patients with T-ALL developed a CNS relapse, of which one had CNS leukemia at diagnosis and the other eventually developed a combined bone marrow and CNS relapse.
Conclusion: In conclusion, pCRT can be avoided in most children with ALL including those with T-cell immunophenotype. Our results demonstrate an excellent CNS control of leukemia just by using IT performed by strict adherence to recommended protocols.

LM-1_V1.19
CNS COMPLICATIONS DURING TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA/LYMPHOBLASTIC LYMPHOMA

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Background: Leukemia & lymphoma together cause 41% of childhood malignancies .Acute lymphoblastic leukemia (ALL) accounts for 80% of childhood leukemia, lymphoblastic lymphoma (LL) accounts for nearly 30% of pediatric lymphomas .
The treatment of both is similar and depends on a handful of drugs with a high toxicity profile.
Current treatment outcomes have improved due to risk stratification and treatment intensification. Intensified chemotherapy targets systemic disease, as well as tumor cells from the sanctuary sites especially the CNS. This entails usage of intrathecal therapy and high dose systemic chemotherapy. This has also increased the incidence and severity of CNS complications . Around 10-20% of children develop acute neurotoxicity while receiving treatment for ALL .Others report MRI abnormalities in nearly 20% children on chemotherapy for ALL, indicating that neurological complications might be under diagnosed.
The commonly reported acute CNS complications include stroke, posterior reversible encephalopathy syndrome, peripheral neuropathy, meningitis and cerebral sinus venous thrombosis (CSVT) .Long term neuroendocrine and neurocognitive decline cause substantial morbidity.
Discrimination of CNS complications secondary to infection, drug toxicity or disease per se is perplexing yet essential as treatment in each case is diametrically opposite. Neuroimaging forms an important tool in reaching a diagnosis. Management is often shrouded in doubt as no clear guidelines exist regarding reduction, delay or termination of further chemotherapy.
Methods:
Objective: To analyze the clinical and neuroradiological features of acute neurotoxicity in children diagnosed with ALL /LL and treated under the ICICLE protocol.
Study type: Retrospective analysis
Study period: 1 December 2013 to 30 June 2015
Study place: Single pediatric tertiary care centre, North India
Study population:
- 77 consecutive children aged <17 years with ALL/LL who were eligible for and treated under the ICICLE protocol.
- Diagnosis of ALL and LL was based on morphological, cytochemical, immunophenotypic and cytogenetic characteristics of bone marrow aspirates and lymph node biopsy respectively.
- All children with neurological complications during treatment were included.
- Based on clinical findings, further lumbar puncture, metabolic examination, CT scan and MRI were done.
Results
- Seven out of 77 children (9%) developed CNS complications during therapy. None of these 7 children had any baseline neurological disease or malignant CNS involvement. Summary is shown in table 1 and 2.
- Five of these children showed gradual improvement of which four are still on chemotherapy while one withdrew from treatment. The remaining two succumbed to their CNS complications (PRES and hypothalamic syndrome).
Conclusion: Intensified chemotherapy has improved survival rates for ALL/LL patients, but also the incidence and severity of CNS complications. Prompt diagnosis and treatment is essential to prevent mortality and limit long term disability

LM-1_V1.20
CYCLIN DEPENDANT KINASE INHIBITOR 2A/B AND IKZF-1 GENE DELETIONS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Cyclin dependant Kinase Inhibitorand IKZF-1 genes are tumour suppressor genes implicated in many leukemia though its role in acute lymphoblastic leukemia has not been studied much. CDKN2A/B are the cell cycle inhibitor genes while IKZF1 is an important gene lymphoid development and differentiation.
Design and Methods: 104 pediatric acute lymphoblastic leukemia patients were investigated for deletion of CDKN2A/2B genes and related Cytogenetic prognostic factors. CDKN2A/2B and IKZF1 deletions were investigated by MLPA SALSA kit 0335.
Results: CDKN2A/B deletions were seen in 19.7% of B- lineage ALL and 38.4% of T lineage ALL. 59% were bi allelic deletions. Monoallelic deletions were found in 50% of B- lineage while all the T-lineage ALL were found to be bi allelic deletions for both CDKN2a and CDKN2B genes.
Interpretation and conclusions: CDKN2A deletions in T-ALL were seen to be associated with higher risk group and poor prognostic outcome in T-ALL. IKZF-1 deletions were seen in 10.9% of B-ALL while no IKZF1 deletions were not seen in any of the T-ALL cases.
There was no difference in the age group while CDKN2A deletions were found to be commoner in high risk group and high leukocyte count. Analysis on survival outcome were also done. IKZF1 gene deletions were seen associated with total leukocyte count, integrated cytogenetics and minimal residual disease.

