and they also carry a high risk of transfusion transmitted infections. This led to the discovery of clotting factor concentrates. Good manufacturing practices have resulted in the availability of products with high degree of purity and safety. Plasma derived single factor concentrates are available for all factors except for factor II and factor V. Advances in genetic engineering led to the discovery of recombinant factors which have very high safety profile. Currently recombinant forms of factor VIIa, factor VIII, factor IX and factorXIII are available. The standard of care for factor deficiencies is to replace the missing factor using clotting factor concentrates to enable patients to lead a completely normal life. This article deals with factor replacement therapy for the common and rare bleeding diatheses.*

Keywords: *Factor replacement therapy, Clotting factor concentrates, Fresh frozen plasma,Cryoprecipitate.*

The human body maintains a fine balance between the naturally occurring anticoagulants, fibrinolytics and the coagulation factors so that there is neither excessive bleed nor thrombosis.Traditionally there are 13 coagulation factors which help in the clotting mechanism (Table I). The terms factor III, IV and VI are no more used as they are not independent proteins.

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Table I. Coagulation factors[1]

Clotting factor	Synonym
I (FI)	Fibrinogen
II (FII)	Prothrombin
V (FV)	Labile factor, proaccelerin
VII (FVII)	Stable factor, proconvertin
VIII (FVIII)	Anti hemophilic factor
IX (FIX)	Christmas factor
X (FX)	Stuart - Prower factor
XI (FXI)	Plasma thromboplastin antecedent
XII (FXII)	Hageman factor (deficiency does not produce bleeding)
XIII (FXIII)	Fibrin stabilizing factor

The deficiency of one or more factors can lead to bleeding diathesis. Congenital deficiency due to genetic abnormalities has been described for all the above factors and von Willebrand factor (vWF).

In the past, these diseases were treated with fresh frozen plasma (FFP) or cryoprecipitate. Plasma contains all coagulation factors whereas cryoprecipitate contains factor XIII, fibrinogen, vWF and factor VIII. However plasma therapy requires a large dose and carries the risk of transfusion transmitted infections (TTI). To overcome these limitations researchers worked to produce clotting factor concentrates (CFC). Advances in the purification methods of plasma and recombinant technology have resulted in factor replacement therapy (FRT) as the treatment of choice for most clotting factor deficiencies. History of FRT is shown in Box 1. Currently plasma derived concentrates are available for the majority of deficient factors except for FII and FV. Recombinant forms of FVIIa, FVIII, IX and FXIII are available.

Fresh frozen plasma

Plasma is obtained by centrifuging whole blood or by plasmapheresis. One mL of plasma contains one international unit of each factor. Fresh frozen plasma is

Box 1. History of FRT

1840- Whole blood was used in hemophilia A.

1929- Technique to separate plasma from whole blood was developed.

1963-Fibrinogen concentrate was first approved for use in Brazil on March 4, 1963.

1965 –Cryoprecipitate was used for hemophilia A.

1968- First plasma derived factor VIII concentrate was produced on an industrial scale.

1972- Lyophilized clotting factor concentrates FVIII was produced.

1970s-Prothrombin complex concentrate was developed.

Between 1985 and 1992- All available plasma-derived factor concentrates became virally inactivated against lipid-enveloped viruses, including hepatitis C, hepatitis B and HIV.

1987- Recombinant FVIII was first used for a patient.

1992 - FDA approves first recombinant FVIII product.

1992- Virally safe products for hepatitis C were available.

1992- First human plasma-derived factor XI concentrate was made available in France.

1993- Highly purified, pasteurized, plasma-derived concentrate factor XIII concentrate was made available in Europe.

1995- Hemophilia prophylaxis was started in US.

1997- Recombinant factor IX was licensed and approved for sale in the U.S.A.

1998 - rFVII was used for FVIII inhibitor patients.

2004 - A synthetic recombinant FXIII (rFXIII) was developed.

2015- Factor X concentrate was approved for use.

prepared by freezing the plasma to -30° C within 8 hours. Plasma frozen within 24 hours of collection is also available but it contains less FVIII and FV. Transfusion of plasma is useful for the treatment of deficiencies of clotting factors F II, V, X and XI.[1] It is not recommended for the management of hemophilia A, B, Von Willebrand disease (VWD) or FVII deficiency because safer factors are available. It is generally difficult to achieve FVIII levels higher than 30 IU/dL (and F IX levels above 25 IU/dL) with FFP alone.[2]

Since FFP or fresh plasma contains all clotting factors, it can be used in an emergency for any coagulation disorder with bleeding if the specific factor is not available and in complex acquired coagulopathies with deficiencies of multiple clotting factors as in bleeding from disseminated intravascular coagulation (DIC) or liver disease.[3]

Dose

For most coagulopathies with bleeds an acceptable starting dose is 15-20 ml/kg. This will raise factor levels by approximately 20%.

Cryoprecipitate

Cryoprecipitate is prepared by slow thawing of FFP at 2° to 4°C for 10-24 hours and the precipitate is collected by centrifugation. It contains fibrinogen, fibronectin, factor VIII, factor XIII and von willebrand factor. Cryoprecipitate is used in afibrinogenemia and factor XIII deficiency if specific factor concentrates are not available. The advantage of cryoprecipitate is the smaller volume required compared to FFP. Each bag of cryoprecipitate contains 100-150 mg of fibrinogen.[1] Cryoprecipitate contains about 3-5 IU FVIII coagulant activity per ml. A bag of cryoprecipitate made from one unit of FFP (200-250ml) may contain 70-80 units of FVIII in a volume of 30-40 ml.[3] If factor VIII is not available, cryoprecipitate is preferred over FFP in bleeding hemophilia A patients.

Whenever FFP or cryoprecipitate is used, a pathogen-inactivated form is recommended.

Clotting factor concentrates (CFC)

CFC are fractionated preparations of individual clotting factors or groups of factors which are freeze-dried.

They provide convenient high doses of clotting factor for the rapid treatment of bleeds.[4]

They are produced by fractionation from plasma. The process of separating the plasma into its different components is called fractionation. Efficient viral inactivation processes have virtually eliminated most enveloped viruses including HIV and Hepatitis B and C irrespective of the type of plasma used.[5]

Purity of a factor describes the concentration of the desired ingredient relative to other ingredients in the CFC and safety refers to the removal of viral infection from the product. Highly purified factor refers to high concentration of the factor but is useless if safety is not taken care of.[4] Recombinant factors in general have very high safety profile

A comparison of plasma, cryoprecipitate and CFC is given in Table II.

The goal of factor replacement therapy is to enable a completely normal life for the person with factor deficiency. This is possible in many developed countries due to access to CFC for regular prophylaxis programs and home therapy. However this may not be feasible in developing countries due to resource constraints and logistical issues. Hence on demand therapy is usually followed for hemophilia and other rare bleeding disorder patients in most parts of our country. In India, prophylaxis program for children has been initiated in many centres recently with modified doses for resource limited setting.

Factor VIII

Factor VIII is found in order of increasing concentration in fresh whole blood, fresh frozen plasma, freshly separated plasma, dry (lyophilized) fresh plasma, cryoprecipitate, plasma-derived factor VIII concentrate (pdFVIII) and recombinant factor VIII(rFVIII). The amounts available in fresh whole blood and plasma are insufficient to control major bleeds or to cover surgery.[4]

Hemostatic level of FVIII is >30-40%. The lower limit in normal individuals is approximately 50%.

Both plasma derived and recombinant FVIII are available for hemophilia A patients.The choice of using either plasma derived or recombinant factor is decided by the availability of the product and cost. The differences are given in Table III.

There are 2 types of factor replacement therapy for hemophilia.

1. On demand therapy refers to administering the factors following a bleed.
2. Prophylaxis refers to replacing the deficient factors in severe hemophiliacs to prevent bleeding episodes.

In general, one unit of Factor VIII /kg increases the level by 2 units/dL. The half life of factor VIII is 8-12 hours. Hence the drug should be given every 12 hours.

The dose is calculated as follows - Weight in Kg x % desired rise x 0.5.

Table II. Comparison of plasma, cryoprecipitate and CFC

	FFP	Cryoprecipitate	Clotting factor concentrate
Available factors	All factors	I, VIII, VWF, FXIII	Only the specific factor/s
Viral inactivation	No	No	Yes
Transfusion transmitted infection	Yes	Yes	Very minimal risk
Dose	Exact dosing not possible	Exact dosing not possible	Exact dosing possible
Volume to be infused	Very high	High	Less
ABO compatibility/ cross matching	Essential	Essential	Not essential
Preparation	Thawing required	Thawing required	Available as lyophilised powder. Can be easily reconstituted.
Transfusion associated lung injury	Yes	Yes	No

Table III. Differences between plasma derived and recombinant factor VIII

Plasma derived factor VIII	Recombinant factor VIII
Derived from plasma pool	Genetically engineered
Theoretical risk of TTI more especially prion* disease. Viral inactivation methods have eliminated the risk of enveloped viruses like HBV, HCV and HIV	Higher safety profile
Allergic reactions can be more with low purity preparations	Rare
Cheap	More expensive
Supply may be limited	No such issues
May contain variable amounts of VWF	Only factor VIII

**Prion disease: A prion is a type of protein that can trigger normal proteins in the brain to fold abnormally. Prion diseases can affect both humans and animals and are sometimes spread to humans by infected meat products. E.g. Creutzfeldt-Jakob disease (CJD).*

The calculated dose is always rounded off to the nearest vial strength to avoid wastage.

Example : A 5 year old severe hemophilia A child weighing 20 kg presents with hemarthrosis. He needs atleast 20% rise (in resource limited setting) in his factor level for 2 days.

Dose =20x20x0.5=200 units. The calculated dose is rounded off to the nearest vial strength of 250 units. He will need 250 units q12h for 2 days.

On-demand therapy

Dose for on demand therapy depends upon the site of bleed, severity of bleed, weight of the child and duration of bleed. If the treatment is started within an hour of the bleed the total duration of treatment can be reduced. For mild to moderate bleeds hemostatic levels of 35-50% activity is usually sufficient. Certain life threatening bleeds like intra cranial bleed or surgery needs almost 100% of factor levels.

Method of administration

Factor VIII comes as a lyophilized powder with the diluent and a syringe. It is infused intravenously slowly at a rate not to exceed 3 ml per minute in adults and 100 units per minute in young children or as specified in the product information leaflet.[2]

Continuous infusions can also be used for life threatening bleeds. This may lead to more stable levels and may reduce the total factor requirement. A stat dose is given to increase the levels to a desired level followed by a continuous infusion at the rate of 2-4 units/kg/hour in a syringe pump.[6] The diluent is 0.9% normal saline. The contents should be infused within 24 hours. Ideally the factor levels have to be frequently monitored and further doses are adjusted based on patient's levels.

Side effects[7]

Headache, fever, urticaria, nausea, vomiting can occur and very rarely anaphylaxis.

There is a theoretical risk of pathogen transmission and risk of thromboembolic event with each use. Lower-purity plasma derived products may have a higher incidence of allergic reactions. In such patients administering an antihistamine before infusing FVIII may help. Sometimes a higher purity product or recombinant factor may be needed.

Hypersensitivity to mouse or hamster protein, or intolerance or allergic reaction to any components is a contraindication for rFVIII.

Factor IX

Factor IX is present in fresh whole blood, freshly separated plasma, fresh frozen plasma, dry (lyophilized) fresh plasma, cryoprecipitate-poor plasma, plasma derived factor IX (pdFIX) concentrates, recombinant factor IX(rFIX) concentrates and in prothrombin complex concentrates (PCC).

Hemostatic level of FIX is >25-35%. The lower limit in normal individuals is approximately 50%.

For hemophilia B, the product of choice is pdFIX or rFIX.

One unit of Factor IX increases the level by 1unit/dL. It is important to remember that recombinant factor IX will increase the level by only 0.8 international units/dL in adults and 0.7 international units/dL in children as they have a low recovery.[6]

The half life of factor IX is 18-24 hours. So it is given once a day.

The dose is calculated as follows:

Weight in Kg x % desired rise x 1.4 for rFIX.

For pdFIX Weight in Kg x % desired rise.

FIX concentrates should be infused by slow intravenous injection at a rate not to exceed a volume of 3 ml per minute in adults and 100 units per minute in young children, or as recommended in the product information leaflet.

Allergic reactions are reported with factor IX infusions. These reactions are often seen in patients who have developed inhibitors. If mild it can be used under cover of hydrocortisone. Rarely anaphylaxis can occur. In such patients bleeding is managed with factor VII.

The World federation of hemophilia recommendations for desired factor level and duration in various bleeds for resource constrained and no resource constraint nations is given in Table IV.[2,8]

Prophylaxis for hemophilia

The standard of care for hemophilia patients in developed countries is primary prophylaxis. The idea of prophylaxis is based on the observation that moderate hemophiliacs (factor levels between 1 and 5%) do not bleed spontaneously like severe hemophiliacs (levels <1%). Prophylaxis is aimed at continuous replacement of factors to maintain the levels >1% in severe hemophiliacs to prevent spontaneous joint bleeds and joint disease.

Table IV. World federation of hemophilia recommendations for desidered factor level and duration in various bleeds

Site of bleed	Hemophilia A		Hemophilia B	
	No resource constraints	Resource constrained	No resource constraints	Resource constrained
Joints	40-60% for 2 days	10-20% for 2 days	40-60% for 2 days	10-20% for 2 days
Superficial muscles	40-60% for 2-3 days	10-20% for 2-3 days	40-60% for 2-3 days	10-20% for 2-3 days
Iliopsoas/deep muscles; neurovascular compromise; significant blood loss	80-100% (days 1-2), 30-60% for days 3-5 or longer	20-40% initially, then 10-20% for days 3-5 or longer	60-80% (days 1-2), 30-60% for days 3-5 or longer	15-30% initially, then 10-20% for days 3-5 or longer
Brain, spine, head	80-100% (days 1-7); 50% (days 8-21)	50-80% (day 1-3); 30-50% (day 4-7); 20-40% (day 8-14)	60-80% (day 1-7); 30% (days 8-21)	50-80% (day 1-3); 30-50% (day 4-7); 20-40% (day 8-14)
Throat and neck	80-100% (days 1-7); 50% (days 8-14)	30-50% (day 1-3); 10-20% (day 4-7)	60-80% (days - 7); 30% (days - 14)	30-50% (day 1-3); 10-20% (day 4-7)
Gastrointestinal	80-100% initially, then 50% (total 7-14 days)	30-50% (day 1-3); 10-20% (day 4-7)	60-80% initially, then 30% (total 7-14 days)	30-50% (day 1-3); 10-20% (day 4-7)
Renal	50% for 3-5 days	20-40% for 3-5 days	40% for 3-5 days	20-40% for 3-5 days
Deep laceration	50% for 5-7 days	20-40% for 5-7 days	40% for 5-7 days	20-40% for 5-7 days
Major surgery	80-100% pre-operatively; 60-80% (post-op day 1-3); 40-60% (day 4-6); 30-50% (day 7-14)	60-80% pre-operatively; 30-40% (post-op day 1-3); 20-30% (day 4-6); 10-20% (day 7-14)	60-80% pre-operatively; 40-60% (post-op day 1-3); 30-50% (day 4-6); 20-40% (day 7-14)	50-70% pre-operatively; 30-40% (post-op day 1-3); 20-30% (day 4-6); 10-20% (day 7-14)
Minor surgery	50-80% pre-operatively; 30-80% 1-5 post-op days (as required)	40-80% pre-operatively; 20-50% 1-5 post-op days (as required)	50-80% pre-operatively; 30-80% 1-5 post-op days (as required)	40-80% pre-operatively; 20-50% 1-5 post-op days (as required)

Continuous replacement is defined as replacement of factor for atleast >85% of the year.

Primary prophylaxis - Starting prophylaxis before second episode of joint bleed.[8]

Secondary prophylaxis-Starting prophylaxis after 2 or more joint bleeds but before development of joint disease.

Tertiary prophylaxis-Starting prophylaxis after development of joint disease.

Doses for prophylactic replacement of factor concentrates vary between different countries and also among centres in the same country. Commonly used dosage for prophylactic factor replacement is 25-40 IU/kg 3 times weekly for FVIII deficiency and 2 times a week for hemophilia B in countries with no resource constraints (Malmo protocol). In resource limited settings, prophylaxis may be initiated with lower doses of 10-20 IU/kg two times per week for hemophilia A and 25-40 IU/kg once a week for hemophilia B.[9]

Inhibitors

The most important complication of factor replacement therapy (FRT) is the development of alloantibodies or inhibitors to the factor infused. The incidence of inhibitors in hemophilia A can be as high as 25-40% within the first 50 exposure days and about 10% in hemophilia B.[10] Once inhibitors develop, bleeding becomes more difficult to treat resulting in increase in morbidity and mortality. The cost of treatment also increases exponentially.

Presence of inhibitor is confirmed by Nijmegen modified Bethesda assay. Patients with inhibitors can be low responders (titre<5 Bethesda Unit/L) or high responders(>5 Bethesda Unit/L). Bleeding in low responders can be managed with high dose of FVIII. Bleeding in high responders need treatment with bypassing agents FEIBA (FVIII inhibitor bypassing activity) or recombinant FVIIa (rFVIIa).

These factor products bypass the inhibitor of FVIII or FIX by providing an activated factor downstream in the clotting cascade, restoring hemostatic function. They have a higher thrombotic risk and are more expensive. They are usually used for on demand therapy. FEIBA is believed to act due to the presence of prothrombin and Xa.[3]

Table V shows factor concentrate products used in patients with inhibitors.

Other treatment for inhibitors

Immune tolerance induction (ITI) is used to eradicate high titre FVIII inhibitors. ITI involves regular infusion of high dose FVIII to induce FVIII antigen-specific tolerance.[11] Before ITI therapy, high-responding patients should avoid FVIII products to allow inhibitor titres to fall and to avoid persistent anamnestic rise. Immunosuppressants and rituximab have also been tried. Emicizumab, a bispecific antibody, a novel therapy, is found to be very useful in these patients. It can be administered subcutaneously once a week.

Extended half-life recombinant factor therapy

The currently available standard half life factors have to be given 2-3 times a week for FVIII prophylaxis and twice a week for hemophilia B. Reducing the frequency of injections or changing the mode of delivery subcutaneous instead of intravenous route are likely to improve the compliance and there by outcomes. In the past 5 years, new recombinant products with extended half-lives have been approved. Fusion with protein conjugates especially IgG or albumin which have long half life, chemical modification by PEGylation, protein sequence modification and expression in human cell lines are some of the methods used to extend half-life of factors.[10] The half-life extension of extended half life-rFVIII products is in the range of 1.4 to 1.6 fold and extension of rFIX half-life 3.8 fold (ranging from 2.4 to 4.8 fold).[12]

Table V. Factor concentrate products used in patients with inhibitors[6]

Product	Source	Factor	Initial dose
Novoseven	Recombinant	VIIa	90mcg/kg every 2-3 hours
FEIBA or anti inhibitor coagulant complex	Plasma derived aPCC	II, IX, X,VIIa	50-100units/kg q 6-12hours. Not to exceed 200units/kg/day
Obizur	Recombinant porcine	F VIII	200 units/kg; then titrate on the basis of FVIII concentrations • Usual interval = every 4-12 hours

Subcutaneous recombinant FVIII

SubQ-8is a recombinant FVIII currently under development, derived from a human cell line for subcutaneous administration. A human cell line derived rFVIII product is fused with a portion of low molecular weight vWF protein.[13]

von Willebrand factor (vWF)

vWF is essential for platelet adhesion in primary hemostasis. It is also a carrier protein of factor VIII protecting it from degradation in plasma.

Following injury to a vessel vWF first binds to exposed collagen in the sub endothelial matrix. This is dependent on the high molecular weight vWF (HMW vWF) multimers which have binding sites for collagen, platelet glycoproteins GPIba and GPIIb/IIIa and for FVIII.

Von Willebrand disease (VWD), the most common inherited bleeding disorder can be caused by quantitative (VWD- type I and III) or qualitative defects in vWF (VWD-type II). The disease usually presents as mucosal bleeds. Menorrhagia is a common problem in women.

In VWD the FVIII level is usually normal except in type 3 where the FVIII level is often <10IU/dL. Some type I and IIN patients may also have moderate deficiency of FVIII:CO. In view of the deficient or defective vWF the stability of FVIII is lost and it is cleared faster resulting in very much reduced half-life. For satisfactory hemostasis the ristocetin cofactor activity (VWF:RCo) which is a measure of VWF activity should be more than 0.6 IU/ml (60% of normal).[3]

Source

FFP, cryoprecipitate, plasma-derived FVIII/vWF concentrates, VWF-only concentrate (plasma derived and recombinant).

Indications for Vwf therapy

For bleeding in Type III VWD, Type II VWD and severe type I VWD not responding to desmopressin and type I C (clearance defects). The concentrate has also been used for prophylaxis before surgery.

Plasma-derived FVIII/vWF concentrates

Traditionally VWD patients are managed with both FVIII and vWF. FVIII and vWF are both present in most plasma-derived FVIII/vWF concentrates used in clinical practice.[14] This is especially true for the intermediate purity pdFVIII concentrates. These products are approved for use in both haemophilia and VWD. The use of these products are a boon in the management of VWD; however if repeated doses are given the FVIII activity may reach very high levels due to the cumulative effect of both endogenously present FVIII and exogenous FVIII. This may lead to increased risk of thrombosis. Hence frequent monitoring of FVIII levels is essential especially when used for surgical prophylaxis and it is prudent to keep the FVIII: C<150U/dL.[15]

Plasma-derived concentrates containing vWF are contraindicated in the rare patients with type 3 VWD who develop alloantibodies against VWF, because they often cause life-threatening anaphylactic reactions.[16]

vWF-only concentrate

Clinical studies have shown that vWF-only products correct not only the primary vWF deciency but also the secondary FVIII:Cdeciency; the exogenous replacement of the primarily deficient vWF stabilizes the endogenous FVIII in plasma. The risk of thrombosis is less when compared with plasma-derived FVIII/vWF concentrates.

Currently one plasma derived human vWF (wilfactin), a high purity vWF concentrate is available and widely used in Europe. Another recombinant vWF is approved for use in both Europe and USA. The latter also contains ultra large vWF multimers and HMWvWF multimers. Some patients treated with plasma derived human vWF (wilfactin) prior to surgery required a priming dose of a FVIII-containing product to maintain surgical hemostasis.[17] This may be true for recombinant vWF also.

Phase 3 clinical studies for recombinant vWFare ongoing in children.

When using a product that contains both FVIII and vWF, dosing in VWD should be based on the standardized vWF/RCo available in the product and not the FVIII. It is imperative for the clinician to know the ratio of vWF:RCo, FVIII concentration and high molecular weight VWF multimers in the product used, as different products have different ratio. The doses are shown in Table VI.

Factor VII

It is a vitamin K-dependent serine protease glycoprotein synthesised in the liver. When a vessel wall is damaged the tissue factor is exposed and it binds to circulating FVII and activates it to FVIIa. This in the presence of calcium and phospholipids converts FIX and X to FIXa and FXa. The plasma concentration of factor VII is 0.5 mg/L. It is predominantly in the inactive form.

