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Minimal Residual Disease in ALL: Clinical challenges and Indian perspective

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Abstract

Minimal residual disease (MRD) represents the residual disease diagnosed following therapy for acute lymphoblastic leukemia (ALL) by molecular methods of multiparameter flow cytometry or quantitative real-time polymerase chain reaction. Assessment of MRD is becoming a standard of care, considering its predictive implication in identifying high-risk patients to intensify the therapy. MRD is an independent prognostic factor and interpretation of MRD results is complicated. Developing and refining MRD assays to define the value of MRD testing to assess response to treatment and predict relapse is the need of the hour. However, the availability of high-end technologies is limited in developing countries. Flow cytometry is the mainstay of evaluating MRD in a developing country like India.

Keywords: Acute lymphoblastic leukemia; Minimal residual disease; Diagnosis; Polymerase chain reaction; Flow cytometry; India

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Introduction

Acute lymphoblastic leukemia (ALL) is a clonal disease that affects early lymphoid progenitor cells in bone marrow, blood and extramedullary sites. The incidence of ALL is estimated to be 0.62 per 100,000 adult populations [1]. ALL is commonly diagnosed in children (80% of ALL) but is a devastating disease when it occurs in adults. Despite advances in the management, the first line of therapy is multiagent chemotherapy which elderly patients are unable to tolerate, and prognosis remains poor. Survival rates for ALL have steadily improved in children, nevertheless, 40% to 50% of adult patients relapse [2]. The survival rate has improved remarkably in the high-income countries (90%) with the progress in diagnostic and treatment methods; however, it is only 60% in India [3]. Multiple factors are attributed to poor outcomes in India like treatment abandonment, relapse and toxic deaths [4].

Evidences have confirmed that minimal residual disease is a strong prognostic indicator in both children and adults with ALL [5]. Minimal residual disease (MRD) represents a low level or residual disease after induction chemotherapy usually not detected by conventional cytomorphology but by sensitive molecular and flow cytometry-based methods. To be clinically relevant, an MRD diagnostic assay must be able to detect and quantify low frequencies of leukemic cells as less as 1 ALL cell in 10,000 normal cells, with a high level of sensitivity and specificity virtually in all ALL patients. Discriminating features of the ALL cells such as aberrant immunophenotypes, specific genetic aberrations, specific immunoglobulins (IG) or T-cell receptor (TR) gene arrangements are exploited by highly sensitive and accurate molecular techniques to quantify MRD and in tailoring treatment in adult ALL [6]. Monitoring MRD aid in interpreting disease kinetics during and after treatment and to stratify patients who can be spared further therapy and whom therapy needs to be intensified. MRD-based risk stratification is also useful in assessing the disease burden in patients undergoing stem cell transplantation for early recognition of impending relapse and as a surrogate endpoint in clinical trials [7]. A large population of adult ALL cases and all the pediatric ALL are being monitored with MRD techniques to assess the effectiveness of the treatment, however, limited evidence are reported from India on risk-directed treatment [8].

Clinical trial studies reveal that MRD directed therapy will be the standard of care for ALL in the coming years. However, the technology is limited in low and middle-income countries. Developing cost-effective assays with high sensitivity and

standardization is desirable in India to rationalize therapy. This mini review will primarily focus on the methods of measuring MRD, clinically relevant technical issues with a brief of therapeutic and laboratory evaluation of MRD from resource constraint Indian subcontinent.

Methods for Assessing MRD in ALL

The early studies on the prognostic significance of MRD in newly diagnosed childhood ALL were independently published in 1998 by three research groups [9-12]. These studies shed light on improving the design of treatment protocols in which risk assignment was largely based on MRD measurement. Janossy and colleagues developed a method of antibody staining of terminal deoxynucleotidyl transferase (TdT) to differentiate lymphoblasts from normal lymphocytes in cerebrospinal fluid of patients with ALL. The method was improved by adding anti-T-cell antibody along with anti TdT to assess MRD in the bone marrow of patients with T-All in morphologic remission [13,14]. Further technological improvements with the advent of flow cytometry to identify leukemic cells using specific cell marker antibodies and PCR based methods to amplify fusion transcripts in ALL cells were developed [12].