LM-1_V1.21
A COST-EFFECTIVE, HIGH SENSITIVITY 10-COLOR SINGLE TUBE FLOW-CYTOMETRY (FC) BASED B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (BCPALL) MINIMAL RESIDUAL DISEASE (MRD) ASSAY

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Introduction: Minimal residual disease (MRD) has been proven to be the most important indicator of relapse in BCPALL. Currently, it is widely used to monitor the treatment effectiveness and MRD -based risk stratification. Hence, the methodology for MRD assessment needs to be fast, highly sensitive and most importantly, affordable & widely applicable. Studies have shown that flow-cytometry based MRD (FC-MRD) technique cannot reach beyond the 1 in 10^4 sensitivity and PCR-based MRD monitoring technique is more sensitive. However, PCR based MRD assay is expensive, time consuming, laborious and has lower applicability. We present a study of the cost-effective high-sensitivity 10-color single tube FC-MRD assay in BCPALL.
Methods: We studied 130 cases of pediatric (<15 year) BCPALL diagnosed as per WHO criteria. FC-immunophenotyping was performed on Navios flow-cytometer using bulk-lysis-and-stain method and data was analyzed with Kaluza-software. MRD was monitored in 164 bone-marrow (BM) samples at post-induction (PI, day 29-35), post-consolidation (PC, day-90) and subsequent follow-up time-points (SFU) using 10-color single tube FC-MRD assay with an additional 4-color nuclear-dye (SYTO13) tube (Table 1). Samples with cluster of ≥20 and ≥2 leukemia associated phenotypes (LAIPs) were called MRD-positive. Wherever possible, high number of events (>1.5 million) were acquired. To evaluate the applicability of assay, number of LAIPs were determined in diagnostic and MRD samples and post-induction modulation of antigen-expression was also studied.
Results: We studied 130 pediatric (<15 year) BCPALL cases for FC-MRD monitoring. High number of events were acquired for MRD-assay with median-events 2341500 (range, 218000 to 3152800). Of 164, MRD was positive in 69 (42%) samples with median of 0.135% and range of 0.0006% to 48.3%. We categorized positive MRD results into samples with MRD <0.001%, 0.001- <0.01%, 0.01- <0.1%, 0.1- <1.0%, 1.0- <10% and >10% and they were respectively 2.4%, 7.1%, 34.5%, 27.4%, 14.3%, and 14.3%. Of 164

MRD samples, PI-MRD were 130 (79.3%), PC-MRD were 24 (14.6%) and SFU were 10 (6.1%). Of 130 PI-MRD, 40% samples were positive with median of 0.21% and range 0.0006% to 48.3%. Of 52 PI-MRD-positive cases, PC-MRD was performed in 46.2% cases and was positive in 62.5% cases (median, 0.075% & range, 0.007-21.5%). Of 10 SFU samples, MRD was positive in 2 cases. Of 130 diagnostic samples, < 2 LAIPs were seen in only 1% and >6 LAIPS in 2% (median LAIPs, 4 & range, 1-8). Similarly, of 164 MRD samples, four had <2 LAIP (2.4%) and labelled as "suspicious" and in 69 MRD-positive samples 2-LAIPS were only 7.1%. Furthermore, in 15 samples with MRD-positive ≤0.01% and >1.5 million acquired-events, the results were compared between time-gated initial 500000-events, 1000000 events and all events acquired. In these 10 samples, eight samples found to be negative in initial 500000-events and four in initial 1000000-events (as the number of MRD-events were < 20) highlighting the importance of acquisition of >1.5 million cells to increase the sensitivity of FC-MRD assay.
Conclusion: We established a cost-effective 10-color single tube FC-MRD assay with high sensitivity of 1 in 10^5 and applicability in >97% MRD samples. Our study showed that acquisition of events less or equal to 1 million cells can reduce the sensitivity of FC-MRD assay.