Table VI. Dosing recommendations for von Willebrand factor concentrate replacement for prevention or management of bleeding[18]

	Major Surgery/Bleeding	Minor Surgery/Bleeding
Loading dose	40-60 vWF:RCo IU/kg	30-60 vWF:RCo IU/kg
Maintenance dose*	20-40 U/kg every 8-24 hours	20-40 U/kg every 12-48 hours
Therapeutic goal	Trough vWF:RCO and factor VIII >50 IU/dL for 7-14 days	Trough vWF:RCo and factor VIII >50IU/ dL for 3-5 days

**Can alternate maintenance factor replacement with desmopressin(DDAVP) for later part of treatment.*

Adapted from - Manco-Johnson MJ, Montgomery RR, Ortel TL, et al. von Willebrand disease (VWD): evidence-based diagnosis and management guidelines, the National Heart, Lung and Blood Institute (NHLBI) Expert Panel report (USA). Hemophilia 2008;14(2):171-232

About 1% circulates in the active form (factor VIIa). Its half life is only 3-5 hours. The reference range for factor VII is 65-140% of normal.[19] Newborns may have lower levels ≥25% and reach adult levels around 6 months.

Source of factor VII

FFP, plasma derived FVII(pdFVII), rFVIIa and prothrombin complex concentrate. rFVIIa is most commonly used for treatment, if available.

Indications for factor VII therapy

1. For bleeding in hemophilia A and B patients with inhibitors.
2. For bleeding in congenital factor VII deficiency.
3. In Glanzmann's thrombasthenia refractory to platelet infusions with or without antibodies.[20]
4. For bleeding in adults with acquired hemophilia.[20]
5. To control severe obstetrical bleeding in women without hemophilia.[21]

Off-label use of FVII in children

rFVIIa has been used in children to control bleeding related to cardiac surgery, liver failure and transplantation, platelet disorders, neurosurgery and disseminated intravascular coagulation based on case reports and small clinical trials though the benefits are uncertain.[22]

For prophylaxis

rFVII is also recommended as prophylaxis for congenital factor VII deficiency in children with severe bleeding (intracranial and gastrointestinal bleed) following the first bleed. However the cost of the drug is a limiting factor.

Mechanism of action

i) In hemophilia patients with inhibitors: Factor VIIa can act both in the presence or absence of tissue factor. FVIIa-TF activates FX in the absence of the FIXa/FVIIIa complex, thus bypassing the need for FIX or FVIII.[23] Bom and Bertina demonstrated the activation of FX by FVIIa on a negatively charged phospholipid surface, independent of TF.[24]

ii) In patients with Glanzmann's thrombasthenia syndrome: Action of rFVIIa is probably due to thrombin generation on the surface of platelets, resulting in faster platelet activation and aggregation.[25]

Congenital deficiency of FVII is a rare autosomal recessive disease. Only homozygote (usually levels <20%) or compound heterozygote patients with factor VII deficiency are symptomatic. The severity of symptoms may not correlate with factor levels. Heterozygotes may have low levels ≤ 50% but generally do not bleed even following trauma. Patients with FVII deficiency with levels < 1% clinically present like severe hemophiliacs with spontaneous joint bleeds and are at risk of intra cranial bleeds. Children with levels >5% have milder symptoms and present with epistaxis, gum bleeds and menorrhagia. It is interesting to note that these patients may have bleeding following dental, urogenital procedures and tonsillectomy but they do not have bleeding following laparotomy, herniorrhaphy, appendicectomy or hysterectomy (probably due to local fibrinolysis in the former procedures).

Dose varies with etiology. Congenital factor VII deficiency requires a smaller dose when compared to hemophiliacs with inhibitors.

For hemophiliacs with inhibitors FVII is given in a dose of 90mcg/kg every 2-3 hours.[6]

A single bolus of 270 μg/kg dose of rFVIIa was found to be at least as effective and well tolerated as standard 90 μg/kg × 3 dosing.[26]

A continuous infusion of rFVIIa as a replacement therapy prior to surgery and bleeding episodes has also been tried.[20]

Replacement therapy for congenital deficiency of FVII

Levels of more than 10% are usually hemostatic, although higher levels may be advisable in the event of a severe bleeding episode. Maintaining factor VII levels of at least 15-25% provides adequate hemostasis levels for most surgical procedures unlike in hemophilia A and B where 100% is needed.[27]

One IU of pd-FVII increases plasma level by 1.9%. The average pd-FVII dosages used are 15-20 IU/kg for mucosal bleeding and 30-40 IU/kg in severe or life-threatening hemorrhages.[28]

The recommended dose of rFVIIa for treatment of hemorrhage is 15-30 mcg/kg every 4-6 hr until hemostasis is achieved. For perioperative replacement therapy, a dose of approximately 20 mcg/kg has proved effective in over 95% of cases; the infusion should be repeated approximately 8 times in patients at high risk (ie, those with a history of major bleeding).[29] Most bleeding episodes can be successfully treated with one day replacement therapy with daily mean rFVIIa dose of 30- 60μg/kg.

Adverse effects

Headache, hematoma or discomfort at the site of infusion, fever can occur in 1-10% patients.

Serious arterial and venous thrombotic and thromboembolic events have been reported. It is more in adults. Risk is increased if used along with PCC/aPCC or factor XIII, history of atherosclerotic disease, coronary artery disease, cerebrovascular disease, crush injury, septicemia or thromboembolic event.

rFVIIa is contraindicated in patients with known hypersensitivity to mouse, hamster or bovine proteins.

Inhibitors to exogenous factor VIIa though rare is reported.[30]

It is mandatory to read the product information before administering the drug.

Note: rFVII a requires a fibrinogen level of ≥1 g/L as a pre-condition for optimal haemostatic activity.[31]

Fibrinogen

Fibrinogen, factor I, an important component of coagulation cascade, is synthesised in the liver. Platelet alpha granules also contain fibrinogen. It binds with the platelet fibrinogen receptor glycoprotein GPIIb/IIIa and activates the platelets and induces platelet aggregation. Fibrinogen is also part of secondary hemostasis where thrombin acts on fibrinogen and converts it into fibrin. Normal fibrinogen level is 150-450mg/dL. The hemostatic level of fibrinogen is >60mg/dL.[1] The plasma half-life of fibrinogen is 2-4 days.

Indications

1. Replacement therapy for bleeding in patients with congenital deficiency of fibrinogen.
2. Secondary prophylaxis in patients with congenital deficiency of fibrinogen following life threatening bleeds like intra cranial bleed.
3. Acquired hypofibrinogenemia (loss or dilution coagulopathy, trauma, cardiac and thoracic surgery, following L-aspasraginase therapy, acute promyelocytic leukemia).

Source

FFP, cryoprecipitate, plasma derived fibrinogen concentrates.

Congenital fibrinogen disorders are rare. They include afibrinogenemia, hypofibrinogenemia (<150mg/dL) and dysfibrinogenemia which is a qualitative defect. The affected persons may be asymptomatic, can present with bleeding, thrombosis or both.

Afibrinogenemia patients usually manifest in neonatal period with prolonged umbilical bleed. They can have spontaneous bleeding in all tissues. They are also prone for arterial and venous thrombosis in large vessels. Pregnant women can present with abruptio placentae, postpartum haemorrhage and early fetal loss. They have a predilection for spontaneous splenic rupture, painful bone cysts and poor wound healing.[28] Hypofibrinogenemia patients have milder symptoms. They usually bleed following trauma. Major bleeding can occur in severe hypofibrinogenemia (fibrinogen< 50mg/dL).

Fibrinogen replacement therapy

Plasma derived fibrinogen concentrate is preferred over cryoprecipitate and FFP. There are 4 plasma derived fibrinogen concentrate products available. The goal of therapy is to maintain fibrinogen level >100mg/dL until hemostasis is achieved.

Dose

Pharmacokinetic studies in patients with afibrinogenemia have shown that a single dose of 60 to 70 mg/kg results in a fibrinogen activity level of 1.3 to 1.45 g/L within 40 minutes to 1 hour and that the half-life is around 80 hours.[32]

For prophylaxis, the usual regimen is 20 to 30 mg/kg of fibrinogen concentrate every 10 to 14 days, increasing the dosing frequency to every 7 days if necessary to maintain a level greater than 0.5 g/L.[33]

There is also a formula for calculating the dose of fibrinogen.[34]

Dose (g) = desired increment (g/L) × plasma volume (0.07) × (1- hematocrit) × weight (kg).

Cryoprecipitate is used if fibrinogen concentrate is not available. Infusion of 1 unit of cryoprecipitate per 5 kg of patient's weight followed by 1 unit/15 kg to maintain hemostasis is recommended.[35]

Adverse effects: Risk of thrombosis, inhibitor development, allergic or hypersensitivity reactions including urticarial rash and anaphylaxis though rare have been reported following administration of fibrinogen concentrate.

Factor XIII

Factor XIII is a protransglutaminase which is essential for the last step in coagulation to form a stable clot. It is a tetramer with 2 catalytic Asubunits and 2 carrier B subunits (A2B2**)**. FXIIIA subunit is synthesized by megakaryocytes, monocytes and macrophages and FXIIIB subunit is synthesized by hepatocytes. The half life of FXIII is 9-14 days.[36] The hemostatic level is 3-10%.[37]

FXIII is converted into an active transglutaminase (FXIIIa) by thrombin and Ca (2+). FXIIIa converts loose fibrin polymers into an organized structure by cross linking of the fibrin clot.

In addition to its role in coagulation it plays a role in wound healing**,** osteoblast matrix secretion and deposition, maintaining pregnancy, cardioprotection, vascular remodelling and angiogenesis.

FXIII deficiency can be inherited (very rare) or acquired secondary to immune mediated disorders like SLE or non-immune mediated due to decreased synthesis or increased consumption. Acquired FXIII deficiency is more common in adults.

Congenital FXIII deficiency is classified as FXIII-A(more common) and FXIII-B deficiency. FXIII-A can further be sub-classified as type 1(quantitative) or type 2 defects (qualitative). These children often present with delayed cord separation and prolonged bleeding from umbilical cord stump (80%). Intracranial bleed (25-30%) is common and often the cause of death. Delayed bleeding after trauma or surgery (12-36 hrs later) and poor wound healing are characteristic of the disease. Repeated miscarriages in pregnancy is another feature of the disease. Skin, mucous membrane, soft tissue and joint bleedings can also occur.

Source

FFP, cryoprecipitate (3IU/mL), plasma derived FXIII (pdFXIII) and recombinant FXIII(rFXIII) concentrate.

In most countries cryoprecipitate is only used for these patients. 1 unit of cryoprecipitate per 5kg patient weight will provide 10 U/kg of factor XIII. Number of bags = 0.2 x weight (kg).[38]

rFXIII contains only FXIII A2; hence it is only indicated for patients with FXIIIA subunit deficiency. Its use in acquired FXIII deficiency has not been studied.

FXIII replacement therapy

Indications

1. Bleeding in congenital FXIII deficiency (on demand).
2. Prophylaxis for those with severe FXIII deficiency to prevent life threatening bleeds like intra cranial bleed.
3. Acquired FXIII deficiency (may need high dose and immunosuppressive therapy in addition).
4. Prophylaxis throughout pregnancy to prevent miscarriages.

Dose[37,39,40,41]

1 U/kg body weight of factor XIII leads to an increase in plasma activity by 1-2%.

Dose for prophylaxis is given in TableVII.

Table VII. Dose of prophylactic therapy

FFP	10 ml/kg every 4–6 weeks
Cryoprecipitate	one bag/10 kg every 4-6 weeks
pd factor XIII	10-20 U/kg every 4-6 weeks
rFXIII A	35 IU/kg monthly

Table VIII. Clinical manifestations and treatment of prothrombin, FV, FX, FXI deficiency[43]

Deficient factor	Hemostatic level	Half life	Main clinical symptoms	Concentrate available	Treatment on demand	Dose	Long term prophylaxis # dose
II	20-30%	3-4 days	Umbilical cord, joint and mucosal bleeding	PCC	PCC FFP	20-40IU/Kg 15-20ml/Kg	PCC-20-40IU/Kg once a week
V*	15-20%	36 hours	Mucosal bleeding-epistaxis, menorrhagia, oral cavity bleeds and post operative bleeding	Under development	FFP	15-25mL/Kg	FFP 20-30ml/Kg twice a week
X	15-20%	40-60 hours	Umbilical cord, joint and muscle bleeding, gastro intestinal and intracranial bleeding	PCC, pd FX	pd FX PCC FFP	20-30IU/Kg 20-30IU/Kg 10-20mL/Kg	pd FX 20-40IU/Kg twice a week
XI	15-20%	40-70 hours	Post surgical and traumatic bleeding	Plasma derived concentrate	FFP pdFXI	15-20mL/Kg 15-20IU/Kg	

PCC-prothrombin complex concentrate; pd-plasma derived

 # Prophylaxis may be considered in individuals with recurrent serious bleeding and especially after life-threatening bleeding episodes.

**Platelet transfusions also have been used because FV is stored in the alpha granules of platelets in a preactivated form which has a greater procoagulant activity than plasma FV and is released locally at the site of vascular injury but there is a risk of allo immunization.[44]*

FV and VIII deficiency can rarely co-exist for which FFP and FVIII have to be administered.

For on-demand treatment in emergencies 25-35 IU/Kg of either plasma derived or recombinant FXIII concentrate will be sufficient to achieve hemostasis.[2]

In patients with factor XIII deficiency and intra cranial hemorrhage, FXIII level should remain in the normal range for a minimum of 2 weeks by regular replacement therapy. The initial dose is 30IU/kg for 4 days followed by 10IU/Kg for 10 days followed by routine prophylaxis regimen may be required.[42]

For major surgery dose of 20-30 U/kg/day is used to maintain a plasma concentration of higher than 5%. Replacement therapy should ideally be administered immediately prior to surgery and should be continued until complete recovery is made. A dose of 10-20 U/kg/day for 2-3 days should be sufficient for minor surgeries.

If FXIII concentrate or plasma is not available in bleeding emergencies, platelet transfusion can be used as an alternative as FXIII is contained in platelets.[37]

Adverse effects

Though rare, hypersensitivity reactions,anaphylaxis, thrombosis and inhibitor development are reported.

Factor replacement therapy for FII, FV, FX, FXI deficiency

FII, FV, FX, FXI deficiency are very rare bleeding disorders.The clinical manifestations, treatment of prothrombin, FV, FX, FXI deficiency, half life and hemostatic level of the factors are summarised in Table VIII.

Table IX. Commonly used CFC in India

CFC	Trade name	Company	Available strength in units	MRP in rupees
FVIII	HemoRel	Reliance	250 500	3588 8732
Third generation rFVIII (MoroctocogAlfa)	Xynthophilia	Pfizer	250 500 1000	11250 22500 35000
rFVIIITuroctogog Alfa	Novoeight	Novonordisk	250 500 1000 1500	3675 (Government rates) 7500 15000 19167
rFVII	Novoseven	Novonordisk	1mg	45750
Plasma derived FVIII	Hemofil-M NF	Takeda	250 500 1000	3489 7116 13556
rFVIIIOctacog alfa	Advate	Takeda	250 500 1000 1500	10710 20790 41580 59400
First generation rFVIII	Recombinate (RAHFVIII)	Takeda	250IU 500IU 1000IU	8925 37800 30975
Third generation rFIX	Rixubis	Takeda	250 500 1000	18900 37800 72000
Plasma derived FVIII&vWF	Immunate	Takeda	250 FVIII &190vWF	3489
Plasma derived FIX	Immunine	Takeda	600	11739
Anti-inhibitor coagulant complex	FEIBA STIM4	Takeda	500	36300

Single factor concentrates for fibrinogen, X, XI and XIII are currently not available in India.

Prothrombin complex concentrates (PCC) are highly purified plasma derived concentrates which may contain FII, X,IX (3 factor PCC) and VII (4 factor PCC).[45] They are safer than FFP as they are virally inactivated. The potency of most PCC is expressed as FIX activity but the other factors are present in varying concentrations in the different products available. It is important to read the product information before using the concentrate. Tranexamic acid should not be used with PCC.

As a rule, 1 U of PCC/kg body weight increases the activity of factors VII and IX by 0.5-1% and the activity of factors II and X by 1-2%.[31]

Indications

1. For prevention and treatment of bleeding in prothrombin deficiency.
2. For prevention and treatment of bleeding in FX and FVII deficiency if the specific factor concentrates are not available.
3. Bleeding or for surgical coverage in vitaminK-dependent coagulation factors deficiency (VKCFD) in addition to maintenance dose of Vit.K.
4. For anti coagulation reversal in case of severe bleeding (coumarin).

5. PCC has been used instead of FFP in dilutional coagulopathy from massive transfusion, bleeding after cardio-pulmonary bypass surgery and the coagulopathy of acute fulminant and chronic liver failure.[3]

Repeated use of PCCs may predispose to thrombosis especially in patients requiring prophylaxis. To prevent this, most PCCs include one or more coagulation inhibitors such as heparin, antithrombin. They also contain proteins C, S and Z.[46]

Activated prothrombin complex concentrate (aPCC) contains activated FVII and inactive form of FII, V and X(FEIBA).[6] This is used in hemophilia patients with inhibitors.

Table IX shows commonly used CFC which are available in India with the rates as on April 2020.

Points to Remember

- ***Clotting factor concentrates are available for almost all factor deficiencies except FV and they are the drug of choice for congenital factor deficiencies.***
- ***FFP contains all coagulation factors and hence can be used in a coagulopathic child with bleeds if specific factor concentrates are not available.***
- ***Cryoprecipitate contains fibrinogen, FVIII, FXIII and von Willebrand factor and can be used in deficiencies if specific factor is not available.***
- ***Recombinant FVIIa and activated prothrombin complex concentrate (aPCC) are useful in arresting bleeding in hemophilia children with inhibitors.***
- ***Prophylaxis using continuous factor replacement is recommended as the standard of care in haemophilia patients.***
- ***Tranexamic acid should be avoided in patients receiving prothrombin complex concentrates (PCC).***

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HEMATO-ONCOLOGY

HEMATOPOIETIC STEM CELL TRANSPLANTATION - WHERE WE ARE AND THE WAY FORWARD

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Abstract: *Hematopoietic stem cell transplantation is potentially curative in several stem cell disorders. The process involves HLA typing, donor selection, conditioning, harvesting stem cells , infusion , supportive care, engraftment and immunosuppression to prevent graft versus host disease and graft rejection. A team of experienced pediatric intensivists, dedicated nurses, antibiotic stewardship and infection control measures are essential components for providing optimal care. With advances in molecular diagnosis and whole-exome sequencing, the indications for hematopoietic stem cell transplantation-are expanding and several hitherto unrecognized life-threatening conditions have a potential for cure. Pediatricians are the key personnel to maintain the shared care and follow up for late effects, thus ensuring intact and quality survival.*

Keywords: *HSCT, Children, Survival, Cure.*

Hematopoietic stem cell transplantation (HSCT) is a potentially life-saving procedure for several stem cell disorders. HSCT involves the replacement of the entire hematopoietic system, which is defective or dysfunctional, with healthy stem cells. During the development of the embryo, totipotential stem cells multiply to form the various organs in the body. In contrast, the pluripotential stem cells divide to form specific systems, namely the hematopoietic system. The bone marrow milieu could be considered to be similar to the concept of a seed and the soil, the seed being the stem cells which divide to form the red blood cells, white blood cells, platelets and the ground being the mesenchymal marrow tissue. HSCT is a technique to replace the stem cells in the above milieu.

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Types of HSCT

There are two types of transplantations - autologous and allogenic HSCT. Autologous involves the use of the patient's own stem cells and allogeneic consists of the use of a healthy donor's stem cells.

a) Autologous stem cell transplantation (ASCT)

ASCT is indicated in refractory or relapsed solid tumors, commonly Hodgkin lymphoma and neuroblastoma. Here high dose chemotherapy is delivered to achieve complete tumor kill. This is followed by myelosuppression and reinfusion of the patient's own stem cells, which were collected prior to chemotherapy for rescuing the marrow.

b) Allogenic stem cell transplantation

All the discussions in this article are about allogenic SCT.

Indications of allogenic stem cell transplantation

Table I outlines the current standard indications for allogenic HSCT.

Steps in allogenic stem cell transplant

The seven steps in allogenic stem cell transplantation are given in Box 1.

Box 1. Steps in allogenic stem cell transplantation

- Right patient
- Right donor - HLA typing
- Conditioning
- Stem cell infusion
- Supportive care
- Immunosuppression - Balance between graft rejection and graft versus host disease
- Long term follow up

Table I. Common conditions where HSCT is potentially curative

Disorders of different cell lines and systems	Conditions
Red cell disorders	Thalassemia major Sickle cell anemia Pure red cell aplasia
White cell disorders	Relapsed/refractory/high risk leukemia Relapsed/refractory Hodgkin lymphoma Stage IV neuroblastoma Rarely in high risk medulloblastoma, relapsed/metastatic retinoblastoma, relapsed germ cell tumours Juvenile myelomonocytic leukemia Chronic myeloid leukemia in blast crisis Primary immune deficiency disorders including disorders of immune dysregulation
Platelet disorders	Glanzmann thrombasthenia
Stem cell disorders	Acquired aplastic anemia Inherited bone marrow failure syndromes including Fanconi anaemia, Schwachman Diamond syndrome Myelodysplastic syndromes
Metabolic disorders	Mucopolysaccharidosis Lysosomal storage disorders – Gaucher's disease X linked adrenoleukodystrophy Metachromatic leukodystrophy Krabbe disease
Osteoclast disorders	Osteopetrosis

Patient and donor selection

Disease status of the patient

It is imperative to note the patient's disease status before HSCT, which varies based on the underlying condition. Prerequisite in malignancies is that the patient should be in remission with no minimal residual disease (MRD) before HSCT.[1] Patients, therefore, need to be given adequate salvage chemotherapy to eliminate the leukemic stem cells as best as possible. Similarly, in diseases such as hemophagocytic lymphohistiocytosis and juvenile myelomonocytic leukemia (JMML), it is essential to achieve adequate disease control for a favorable outcome. Patients with thalassemia major need to be transplanted after optimizing transfusion and chelation. Pesaro classes are defined based upon the ferritin levels, hepatomegaly and liver iron content to classify patients into Class 1, 2 and 3. (Pesaro classes - It is a scoring system developed by Pesaro group in late eighties to predict the outcome of allogeneic hematopoietic stem cell transplantation in thalassemia). Several studies have demonstrated poor outcomes in class 3 thalassemia patients as compared to those taken up after optimal transfusion and chelation.[2]

Certain conditions require patients to be transplanted as soon as possible without delay. There may sometimes be challenges to achieve stabilization, namely primary immune deficiencies and severe aplastic anemia, including Fanconi anemia. In particular, children diagnosed to have severe combined immune deficiency need to be treated as an emergency. Control of their infection may not always be possible without providing the required immune system. In aplastic anemia, published literature has proven that children taken up for HSCT after having received more than 15 to 20 transfusions have a higher risk of rejection due to alloimmunization.[3]

Donor selection

The process of allogeneic transplantation begins with finding a compatible donor through human leukocyte antigen (HLA) typing located on chromosome 6. There are three HLA class I antigens namely A, B and C, and three class II antigens namely DP, DQ and DR. Full HLA match entails complete match between A, B, C, DRB1 and DQB1 with two alleles in each group, thereby requiring a 10/10 match.[4] The significance of DPB1 is evident in malignancies, whereas a DPB1 mismatch is associated with a decreased risk of relapse.[5] HLA typing involves an analysis of the patient and donor HLA from either a blood sample or a buccal swab.