Current strategies for detecting MRD represents a 100-fold increase in sensitivity compared to conventional optical microscopy [15]. Available methods include 1) detection of Leukemia-associated immunophenotype (LAIP) by multiparametric flow cytometry 2) polymerase chain reaction (PCR) of leukemia-specific rearrangement of immunoglobulin and T-cell receptor (IG/TR) genes 3) detection of fusion gene transcripts by real-time quantitative polymerase chain reaction (RT-qPCR)

Detection of MRD by flow cytometry

Evaluation of MRD by flow cytometry emerged in the late 1980s. The technique started with a basic panel of antibodies or a combination of cell markers to distinguish T lineage ALL cells from normal immature and mature lymphocytes. The method harnesses the fact that leukemic cells express abnormal marker profile different from normal hematopoietic cells due to altered genetic and cellular features. LAIP can be identified in more than 90% of patients with ALL, moreover, immunophenotyping of leukemic cells at the time of diagnosis can help to classify the disease by cell lineage and maturation and provide information on treatment response [16]. Flow-based detection of MRD requires a cluster of 10 to 40 events during acquisition and knowledge of the immunophenotypic profiles of bone marrow and peripheral blood under various conditions and selection of best markers to use in each case. A sensitivity of 10^{-3} to 10^{-4} can be achieved for MRD assessment by flow cytometry [17].

The strength of this technique is that the detection of aberrant LAIP is less laborious and faster compared to other molecular methods. Flow cytometry based MRD assessment can assess the normal hematopoietic cell maturation status simultaneously along with accurate quantification of MRD [18]. Detection of MRD at early stages of remission induction chemotherapy using a restricted panel of antibodies provides information of the patient response to therapy at an early stage. The pitfall is that the sensitivity is about 1 log lower than that of molecular methods and requires high cell numbers to quantify [19]. Therapeutic approaches can alter the phenotypic features of residual and normal leukemic cells throughout the treatment [20]. In addition, false positive results are reported due to phenotypic similarities between leukemic lymphoblasts and non-malignant lymphoid precursors at the stage of bone marrow regeneration during and after chemotherapy [21]. Further, the immunostaining protocols, antibody panels, and gating strategies differ between centers [22].

Impressive technological and methodological advances have evolved in the evaluation of MRD using flow cytometry. The development of new marker antibodies and fluorochromes, the ability to test multiple parameters simultaneously in individual cells. The Euro flow consortium focusing on the development, standardization, and validation of MFC assays for MRD detection recently introduced high throughput concepts based on multivariate analysis in flow cytometric MRD detection [23,24].

Detection of MRD by quantitative PCR of IG/TR targets

During the early stages of B and T- lymphoid cell differentiation antigen receptor genes (IG and TR) undergo variable rearrangements. IG and TR diversity are generated by the random joining of V(D)J exon and the sequence is unique to each lymphocyte [25]. Conventional PCR methods developed in the 1990s helped the analysis of IG/TR gene rearrangements to assess clonality and MRD detection. Oligonucleotide primers designed complementary to individual junction region sequences which are highly diverse and sensitive used target cell DNA. PCR based MRD detection methods can be classified as qualitative or quantitative approaches. Qualitative strategies use nested or semi-nested PCR, whereas the quantitative real-time methods enable accurate assessment of residual tumor cells at consecutive time points [26]. Introduction of quantitative real-time PCR (RT-qPCR) using fluorescent probes as a reading system further improved the technology. Detection of IG/TR by RT-qPCR is currently the gold standard in MRD detection in ALL [27].

IG/TR RT-qPCR is used to detect MRD in more than 95% of patients with ALL with higher levels of sensitivity (10^{-4} to 10^{-5}) and standardization [6]. Characterization of IG/TR rearrangements using a panel of screening PCRs, sanger or next-generation sequencing of the PCR products and optimization of the RT-qPCR assays is essential for implementing IG/TR into practical applications. EuroMRD consortium (www.EuroMRD.org) consisting of 57MRD PCR laboratories is involved in extensive optimization and standardization of RT-qPCR based MRD detection [6].

In addition to high sensitivity and accurate quantification, false results appear [26]. Massive bone marrow regeneration after treatment can cause nonspecific primer annealing, presence of contaminating DNA, nonspecific hybridization to amplified DNA from normal lymphocytes all lead to false positive results. Clonal evolution during the disease might lead to loss of leukemia-specific IG/TR sequence and false negative MRD results [27].