Table 1

	Main-MRD-tube			Nuclear-dye-tube		
Fluorochrome	Antibody	Clone	Company	Antibody	Clone	Company
BV421	CD123	9F5	BD			
BV510	CD20	2H7	BD			
FITC	CD58	AICD58	BC	SYTO13	–	INVITROGEN
PE	CD86	FUN-1	BD			
PE-CF594	CD25	M-A251	BD			
PC5.5	CD19	J3-119	BC	CD19	J3-119	BC
PC7	CD10	ALB1	BC			
APC	CD34	581	BD			
APC-A700	CD45	J.33	BC	CD45	J.33	BC
APC-A750	CD38	LS198-4-3	BC			

Myeloid Malignancies

MM-1_V1.1

COMBATING BLOOD STREAM INFECTIONS DURING INDUCTION CHEMOTHERAPY IN CHILDREN WITH ACUTE MYELOID LEUKAEMIA – SMART BUGS NEED SMARTER SOLUTIONS

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Background: Optimal management of infectious complication is the biggest challenge in children receiving chemotherapy for acute myeloid leukemia (AML) especially during the induction phase. Significant improvement in overall survival worldwide has been due to advances in supportive care. The aim of this study is to assess the changing pattern of blood stream infections (BSI) during AML induction chemotherapy over the last decade and to analyse the burden of supportive care in managing these infections.
Material and Methods: The study is a retrospective analysis of children undergoing AML induction chemotherapy at our centre from 2002 to 2015.
Results: A total of fifty four children had received induction chemotherapy as per UK MRC protocol were included in the study. Ninety six episodes of febrile neutropenia were studied. Fifty septic events were recorded and in twenty seven such episodes children had to be shifted to paediatric intensive care for either ventilatory or ionotropic support. Blood culture proven septic episodes were seen in 37% of the children, of which 87% were due to gram negative organisms. High end antibiotics like colistin / tigecycline were used in 38% of these children. Neutropenic enterocolitis was the most common focus of infection in these children. Remission was achieved in 85% of children at end of induction and the mortality rate was 10%.
Between the years 2012 to 2015 the incidence of drug resistant gram negative sepsis had risen to 87% when compared to 20% during the 2002 to 2011 period. Though the mortality rates had remained the same in both groups, the morbidity due to therapy including the duration of hospital stay, the need for paediatric intensive care support, the use of colistin for carbapenam resistant infections and the use of granulocyte transfusions to help tide over the sepsis had dramatically increased.
Conclusion: Western data has shown a rising trend in gram positive infections during AML induction. However, our study has shown predominantly gram negative infections. The last 5 years has shown a clear increase in carbapenam resistant Klebsiella infections. This has increased the cost of therapy significantly. Several interventions have been introduced at our unit to reduce the incidence and mortality due to drug resistant gram negative sepsis. An active infection control policy with strict surface cleaning, education of personnel on hand hygiene and restricting unnecessary use of high end antibiotics had been the first measure. Surveillance of children for Carbapenem resistance (Carba-R) organisms at the start of induction therapy has helped identify children at risk. Early introduction of colistin in Carba-R positive children during febrile neutropenic episodes has been the main intervention to reduce mortality. The introduction of neutropenic diet which is lactose and gluten free during the mucositis phase has helped reduce translocation of intestinal bacteria. Early use of granulocytes in the first 48 hours of onset of septic shock has helped as a bridge till neutrophil recovery.
These measures have kept the mortality rates constant at 10% over the last decade. However, it comes with a significant increase in the cost of supportive care during AML induction.