The chance of finding a fully HLA matched family donor is approximately 20-30%, thereby requiring alternative donor HSCT, namely unrelated and haploidentical stem cell transplantation (half- matched donor).[6] Alternative donors are needed in 70% of the cases. There are several registries that recruit unrelated voluntary donors where their HLA typing is recorded and stored in the database. In India, Datri and Marrow Donor Registry India (MDRI) are some of the largest registries, with over 400,000 donors registered with Datri as of 2019. Similar large and moderate scale registries exist the world over with some of the biggest being DKMS in Germany and the National Marrow Donor Program (NMDP) in the United States of America. Unrelated donor searches may sometimes fail to identify a compatible donor or may take up to 2-3 months. Haploidentical transplantation is a significant advance in this field and has opened up new doors and possibilities.

Haplo-transplants are defined as HSCT from a half match; generally, 3/6 or 5/10 matched graft from a related donor. The grafts have the advantage of speed because relatives are usually easy to contact for stem cell collection. The cost of procuring stem cells is lower than in unrelated transplants and umbilical cord blood products.

Conditioning

Donor selection is followed by conditioning, which involves the use of chemotherapy to prepare the soil in the bone marrow. Stem cells are known to arise in the form of niches. The purpose of conditioning is threefold - a) to eliminate the patient's stem cells, b) to create space for the new donor-derived stem cells and c) to provide adequate immune suppression in the host. Conditioning not only helps to reduce the risk of graft rejection and graft versus host disease (GVHD) but also eradicate residual leukemic stem cell clones in malignancies. The underlying condition determines the combination of chemotherapy used in conditioning. In general, the host's underlying immune status determines if myeloablation or immune suppression or both are required.[7]

Myeloablative conditioning with high doses of chemotherapy or radiotherapy is required for conditions with a robust yet dysfunctional marrow, for example, acute lymphoblastic leukemia, thalassemia major, JMML and osteopetrosis. Reduced-intensity conditioning (RIC) includes a lower dose of chemotherapy, whereby immune suppression would be higher than myeloablation. RIC is used in conditions where the host has an already compromised immune system, namely in aplastic anemia or severe combined immune deficiency. Conditioning regimens include a combination of cyclophosphamide, busulphan, treosulphan, melphalan, thiotepa, fludarabine and total body irradiation.[8]

Sources of stem cells, stem cell infusion and engraftment

Stem cells can be harvested from the peripheral blood (Peripheral blood stem cell-PBSC), bone marrow, or cord blood. There are several advantages and disadvantages in each and their indication depends on the underlying disease. Engraftment is the earliest with PBSCs (10-14 days), followed by bone marrow (14-21 days) and cord blood (21-28 days) as a result of the varying amounts of CD34 stem cells in each with PBSCs having the highest. Engraftment is a process in which transplanted stem cells travel through the blood to the bone marrow, where they begin to make new white blood cells, red blood cells, and platelets.

Graft versus host disease (GVHD) and immune suppression

Post-infusion, patients need to be maintained on immune suppression to prevent GVHD and graft rejection. Drugs used for GVHD prophylaxis include tacrolimus, cyclosporine, methotrexate, anti-thymocyte globulin, alemtuzumab and mycophenolate mofetil. GVHD affects mainly the skin, gut, and liver and steroids are used as the first-line agents for treatment. In the case of steroid-refractory GVHD, second-line agents used include etanercept, tocilizumab, ruxolitinib, rituximab, basiliximab and extracorporeal photopheresis.[9]

Supportive care and the problem of drug-resistant infections

Optimal outcomes post-HSCT is highly dependent on the supportive care provided, particularly with the rise of

gram-negative bacterial sepsis.[10, 11] One to one nursing is the key with nurses being the pillars. High-efficiency particulate absorbent filters are recommended so as to maintain positive pressure within the rooms. Patients need to be on antifungal and antiviral prophylaxis and appropriate antibiotics need to be started when needed. The hospital infection control and antibiotic stewardship are essential components of care. Precautions taken to prevent infections include neutropenic care and neutropenic diet. The neutropenic diet is an eating plan for people with weakened immune systems. It involves choosing foods and preparing them in a way that lowers the risk of infection by preventing bacterial translocation of gut microbes, which limits the exposure to harmful microbes and bacteria. Alternate name is low-microbial diet.

Post HSCT

In thalassemia major and PIDs, overall survival of over 90% and 70% can be achieved with intact survival. In malignant disorders, overall survival is usually in the range of 60% to 70% depending upon the underlying condition, remission status prior to HSCT and graft versus leukemia effect post HSCT. Over 90% of the children can resume school in their full capacity approximately 6 to 9 months post HSCT.

The current scenario in India

The first HSCT in India was performed in 1983 and the field has grown by leaps and bounds since then. The Indian stem cell transplant registry (ISCTR), now renamed as the Indian Society of Blood and Marrow Transplantation (ISBMT), was established in 2004 with a handful of transplant centers and had over 65 transplant centers reporting data as on 2019.[12] Pediatric HSCTs have similarly grown in terms of facilities, technology available and access to care.[13]

Role of the pediatrician – shared care and follow up

The most advanced technology, facilities, trained and experienced teams for HSCT are now available in India. Physicians and patients need to be sensitized regarding the availability and accessibility of a curative option and the importance of early referral. The impact of shared care with pediatricians over ensuring monitoring for late effects, maintaining intact survival and improved quality of life in children post HSCT cannot be over emphasized.

Financial implications

The cost of HSCT continues to be a major challenge and public-private partnership is the way forward. Several government and non-governmental organizations now support HSCT. Tamil Nadu is one of the states in India, where the Chief minister's comprehensive health insurance scheme supports HSCT entirely, whilst other states partly fund the therapy. Similar funds, including the Prime minister's relief fund and inter-state government funds, have helped in increasing access to care. The average cost of autologous HSCT is about INR 800,000. Sibling allograft costs about INR 15,00,000 and unrelated or haploidentical about INR 30,00,000.

Our experience

At our center, over 1392 HSCTs have been performed to date, with 901 being pediatric HSCTs. The most common indications have been thalassemia major (336), leukemia (235), primary immune deficiency disorders (131) and Fanconi anemia (56). With advances in supportive care, early use of granulocyte transfusions to combat sepsis, antibiotic stewardship, experienced and trained pediatric intensivists, morbidity and mortality can be minimized, thus optimizing outcomes and providing intact survival.

Points to Remember

- ***Hematopoietic stem cell transplantation (HSCT) is potentially curative in several congenital and acquired stem cell disorders including thalassemia major, primary immune deficiency disorders, Fanconi anemia and malignancies.***
- ***HLA typing of Class I (A, B, C) and Class II (DP, DQ, DR) antigens is the key to determining the compatibility of the donor and in planning the type of HSCT namely matched related, matched unrelated, mismatched related or unrelated and haploidentical stem cell transplantation.***
- ***Although 30% of patients can find a compatible match within the family, alternative donor transplantation is an option in the remaining 70%, including unrelated and haploidentical transplants.***
- ***The source of stem cells could be peripheral blood, bone marrow or cord blood and donation of stem cells is safe for the donor.***
- ***Supportive care is the key to ensuring optimal outcomes.***
- ***Teamwork between experienced pediatric intensivists and nursing groups, antibiotic stewardship and infection control measures are the essential components of care.***
- ***Immunosuppression is only for a short duration of***

one year on average unlike solid organ transplantation where the children are on lifelong medications. However, follow up for late effects of chemotherapy utilizing shared care with pediatricians is essential for optimal outcomes.

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CLIPPINGS

Elevated international normalized ratio (INR) is associated with an increased risk of intraventricular hemorrhage in extremely preterm infants.

The international normalized ratio (INR), a standardized method of reporting the prothrombin time, can be a surrogate marker of the vitamin K-dependent coagulation pathways. The study was done to evaluate the relationship between INR measurements in the first 48 hours of life and subsequent development of intraventricular hemorrhage (IVH) in extremely preterm infants. It was concluded that an elevated INR in the first 48 hours of life may be useful to identify preterm infants at risk of severe IVH and may guide strategies to prevent the development, or limit the extension, of IVH.

Glover Williams A, Odd D Bates S , Russell G, Heep A. Elevated international normalized ratio (INR) is associated with an increased risk of intraventricular hemorrhage in extremely preterm infants. Journal of Pediatric Hematology / Oncology 2019; 41(5):355-360.

DRUG PROFILE

DRUGS IN PEDIATRIC RHEUMATOLOGY

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Abstract: *Various factors, including disease activity and severity, co-morbidities and patient preference (including cost, route of administration and frequency of monitoring) need to be factored in deciding the optimal treatment of various rheumatic diseases in children. Non-steroidal anti-inflammatory drugs and steroids may be used to provide symptomatic relief whereas the arrest of progression of disease is achieved using disease modifying drugs. Treatment goals include achievement of remission or low disease activity, and the prevention of radiographic progression of the disease.*

Keywords: *Juvenile idiopathic arthritis, Rheumatic, NSAIDs, Steroids, Disease modifying anti rheumatic drugs, Methotrexate, Biologicals, Children.*

Pediatric rheumatic diseases include a spectrum of musculoskeletal and connective tissue disorders which begin during childhood. Although they share many common symptoms such as joint pain, swelling, redness and warmth, each disorder is distinct and each has its own set of signs and symptoms. Extra-articular organ involvement includes eyes, skin, muscles and gastrointestinal tract. Pediatric rheumatic diseases include juvenile idiopathic arthritis (JIA), juvenile dermatomyositis (JDM), systemic lupus erythematosus (SLE), juvenile scleroderma, Kawasaki disease (KD), fibromyalgia, Behcet's disease, Henoch-Schonlein purpura, rheumatic fever, post-streptococcal reactive arthritis, Lyme arthritis and auto inflammatory diseases.[1]

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Juvenile idiopathic arthritisis the most common type of chronic arthritis in children under the age of 16. It can cause persistent joint pain, swelling and stiffness. Symptoms can be short lived for few months or may be lifelong. It is an autoimmune disorder causing non-suppurative proliferative synovitis resulting in articular cartilage destruction and disabling arthritis. Cytokine release due to activation of complement pathway initiated by IgM complexes is thought to be the pathology. The inflammatory cells secrete lysosomal enzymes resulting in cartilage damage and erosion of bones. The prostaglandins produced causes vasodilatation and pain.[2] Some types of juvenile idiopathic arthritis can cause serious complications such as growth problems, joint damage and eye inflammation, especially, when not treated early and adequately. Treatment goals include relief of pain, reduction of swelling and stiffness, protection of articular structures, preventing cartilage damage, maintenance of joint function and control of systemic disease.

Classification of anti-rheumatic drugs[3]

Currently available anti-rheumatic drugs include the following (Box 1).

Box 1. Classification of anti-rheumatic drugs

1. Non-steroidal anti-inflammatory agents (NSAID): Naproxen, ibuprofen, diclofenac, indomethacin, aspirin
2. Glucocorticoids
3. Disease modifying antirheumatic drugs (DMARDs)
   a) Synthetic DMARDS
      i) Conventional: methotrexate, hydroxychloroquine, sulfasalazine, leflunomide
      ii) Latest targeted drugs: JAK1 and JAK3 inhibitor such as tofacitinib
   b) Cytotoxic immunomodulatory agents: Cyclophosphamide, mycophenolate mofetil, cyclosporine, azathioprine, intravenous immunoglobulin (IV Ig)
   c) Biological DMARDS: Etanercept, Adalimumab, Infliximab, Tocilizumab, Anakinra, Rituximab

Table I. Commonly used NSAIDs[5]

NSAID	Dosage
Ibuprofen	Child 3 months-17 years: 30-40 mg/kg daily oral in 3-4 divided doses; maximum 2.4 g per day
Naproxen	Child 2-17 years: 5-10 mg/kg twice daily; maximum 1g per day
Indomethacin	1.5-3 mg/kg/day oral in 3 divided doses; maximum 150 mg per day
Etoricoxib	Child 16-17 years: 30 mg once daily orally, then increased if necessary to 60-90 mg once daily. May be considered in only in one situation, as an alternative in older children who showed reactions to multiple anti-inflammatory agents

Other NSAIDs like meloxicam are not used in children.

NSAIDs (Table I)

They are the first line drugs in the treatment of JIA and may be given while awaiting investigations. Although it provides relief from pain, swelling and morning stiffness, it has little effect on the progression of bone and cartilage destruction. Currently used NSAIDs inhibit the activity of cyclooxygenases 1 and 2 (COX-1 and COX-2, respectively). Indomethacin and diclofenac are not commonly used in children. Recent observations in clinical trials involving adults have suggested an increased risk of cardiovascular events secondary to Cox-2 inhibitors like meloxicam. Aspirin use is only restricted to rheumatic fever because of their side effects and association with Reye Syndrome. Naproxen and ibuprofen are the two commonly used NSAIDs in children. The lowest NSAID dose possible should be prescribed and the dose should be reduced and withdrawn following good response to DMARD.[4]

Corticosteroids

Steroids are potent immunosuppressant and anti-inflammatory agents which can be used at any stage of the disease either as a first line or second line agent. Usual indication for steroid is as a bridge therapy in JIA, for immediate control of disease activity or while waiting for DMARDs to take effect. They can also be used in combination with DMARDs or NSAIDs. They can be administered systemically or locally at the affected areas. Similar to NSAIDs, they provide symptomatic relief and slow down bony erosion and joint destruction but do not arrest the rheumatic process.[6]

Systemic corticosteroids

Systemic corticosteroids can be used through many routes, intravenous and oral, they may be considered for the management of juvenile idiopathic arthritis with systemic disease or when multiple joints are affected. Systemic corticosteroids may also be considered in severe, possibly life-threatening conditions such as systemic lupus erythematosus, systemic vasculitis and macrophage activation syndrome. In severe conditions, short courses ('pulses') of high dose intravenous methylprednisolone or a pulsed oral corticosteroid may be particularly effective for providing rapid relief and has fewer long-term adverse effects than continuous treatment. Complications related to long-term corticosteroid treatment such as growth retardation and other adverse effects should be monitored.[7]

Dosage of methylprednisolone pulsing: By intravenous injection (1 month to 18 years): 10-30 mg/kg (maximum 1gm) once daily or on alternate days for up to 3-5 doses.[8]

Intra-articular corticosteroids

Corticosteroids are sometimes injected locally for an anti-inflammatory effect. They are given by intra-articular injection as monotherapy, or as an adjunct to long-term therapy to reduce swelling and deformity in one or a few joints under asepsis with appropriate anaesthesia. Occasionally an acute inflammatory reaction develops after an intra-articular or soft-tissue injection of a corticosteroid which may be a reaction to the microcrystalline suspension of the corticosteroid used, but must be distinguished from sepsis introduced into the injection site.[9]

Triamcinolone hexacetonide is preferred for intra-articular injection because it is almost insoluble and has a long-acting (depot) effect. Triamcinolone acetonide and methyl prednisolone may also be considered for intra-articular injection into larger joints, while hydrocortisone acetate should be reserved for smaller joints or for soft-tissue injections. Each joint should usually be treated no more than 3-4 times in one year.[10, 11]

Dosage

Triamcinolone hexacetonide

By intra articular injection (1-18 years) on larger joints: 1mg/kg (maximum 20mg), higher doses may be used according to size of the joint.

Triamcinolone acetonide

By intra articular injection (1-18 years) on larger joints: 2mg/kg (maximum 40mg).[5]

Methylprednisolone acetate (Depo Medrone) : 1mL (40mg) intra articular injection.[11]

Hydrocortisone acetate

By intra articular injection

- Child 1 month to 12 years: 5-30mg according to size of child and joint
- Child 12 years to 18 years: 5-50mg according to size of child and joint.[5]

Disease modifying anti rheumatic drugs (DMARDs)

These are drugs which can suppress the rheumatoid process and bring about a remission, but do not have nonspecific anti-inflammatory or analgesic action. They are known as disease modifying anti-rheumatic drugs (DMARDs) which can be synthetic or biologic. Synthetic DMARDs are further categorized as conventional ones and the latest targeted ones which act by janus kinase pathways, called the small molecules (Table II).

Conventional synthetic DMARDs

Methotrexate, sulfasalazine, leflunomide and hydroxychloroquine (HCQ) are the commonly used DMARDs. DMARDs, whenever used should be under specialist supervision with monitoring for side effects (Table III). They should be administered for 3-6 months for a full therapeutic response. The dose of NSAIDs and or steroids can be reduced once there is a response to DMARDs. Methotrexate is the most frequently used and highly effective agent in JIA. Sulfasalazine is an alternative but should be avoided in systemic onset JIA. Other DMARDs such as cyclosporine and azathioprine have an adverse risk- benefit ratio and should be used with caution. Antibiotic DMARDs, such as doxycycline or minocycline are not recommended due to the availability of more effective drugs. DMARDs can improve not only the symptoms of inflammatory joint disease but also the extra-articular manifestations.[13]

Table II. Commonly used synthetic DMARD[12]

Conventional	Methotrexate
	Sulfasalazine
	Leflunomide
	Hydroxychloroquine
	Azathioprine
	Cyclosporine
	Gold and penicillamine
Kinase inhibitors	Tofacitinib

Methotrexate

Methotrexate primarily kills cells in S phase, inhibiting the DNA synthesis and thus synthesis of RNA and proteins. Basically it is an inhibitor of dihydrofolate reductase enzyme and affects lymphocyte and macrophage function. It also inhibits amino imidazole carboxamide ribonucleotide (AICAR) transformylase and promotes the accumulation of extracellular adenosine. Adenosine is responsible for site specific anti-inflammatory effects. The anti-rheumatic action is by exerting inhibitory effects on proliferation and it stimulates apoptosis in immune inflammatory cells. It also inhibits pro-inflammatory cytokine production, chemotaxis and cell mediated immune reactions.[14]

Methotrexate is the most commonly prescribed DMARD for juvenile idiopathic arthritis.[15] It is now recommended as the first-line treatment in oligoarthritis that persists despite non-steroidal anti-inflammatory drugs and intraarticular steroid therapy and in polyarticular disease.[16]

Dosage: In JIA, juvenile dermatomyositis, SLE, sarcoidosis, localised scleroderma, by oral, subcutaneous injection or intramuscular injection: 10-15 mg/m^2 once a week initially, increased if necessary to maximum of 25 mg/m^2 once weekly.

Side effects: Bone marrow suppression can occur abruptly especially if there is renal impairment and if there is concomitant use of another anti folate drug like trimethoprim. Other issues include gastrointestinal disturbances, hepatotoxicity and pulmonary toxicity.[17]

Sulfasalazine

Sulfasalazine has a beneficial effect in some forms of JIA but generally not used in systemic onset disease. Anti-inflammatory properties of sulfasalazine may be related to inhibition of bacterial growth, interference with production of prostaglandins and leukotrienes and accumulation of adenosine. It is a recommended treatment for enthesitis-related JIA following a NSAID trial and/or glucocorticoid joint injection.[18] A randomized, placebo-controlled study demonstrated the safety and efficacy of sulfasalazine in the treatment of children with oligoarticular and polyarticular JIA.[19]

Dosage: Orally in children between 2-18 years: Initially 5mg/kg twice daily for 1 week, then 10mg/kg twice daily for 1 week, then 20mg/kg twice daily for 1 week, maintenance dose 20-25mg/kg twice daily (Maximum

2gm/day in children 2-12 years and 3gm/day in children 12-18 years).[20]

Side effects: Hematological abnormalities like neutropenia and thrombocytopenia, hepatitis and rash.

Leflunomide

Even though the gold standard of therapy in JIA is methotrexate, leflunomide has shown to have comparable efficacy and usually well tolerated in various studies. Leflunomide is a prodrug that is quickly metabolized to an active metabolite which reversibly inhibits the enzyme dihydroorotate dehydrogenase, which is required for pyrimidine nucleotide synthesis. Though this drug has an anti-proliferative effect on T cells in vitro, mechanism of action in inflammatory arthritis is not known.[21]

Dosage: (as oral dose)

< 20kg: 10 mg on alternate days

20-30kg: 10 mg once daily

30-40kg: 10 mg alternating with 20 mg or 15 mg per day

>40kg: 20 mg once daily

Side effects: GI complaints like abdominal pain, nausea, diarrhoea, rashes, loss of hair, thrombocytopenia and leucopenia are noted.[22]

Hydroxychloroquine

The antimalarial hydroxychloroquine (HCQ) is less commonly used in treatment of JIA. It is regularly used in systemic/ discoid lupus erythematosus, particularly involving skin and joints and in sarcoidosis. It can be a useful adjunctive agent for treating chronic arthritis in older children. The therapeutic effect of this drug is usually subtle and is rarely evident before 2 to 3 months of therapy. It may be used as an add-on medication in treatment of JIA. In patients with SLE, HCQ is recommended to be continued indefinitely.

Dosage: Orally based on ideal body weight - 5-6.5 mg/kg (Maximum 400mg) once daily in children between 1 month and 18 years.

Side effects: It should be used with caution in neurological disorders, severe gastrointestinal disorders, G6PD deficiency and in acute porphyrias. It may exacerbate psoriasis and aggravate myasthenia gravis. It can also cause ocular toxicity and skin rashes. An ophthalmologic examination, including testing of colour vision and visual fields, is usually performed before therapy is started and traditionally every 12 months thereafter.[23]

Azathioprine

Azathioprine is a purine antimetabolite which acts after getting converted to 6-mercaptopurine by enzyme thiopurine methyl transferase (TPMT). It selectively affects differentiation and function of T-cells and natural killer cells thus suppressing cell-mediated immunity and inflammation. It is less sparingly used in the treatment of JIA as the success rate is not so promising. It may be reconsidered in the stepladder approach for the treatment of JIA-associated uveitis. The addition of azathioprine may also be beneficial for patients not responding properly to methotrexate.[24]

Dosage: In SLE, vasculitis and other autoimmune conditions usually when steroid therapy alone has proved to be inadequate, azathioprine may be started with 1mg/kg/day orally, and adjust according to response to maximum of 3mg/kg /day. Consider withdrawal of drug if no improvement is seen within 3 months.

Side effects: Bone marrow suppression (monitor blood counts at 2 weeks of initiation and thereafter at least once in every 3 months), GI disturbances, hepatic and renal impairment, infection risk and hypersensitivity reactions.