Detection of MRD by real-time quantitative PCR of fusion gene transcripts

Approximately 30-40% of B-cell precursor ALL (BCP-ALL) and 10-20% of T cell ALL (T-ALL) express chimeric transcripts arising from chromosomal translocation representing specific markers for leukemic clones [22]. Chromosomal abnormalities and their corresponding gene fusions such as BCR-ABL, MLL-AF4, TEL-AML1 occur in less than one-third of ALL patients limiting the value of this approach. In childhood ALL, this approach is much less used, however, it might add value to a specific subgroup of patients such as BCR-ABL-ALL. The advantage of this approach over IG/TR rearrangement detection is that the primer sets are not patient specific. The method is highly sensitive (10^{-4} to 10^{-6}) and relatively easy to perform with standardized protocols and primer-probe sets [28].

Advanced MRD technologies

Clinical studies on the use of PCR-based MRD techniques are standardized by European countries whereas flow-based approaches are preferred in the United States and South Asian countries. Novel technologies like high through put PCR sequencing and flow MRD techniques have been developed from the basic knowledge and experience of classical MRD techniques. The principle and characteristics of assays used to monitor MRD are summarized in **Table 1.**

EuroFlow-based next generation Flow MRD: Within the last decade, the 3-4 color flowcytometres are replaced by 8-10 color, the introduction of new fluorochromes, 4-6 lasers have enabled detection of more than 15 colors, contributing to improved applicability and specificity of flow MRD measurements. The EuroFlow based next generation flow (NGF-MRD) strategies clearly illustrates the difference between the normal and aberrant cells such as treatment induced phenotypic shifts within ALL cell population. The high throughput concept in flow MRD is based on multivariate analysis and reaches a sensitivity of 10^{-5} to 10^{-6} [29]. However, efforts are aimed at making it the global standard.

Droplet Digital PCR (ddPCR): The third generation PCR technology is based on partitioning and poisson statistics, has the potential to overcome the limitations of RT-qPCR. The technology can quantify nucleic acid targets without the need of calibration curves, with a high degree of efficiency, sensitivity, and accuracy. There is a high level of concordance in the detection of MRD by ddPCR and RT-qPCR [30,31]. However, published guidelines for interpretation of ddPCR data and clinical confirmation are not yet available.

High throughput sequencing (HTS) of IG/TR targets (DNA): The RT-qPCR results should always interpret cautiously particularly for samples after the end of therapy or after HSCT as there are chances of nonspecific amplification. PCR based high throughput sequencing (HTS) of IG/TR rearrangement is a promising improvement in this area. HTS can detect clone specific IG/TR index sequences and can be applied to follow up samples, relapse prediction in ALL patients after HSCT and as well as after induction [32]. The use of universal primers allows monitoring of all IG/TR gene arrangements at the same time, thus provide insights into the normal immune repertoire along with residual leukemia [27]. Nevertheless, HTS MRD applicability of HTS MRD is limited due to high cost, complex protocol, and limited standardization.

Challenges for Clinical Applications of MRD

MRD certainly matters in ALL and monitoring MRD has become a routine clinical practice in the management of patients with ALL. However, the treatment time points and therapeutic settings influence the sensitivity and specificity of this diagnostic method.

Sample requirement

Peripheral blood or bone marrow can be used for quantifying MRD, however large-scale clinical studies evaluating MRD levels in paired blood/bone marrow samples confirmed that MRD

Table 1 Characteristics of assays used to monitor minimal residual disease in acute lymphoblastic leukemia.

Detection method	Flow cytometry	RT PCR (Fusion genes)	RT qPCR (*Ig/TCR*)	Digital PCR (*Ig/TCR*)	HTS *Ig/TCR*
Target	Leukemia-associated immunophenotype	Fusion gene transcripts	*Ig/TCR* gene rearrangements	*Ig/TCR* gene rearrangements	*Ig/TCR* gene rearrangements
Percentage of patients that can be monitored	>90%	30-40%	90%	>90%	>95%
Sensitivity	10^{-3} to 10^{-4}	10^{-4} to 10^{-6}	10^{-4} to 10^{-5}	10^{-4} to 10^{-5}	10^{-4} to 10^{-6}
Pros	Widely applicable, rapid, information on whole sample cellularity, distinguish between viable and apoptotic cells, cost-effective	Sensitive, applicable for specific leukemic subgroups, Stable throughout the treatment	A standardized method, high sensitivity, widely applicable, accurate quantification	Highly sensitive, rapid, widely applicable	Highly sensitive, widely applicable
Cons	Phenotypic shifts, require more experience, sensitivity depends on the antibody panel used, lack of standardization	Cross contamination, RNA stability, limited standardization, and applicability, high cost	Time-consuming, target instability, more than one target reduces applicability, requires more experience	Limited standardization, available only in few labs, no guidelines for data analysis, relatively expensive	Limited standardization, available only in few labs, no guidelines for data analysis, relatively expensive