Gold and D-Penicillamine

Before the advent of methotrexate, gold therapy was very popular. D-Penicillamine also has gold like action but gold is not used nowadays, due to fear of toxicity.[25]

Table III. Quick reference guide for monitoring of DMARD therapy

Conventional DMARD	Monitoring parameters
Methotrexate	Complete blood count (CBC) fortnightly until 6 weeks after last dose increase; if this remains stable, then monthly thereafter monitoring may be reduced in frequency, based on clinical judgement. Liver function tests (LFT): 3 monthly renal function test: 6-12 monthly
Sulfasalazine	CBC and LFTs monthly for 3 months and 3 monthly thereafter
Hydroxy-chloroquine	12 monthly review by an ophthalmologist
Leflunomide	CBC, LFTs every 6 months and if stable 12 monthly thereafter

Targeted synthetic DMARD/ Janus kinase inhibitors

Janus kinase inhibitors are needed for a small subset of patients who are refractory to all DMARDs. JAK inhibitors (JAKinibs) are small molecule inhibitors of the Janus Kinase family of receptors. Cytokines are key mediators of the development and homeostasis of haematopoietic cells. They are not only critical for host defence, but are also implicated in the development of autoimmune and inflammatory diseases such as psoriasis, rheumatoid arthritis (RA), inflammatory bowel disease and several other immune-mediated inflammatory diseases. Blocking cytokine activity by interfering with the ligand–receptor association has been successfully employed to treat several immune disorders. Fig.1 depicts a subgroup of cytokines that signal through receptors requiring the association with a family of cytoplasmic protein tyrosine kinases known as Janus kinases (Jaks). Jaks have recently gained significant attention as therapeutic targets in inflammation and autoimmunity, and several Jak inhibitory small molecules have been developed. These small molecule inhibitors provide a novel mechanism of action in treating these conditions by affecting intracellular signalling pathways. JAKinibs have now been approved for use in adults with rheumatoid arthritis and psoriatic arthritis, and phase 3 studies are underway to establish the role and efficacy of these molecules in the treatment of JIA.[26,27] The most studied molecule of JAKinib is tofacitinib. Studies have shown that tofacitinib is effective for reducing the risk for disease flare in patients with polyarticular juvenile idiopathic arthritis.[28]

The effectiveness of tofacitinib treatment in a case of refractory systemic JIA with inadequate response to nonsteroidal anti-inflammatory drugs, methotrexate, glucocorticoids and etanercept has also been reported.[29] Other agents in this category are baricitinib and ruxolitinib which are also under therapeutic trials but no conclusive pediatric data is available as of now.[29,30]

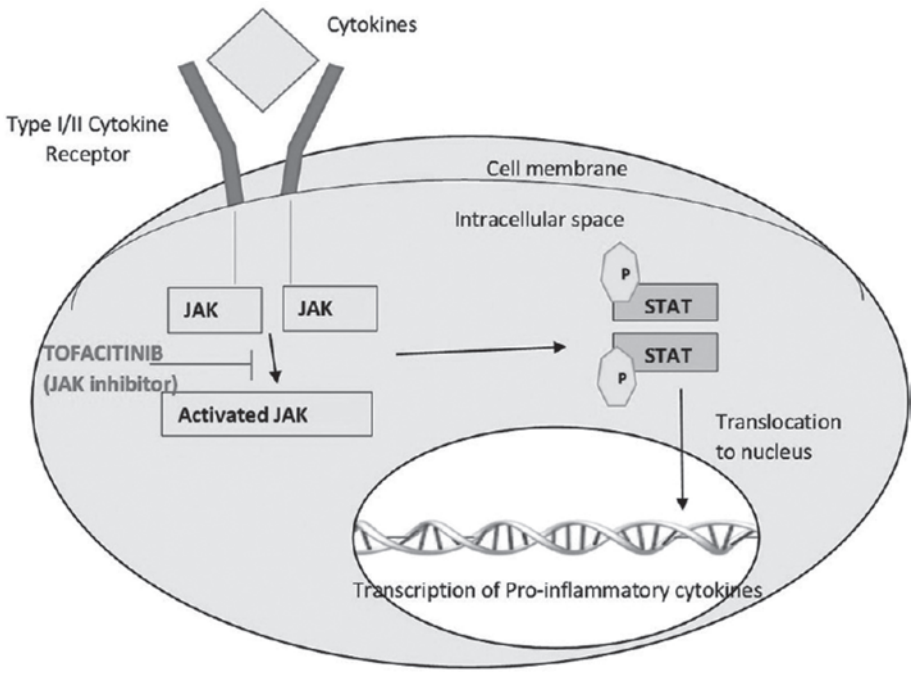

Fig.1. Type I/II cytokine receptors lack intrinsic kinase activity and signal through the cytoplasmic Janus kinases (JAK1, JAK2, JAK3, and TYK2) along with the DNA-binding proteins called signal transducers and activators of transcription (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6). Upon engagement of extracellular ligands with the receptors, intracellular JAK proteins become activated and phosphorylate STAT proteins which dimerize and translocate to the nucleus to regulate gene expression. Jakinibs interfere with the cytokine signal transduction to bring about their anti-inflammatory effects

(Source: Sonthalia S, Aggarwal P. Oral tofacitinib. Contemporary appraisal of its role in dermatology. Indian Dermatol Online J 2019;10:503-518).

Table IV. Classification of biologic DMARDs based on their mechanism of action

Drug class	Examples
TNF α inhibitors	Adalimumab Etanercept Infliximab Golimumab Certolizumab
CTLA4-Ig	Abatacept
IL-6 inhibitors	Tocilizumab
IL-1 inhibitors	Canakinumab Anakinra Rilonacept
Anti CD-20	Rituximab

Biologic DMARDs (Table IV)

Biological agents are the drugs which target specific components of the immune system like cytokines. They are increasingly used after conventional DMARD therapy fails or when the course of the disease is aggressive. They may have serious side effects like major infections or malignancies, hence must be prescribed only by a pediatric rheumatologist.[31]

TNF-α inhibitors

Adalimumab, etanercept and infliximab are commonly used TNF-α inhibitors. Adalimumab and etanercept can be used for the management of refractory polyarticular JIA, psoriatic arthritis and enthesitis related arthritis. In patients with refractory uveitis, adalimumab is the preferred agent. Infliximab is also used in management of refractory uveitis; however, as this requires intravenous administration, one would prefer adalimumab (given as subcutaneous injections).[32]

Side effects: They are generally associated with increased risk of infections, sometimes severe, including latent TB reactivation, septicemia and hepatitis B reactivation. Other side effects include nausea, abdominal pain, worsening heart failure, hypersensitivity reactions, fever, head ache, depression, SLE like syndromes and blood dyscrasias.[33]

Adalimumab

Adalimumab is a monoclonal antibody produced by DNA recombination technology, and is the first human monoclonal antibody against human tumor necrosis factor (TNF-α) in the world. Adalimumab binds with high affinity and specificity to soluble TNF-α and normalizes its biological action. It has been now approved for the use in psoriatic arthritis, ankylosing spondylitis, Crohn's disease and juvenile idiopathic arthritis (JIA).[34] Adalimumab is safe in children with active polyarticular JIA and the pharmacokinetics and its effectiveness were similar to that seen in older pediatric patients with JIA.[35]

Dosage: By subcutaneous injection[36]

Children 2-4 years: 24 mg/m^2 (maximum 20 mg) on alternate weeks.

Children 4-13 years: 24 mg/m^2 (maximum 40 mg) on alternate weeks.

Children 13-18 years: 40 mg on alternate weeks.

Review treatment if no response within 12 weeks.

Etanercept

Etanercept is a human soluble TNF-α receptor, attached to human IgG. It neutralizes TNF by binding with an affinity 50-1000 times that of the naturally occurring TNF receptors and may also exert its effect by binding other cytokines, including IL-1α and TNF-β. It is administered by subcutaneous injection twice weekly or weekly, for an indefinite period and may be used with or without methotrexate. Early safety and efficacy data in children are encouraging.[37]

Dosage: By subcutaneous injection

Children 2-17 years: 0.4 mg/kg (maximum 25mg) twice weekly, with an interval of 3-4 days between doses or 0.8 mg/kg (maximum 50mg) once weekly. Consider discontinuation if no response after 4 months.[38]

Infliximab

Infliximab is a chimeric human-murine monoclonal antibody that binds both soluble and cell bound TNF-α. It was the first commercially available agent for blocking TNF-α. There are some anecdotal reports of its success in JIA but no large scale studies on the efficacy of infliximab have been published in children. It is administered as intravenous infusion and in combination with methotrexate to avoid tachyphylaxis to the murine component.[39,40]

Dosage: 6-10 mg/kg given intravenously at weeks 0, 2, 6, and thereafter at four to eight week intervals.[41]

Other TNF-α blockers

Golimumab is a completely humanized monoclonal anti-TNF antibody for subcutaneous administration at the dose of 50 mg once per month. It is approved for the treatment of rheumatoid arthritis, psoriatic arthritis and

adult ankylosing spondylitis, while its efficacy in JIA is still under research. Certolizumab pegol is a PEGylated Fab fragment of a humanized anti-TNF antibody. The PEGylation of the antibody delays the elimination and thus provides a longer half-life. It was tested in Phase III trials in Crohn's disease and in rheumatoid arthritis.[42]

Abatacept

Abatacept is a recombinant fusion protein comprising the extracellular part of human Cytotoxic lymphocyte-associated antigen-4 (CTLA-4) connected to a modified Fc part of IgG1. CTLA-4 is a potent inhibitor of the co stimulation pathway necessary to activate T cells and thus abatacept prevents the full activation of T lymphocytes. It has been found to be effective in management of active polyarticular JIA and generally well tolerated but is not recommended to be used in combination with TNF inhibitors.[43]

Dosage: In moderate to severe active polyarticular JIA (in combination with methotrexate) and in children who have not responded adequately to other DMARDs (including at least one TNF inhibitor): By intravenous infusion (6-17 years)

Body weight: less than 75kg-10mg/kg; 75-100 kg - 750mg; >100 kg - 1gm.

The doses are repeated at 2 weeks and 4 weeks after initial infusion, then every 4 weeks.[44]

Tocilizumab

In patients with severe disease that do not respond to methotrexate and anti-TNF drugs, strong evidence supports the approach of targeting interleukin-6 (IL-6).[45] Tocilizumab antagonizes the actions of IL-6. It can be used for the management of active systemic JIA when there has been inadequate response to NSAIDs and systemic corticosteroids. Tocilizumab can be used in combination with methotrexate, or as monotherapy if methotrexate is not tolerated or is contraindicated. It is not recommended for use with other cytokine modulators. Subcutaneous tocilizumab was less effective than intravenous tocilizumab for juvenile idiopathic arthritis uveitis in a small case series, but has shown similar efficacy to intravenous tocilizumab in randomised controlled studies of rheumatoid arthritis.[46] Subcutaneous therapies are more desirable than intravenous infusions for patient convenience, maximising school attendance and using fewer health-care resources.[47]

Dosage in children with systemic JIA:[48]

By intravenous infusion (2-18 years).

Body weight <30kg: 12mg/kg once every 2 weeks.

Body weight >30kg: 8mg/kg once every 2 weeks; review treatment if no response within 6 weeks.

By subcutaneous injection

Body weight <30kg: 162mg once in every 2 weeks

Body weight >30kg: 162mg once in every week

Dosage in children with polyarticular JIA

Body weight <30kg: 162mg once in every 3 weeks

Body weight >30kg: 162mg once in every 2 weeks

Canakinumab

Canakinumab, a fully human monoclonal antibody against interleukin-1β, is a relatively new medication approved for treatment of systemic juvenile idiopathic arthritis.[49] When administered, the response to canakinumab treatment was sustained and associated with substantial glucocorticoid dose reduction or discontinuation and a relatively low retention-on-treatment rate. No new safety findings were observed on long-term use of canakinumab.[50]

Dosage:[51]

By subcutaneous injection

In cases of Tumor Necrosis Factor Receptor Associated Periodic Syndrome (TRAPS), hyperimmunoglobulin D Syndrome/ Mevalonate Kinase Deficiency and familial Mediterranean fever.

Body weight ≤ 40 kg: Start with 2 mg/kg every 4 weeks. The dose can be increased to 4 mg/kg every 4 weeks if the clinical response is not adequate.

Body weight ≥ 40 kg: The recommended starting dose is 150 mg every 4 weeks. The dose can be increased to 300 mg every 4 weeks if the clinical response is not adequate.

In cases of Systemic juvenile idiopathic arthritis: 4 mg/ kg (maximum 300 mg) for patients with a body weight ≥7.5 kg, administer subcutaneously every 4 weeks.

Anakinra

Anakinra is recombinant IL-1β receptor antagonist which has shown be effective in small cohorts of therapy-resistant adult and pediatric Still's patients. IL-1β has been shown to be a main contributor to the pathogenesis of systemic JIA.[52] Various studies have shown that earlier treatment with anakinra is associated with a better short-term outcome.[53]

Dosage: By subcutaneous injection: Initial: 1 to 2 mg/kg/dose once daily; (maximum initial dose - 100 mg; if no response, may titrate typically at 2-week intervals by doubling dose up to 4 mg/kg/dose once daily; maximum dose: 200 mg.[54]

Rilonacept

Rilonacept (IL-1 Trap) is an IL-1 neutralizer incorporating into one molecule of the extra-cellular domain of two human cytokine receptors required for IL-1 signalling, combined with the Fc portion of human IgG1. Rilonacept binds to IL-1β with high affinity and specificity, and blocks inflammation caused by overproduction of IL-1.[55] Studies have shown achievement of sustained improvements in clinical and laboratory measures of the articular and systemic manifestations of systemic JIA in >50% of rilonacept-treated patients over 2 years. Treatment with rilonacept had a substantial steroid-sparing effect and was generally well-tolerated.[56]

Dosage: By subcutaneous injection

Loading dose of 4.4 mg/kg on week 0 followed by maintenance weekly doses of 2.2 mg/kg.[57]

(NOTE: At present, IL-1 inhibitors are not being marketed in India).

Rituximab

Rituximab (RTX) may be considered as a treatment option for children and young people with JIA, although it is not licensed for this indication. Depletion of B cells has emerged as a new approach for the treatment of autoimmune diseases, including JIA. Rituximab is sparingly used in the treatment of JIA; however, there are many rheumatic diseases where rituximab is highly effective. It is used in the management of lupus nephritis (refractory to conventional therapy), vasculitis (e.g. ANCA associated vasculitis), steroid-refractory autoimmune hemolytic anemia, IgG4 related disease, etc.[58,59]

Box 2. Algorithm for stepwise use of drugs in juvenile idiopathic arthritis

1. Management of oligoarticular JIA

Oligoarticular JIA
Intra-articular steroid injections
↓ (Persistent disease activity)
Weekly methotrexate oral/subcutaneous injection
↓ (Persistent disease activity)
Injection adalimumab/etanercept

2. Management of polyarticular JIA

Polyarticular JIA
NSAIDs + Methotrexate
↓ (Persistent disease activity)
Add leflunomide
↓ (Persistent disease activity)
Injection adalimumab/etanercept

3. Management of Systemic onset JIA

Systemic onset JIA
Systemic features predominant Steroids
↓
(Persistent disease activity)

Injection tocilizumab/anakinra

Systemic onset JIA with prominent arthritis Steroids + Methotrexate
↓
(Persistent disease activity)
Injection tocilizumab/anakinra
(NSAIDs may be used to provide immediate relief in children with all types of JIA)

Dosage: By IV infusion 375 - 500 mg/m^2once in 2 weeks for 2 doses.

Algorithms for management of JIA

An example of the step wise use of drugs in JIA is given herewith (Box 2).

Conclusion

Advances in the therapy of childhood rheumatic diseases have improved the prognosis for affected children. Evidence suggests that early diagnosis and rapid disease control would improve the course of the disease and long-term outcomes. This emphasises the need for effective and aggressive treatment during the critical therapeutic window of opportunity. The past decade has seen increasing use of biologic drugs for the treatment of pediatric rheumatic diseases. The widest range of such treatments is used for juvenile idiopathic arthritis, although biologics are sometimes given in more refractory cases of juvenile systemic lupus erythematosus, juvenile dermatomyositis and vasculitis.

Points to Remember

- ***Numerous medications are currently available for the treatment of rheumatic diseases apart from NSAIDs and steroids.***
- ***NSAIDs and steroids can be used as a stopgap measures till optimum effects of disease modifying drugs start appearing.***
- ***Methotrexate is the most commonly used agent for initial treatment of juvenile idiopathic arthritis.***
- ***Combination therapy has been shown to have better outcome than monotherapy but the choice of medications should be tailored for each patient.***
- ***Most of these medications require periodic monitoring by specialists for possible major adverse effects.***

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CLIPPINGS

A Note from History : The Discovery of Blood Cells

Invention of microscope in Holland (1590) by Hans and Zacharias. Dutch naturalist, Jan Swammerdam was the first person to observe RBC under the microscope (1658). Dutch microscopist, Leeuwenhoek, rendered the first illustration of them in 1695. Gabriel Andral and William Addison, reported simultaneously the first descriptions of leukocytes (1843).

Hajdu SI. A Note from History: The Discovery of Blood Cells. Ann Clin Lab Sci Spring 2003; 33(2):237-238

SURGERY

ACUTE PAIN MANAGEMENT - REVIEW OF CURRENT CONCEPTS

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**Gopa Das Majumdar

Abstract: *Perception of pain in children is complex and often remains underrated and untreated. It entails many physiological, psychological, behavioral and developmental factors. Pain management requires identification of the source and assessment of the intensity of pain.*

This review article discusses some of the common age specific pain assessment tools used in practice to grade the severity of pain and how to plan the patient specific analgesic regimes. It has also reviewed the different methods currently used for acute pain management and the pharmacological aspects of various analgesics used in children.

Keywords: *Acute pain, Management, Children.*

The International Association for the Study of Pain defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Perception of pain in children is complex and entails physiological, psychological, behavioural and developmental factors.[1]

Pain in children is underrated and undertreated.[2] Many misconceptions plague the pain scenario in children - from beliefs that 'children do not feel pain like adults' to the 'Neonates do not feel pain' theory. The truth is that many of the nerve pathways essential for the transmission and perception of pain are present and functioning by 24-29 weeks of gestation. As health care professionals, it becomes our responsibility to make sure that the pain is assessed appropriately and treated effectively for all children under our care, while maintaining safety.

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Irrespective of the source, management of pain in children involves identification and assessment using various tools based on the age and cognitive ability of the child, followed by prompt control through pharmacological and non pharmacological methods, along with resolution of the underlying cause. Unmanaged, it can lead to anxiety and stress and in the long-term this can impact the psychosocial health and development of the child. It is difficult to confirm and assess the severity of pain in infants and preverbal children, who exhibit both hunger and need for comfort in a similar manner.

Evaluation of pain

First step in the treatment of pain is to identify the source (Table I). Details of the primary illness help to identify the source and type of pain.

Pain assessment scale

Next step in management of pain is assessment of severity and type of pain. It is important to have tools to quantify the pain in children based or their age and cognitive ability.

Three main methods are currently used to measure pain intensity: i) self report, ii) behavioral and iii) physiological measures. Self-report measures are optimal and the most valid. Both verbal and nonverbal reports require a certain level of cognitive and language development for the child to understand and give reliable responses. Children's capability to describe pain increases with age and experience and changes throughout their developmental stages.[4]

Children older than 8 years will be able to communicate the nature and amount of pain they have.

Behavioural measures consist of assessment of crying, facial expressions, body postures and movements. They are more frequently used with neonates, infants and younger children where communication is difficult.[8]

Physiological measures include assessment of heart rate, blood pressure, respiration, oxygen saturation, palmar sweating and sometimes neuro-endocrine responses.[9] These are however used in combination with other pain scores and vary with age of the child. The physiological

Table I. Causes of pain in hospitalized children[3]

Procedures causing pain	
Therapeutic interventions	IV insertion, blood sampling procedures, catheter insertion such as nasogastric tube, drain tubes, physiotherapy
Diagnostic procedures	Bone marrow aspiration, biopsy, lumbar puncture, micturating cystourethrogram
Illness related pain	Injury, urinary tract infection, inflammatory disorders-arthritis, pancreatitis, pleuritis, malignancy, organ distension like hepatic enlargement, bladder distension; neuropathic pain
Colonoscopy, Upper GI scopy	
Surgical causes of pain	
	Underlying surgical problems
	Positioning during surgery
	Incision
	After surgery care
	Mobilisation and physiotherapy
	Complications

Chronic pain is not dealt with in this review.

Table II. FLACC scale for pain[10]

Criteria	**Score 0**	**Score 1**	**Score 2**
Face	No particular expression or smile	Occasional grimace or frown withdrawn, uninterested	Frequent to constant quivering chin, clenched jaw
Legs	Normal position or relaxed	Uneasy, restless, tense	Kicking, or legs drawn up
Activity	Lying quietly, normal position, moves easily	Squirming, shifting, back and forth, tense	Arched, rigid or jerking
Cry	No cry (awake or asleep)	Moans or whimpers, occasional complaint	Crying steadily, screams or sobs, frequent complaints
Consolability	Content, relaxed	Reassured by occasional touching, hugging or being talked to, distractible	Difficult to console or comfort

parameters may change due to other factors like stress, metabolic or other derangements in the child.

Premature infants and acutely ill children may not however, possess the maturity or energy to express pain. The composite pain scales take into account behavioural and physiological parameters. The premature infant pain profile (PIPP), crying requires increased vital signs expression sleeplessness (CRIES) and the maximally discriminate facial movement coding system (MAX) are some of the scales used.

FLACC scale is a scoring system for pain, used in children of 2 months to 7 years. It looks at behavioural and motor responses to pain. The parameters assessed are face, legs, activity, cry and consolability. Each variable has a score of 2 with a maximum score of 10 for severe Pain (Table II).

Following identification of source and assessment of severity of pain, the final step is management with drugs and other methods.

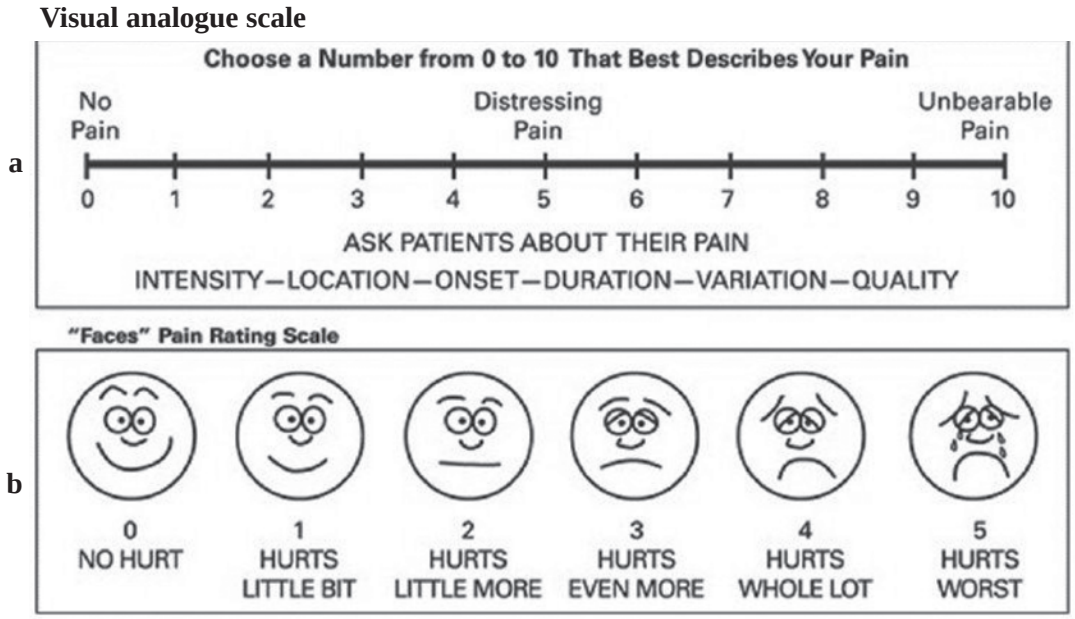

Fig.1a. Visual analog scale, 1b. Faces pain scale

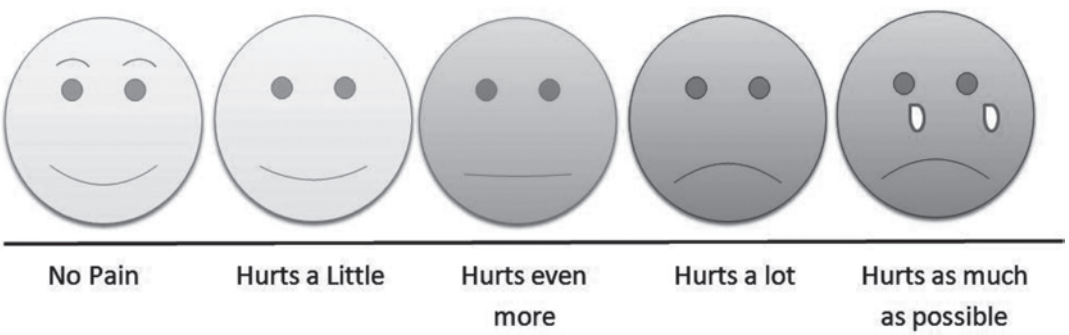

Fig.2. Colour analog scale (darker shades mean more Pain)

Visual analog scale (VAS) Fig.1a. It is a horizontal line, 100 mm in length, attached to word descriptions at each end, "not hurting" or "no pain" to "hurting a whole lot" or "severe pain". The children are asked to mark on the line the point that they feel represents their pain at this moment.[5]

Faces pain scale Fig.1b. It was developed by Wong and Baker and is recommended for children aged 3 and older. The scale requires health care professionals to point to each face and describe the pain intensity associated with it and then ask the child to choose the face that most accurately describes his or her pain level.[6]

Fig.2. Color and analog scale can also be used, where darker more intense colours (i.e., red) represent more pain.[7]

Different methods of pain management

The New York society for regional anesthesia outlines the broad principles in managing pain in the acute setting of surgery.[3]

- The aim of acute pain management is providing a comfortable/pain-free perioperative period in order to facilitate early ambulation and rehabilitation.
- During the preoperative assessment (in case of surgical or other procedure), discussion with the child and his /her parents regarding the amount of pain expected, various available pain management modalities and what to expect, play an important role in achieving a satisfactory outcome for all concerned.
- A multimodal approach to pain management achieves the best results.

If possible, regional anesthesia/analgesia should be part and parcel of any multimodal analgesia regime.

Mild pain : Mild Pain can usually be treated with paracetamol alone in the outpatient setting. If pain persists, one analgesic (ibuprofen) is used round the clock as fixed interval dosing for 48 hours, while another (paracetamol) is used for breakthrough pain as needed.

Moderate to severe pain: Once pain is not controlled with NSAIDS or paracetamol, the next step is to add a weak opioid. IV infusion of opioid-continuous or fixed interval dosing and regional techniques are other options to manage moderate to severe pain (Table III).

Weak opioids are available as oral preparations and can still be used in the outpatient setting in certain groups of patients. In patients coming for surgery, most pediatric anaesthetists would include a regional anaesthetic as part of the plan which takes care of analgesia in the intraoperative and immediate postoperative period.

Analgesic pharmacotherapy

Acute pain management in pediatrics is increasingly characterized by a multimodal or "balanced" approach in which smaller doses of opioid and nonopioid analgesics, such as nonsteroidal anti-inflammatory drugs (NSAIDs), local anaesthetics, N-methyl-D-aspartate (NMDA) antagonists and α2-adrenergic agonists are combined to maximize pain control with minimal adverse side effects.[3] The drug administration is individualized so that a favourable balance between pain relief and adverse pharmacological effects is achieved and maintained.[12]

Table III. The analgesic ladder for the management of acute pain (modified)[11]

Pain rating	Recommendation
Mild pain	• Oral drugs - NSAID, acetaminophen or salicylate
Moderate pain	• Oral drugs - NSAID or acetaminophen with weak opioid • Intravenous opioids (with addition of fixed-interval dosing of NSAID or acetaminophen) a. Continuous infusion of opioid with as-needed rescue doses of opioid b. Fixed-interval dosing of opioid • Regional anesthetic techniques
Severe pain	• Continuous fixed-interval dosing of NSAID or acetaminophen, • Intravenous opioid • Regional anesthetic techniques • Add adjuvant agents where suitable

Analgesic drugs may be broadly divided into 3 groups- a) non opioid analgesics, b) opioid analgesics and c) adjuvant drugs.

Non opioid analgesics

Non opioids include acetaminophen (paracetamol) and non steroidal and anti inflammatory drugs. Aspirin (acetylsalicylic acid) is not recommended for children less than 16 yrs of age for fear of Reye's syndrome.

Acetaminophen (paracetamol): Routes: oral, IV, rectal.

Paracetamol has analgesic and antipyretic effects and is an effective analgesic in the outpatient setting. It does not have the side effects common to NSAIDS. In doses less than 60mg/kg/day, it does not produce hepatotoxicity in healthy children. Appropriate timing and route of administration is important for good analgesic effect. The rectal route is unreliable and has variable absorption characteristics. Combining the oral premedications (midazolam) with either syrup paracetamol (15 mg/kg) or syrup ibuprofen (10 mg/kg) allows the patient to have some analgesia on board upon awakening-preemptive analgesia (useful for short surgeries). It is now common practice to include a rectal suppository of paracetamol as analgesic supplement or for postoperative cover (inserted after induction of anaesthesia) for most daycare procedures. Paracetamol can be prescribed for therapy at home for surgical patients undergoing orthopaedic or other procedures for postoperative pain (combination of ibuprofen 100mg/5ml and paracetamol -120 mg/5ml) at a dose of 5-10mg/kg PO of ibuprofen to be given after food. NSAIDs are relatively safe, not producing tolerance or dependence. They have a ceiling effect for analgesia. Caution: In certain febrile illnesses like dengue and COVID-19, NSAIDS should not be used and only paracetamol is preferred.

The mechanism of action of the NSAIDs is inhibition of the enzyme cyclooxygenase which prevents production of prostaglandins (pain modulators). Most common side effects are gastritis, erosions, ulcerations and reduced renal perfusion. Inhibition of cyclooxygenases in platelets reduces the synthesis of thromboxane A_2, which normally contributes to platelet aggregation. So, NSAIDs (Aspirin) can cause abnormal platelet function and increase bleeding tendency in surgical patients. By inhibiting cyclooxygenase, NSAIDs shunt the arachidonic acid pathway towards leukotriene synthesis. Leukotrienes mediate bronchospasm and anaphylaxis which may be seen in some sensitive patients.

While NSAIDs like ibuprofen in lower doses produce antipyretic and analgesic effects, higher doses have anti inflammatory action. So it is appropriate to treat pain associated with inflammation with the higher dose range. Other nonselective NSAIDs are Naproxen (longer duration of action - so twice daily dosing is sufficient) and Ketorolac (which has quicker onset of action and can be given IV - useful in hospital setting). Non opioid drugs have been summarised in Table IV.

Opioids

Opioids are drugs that act by interaction with specific receptors in the brain (mu) and spinal cord (kappa) to produce their effects.

Opioids may be largely grouped as agonist, partial agonist and agonist-antagonist.

The pure agonists - morphine, fentanyl, sufentanyl, meperidine, have no ceiling effect for analgesia. As dose increases analgesic effect increases or the patient loses consciousness/ goes for respiratory depression. It is useful to titrate the dose for effect especially in children for eg: morphine dose of 0.05mg may be given IV every 15 min (till analgesia is achieved; in the postoperative setting).

The mu receptor (of which morphine is an agonist) is responsible for mediating analgesia and two of the most undesirable side effects attributed to opioids: respiratory depression and dependence. Mu effects increase proportionately with the dose.

Kappa receptors are responsible for analgesia and respiratory depression but the effects do not increase with dose and there is a ceiling effect. This led to the birth of various drugs which are now called agonist-antagonists. They are agonists at kappa receptors producing limited analgesia and antagonists at mu receptors. They have less chance of respiratory depression at higher doses (mu effect) and do not produce constipation, also having less potential for abuse. They can produce withdrawal effects because

Table IV. Non-opioids as analgesics

Non opioid analgesics	Preterm infants 32-36 wks	Term neonates >36 wks to 44 wks	Infants> 44 wks and children < 50 kg	Children >50 kg, > 12 yrs
Paracetamol oral	15 mg/kg PO/PR every 8 hours (max 60 mg/kg/day)	15 mg/kg PO/PR every 6 hours (max 60 mg/kg/day)	15 mg/kg PO/PR every 4-6 hrs (max 90 mg/kg/day)	1 g per dose PO/PR every 4-6hrs (max 4g/day)
Paracetamol IV	7.5 mg/kg every 8 hours (max 25 mg/kg/day)	7.5 mg/kg every 6 hours (max 30 mg/kg/day)	15 mg/kg every 6 hours (max 60 mg/kg/day)	15 mg/kg every 6 hours (max 1 gm every 6 hours)
NSAIDS - prescribe any one drug only				
Ibuprofen	Not recommended	Not recommended	Beyond infancy 10mg/kg (max 400 mg) Every 8 hours (max 30 mg/kg/day)	400 mg PO every 8 hours
Diclofenac	Not recommended	Not recommended	Not recommended	50 mg PO/PR every 8 hours
Naproxen	Not recommended	Not recommended	Not recommended	5 mg/kg every 12 hours (max 1 g/day)
Ketorolac			Bolus: 1-3 mg/kg every 8 hours	10-30 mg every 4-6 hrs im or iv (max 90 mg/day)

(Modified from (New York school of regional anesthesia) NYSORA - acute pain management in children[3])

All NSAID's are not commonly used in children in Asian countries because of high prevelance of dengue.

of antagonist effects (at mu receptor) and should be used with caution. Nalbuphine and buprenorphine belong to this group of drugs. Both have limited use in children.

Physical dependence is a pharmacological property of opioid drugs defined by the development of an abstinence (withdrawal) syndrome following either abrupt dose reduction or administration of an antagonist. A tapering schedule is used if treatment cessation is indicated and opioid antagonist drugs (including agonist-antagonist analgesics) are avoided.[13]

Opioid prescribing practice

Codeine, oxycodone, hydrocodone and tramadol are weak opioids. They are used in the outpatient setting for moderate pain. Codeine is metabolized by a hepatic microsomal enzyme to morphine for a significant part of its analgesic effect. Some patients rapidly metabolise codeine resulting in plasma morphine concentration high enough to cause respiratory depression and death, even at recommended doses.Tramadol has a dual mechanism of action including agonism at the mu-opioid receptor and inhibition of norepinephrine and serotonin reuptake in the CNS. Tramadol has a half life of 6-7 hours and its active metabolite lasts 10-11hours. It can be used as an oral preparation (tablet) - 50 mg prescribed at 0.5-1mg/kg twice daily. It produces less respiratory depression than other weak opioids at equipotent doses. It is metabolised by the hepatic CYP450 system and has similar issues as codeine with fast metabolisers. Hence, codeine and tramadol are not recommended for children under 12 years of age. Metabolites of tramadol undergo renal excretion so it is not recommended in patients with compromised renal function. Nausea,vomiting and giddiness are common side effects with tramadol.

A brief overview of the commonly used opioids is given below.

Morphine is the most commonly used opioid in children for moderate to severe pain. It is given IV, as an infusion. Other possible routes are oral, intrathecal or subcutaneous.[14] Following epidural or intrathecal administration , the duration of action is significantly longer - about 24 hrs and needs monitoring .

Morphine is given in a bolus dose as 0.1 to 0.2mg/kg IV for intraoperative analgesia. 10-20 mcg/kg/hr IV in the postoperative period as infusion. Weight in kg (as mg of morphine) in 50 ml NS at 1 ml/hr gives 20mcg/kg/hr.

For a 10 kg child, add 10mg morphine in 50ml NS - deliver at 1ml/hr as a continous infusion. This is usually continued for 24 to 48hrs in post op surgical patients. In combination with Paracetamol it forms an excellent analgesic regime.

Fentanyl has a shorter onset and duration of action. It is thus useful for short invasive procedures. Fentanyl is given as bolus dose of 0.5mcg/kg to 1mcg/kg IV every 1-2 hrs intraoperatively. For post op infusion, the dose is 0.5 to 1mcg/kg /hr.[3]

Dosage schedule for the opioids is shown in Table V.

Patient-controlled analgesia:[15,16] Patient-controlled analgesia (PCA) generally is a technique of parenteral drug

Table V. Opioid analgesic initial dosage guidelines

Drugs	Usual Starting IV dose and Interval		Usual starting Oral dose and interval	
	<50kg	>50kg	<50kg	>50kg
Codeine	NA	NA	0.5-1 mg/kg every 4-6 hours	0.5-1 mg/kg every 4-6 hours
Oxycodone	NA	NA	0.1mg/kg every 3-4 hours	5-10 mg every 3-4 hours
Morphine	Bolus: 0.03-0.1 mg/kg, every 0.5-2h Infusion: 20 mcg/kg/h The formula for preparing morphine infusion. Mix 1mg/kg of morphine in 50ml NS.1ml/hr will deliver 20mcg/kg/hr	Same	0.2-0.3 mg/kg every 4-6 hours	15 mg/kg every 4-6 hours
Fentanyl	Bolus: 0.5-1 mcg/kg,every 0.5-2h Infusion: 0.5-1 mcg/kg/h	Same	NA	NA

administration in which the patient controls an infusion device that delivers a background rate of the drug along with bolus drug "on demand".

Inability to push the bolus button and inability to understand how to use the pump may be reasons for not using PCA in a child.

Nurse-controlled analgesia (NCA): In patients considered below "competent" age, neonates, toddlers and patients with complex needs, the practice of allowing surrogates such as nurses to initiate a PCA bolus is called nurse-controlled analgesia (NCA).

When treating pain with opioids, it is important to anticipate and manage their adverse effect to assure continued use. Adverse effects and management is summarised in Table VI.

Risk factors for opioid related side effects are[17]

1. Infants younger than 6 months of age
2. Patients with severe underlying systemic illness: Cardio respiratory dysfunction, hepatic insufficiency, renal insufficiency, altered mental status, airway obstruction or obstructive sleep apnea (OSA)
3. Concomitant use of other medications: barbiturates, phenothiazines benzodiazepines.

Table VI. Adverse effects of opioids and treatment strategies (modified)[17]

Adverse effects	Treatment strategies
Respiratory depression	• Stop opioid administration. • Airway management with supplemental oxygen or bag-mask ventilation as needed. • Naloxone 1 μg/kg every 3 minutes up to 10 μg/kg; naloxone infusion may be needed for longer-acting opioids (0.25-1mcg/kg/hr)
Constipation or ileus	• Stool softeners. • Cathartic agents. • Motility agent (metoclopramide).
Nausea or vomiting	• Change opioid. • Phenothiazine (promethazine 0.25 mg/kg up to 12.5 mg). • 5-HT3 antagonist: ondansetron (0.1 to 0.2 mg/kg max. upto 4mg). • Neurokinin antagonist - (Aprepitant).
Pruritus	• Diphenhydramine (0.5 mg/kg). • Change opioid.
Urinary retention	• Bladder catheterization.

Adjuvant agents

The term "adjuvant analgesic" describes a drug that has a primary indication other than pain but is used as analgesic in some conditions.[18] These drugs can be added to other analgesics. Adjuvants can enhance the analgesic efficacy and treat the concurrent symptoms. Sometimes they are used as independent analgesics in specific types of pain. They are started with low initial doses to avoid early side effects. Adjuvants like tricyclic anti depressants, clonidine can improve comfort in nonverbal children with neurological impairment.

Adjuvant therapy in children includes the following classes of drugs.[14]

- Antispasmodics
- Antidepressant for neuropathic pain (tricyclic anti depressant)
- Anticonvulsants for neuropathic pain (gabapentin, carbamazepine)
- Glucocorticoids (dexamethasone)
- Bisphosphonates for bone pain due to tumour
- Radiation therapy for bone pain

Regional Anesthesia Techniques: These form an integral part of any surgical procedure today. With the advent of ultrasound guidance for performing nerve blocks - the peripheral nerve blocks have become very safe in children. Its still not uncommon to find an epidural (central neuraxial block) infusion for postoperative pain relief in major abdominal or pelvic surgeries.

Doses used for epidural infusion

Bupivacaine - 0.2-0.4mg/kg/hr, usually a 0.125% bupivacaine is used for postoperative analgesia.

To prepare a 0.125% solution - 25 ml of 0.25% bupivacaine is diluted with 25 ml of NS.

For a 10 kg child, 2.5 ml /hr infusion is started and continued for 48-72 hrs in the postoperative period.

Ropivacaine - 0.5mg/kg/hr in children older than 4-6 months.

Table VII. Maximum local anesthetic dosing guidelines (modified)[3]

Drug	Dose mg/kg Without Epinephrine	Dose mg/kg with Epinephrine	Duration in hours	Comments
Bupivacaine	2.5	3	3-6	Reduce dose by 50% in neonates
Lignocaine	5	7	1	
Ropivacaine	3	Never mixed	3-6	Less cardiotoxicity than bupivacaine

Dosage schedules are shown in Table VII.

Local anaesthetic toxicity : Light headedness, convulsions, arrhythmia and cardiac arrest may occur if toxic dose is exceeded. Since dedicated colour coded infusion pumps are not available in most places in India for local anaesthetic infusion, care must be taken to label the syringe pump properly to prevent mishaps.

Topical and local anaesthetics

Local anesthetics are widely used drugs nowadays for topical analgesia, EMLA-eutectic mixture of 2.5% lignocaine and 2.5% prilocaine is an excellent surface anaesthetic. The cream is applied over the site where the IV access is planned (e.g. dorsum of hand) 1 hour before.

It can be used for Lumbar puncture, superficial suturing or ear piercing along with sedation as needed.

Non-pharmacological management of pain

Non-pharmacological techniques should be included in the management of children with pain in certain situations like IV insertion, burn dressing changes, immunizations etc. Distraction and comfort can be provided by parents with physical touch (e.g. cradling, cuddling), books, toys, singing, story telling or engaging in conversation.[19]

Engaging postoperative children with their favourite TV shows, cartoons or games markedly reduces the requests for rescue medications.

Points to Remember

- ***Pain in children is underrated and undertreated.***
- ***The source of pain must be identified, followed by assessment of severity.***
- ***Analgesic drugs can be broadly divided into-opioid, non opioid analgesics and adjuvant drugs.***
- ***Optimal combinations of opioid and non opioid analgesics are used to maximise pain control with minimal drug induced side effects.***
- ***Paracetamol either alone or along with NSAIDS form the mainstay of treatment for mild to moderate pain and weak opioids (codeine, oxycodone, hydrocodone and tramadol) for outpatient management of moderate pain.***
- ***Severe pain is ideally treated with opioids like morphine in the hospital setting where it can be used with precautions.***
- ***Opioids may be largely grouped as agonist, partial agonist and agonist-antagonist. The latter agents have less potential for side effects like respiratory depression and lesser potential for abuse.***
- ***Adjuvant analgesics derived from diverse pharmacologic classes like antispasmodics, clonidine etc. are now used to manage non-malignant pain.***
- ***Local anaesthetics are widely used nowadays for topical analgesia, intraoperative pain management and post operative pain.***
- ***Non-pharmacological techniques of pain management should be utilized in children in appropriate situations.***

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CLIPPINGS

Guidelines for surviving pediatric sepsis.

The authors recommend the following: A protocol /guideline for management of children with septic shock or sepsis associated organ dysfunction (SAOD) is necessary. Blood cultures should be obtained before initiating antimicrobial therapy. Empiric broad spectrum antimicrobials to cover all the likely pathogens is justified. Recommendations include using antimicrobial dosing strategies that have been based on published pharmacodynamic / pharmacokinetic principles. The duration of antimicrobial therapy is determined according to site of infection, microbial etiology, response to treatment and ability to achieve source control. Emergent source control intervention should be implemented. Intravascular devices that are confirmed to be the source of sepsis or septic shock need to be removed. In the health care systems with no availability of intensive care and in the absence of hypotension, they recommend against fluid boluses with maintenance fluid. They recommend against using starches in the acute resuscitation of septic shock or SAOD, the routine use of inhaled nitric oxide in all children with sepsis induced PARDS and the use of insulin to maintain the target blood glucose $\leq$ 140mg/dL.

Weiss SL, Peters MJ, Alhazzani W, Agus MSD, Flori HR, Inwald DP. Surviving sepsis campaign international guidelines for the management of septic shock and sepsis-associated organ dysfunction in children. Pediatr Crit Care Med 2020 Feb;21(2):e52-e106. doi: 10.1097/PCC.0000000000002198.

ADOLESCENCE

OFFICE MANAGEMENT OF SUBSTANCE USE IN ADOLESCENCE

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Abstract: *Substance use in adolescents begins in the critical phase of growth. Adolescents are "biologically wired" to seek new experiences and take risks, as well as to carve out their own identity. Substance use during adolescence has been associated with a greater risk of substance use disorders in adulthood. Efforts should be focused on early identification, awareness and prevention programs, and routine monitoring of adolescent health. Pediatricians should screen for nonspecific flag signs and specific indicators of substance use and underlying mental health disorders should be diagnosed in these adolescents. Behavioural interventions, family, school and community support groups need to be created for their management*

Keywords: *Substance use, Drug addiction, Adolescence, Adolescent behaviours, Screening.*

Adolescence is a period of exploration and experimentation. Many risky behaviours like substance use have their onset in adolescence. These habits usually track into adulthood. Substance use before the age of 18 years is associated with an eightfold greater likelihood of developing substance dependence in adulthood.[1] Even the first use of a psychoactive substance may result in tragic consequences of injury, victimization or even fatality. Substance use disorder (SUD) is associated with problems in all spheres of an adolescent's life namely individual, family, school and society. It can cause fall in academic performance, juvenile delinquency, rape, promiscuous sexual behaviour, HIV, hepatitis, family conflict, run away behaviour, depression or suicide attempts.[2] Drug-crime correlation has been noted with the consumption of substances-for example, cannabis intake is linked with murder, inhalants with rape and opioids with snatching-related crimes.[3]

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Situational analysis

In India, as per national survey on extent and pattern of substance use 2019, the prevalence of various substance use in children between 10-17 years were alcohol (1.3%), cannabis (0.9%), opioid (1.8%) and sedatives (1.7%).[4] In the USA, 21% of teens between 12 to 17years have tried a tobacco product, including use of traditional cigarettes (13%), electronic cigarettes (11%), cigars (8%), hookahs (7%) and smokeless tobacco (4%).[5] A major source of information about these products is available online, especially on the widely accessed YouTube video platform. 55% to 88% of adolescents in treatment for SUD meet criteria for a psychiatric disorder, namely conduct disorder, ADHD, depression, and anxiety.[6,7]

Categories of substances used by adolescents

Based on effects on the central nervous system, drugs are classified as depressants, hallucinogens and stimulants.[8]

1. Depressants: These drugs include alcohol, oxycontin, opioids, marijuana, tranquillisers, barbiturates, solvents and inhalants including petrol, glue, paint thinners and lighter fluids like gasoline, nail polish remover, aerosols present in hair sprays, deodorants, room fresheners, etc.