levels in BCP-ALL tend to be 1 to 3 logs lower and in T-ALL up to 1 log lower in peripheral blood than in bone marrow [33]. Hence, for monitoring of MRD invasive bone marrow sampling is a prerequisite.

The sensitivity of each technique is determined by several factors including the number of cells in the bone marrow aspirate. To achieve sensitivity more than 10^{-4}, flow cytometric method requires more than 5×10^6 cells and RT-qPCR methods require more than 2×10^6 cells [22].

Timing of MRD measurement

Studies reveal that assessment of MRD at different time points (early during therapy, end of induction chemotherapy, end of consolidation therapy) have different prognostic value for relapse [34]. Assessment of MRD at the end of induction therapy is helpful in identifying a subgroup of patients at low risk of relapse while persisting MRD at the end of consolidation therapy identifies high-risk relapse patients [10]. In addition, the treatment protocols (type and number of drugs and their dosages) also influence the prognostic significance of MRD. Evidence from St Jude children research hospital suggests MRD measurement on day 15 and 42 for treatment assignment. Patients with undetectable MRD (less than 10^{-4}) on day 15 receive less intensive reduction therapy and MRD higher (1×10^{-2} to 5×10^{-2}) receive intensified remission induction therapy. Patients with 10^{-4} or higher MRD at day 42 is reclassified as high risk. Patients with MRD of 10^{-2} or higher on day 42 is a candidate for hematopoietic stem cell transplantation in first remission [7]. In routine clinical practice at our centre we follow AIEOP-BFM guidelines, MRD measurement is done by multicolour flowcytometry on day 33 of induction chemotherapy Thus, MRD is a time point dependent variable and MRD results of different clinical trials should be interpreted cautiously.

Best method of MRD measurement

Over the last few decades, MRD was evaluated by immunophenotyping by flow cytometry and real-time polymerase chain reaction at different time points during treatment. Both the methods are highly sensitive (10^{-3} to 10^{-5}), expensive, and complex, require highly trained staff [35]. The overall cost of the two methods are similar, however, flow cytometry is readily available virtually in every cancer care center. Flow cytometric measurements of MRD studies at an early point of therapy has an advantage over PCR. However, MRD assessment at the end of therapy or post stem cell transplantation is done using PCR as it has higher sensitivity [36]. Knowledge of the technology used and the type of expertise of the laboratory both are critical for correct interpretation of the results.

MRD negativity

MRD assessment techniques have a lower limit of detection and quantification. Currently, available treatment protocols require a sensitivity of 10^{-4} which both the flow and PCR based methods claim. Defining MRD negativity is necessary to identify low-risk patients with less chance of relapse (3-5%) for considering a reduction in therapy [22]. Some recent high throughput technologies claim to reach sensitivity as low as 10^{-7}, however, it is necessary to understand the cellularity limits of bone marrow samples, especially in the aplastic bone marrow and the amount of DNA used for MRD assessment [6]. Therefore, MRD negativity is not the absence of residual disease rather "measurable residual disease" and MRD reports should essentially include the techniques used, cut off levels, sampling time point, markers probed, the limit of detection and quantification of the assay used.

Improved high throughput molecular methodologies are introduced to monitor MRD to widen clinical applications. Droplet digital PCR can overcome some of the drawbacks of RT-qPCR and has shown accurate, sensitive and reproducible results in evaluating MRD [37]. Next-generation sequencing is another important tool, aid in better understanding of the disease by giving a clear picture of the mutational landscape of subtypes of ALL, clonal heterogeneity and the kinetics of the disease [38]. Further, multi-parameter (more than 8 colors) flow cytometry with automated data analysis will improve the clinical feasibility of molecular assessment of MRD [24].