2. Hallucinogens: This group of substances contains psychoactive drugs that distort reality by triggering hallucinations, delusional thinking and/ or skewed experiences of time and space. These substances include LSD (d-lysergic acid diethylamide), peyote, mescaline, mushrooms (psilocybin), DMT (dimethyltryptamine).

3. Stimulants: These include methylphenidate, cocaine, narcotics like pentazocine, buprenorphine, morphine, pethidine, spasmoproxyvon, codeine containing cough syrups, cocaine, amphetamine, club drugs, anabolic steroids, electronic cigarettes and hookahs that are commonly used by adolescents.

Alcohol is the most commonly abused substance. In adolescents there are genetic influences, familial and

environmental factors which contribute to alcohol initiation, use and dependence. Alcohol use by adolescents is much more likely to be episodic and in larger volumes (binge drinking). Rapid binge drinking puts the teenager at even higher risk of alcohol overdose or alcohol poisoning, in which suppression of the gag reflex and respiratory drive and hypoglycemia can be fatal. Binge drinking and its sequelae of elevated blood alcohol concentration (BAC) are especially dangerous for young people who, when compared with adults, may be less likely to be sedated and therefore, more likely to engage in activities such as driving despite impairment in coordination and judgement which can lead to road traffic accidents. The Advertising Standard Council of India (ASCI) regulates alcohol advertising in India. Although it bans both direct and indirect alcohol advertising in traditional media, it does not cover online alcohol advertising. Therefore, alcohol advertising on social networking sites remains unfettered and is thus extensive in India. Private channels often permit alcohol companies to advertise using surrogate means such as soda, water or music. Most of the movies and web series in OTT(over-the-top) platforms (like Netflix, Amazon prime, Hotstar etc) depict alcohol as a soul soother during stress, relationship breakups and much needed substance in times of celebrations. Associating alcohol use with social, sexual, and financial success with little depiction of the hazards of drinking such as poor cognition, memory, judgement or discouragement of drinking, in the media must be avoided. Much of the content features youth-oriented themes, in the interest of creating "intoxigenic" or "alcogenic" environments that normalize youth drinking behavior and promote a culture of alcohol use.[9]

Pediatrician's Role: Primary prevention by providing anticipatory guidance to adolescents regarding hazards of alcohol use and to educate parents regarding digital media literacy so that they can decode media images/messages. Also have an important advocacy role in health system changes as well as legislative efforts, such as increasing alcohol taxes and efforts to weaken minimum drinking age laws.[10] The legal age for drinking alcohol in different states of India is depicted in Table I. Secondary prevention is helping to identify and screen adolescents with alcohol use and treating them appropriately with multidisciplinary approach. One of the tool specific for alcohol use disorder (AUD) management is AUDIT (The Alcohol Use Disorders Identification Test).[11] Tertiary prevention by providing social reintegration facilities for severe AUD.

Electronic nicotine delivery systems (ENDS) or e-cigarettes have become popular among adolescents. Their use has been increasing globally at an alarming rate and the facts related to ENDS are listed above in Box 1.

Table I. The legal age for drinking alcohol in different states of India

Age in years	States and Union Territories
> 18	Sikkim, Puducherry, Karnataka, Himachal Pradesh, Goa
> 21	Andhra Pradesh, Arunachal Pradesh, Assam, Chhattisgarh, Jammu and Kashmir, Jharkhand, Kerala, Maharashtra, Orissa, Rajasthan, Tamil Nadu, Uttarakhand, Uttar Pradesh
> 25	Punjab, Meghalaya, Delhi, Haryana, Chandigarh
Ban On consumption Alcohol	Gujarat, Lakshadweep, Manipur, Nagaland, Bihar

Box 1. E-cigarettes

- E-cigarettes are battery powered devices used to smoke or vape.
- Contain nicotine and other harmful chemicals as flavouring agents.
- Result in nicotine addiction, nicotine toxicity.
- Harmful long-term effects to the developing brain, negative effects on cognition.
- E-cigarette or vaping, product use-associated lung injury (EVALI) and death, depending on the ingredients included in e-cigarette fluids.
- Use of ENDS can open a gateway for new tobacco addiction which is a potential threat to the country's tobacco control laws and on-going tobacco control programmes.
- US Preventive Services Task Force (USPTF) behavioural interventions may reduce the likelihood of smoking initiation in non-smoking children and adolescents.[12]
- Indian council of medical research has completely prohibited (including procuring, production, marketing, promotion and sale) ENDS or e-cigarettes.[13]
- Rajya Sabha has completely banned ENDS in India on September 18th 2019.

Vulnerability of adolescents to substance use

Neurodevelopmental vulnerability of adolescents to drug use exists in adolescence. There is a general "imbalance" in functional development across brain regions, with earlier development occurring in posterior regions (reward centre) and anterior regions (prefrontal cortex) progressing later, which leads to underdeveloped connections between mid-brain cortico-limbic (reward) and frontal (inhibitory) region circuits. This "imbalance" enables heightened risk-taking behaviour, particularly when the behaviour results in immediate rewards.[14,15] Adolescents with high alcohol consumption, on long-term drug abuse are noted to have structural changes in the brain associated with cognitive impairment and anxiety-like behaviour.[16] The risk factors and protective factors for substance use is given in Table II.

Having a family member who uses substances, enjoyment and curiosity are cited as major influences in decisions by adolescents to use substances.[17]

Role of Pediatrician

Screening for SUD

The primary care setting provides a unique opportunity to screen adolescents for SUD. The HEEADSSS interview focuses on assessment of the Home environment, Education and Employment, Eating, Activities-peer related, Drugs, Sexuality, Suicide/depression and Safety from injury and violence. During HEEADSSS screening of an adolescent, anticipatory guidance and reinforcing counselling on avoidance of drug use should be given to abstinent adolescents. Non specific red flag signs and specific findings of drug use are enlisted in Box 2 and Table III respectively.

Table II. Risk factors and protective factors for substance use

Risk factors	Protective factors
Experimentation, curiosity, peer pressure	Prosocial peers
Lack of awareness	Home support, school support
Poor parental monitoring	Self-awareness, self-esteem
Family influences	Problem solving capacity
Gang affiliation	Peer caring relationships
Absenteeism, below-average academic grades	Community support, religiosity
Oppositional defiant disorder (ODD), attention deficit hyperactivity disorder (ADHD), or conduct disorder (CD)	School attendance, parental or guardian connectedness and parental supervision
Anxiety or depression, stress, emotional struggles and a desire to escape	Peer support at school

Box 2. Nonspecific red flag signs

- Efforts to mask the smell as evidenced by frequent rinsing and washing hands, use of perfume or deodorant, chewing mint.
- Avoiding eye contact and hug by parents.
- New set of (senior) friends.
- Social isolation with loss of interest in activities which the teenager was enjoying earlier.
- Scholastic deterioration.
- Poor hygiene, altered appetite and sleep pattern.
- Changed preference for movies and music which depict high action and drug abuse.
- Unexplained irritability and increasing conflicts with parents and teachers.
- Spending extra time in toilet.
- Stealing money/valuables from the house.

Therapeutic intervention

Pediatricians should have a high index of suspicion for substance abuse. The gold standard for making a diagnosis of substance use disorders are based on DSM 5 criteria[18] as given in Table IV.

Such an adolescent can present in an outpatient, inpatient or emergency setting. In 2016, American Academy of Pediatrics endorsed the simple to use and implement Screening Brief Intervention Referral to Treatment (SBIRT) model to manage substance use in adolescence. Detailed history of comorbid high-risk behaviour needs to be taken and look for co-occurring mental disorders. Adolescents who abuse drugs, particularly those involved in the juvenile justice system, should be screened for other psychiatric disorders. Specific drug history including type of the drug/s used, extent of use, setting of use and degree of social, educational and vocational disruption should be elicited.

The Screening to Brief Intervention (S2BI) tool uses a stem question and forced-response options (none, once or twice, monthly and weekly or more) in a sequence to

Table III. Drug specific clues

Drug used	Physical symptoms	Look for
Alcohol	Black outs, gastritis, slurred speech, relaxed inhibitions, impaired coordination	Smell of alcohol on clothes or breath, intoxicated behaviour, hangover, glazed eyes
Marijuana	Flu like symptoms, red conjunctiva, abnormal pupils, increased appetite for sweets, gynecomastia, small testes and irregular periods	Rolling papers, pipes, dried plant material, odour of burnt hemp rope, roach clip
Cocaine	Brief intense euphoria, raised blood pressure and tachycardia, restlessness, excitement	Glass vials, glass pipes, razor blades, white crystalline powder, syringes, needle marks
Hallucinogens (Lysergic acid diethylamide, Psilocybin, MDMA (Methylene Dioxy Meth Amphetamine)	Altered mood and perceptions, focus on detail, anxiety, panic, nausea, synaesthesia (e.g. smell colours, see sounds)	Capsules, tablets, microdots, blotter squares
Narcotics (opium, heroin, codeine)	Euphoria, drowsiness, insensitivity to pain, pinpoint pupil, cold moist skin, runny nose	Needle marks on arms, needles, syringes, spoons
Stimulants (amphetamines, nicotine, caffeine)	Alertness, talkativeness, wakefulness, increased blood pressure, chest pain, tachycardia, loss of sleep and appetite, hyperactivity	Pills and capsules
Date rape drugs e.g. Flunitrazepam, Ketamine, Barbiturates	Amnesia	Tablets, ampoules, syringes

- Use the Screening to Brief Intervention Tool (S2BI Tool) given below:[19]

Screening	Brief Intervention	Referral to Treatment
Quickly assess the severity of substance use and identify the appropriate level of treatment.	Increase insight and awareness of substance; motivation toward behavioural change.	Provide those identified as needing more extensive treatment with access to specialty care.

reveal the frequency of past-year use of tobacco, alcohol, marijuana.

Management according to severity of drug use is as follows:

1. No substance use: Pediatricians should give positive reinforcement, encourage being 'drug free' and discuss the risks of drug use and skills to withstand negative peer pressure.

2. No SUD: Pediatrician should give brief advice regarding consequences of drug use. Discuss and deal with 'stressors' that trigger drug usage and reduce other risky behaviour.

3. Mild / moderate SUD: Here the pediatrician should give motivational intervention. The goal of motivational interviewing is to assess the patient's readiness to make a change, help him/her to identify reasons for change and support his/her autonomy to do so. The desired change may be discontinuation of substance use or may focus on risk reduction, depending upon the patient's level and risks of use.[20] This is based on the principles of expressing empathy, developing discrepancy between life goals and the need to use drugs which could be stumbling blocks towards reaching the goals, enhancing self-efficacy to resist drug use and rolling with resistance; if the adolescent refuses to get motivated to decrease or stop drug use.

Table IV. DSM 5 has following criteria for diagnosing substance use disorders

1. Substance is often taken in larger amounts and/or over a longer period than the patient intended.
2. Persistent attempts or one or more unsuccessful efforts made to cut down or control substance use
3. A great deal of time is spent in activities necessary to obtain the substance, use the substance, or recover from effects.
4. Craving or strong desire or urge to use the substance.
5. Recurrent substance use resulting in a failure to fulfil major role obligations at work, school, or home.
6. Continued substance use despite having persistent or recurrent social or interpersonal problem caused or exacerbated by the effects of the substance.
7. Important social, occupational or recreational activities given up or reduced because of substance use.
8. Recurrent substance use in situations in which it is physically hazardous.
9. Substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance.
10. Tolerance, as defined by either of the following: a. In order to achieve intoxication or desired effect, the need to increase the amount of the substance used. b. Markedly diminished effect with continued use of the same amount.
11. Withdrawal, as manifested by either of the following: a. The characteristic withdrawal syndrome for the substance; b. The same (or a closely related) substance is taken to relieve or avoid withdrawal symptoms; **Mild:** 2-3 symptoms present **Moderate:** 4-5 symptoms present **Severe:** 6+ symptoms present

The CRAFFT questionnaire is a brief screening test for adolescent substance abuse. CRAFFT questionnaire is used to identify adolescents with a serious problem of substance use, who need an in depth assessment of staging and motivation level. Each 'Yes' item is scored as 1. A score >2 indicates a high risk use and the need for psychiatric referral (Box 3).

4. Severe SUD: Pediatrician should refer such cases to an adolescent friendly psychiatrist for cognitive behaviour therapy, motivational intervention, family therapy in the form of parental support, love, better communication, reduced blame and encouragement for the change.

Box 3. CRAFFT Screening Questionnaire[21]

C Have you ever ridden in a car driven by someone who was high or had been using drugs?

R Do you ever use drugs to relax, feel better or fit in?

A Do you ever use drugs when you are alone?

F Do you ever forget things while using drugs?

F Do your family / friends ask you to cut down on drug use?

T Have you ever got into trouble while using drugs?

Prevention of drug use: Pediatrician can play a vital role in prevention of substance use by adolescents at office level by screening for substance use. Recent meta-analysis by USPTF (United States Preventive Task Force) suggests that behavioural interventions can prevent substance use but its effectiveness in addicts needs further research.[12] Initiatives at the school level include advocating life skills education programs for students and creating awareness among teachers and at the government level, to press upon formulating better laws against drug use.

Conclusion

Substance use disorder is the most commonly missed diagnosis in adolescent children. Substance abuse is a medico-social economic problem which can start during adolescent life and track into adulthood. Pediatricians

should partner with parents, school, mental health professionals and community to help prevent it. They can play a vital role in early recognition and treatment /referral. Treatment requires a multidisciplinary approach along with parental and peer support.

Points to Remember

- ***Substance use in adolescents begins as a result of curiosity or peer pressure.***
- ***The primary care pediatrician plays an important role and has an unique opportunity to screen adolescents for SUD.***
- ***Creating awareness among adolescents, parents and teachers is the need of the hour.***
- ***Pediatricians should screen every adolescent for substance use.***
- ***Treatment requires a multidisciplinary approach along with parental and peer support.***
- ***Behavioural interventions help in prevention of substance use.***

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RADIOLOGY

RADIOLOGICAL EVALUATION OF GASTROINTESTINAL FOREIGN BODIES

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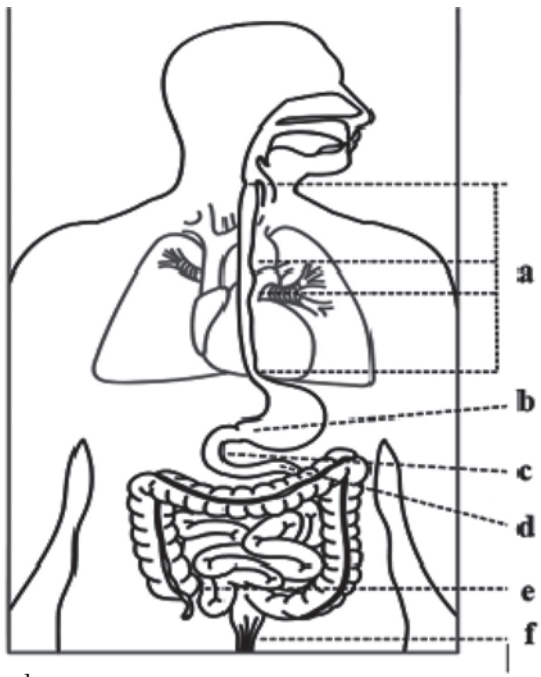
Accidental ingestion of foreign bodies (FBs) is very common in children. Most of the events occur in children of age group between 6 months and 3 years (rare beyond 5 years). Ingested foreign bodies can be either radiopaque or radiolucent. The X-ray is used as initial imaging technique as well as for serial follow up to trace the radiopaque foreign bodies in gastrointestinal tract.

Radiopacity and radiographic visibility are the two factors to be considered in the radiological diagnosis of ingested foreign bodies. Radiopacity is recognized as the intrinsic feature of the ingested object based on its ability to absorb the emitted X-rays. It represents the white area on the X-ray, a tissue or structure within the patient which attenuates the primary beam of X-rays more than the adjacent tissue or structures. Radiographic visibility depends on the X-ray attenuation characteristics of the object and its surrounding structures through which the X-ray photons pass to reach the detector. Plastic and organic foreign bodies (like wood) appear radiolucent. Foreign bodies like stone are usually radiopaque. All glass foreign bodies appear radiopaque of varying degrees of radio density. Metal foreign bodies are always radiopaque (except thin aluminium metal).

Radiopaque foreign bodies can be picked up quite easily by the preliminary radiograph images which help us to recognize the size and shape of FBs as well as the current location in the gastrointestinal tract (GIT). This aids in prompt management. When measuring the size of ingested foreign body we need to take into account the radiographic magnification. Usually the measurements made on radiographs are slightly larger than the actual size.

Fortunately, around 80-90% of FBs pass spontaneously without complications and only few (around 10%) of FBs find it difficult to pass through the pylorus, duodenum, ileocecal valve, Meckel's diverticulum (if present) and anus landmarks (Fig.1). About 10%-20% need endoscopic removal and rarely some 1% require open surgery secondary to complications Parameters to be considered regarding the timing of endosopy in children with ingested FBs are age, body weight of the child, clinical presentation, time lapse since ingestion, time since the last



a) Esophagus –
b) Pylorus
c) Duodenal C cap
d) Ligament of Treitz
e) Ileocaecal valve
f) Rectosigmoid region

Fig.1. Schematic representation of anatomical location of various levels of foreign body impaction in GIT.[1-5]

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meal, type, size, shape of FB and finally its present location in GI tract. If the circumstances are not an emergency and there is no absolute indication for the endoscopic removal of the FB then the risk –benefit ratio ought to be considered.

Depending on the region of interest, the required radiograph is selected as follows.

- Neck with chest radiograph for FB in cricopharynx,
- Chest with upper abdomen radiograph for FB in oesophagus
- Abdomen radiograph for stomach, duodenum, small and large intestinal foreign bodies.

Remember all X-rays taken for detecting FBs require two views (Antero posterior and lateral views) which help to localize in two planes. Serial X-rays are taken for follow up to ensure the spontaneous expulsion of foreign body.

Significance of each anatomical site is given in Box 1.

Box 1. Significance of each anatomical site.

a) Esophagus: Cricopharyngeus muscle is the most common site of esophageal impaction (75%). FB at or above cricopharyngeus muscle warrants otorhinolaryngology consult.

b) Aortic arch, left main bronchus and gastroesophageal junction are also common levels of esophageal impaction. Any FB in the esophagus should be removed, except for coins, which can be observed for 12-24 hours before endoscopic removal.

c) Pylorus: Blunt object with width >2.5 cm have difficulty passing through and should be removed endoscopically.

d) Duodenal C-loop: Blunt object with length >6 cm have difficulty passing through and should be removed endoscopically.

e) Ligament of Treitz: Most common location of perforation by long ingested object.

f) Ileocecal valve: Common site of obstruction.

g) Rectum/sigmoid colon: Objects longer than 10 cm or object located in sigmoid colon are associated with failure by transanal extraction.

Locations and imaging

Cricopharynx and esophagus

Most common location for ingested foreign body impaction is in the upper esophagus at cricopharynx or the narrowest part of esophagus. The pharyngoesophageal junction is the constriction produced by the cricopharyngeal part of inferior constrictor muscle. This cricopharyngeal region is the narrowest part of esophagus.

Ingested foreign body impaction within the upper esophagus, at the level of cricopharyngeus muscle (Fig.2a and 2b) accounts for approximately 75% of all foreign body impaction cases. Coins, meat and fish bones are the commonest foreign bodies stuck in cricopharynx. Most of the cricopharynx and esophageal foreign bodies pass out spontaneously. Endoscopic removal is required for those foreign bodies which do not pass out even after 24 hours. Cricopharynx and esophageal foreign bodies if

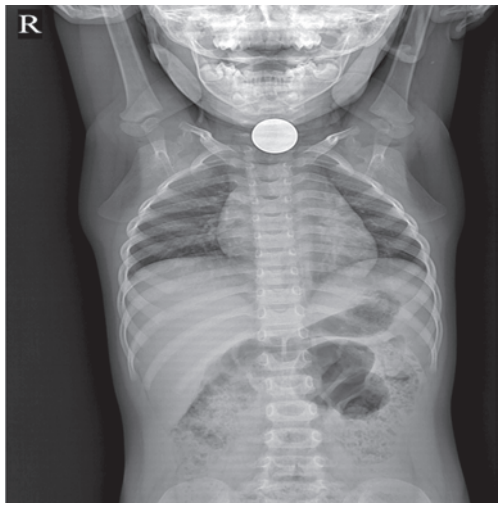

Fig.2a. AP view neck shows round radiopaque foreign body (coin) lodged at level of cricopharynx

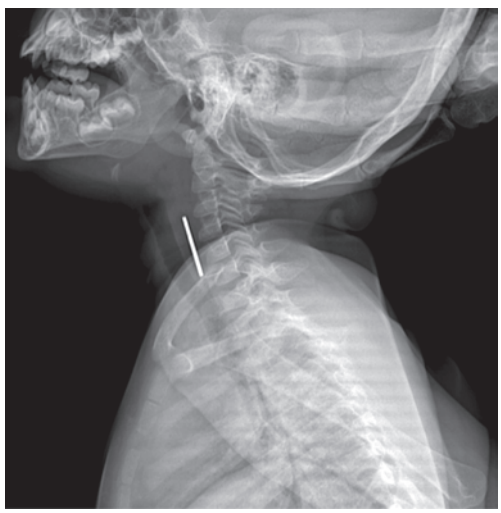

Fig.2b. Lateral neck radiograph shows thickening of prevertebral soft tissue at the C5-C7 level associated with an impacted FB.

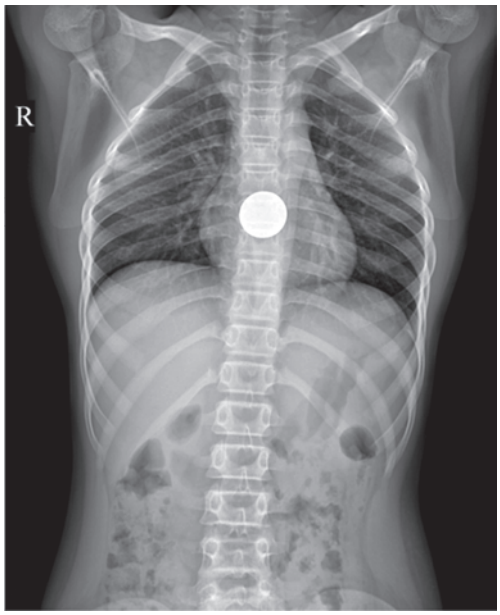

Fig.3a. AP view chest shows round radiopaque foreign body (coin) lodged at level of mid esophagus.