Discussion

In India, ALL is diagnosed in 60-85% of all reported leukemia cases with T-cell ALL being more frequent [3,8,39]. Geographic variations do play a role in the frequency of leukemia with ALL, most common in south India, less common in east India and northern India. Evidence also suggests that of the ALL cases reported 70% are children and 30% are adult patients and predominance is seen in males [40]. A gradual increase in the incidence of ALL is seen in the past 25 years. The five-year survival rate is reported to be more than 90% in developed countries whereas the overall survival rate is reported to be 40% in India [4]. This bad prognosis of ALL patients in India could be due to lack of resources, delay in diagnosis and treatment abandonment, and higher rates of infections during treatment, difficulties in salvaging patients with relapsed disease and probably due to differences in the biology of the disease [4]. Management strategies focusing on collaborative efforts which promote treatment of patients on a risk-directed protocol based on local infrastructure improved supportive care might improve the survival of patients with ALL in India.

Significant improvements in the management of ALL and clinical outcomes are achieved by the intensification of therapy, allogeneic stem cell transplantation, improved molecular risk stratification, and MRD directed therapy. However, reports from developing countries addressing therapeutic or laboratory practices related to MRD directed therapy is largely lacking. Expression of fusion transcripts in ALL is used to risk-stratify patients and decide on treatment and to detect MRD. Chopra et al. determined the frequency of common fusion transcripts BCR-ABL, TEL-AML1, MLL-AF4 and E2A-PBX1 for ALL and SIL-TAL1 for T-ALL by RT-qPCR. Their study concluded that higher incidences of BCR-ABL in ALL patients compared to west and the ALL is biologically different from that of the west [41]. Sudhakar et al. studied the T-cell gamma delta rearrangements and its junctional region characteristics in south Indian T-ALL patients. They concluded that they rely on T Cell Receptor Gamma clonal

markers to design patient-specific primers to quantitate MRD [42]. Patkar et al. from CMC Vellore developed and standardized a flow cytometry-based cost effective MRD assessing panel applicable to 90% of patients in precursor BCP-ALL [43]. Deepak Bansal and team developed an early minimally invasive easily accessible MRD screening option from peripheral blood in pediatric B-ALL. They assessed the role of mid induction (day 15) peripheral blood minimal residual disease detection (PB-MRD) in B-ALL by six-color flow cytometry in 40 patients enrolled in the study [44]. Kumar et al. assessed MRD in children and adults with ALL at day 33 post induction chemotherapy by flow cytometry to risk stratify patients and identify a subset of patients who will benefit from early hematopoietic stem cell transplant [45]. Data from our centre suggests on day 33 MRD was positive in 40% of patients and MRD measurement on day 33 post chemotherapy is an important factor in determining risk of relapse in patients with ALL [45] They conducted the study in 70 patients and concluded that assessing MRD at day 33 helps to take decisions on offering stem cell transplant to MRD positive patients. An effective and useful methodology to assess MRD at day 19 of remission induction therapy for B-lineage ALL was developed by Chatterjee et al. [46]. They proposed measuring MRD by simple 3 colors MRD "Lite" panel, successfully tried in Brazil; however, the panel is not applicable to a subset of infants with B-ALL.

MRD testing is now standard practice in the treatment of adult ALL across France, Germany, Italy, UK with respect to timing and frequency of MRD testing, aligning with the use of national protocol [1]. A multicentric clinical trial to define the national protocol of MRD testing is not there in India. However, the development of specialized centers for cancer in India is inspirational to provide quality care. A standard protocol for the treatment of ALL was developed 15 years ago jointly by 4 cancer centers in India and National Cancer Institute called MCP-841 [47]. The overall survival of ALL patients dramatically increased, and the protocol also helped to reduce the cost of treatment and care. It is time for India to further improve survival by creating protocols for Indian conditions incorporating latest treatment principles like MRD based risk stratification. In addition, specific training on recent diagnostic and therapeutic advances made in the MRD detection and interpretation is extremely important. Simplified methods of MRD detection during the early period of treatment to identify patients at high risk should be developed by several cooperative group studies to develop a standard national protocol. Thus, management of adult and pediatric ALL in India requires evidence-based treatment, which is expensive, resource heavy and needs expertise. Wherever resource permits, clinical correlations along with comprehensive analysis of MRD based on flow cytometry adapting to our system needs to be followed. Further, flow cytometry plots can be reviewed remotely by expert trained technicians using web-based systems. In addition, tertiary centers can partner with academic institutions to develop, standardize and validate flow based MRD diagnostic assays.

Conclusion

ALL is a potentially curable hematologic malignancy. The survival rate of ALL patients has improved over the past few years due to refinement in the treatment protocols, including reduction of therapy for low-risk patients thereby reducing toxicity associated mortality. In addition to clinical and laboratory features like age, gender, initial white blood count, immunophenotyping and cytogenetics, assessment of minimal residual disease is an important prognostic determinant of both childhood and adult ALL. Improved molecular technologies with high sensitivity and accuracy are introduced to monitor MRD to guide risk-directed therapy in developed countries. However, MRD directed therapy with improved technologies is limited due to financial and technical challenges in low-income countries. Collaborative efforts among individuals and institutions regionally, nationally and internationally to develop standard treatment guidelines on evidence-based approaches, prospective multicentre clinical trials should be developed. The MCP 841 protocol is an important landmark in the advancement of treatment and outcomes of childhood ALL in India is developed by international collaborative efforts. It improved survival rates of childhood cancer outcome from less than 20% to nearly 60% [48]. Further, more research on the biology of ALL in an Indian setting to develop novel biomarkers to increase the precision of risk assessment and tailor treatment intensity is also demanded.

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References

1 Pigneux A, Montesinos P, Cong Z (2018) Testing for minimal residual disease in adults with acute lymphoblastic leukemia in Europe: A clinician survey. BMC Cancer 18: 1-8.

2 Gökbuget N, Stanze D, Beck J (2019) Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors and performance of stem cell transplantation. Blood 120: 2032-2042.

3 Kanwar VS (2019) Acute childhood leukemia in India: Where are we going, where have we. South Asian J Cancer 5: 5-6.

4 Arora RS, Arora B, Super M (2019) Acute leukemia in children: A review of the current Indian data. South Asian J Cancer 5: 155-160.

5 Bru M, Raff T, Kneba M (2019) Has MRD monitoring superseded other prognostic factors in adult ALL? Blood 120: 4470-4482.

6 Kotrova M, Brüggemann M (2017) Minimal residual disease in adult ALL: Technical aspects and implications for correct clinical interpretation. Hematology Am Soc Hematol Educ Program 1: 13-21.

7 Campana D (2010) Minimal residual disease in acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Progr pp:7-12.

8 Bibin V, Austoria AJ, Savida P (2018) Five-year survival rate and the

factors for risk directed therapy in acute lymphoblastic leukemia. Leukemia 39: 301-306.

9 CoustanSE, Behm FG, Sanchez J (1998) Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet 351: 550-554.

10 Cave H, Van der Werff ten Bosch J, Suciu S (1998) Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer--Childhood Leukemia Cooperative Group. N Engl J Med 339: 591-598.

11 Van Dongen JJ, Seriu T, Panzer-Grumayer ER (1998) Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 352: 1731-1738.

12 Borowitz MJ, Wood BL, Devidas M (2015) Prognostic significance of minimal residual disease in high risk B-ALL: A report from Children's Oncology Group study AALL0232. Blood 126: 964-971.

13 Bradstock KF (1980) Detection of leukaemic lymphoblasts in CSF by immunofluorescence for terminal transferase. Lancet 1: 1144.

14 Bradstock KF, Janossy G, Tidman N (1981) Immunological monitoring of residual disease in treated thymic acute lymphoblastic leukaemia. Leuk Res 5: 301-309.

15 van der Velden VHJ, Hochhaus A, Cazzaniga G (2003) Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: Principles, approaches, and laboratory aspects. Leukemia 17: 1013-1034.

16 Chatterjee T, Mallhi RS, Venkatesan S (2016) Minimal residual disease detection using flow cytometry: Applications in acute leukemia. Med J Armed Forces India. 72: 152-156.

17 Rocha JM, Xavier SG, De Lima Souza ME (2016) Current strategies for the detection of minimal residual disease in childhood acute lymphoblastic leukemia. Mediterr J Hematol Infect Dis 8:pp: e2016024.

18 Campana D (2010) Minimal residual disease in acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Progr pp: 7-12.

19 Brüggemann M, Schrauder A, Raff T (2010) Standardized MRD quantification in European ALL trials: Proceedings of the Second International Symposium on MRD assessment in Kiel, Germany. 18-20 September 2008. Leukemia 24: 521-535.