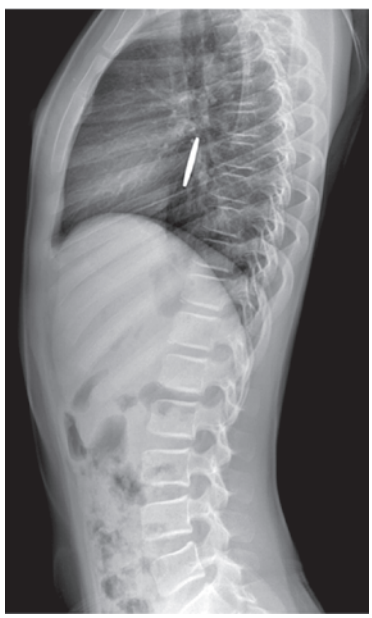

Fig.3b. Lateral view shows coin lodged in mid esophagus level. Note the foreign body is coronally oriented and lies behind the respiratory tract.

retained result in major complications like retropharyngeal / prevertebral abscess, esophageal obstruction / perforation and mediastinitis. Rarely aortoenteric fistula can occur with button battery in esophagus.

Foreign bodies at or above the cricopharyngeus muscle necessitate otorhinolaryngology consultation. If below the

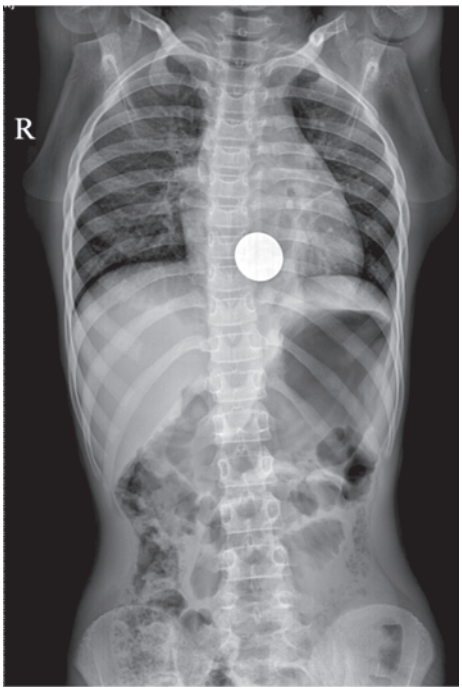

Fig.4. Lower esophagus foreign body. AP view chest shows round radiopaque foreign body (coin) lodged at level of lower esophagus above lower esophageal sphincter.

cricopharyngeus muscle they can be removed by endoscopy.

Other common locations of esophageal foreign body impaction include the level of the aortic arch, left main bronchus (Fig.3a and b), or gastroesophageal junction (Fig.4). Once the objects have made their way through the gastroesophageal junction, they usually have no problem progressing through the remainder of the gastrointestinal tract. In total, less than 10% of impaction occurs distal to the gastroesophageal intestinal junction. Depending on the size and shape of the foreign bodies, other potential regions of the foreign body obstruction in the gastrointestinal tract include the pylorus, duodenal C- loop and ileocecal valve. Many commonly swallowed objects can be radiolucent and may still be invisible on radiographs, such as thin fish or chicken bones, plastic, wood and thin aluminium objects (such as carbonated soft drink tabs). Therefore, negative result radiographs, in the setting of high clinical suspicion for foreign body ingestion, should not preclude further evaluation with either CT or endoscopy.

When a circular coin foreign body appear as a circle on a frontal radiograph and is located on the posterior wall of the cricopharynx on a lateral radiograph, the coin is visualized as remaining in the esophagus. As a rule of thumb, coins visualized in the sagittal plane (acquired while entering through vocal cords) on anteroposterior radio

graphs are in the trachea whereas coins in the esophagus takes the coronal orientation on anteroposterior radiograph.

Lower esophageal foreign body lies just proximal to lower esophageal sphincter and above the diaphragmatic outline (Fig.4). Coins are by far the most common type of ingested foreign body in children. Fortunately, because they lack sharp edges and are generally nontoxic, ingested coins that reach the stomach can be managed conservatively. However, ingested coins that are lodged in the esophagus or stomach and causing symptoms or coins not causing symptoms but fail to exit the esophagus after 24 hours or the stomach after 4 weeks usually require endoscopic removal.

Unlike other blunt objects, button batteries in the esophagus require emergency endoscopic removal even without symptoms of severe impaction.

Stomach

FB in stomach often pass in 4 to 6 days and require conservative outpatient management in majority, although some recommend early endoscopic removal. Children with gastric foreign body like coin should be followed up with advice to take regular diet and observe their stools for evidence of passage of the object in the stool. In the absence of symptoms, weekly radiographs are sufficient to follow the progression of small blunt objects that are yet to pass because this may take as long as 4 weeks (Fig.5a and 5b).

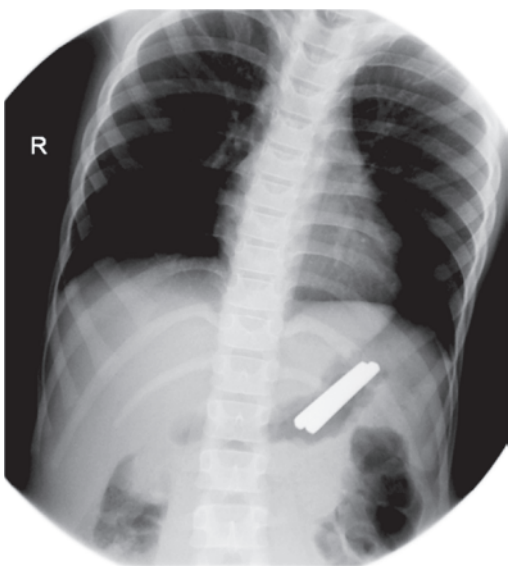

Fig.5a. AP view lower chest with abdomen shows longitudinal blunt radio opaque(metal) foreign body (longitudinal brass dice) lodged within the stomach air shadow.

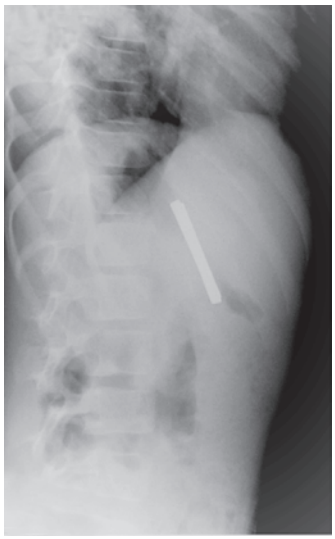

Fig.5b. Lateral view confirms the foreign body is within the stomach air shadow. Note the foreign body is oriented and lies within the stomach lumen.

Button batteries may mimic coins on radiographs. Some helpful ways to differentiate the two are double contour, “halo” sign on frontal projection (Fig.6a) or “step-off” sign on lateral projection (Fig.6b). Step off signs means the asymmetry of both sides of button battery, because negative terminal diameter is smaller than the positive terminal’s diameter.

After button batteries Fig.6c and 6d have passed through the esophagus, the majority of them progress without complications and can be followed with radiographs every 3 to 4 days. The lithium button batteries larger than 2 cms are the ones that cause major fatal complications. Endoscopic removal is recommended. Once batteries pass the GE junction, initial follow-up radiograph is to be done at 48 hrs. When the foreign body crosses pylorus, then radiograph is repeated every 3-4 days to ensure its movement. Magnets retained in the stomach in symptomatic children require removal within 2 hours. In asymptomatic children, they should be removed within 24 hours. Multiple magnets are more dangerous as they can lead to complications like bowel necrosis, obstruction, perforation and hence prompt intervention is important.

Duodenum

Coins and batteries mostly pass through the duodenum. Blunt objects longer than 6 cm proximal to duodenum (Fig.7) usually have difficulty passing the duodenal C-loop and warrant urgent endoscopic removal. The most common site of perforation by the long objects is near the ligament of Treitz. Deliberate efforts are required

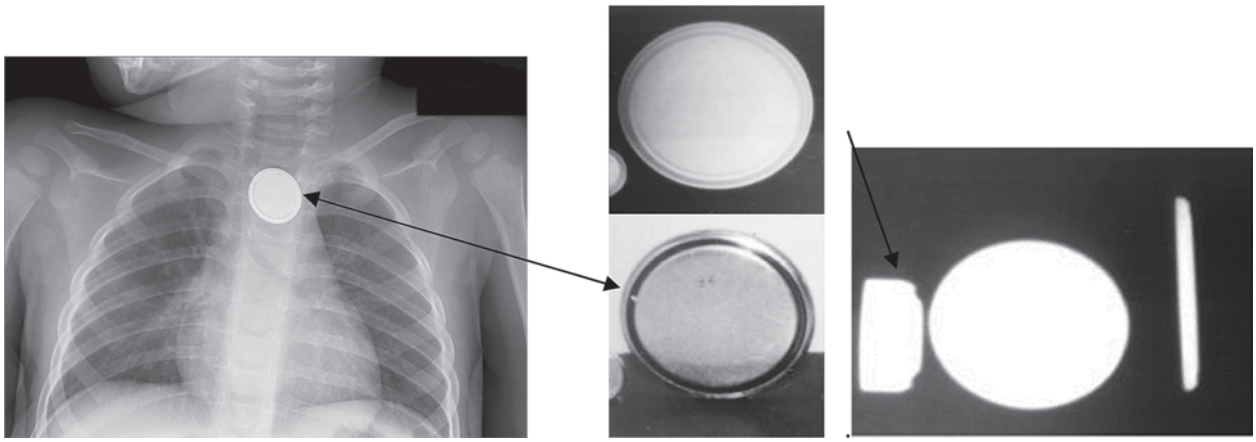

Fig.6a. Double halo or rim sign shown by arrow

Fig.6b. Coins will appear as a singular rectangular opacity on the lateral view, a button battery will have a subtle step-off, because negative terminal diameter is smaller than the positive terminal's diameter

Source: Loren G. Yamamoto, Inaba AS. A Second Look at a Coin in the Stomach. Radiology Cases in Ped Emerg Med 2, Case 9.

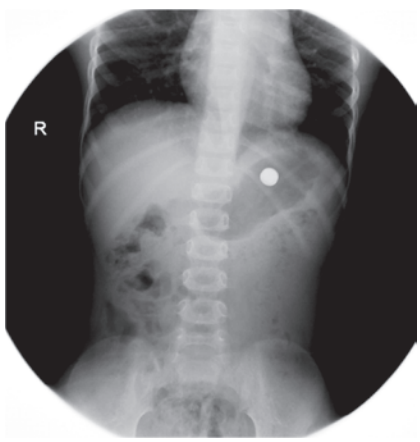

Fig.6c. AP view lower chest with abdomen shows a round radio opaque (metal) foreign body (button battery) lodged within the stomach air shadow.

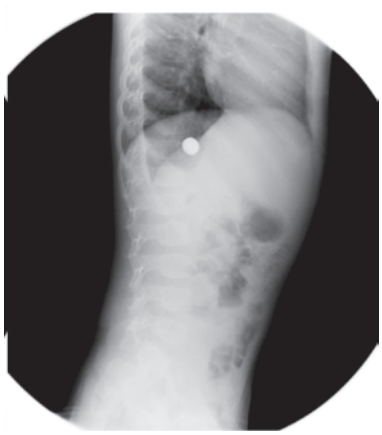

Fig.6. (d) Lateral view confirms the foreign body is within the stomach air shadow. Note the "step - off" sign on lateral projection.

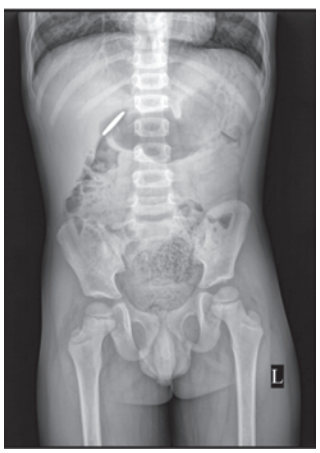

Fig.7. Duodenal foreign body AP view abdomen shows a sagittally oriented radiopaque (metal) foreign body (coin) lodged within the first part of duodenum.

to ingest long foreign bodies, such as toothbrushes, pencils and utensils. Such incidents are often intentional and occur more frequently in patients with psychiatric illness. Both short and long blunt objects share the same conservative management and imaging follow-up.

Small bowel

Foreign bodies in small bowel loops appear to lie in mid line or paramedian location in antero posterior view (Fig.8a). In lateral view, the foreign body lies just anterior to spine and sometimes large foreign bodies overlap spine shadow (Fig.8b).

Most FBs in small bowel are passed spontaneously without complications. Therefore, physicians should reassure the children and / or caregivers and advise them

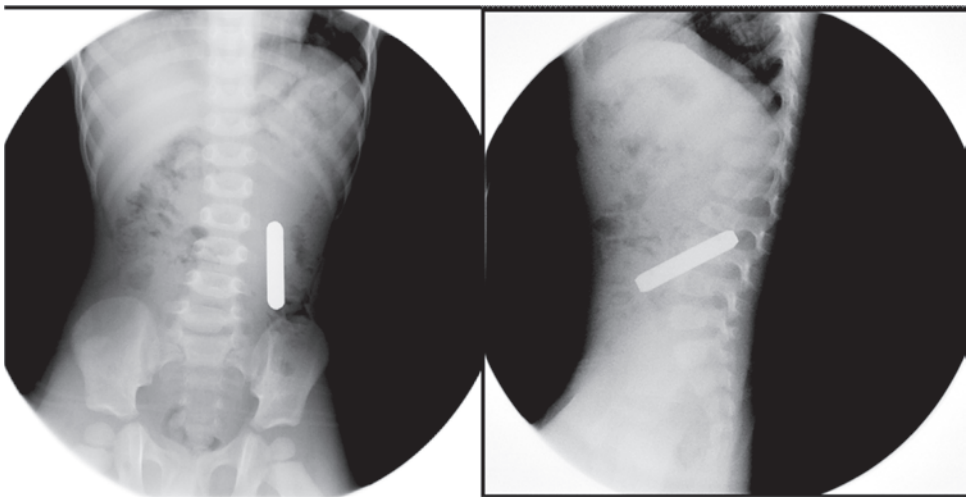

Fig.8a. AP view lower chest with abdomen shows longitudinal blunt radiopaque (metal) foreign body (longitudinal brass dice) lodged within the small bowel loops.

Fig.8b. Lateral view confirms the foreign body is within the small bowel. Note the foreign body is oriented cranio caudal and antero posterior overlapping both the spine (posteriorly) and the small bowel loops (anteriorly).

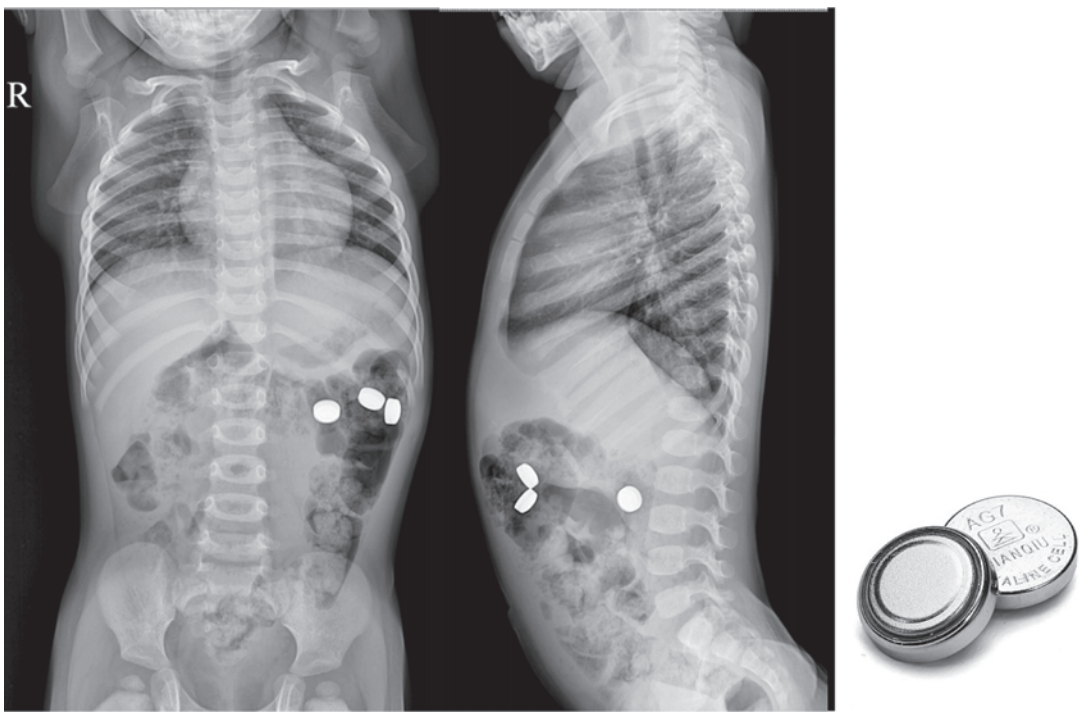

Fig.9a. AP view of abdomen shows a round radioopaque (metal) foreign body (button batteries) lodged within the distal transverse colon and splenic flexure region. All the button batteries are within the large bowel as there is no interposed soft tissue between the two opacities.

Fig.9b. Lateral view confirms the foreign body is within distal transverse colon and splenic flexure region air shadow. Note the foreign body lies within the bowel air anteriorly (transverse colon) and no overlap on spine. Also note the step off sign (both sides of the button battery looks asymmetric unlike coin) on lateral projection.

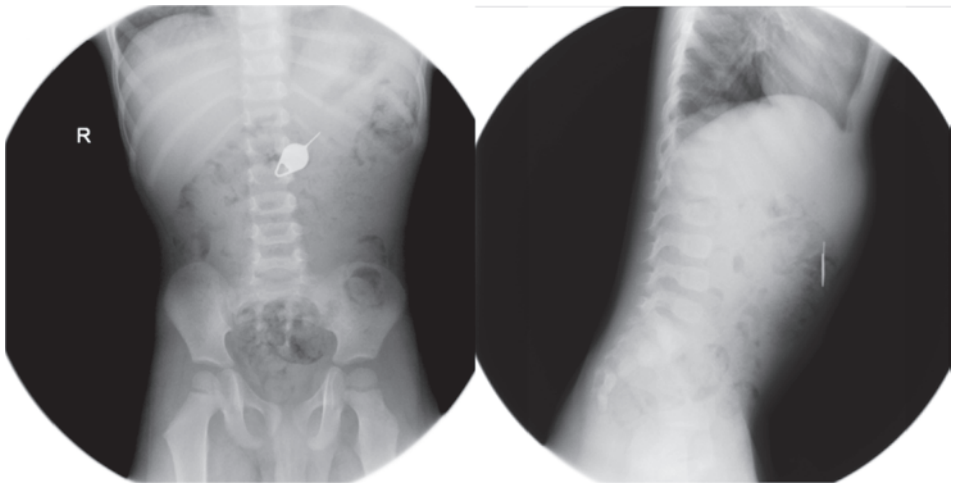

Fig.10a. AP view of abdomen shows a radiopaque (metal) foreign body (Sim card remover) lodged within the transverse colon region.

Fig.10b. Lateral view confirms the foreign body is within transverse colon region air shadow. Note the foreign body lies within the bowel air anteriorly (transverse colon) and no overlap on spine. Also note the foreign body is coronally oriented at present.

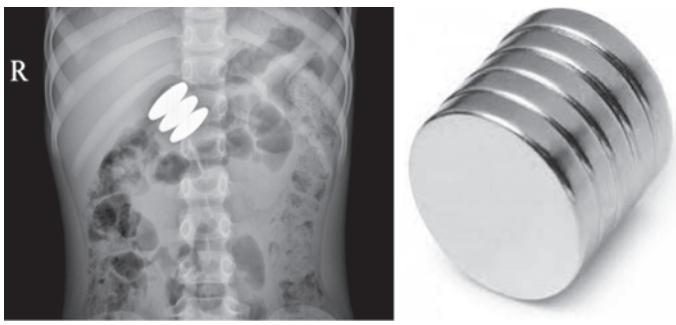

Fig.11. AP view of abdomen shows a radiopaque foreign body (neodymium magnets) lodged within the transverse colon region. The shape and appearance of the foreign body helps us in identifying the object ingested and also idea of sharp or blunt foreign body. Piled up neodymium magnets are carefully monitored by serial radiographs, if no distal progress is noted, then immediate surgical removal is needed

to check the children's stool for the FB. If the FB is not eliminated even after a week, children need to visit the hospital and obtain an x-ray to identify the accurate location of the FB. Children should be strictly advised of the need to visit the hospital earlier if they develop signs of perforation or obstruction of the intestine, such as vomiting, severe abdominal pain, fever or intestinal bleeding.

Large bowel

Foreign bodies are seen within the large bowel shadow (Fig.9a) and confirmed by the lateral view. In lateral view, foreign bodies within the transverse colon are seen anteriorly whereas foreign bodies that lie in ascending or descending colon and flexure are seen posteriorly (Fig.9b). Button batteries in (Fig.9c).

Majority of foreign bodies that reach the large bowel are spontaneously expelled without much complications. The hurdles of foreign body in large bowel loops are hepatic flexure, splenic flexure, recto sigmoid junction and anal canal. Most of the foreign bodies easily pass through these regions with few exceptions. The shape and appearance of the foreign body helps us in identifying the object ingested and also idea of sharp or blunt foreign body. (Fig.10a and 10b).

When multiple magnets or a pair of magnetic and metal objects is ingested, there is a risk for bowel wall pressure necrosis caused by the attractive force between the two objects (Fig.11). Devastating complications such as fistula, perforation, obstruction, volvulus and peritonitis have been reported. It may be difficult to discern the number of ingested objects from radiographs. A summary of recommendations for follow up imaging is given in Table I.

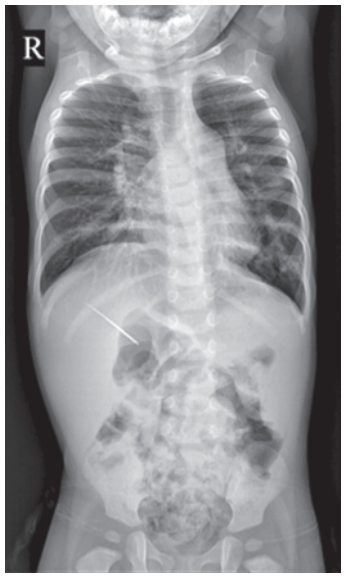

Fig.12. AP view of abdomen shows a radiopaque (metal) foreign body (sharp needle) appear overlapping the liver probably pierced the duodenum and entered the liver parenchyma. Minimal right subdiaphragmatic air pocket is seen. The sharp appearance of the foreign body is clearly depicted in radiograph which helps to decide the further course of management.

Table I. Summary of recommendations for imaging follow-up for various types of ingested foreign bodies.[6]

Foreign body types	Imaging follow up protocol
Sharp foreign body	Daily radiograph for up to 3days Consider CT for radiographically invisible FB or evaluation of complications (e.g. abscess)
Blunt foreign body	Weekly radiograph for up to 4 weeks
Coins	Weekly radiograph for up to 4 weeks
Batteries	Once batteries past GE junction, initial follow-up radiograph at 48 hours. Once past the pylorus, repeated radiograph every 3-4 days
Magnets	Close follow up with frequent serial radiographs to ensure mobility and multiple magnets need immediate referral

Sharp foreign body

When a sharp foreign body is present within the esophagus, emergency endoscopic removal is indicated. If the object has passed through the gastro esophageal junction but remains within reach of the endoscope (such as in the stomach or duodenum), urgent endoscopic retrieval is recommended as long as the object can be withdrawn safely. Once sharp objects pass the duodenum, up to 35% of them can lead to perforation; therefore, they should be followed with daily radiographs to document passage. Surgical removal is recommended if the patient becomes symptomatic or if sharp objects fail to progress after 3 days and are beyond endoscopic reach.

The sharpness of the FB can be easily be made out by radiographs and determines the further course of management (Fig.12).

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CASE REPORT

HERBS AND HEMOLYSIS

**** Shyamala Jayamoorthy***
***** Revathi Raj***

Abstract: *Glucose 6 phosphate dehydrogenase is an important enzyme preventing oxidative damage to red blood cells. While hemolysis induced by exposure to various medications in G6PD deficient individuals is well recognized, less well known is the same phenomenon triggered by exposure to herbs. We present here an infant with this rare clinical presentation.*

Keywords: *Hemolysis, G6PD deficiency, Herbs, Acalypha indica.*

A 7-month-old male infant born of non-consanguineous marriage presented with cola coloured urine following a home made herbal extract given for an upper respiratory infection the previous day. There was no preceding fever or drug intake. The infant was pale, minimally icteric, slightly tachypneic and tachycardic but hemodynamically stable on admission. Abdominal examination revealed a soft splenomegaly 1 cm below left costal margin.

Urine routine showed 1-2 RBCs / HPF, urine for hemoglobin was 602 mgs%, serum LDH was 1600 IU/l. Complete blood count revealed hemoglobin of 6 gms%, platelets count of 3.74 lakhs, reticulocyte count of 10 %. The peripheral smear showed normocytic normochromic red cells with marked polychromasia, red cell fragments with blister and bite cells, absolute neutrophil leucocytosis with shift to left. Platelets were normal. Smear was suggestive of hemolysis compatible with that of oxidant stress (as seen in G6PD deficient patients). Direct Coomb's test was negative. Liver function tests revealed raised indirect bilirubin 3.3 mg/dL (total - 4.8mg/dL), alanine aminotransferase level - 68 U/L, aspartate aminotransferase level - 45 U/L, alkaline phosphatase level - 499 U/L and γ glutamyl transferase 12 U/L. Urine C/S was sterile.

Clinical presentation with hemoglobinuria, anemia, increased indirect bilirubin and increased reticulocyte count pointed towards intravascular hemolytic anemia. Intravascular hemolysis may occur from mechanical trauma (as in prosthetic valves), complement fixation, or other toxic damage to the red blood cells. Free hemoglobin binds to circulating haptoglobin and is degraded and cleared by the liver. When haptoglobin becomes saturated by hemoglobin, unbound free hemoglobin is then excreted by the kidneys.

A negative Coomb's test and typical RBC changes on peripheral smear prompted a serum G6PD level estimation which was found to be very low - 4.3 U/gm Hb (8.8-18.4 U/g Hb in children 3 months - 12 years). He was given a packed cell transfusion and started on folate supplements. Following this, his Hb rose to 9.5 gms % and hemoglobinuria ceased.When we counseled the family on avoidance of drugs and other agents that could precipitate hemolysis, the parents volunteered that the home remedy was extracted from Indian nettle, Acalypha indica - known locally as Kuppameni Keerai (Fig.1).

Fig.1. Acalypha indica - known in Tamil as "Kuppameni Keerai"

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Acalypha indica is an annual perennial herb which is listed in the Indian Pharmacopoeia as an expectorant and also used in Indian traditional medicine as an emetic, laxative and antihelminthic. Phytochemial analysis of the leaves shows the presence of various ingredients like alkaloids, catechols, flavanoids, phenolic compounds, saponins and steroids.[1]

Glucose is the primary source of energy for the red cell, which is metabolized by two major routes-the glycolytic pathway and the hexose monophosphate (HMP) shunt. Glucose-6-phosphate-dehydrogenase (G6PD) is an X-linked enzyme that catalyzes the first step in the HMP pathway of glucose metabolism. It produces NADPH, required for the maintenance of reduced glutathione (GSH). GSH in turn protects red blood cells from oxidative damage.[2] Hence, G6PD is essential in red cell metabolism and its deficiency renders the red cell extremely vulnerable to any kind of oxidative stress.

Intravascular hemolysis could be triggered on ingestion of antimalarials, sulfonamides, INH, nitrofurantoin, fava beans or exposure to naphthalene balls in G6PD deficient individuals. Less commonly known is the fact that certain herbs can also trigger such a reaction. A systematic review in British Journal of Clinical Pharmacology looked at use of few widely used herbal supplements in G6PD deficient individuals. They concluded that incontrovertible evidence for undesirable effects exists only for henna.[3]

However, Acalypha indica induced hemolysis has been reported first from Sri Lanka[4] in 1992 and on occasions, even proving fatal.[5] Since then, other studies have been published including one from Tamilnadu.[6-8] Hemolysis following acalypha indica ingestion must occur due to oxidative damage to RBCs although it is not clear which ingredient causes this. In individuals with normal G6PD levels there is no hemolysis.[6] Senanayake et al reported ingestion of a broth containing acalypha indica by multiple family members, of whom only two needed packed cell transfusions while the rest remained well.

G6PD levels of the afore mentioned child was estimated a few months after the hemolytic episode - it continued to be low (3.5U/gm Hb). He has been under follow up for the last 5 years and has not had any further episodes of jaundice or hemolysis. His Hb electrophoresis was normal. His younger sister had a positive screen for G6PD deficiency in the newborn screen. Her G6PD level was estimated after 6 months of age and is in the deficient range. She has however been asymptomatic.

Use of native medicines for treating ailments is common practice in India. There is no prescribed 'dose or purity' when used for this purpose by household members especially in rural or semi urban settings where herbs are widely available. Following the diagnosis of G6PD deficiency in a child, the family requires to be counselled not only about the list of medications to be avoided but also warned against seemingly innocuous herbs that can cause life threatening hemolysis.

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CASE REPORT

ALLGROVE SYNDROME WITH A NOVEL MUTATION - CASE REPORT IN TWO SIBLINGS

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Abstract: *Allgrove syndrome (AS/Triple A syndrome) is a rare, familial, multisystem, potentially fatal autosomal recessive disorder characterized by achalasia, alacrimia and ACTH-resistant adrenal failure. There is significant heterogeneity in the clinical features and the types of mutations reported in families with Allgrove syndrome. Two siblings (ten- year-old girl and her six-year-old brother) presented with adrenal insufficiency, hyperpigmentation and alacrimia. Genetic exome sequencing revealed a homozygous variant of uncertain significance in exon 6 of the Triple A syndrome (AAAS) gene in the proband which was further confirmed by Sanger validation.*

The parents were found to be heterozygous, and the sibling homozygous for the tested variant of the Achalasia, Adrenocortical insufficiency, Alacrimia Syndrome AAAS gene. There was good response to replacement therapy with hydrocortisone.

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Keywords: *Adrenal insufficiency, Alacrimia, AAAS gene, ALADIN.*

Allgrove syndrome (AS) was described by Allgrove, et al in 1978 in four siblings all of whom had achalasia and ACTH insensitivity, three had impaired lacrimation and one had autonomic dysfunction.[1] There is a wide variation in the spectrum of disease with only two of the cardinal features being expressed in some patients. Associated neuropathy may be seen in some.[2] It is caused by mutations in the AAAS gene which encodes for a protein named Alacrima Achalasia Adrenal Insufficiency Neurologic disorder (ALADIN) located on chromosome 12 q13. The authors report two siblings with AS in whom a novel mutation was identified.

Case report

A 10-year-old girl born to non-consanguineous parents was referred for evaluation of fatigue, anorexia and progressive hyperpigmentation of hands and lips of 6 months' duration (Fig.1). Her milestones of development were normal. There was no history of recurrent vomiting, dysphagia or halitosis. Examination revealed a thin built girl weighing 18 kg (between 10-25th centiles). Her height was 119 cm (between 25-50th centiles), head circumference 51cm, and BMI was between 25-50th centiles as per IAP BMI centile chart. Her blood pressure was 90/60 mm Hg with a postural drop of 26/20 mmHg on standing with no

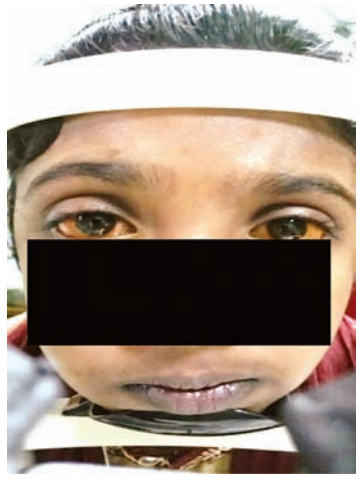

Fig.1. Patient 1. Note pigmented lips, muddy eyes and sparse eye lashes

compensatory tachycardia on repeated occasions. She had striking hyperpigmentation of tongue, lips, buccal mucosa and creases of palms and soles. Her conjunctivae were congested and muddy and eyelashes were sparse. Fundoscopy was normal. She did not have features of polyglandular syndrome.

Her systemic examination was unremarkable. Baseline investigations revealed normal complete blood counts, ESR, calcium, magnesium, creatinine and urea. Thyroid function tests, liver function tests and ECG were normal. Fasting blood glucose was 80 mg/dl, and electrolytes were normal (serum Na 142 mmol/L; serum K 4.1 mmol/L). Her 8 AM serum cortisol was very low (0.41 µg/dL), serum ACTH was very high (853; normal 15-64pg/ml). A negative Mantoux test and normal chest radiograph alongwith absence of adrenal calcification on abdominal CECT (Contrast Enhanced Computed Tomography) helped to exclude tuberculosis as the etiology of adrenal insufficiency.

A provisional diagnosis of Addison disease was made and she was put on replacement dose of oral hydrocortisone (15 mg/day) and topical eye lubricants along with supportive measures and periodic monitoring. Her ACTH became normal after 6 months, lethargy and malaise subsided and pigmentation decreased significantly.

Two years later, the proband's 6-year-old brother was also brought to us with similar complaints (Fig.2). His weight was 11.5 kg, height 93 cm (both < 3rd centile), and BMI was between 25-50th centiles. His head circumference was 46 cm which was in microcephaly range. His 8 AM serum cortisol was 4.4 µg/dL and serum ACTH 840pg/ml. His electrolytes and CECT adrenal were normal. He was also put on oral hydrocortisone and topical eye lubricants and he is also doing well.

Fig.2. Patient 2. Note microcephaly, pigmented lips and sparse eye lashes

The mother gave a vital clue for the diagnosis of AS- both children had red eyes ever since birth and she was happy as she thought that both were very good-mannered kids as they never shed tears even when crying aloud. Besides, both were initially evaluated by an ophthalmologist for absence of tears. Schirmer's test was positive. This is a bed side test which evaluates aqueous tear production in patients with signs and/or symptoms of dry eye. It can determine whether surface dryness is due to reduced tear production from the lacrimal glands as opposed to some other cause such as blepharitis, meibomitis and exposure.

Barium swallow was deferred as they did not have clinical features of achalasia cardia.

The diagnosis of AS was confirmed by clinical exome sequencing. In the proband, a homozygous variant of uncertain significance (VUS) was detected in exon 6 of the AAAS gene [c.530T>G (p. Val177Gly)]. It is a novel missense variant which was further confirmed by Sanger validation (Fig 3). Co-segregation of this variant was done in the parents and the symptomatic sibling by a Mutation Specific Test (Sanger Sequencing). The parents were found to be heterozygous, and the sibling homozygous for the tested variant of the AAAS gene.

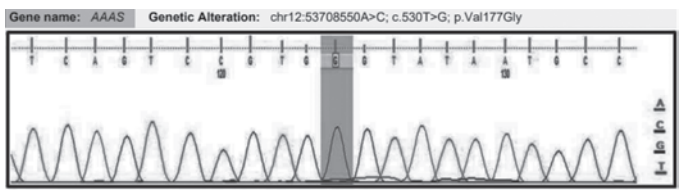

Fig.3. Sanger sequencing data (electropherogram) showing a homozygous nucleotide change 'T>G' at position c.530 in AAAS gene. This variation was confirmed by sequencing with both forward and reverse primers.

The homozygous missense substitution (p. Val177Gly) alters a conserved residue in the protein. It lies in the WD1 repeat region (residues 142-180) of the protein. It is predicted to be damaging by 5 (SIFT, LRT, Mutation Taster, PolyPhen-2 and FATHMM) out of 6 insilico missense prediction tools. The insilico (performed on a computer or via computer simulation) tools are used to predict the pathogenicity of missense variants.

The identified variant seems to be a novel variant, as it has not been previously reported in literature.

The co-segregation of the mutation into the family indicates it is disease causing with highest probability.

Variant c.530T>G (Val177Gly) was neither found in ExAC (Exome Aggregation Consortium) nor 1000G (1000 Genome) databases. Hence the probability that this mutation is a disease-causing one is very high.

Discussion

AS is the combination of adrenal insufficiency, achalasia cardia and alacrimia. As only about 100 cases have been reported so far, the exact prevalence is unknown.[1] The combination of AS with autonomic neuropathy is called 4A syndrome while that with both autonomic neuropathy and amyotrophy is called 5A syndrome.

The mutated Achalasia, Adrenocortical insufficiency, Alacrimia Syndrome (AAAS) gene which was identified by Tullio-Pulletet al and located on chromosome 12q13, is the molecular basis of AS. It codes for ALacrima Achalasia aDrenal Insufficiency Neurologic disorder (ALADIN) protein. This is a 60-KD protein with a 170-AA Domain composed of 4WD repeats. ALADIN is involved in the movement of molecules into and out of the nucleus, which may affect DNA repair mechanisms resulting in cell death.[3] Siblings with AAAS mutations may die suddenly due to undiagnosed adrenal failure.[4,5] A truncated protein is produced by most mutations although missense and point-mutations have also been reported. However, AAAS mutations may be absent in some patients with AS. There is no specific genotype-phenotype correlation.[6] A progressive loss of cholinergic function throughout the body may be the basic pathology of AS. As melanocortin receptors are known to regulate adrenal and skin exocrine gland function, melanocortin receptor signaling dysfunction is also possible.[7] The very low cortisol and very high ACTH in our patients confirmed glucocorticoid deficiency due to ACTH resistance. The normal serum levels of sodium and potassium imply normal mineralocorticoid function.

One of the early manifestations of AS is adrenal insufficiency. Alacrimia also may be an early symptom and dysphagia may occur early or late. This may present as severe hypoglycemic or hypotensive attacks resulting in sudden unexplained deaths during childhood. Patients with AS have a unique form of primary adrenal insufficiency in which mineralocorticoid production is preserved, but it may be impaired in about 15% of patients. The only other cause of primary adrenal insufficiency with intact mineralocorticoid synthesis apart from AS is familial glucocorticoid deficiency in which, however, adrenal insufficiency is the sole manifestation.[8]

A child with AS may present with any one of the four cardinal features like alacrimia, Addison disease, achalasia and progressive neurological dysfunction and the symptoms may evolve over a period of time.[3] Even though alacrimia is not a usual presenting feature, it may sometimes be the earliest and most consistent feature. Both our patients had alacrimia right from infancy.[9] It is important to do molecular analysis of AAAS gene in patients with one or more of the cardinal features of AS.[10]

Neurological manifestations which are relatively rare in Indian children may develop in older age. These include mental retardation, optic atrophy, amyotrophy, ataxia, dysarthria, dementia, and chorea. Parkinsonism is the most common extrapyramidal manifestation.[11] Dysphagia is due to achalasia cardia secondary to dysfunctional esophageal autonomic nerve plexus and degeneration of nerve fibers. Achalasia cardia which usually manifests as dysphagia especially for liquids is seen in about 75% of cases in older children and adults.

Some of the rare associations of AS include microcephaly, short stature, palmar and plantar hyperkeratosis, osteoporosis and long QT interval. Some may have dysmorphic features like long narrow face, long philtrum, down-turned mouth, thin upper lip and sparse eyelashes.[12] Both of our patients had sparse eye lashes and microcephaly.

A high index of suspicion is essential to make an early diagnosis of AS due to its extreme rarity and presentation with isolated features.[13] Molecular analysis of AAAS gene is a gold standard modality to confirm the diagnosis and end the diagnostic odyssey.[14]

Learning points for a general pediatrician

1. When a child presents with a relatively benign but unusual symptom like lack of tearing, one should enquire for malaise and dysphagia and look for pigmentation of skin. This may indicate the presence of an underlying life threatening illness and hence request for specialist consult to diagnose a rare genetic disease that may be amenable to treatment.

2. Advanced genetic tests like clinical exome sequencing are now easily available in our country.

3. The child may need long term follow up for neurological problems.

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CLIPPINGS

Endoscopic Removal of foreign bodies in the GI tract.

The timing of an intervention of foreign body (FB) removal depends on the patient's age, clinical condition, the size, shape, content, anatomic location of the ingested object(s), and the time since ingestion apart from judgment of the risks of complications like aspiration, obstruction or perforation in that child.

- Most of the time spontaneously passed and do not require urgent endoscopy.
- Esophageal FBs should be removed within 24 hours to prevent complications including risk of perforation.
- FB in stomach often pass in 4 to 6 days and require conservative outpatient management in majority, although some recommend early endoscopic removal. Children with gastric foreign body like coin should be followed up with advice to take regular diet and observe their stools for evidence of passage of the object in the stool. In the absence of symptoms, weekly radiographs are sufficient to follow the progression of small blunt objects that are yet to pass because this may take as long as 4 weeks.

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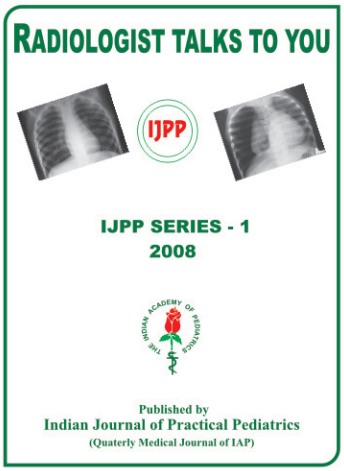

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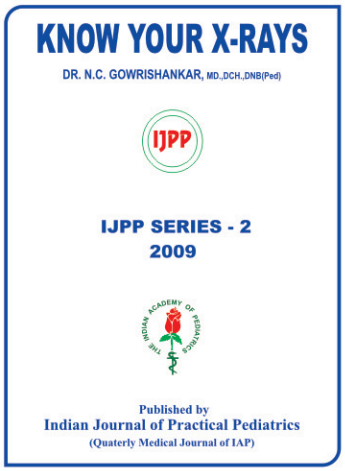

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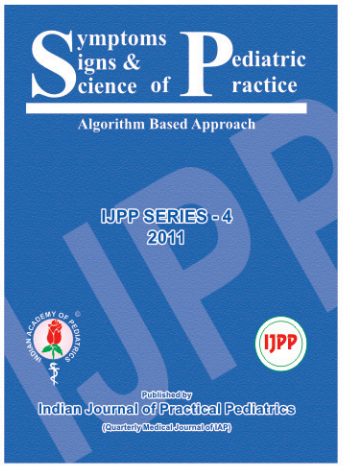

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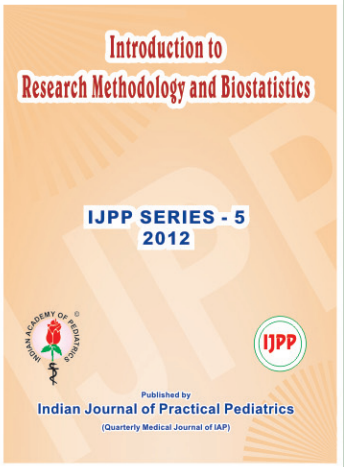

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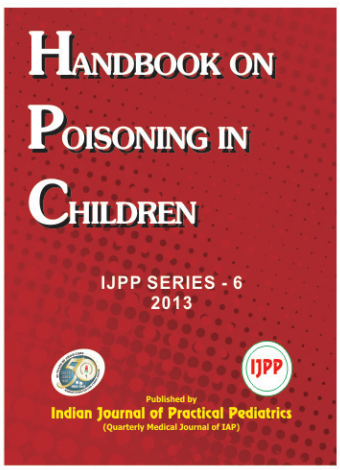

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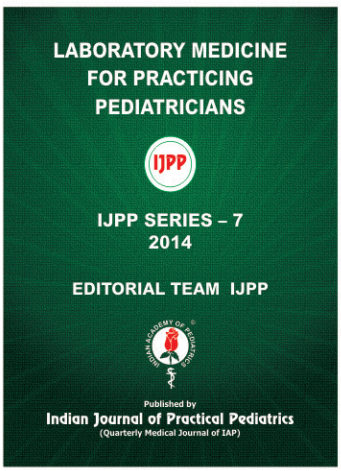

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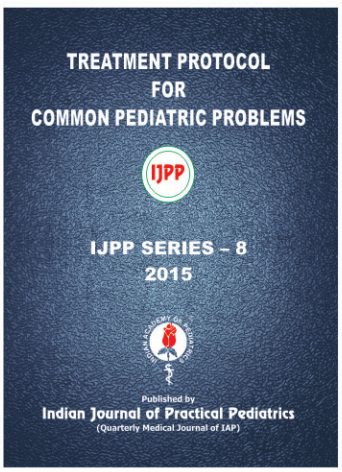

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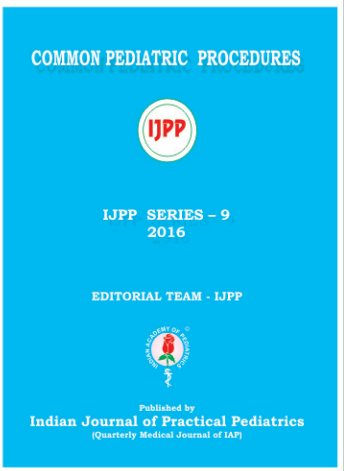

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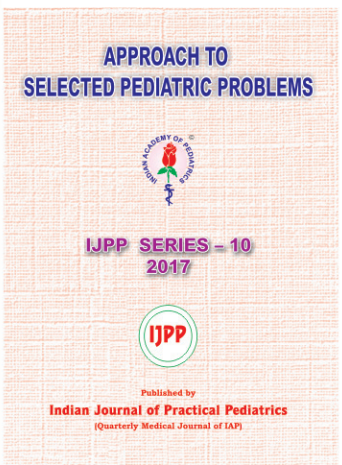

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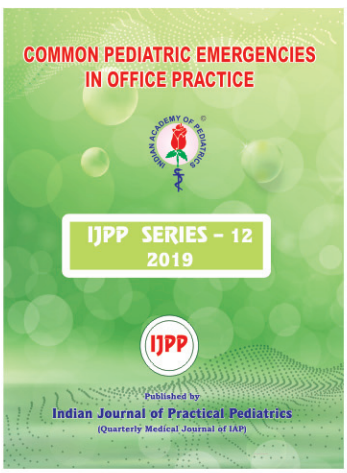

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