20 Dworzak MN, Gaipa G, Schumich A (2010) Modulation of antigen expression in B-cell precursor acute lymphoblastic leukemia during induction therapy is partly transient: Evidence for a drug-induced regulatory phenomenon. Results of the AIEOP-BFM-ALL-FLOW-MRD-Study Group. Cytometry B Clin Cytom 78: 147-153.

21 Gaipa G, Giovanni C, Maria GV (2012) Time point-dependent concordance of flow cytometry and real-time quantitative polymerase chain reaction for minimal residual disease detection in childhood acute lymphoblastic leukemia. J Ferrata Storti Foundation 97: 1586-1593.

22 Van Dongen JJM, Van Der Velden VHJ, Bruggemann P (2019) Minimal residual disease diagnostics in acute lymphoblastic leukemia: Need for sensitive, fast and standardized technologies Classical MRD techniques. Blood 125: 3996-4009.

23 Kalina T, Flores MJ, Van der Velden VHJ (2012) EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia 26: 1986-2010.

24 Theunissen P, Mejstrikova E, Sedek L (2017) Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood 129: 347-357.

25 Cazzaniga G, Biondi A (2005) Molecular monitoring of childhood acute lymphoblastic leukemia using antigen receptor gene rearrangements and quantitative polymerase chain reaction technology. Haematologica 90: 382-390.

26 Van Dongen JJ, Langerak AW, Bruggemann M (2003) Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia. 17: 2257-2317.

27 Nunes V, Cazzaniga G, Biondi A (2017) An update on PCR use for minimal residual disease monitoring in acute lymphoblastic leukemia. Expert Rev Mol Diagn 17: 953-963.

28 van Dongen JJ, Macintyre EA, Gabert JA (1999) Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 13: 1901-1928.

29 Paiva B, Puig N, Bottcher S (2016) Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. 31: 2094-2103.

30 Della Starza I, Nunes V, Cavalli M (2016) Comparative analysis between RQ-PCR and digital-droplet-PCR of immunoglobulin/T-cell receptor gene rearrangements to monitor minimal residual disease in acute lymphoblastic leukaemia. Br J Haematol 174: 541-549.

31 Drandi D, Ferrero S, Ladetto M (2018) Droplet Digital PCR for Minimal Residual Disease Detection in Mature Lymphoproliferative Disorders. Methods Mol Biol pp: 229-256.

32 Kotrova M, Van der Velden VHJ, Van Dongen JJM (2017) Next-generation sequencing indicates false-positive MRD results and better predicts prognosis after SCT in patients with childhood ALL. Bone Marrow Transplant 52: 962-968.

33 Coustan-Smith, Sancho J, Hancock ML (2002) Use of peripheral blood instead of bone marrow to monitor residual disease in children with acute lymphoblastic leukemia. Blood 100: 2399-2402.

34 Jastaniah W, Elimam N, Abdalla K (2018) Early *vs*. late MRD response and risk based treatment intensification of childhood acute lymphoblastic leukemia: A prospective pilot study from Saudi Arabia. 7: 1-15.

35 Chen X, Wood BL (2017) Monitoring minimal residual disease in acute leukemia: Technical challenges and interpretive complexities. Blood Rev 31: 63-75.

36 Kerst G, Kreyenberg H, Roth C (2005) Concurrent detection of minimal residual disease (MRD) in childhood acute lymphoblastic leukaemia by flow cytometry and real-time PCR. Br J Haematol 128: 774-782.

37 Alikian M (2017) RT-qPCR and RT-Digital PCR: A comparison of different platforms for the evaluation of residual disease in chronic myeloid leukemia. Clin Chem 63: 525-531.

38 Kotrova M (2015) The predictive strength of next-generation sequencing MRD detection for relapse compared with current methods in childhood ALL. Blood 126: 1045-1047.

39 Arora RS, Eden TOB, Kapoor G (2009) Epidemiology of childhood cancer in India. Indian J Cancer 46: 264–273.

40 Rathee R (2014) Acute lymphoblastic leukemia: Characterization and

its prognostic values acute lymphoblastic leukemia: Characterization and its prognostic values.

41 Chopra A (2019) Prevalence of common fusion transcripts in acute lymphoblastic leukemia: A report of 304 cases. 11: 1-2.

42 Sudhakar N, Nancy NK, Rajalekshmy KR (2007) T-cell receptor gamma and delta gene rearrangements and junctional region characteristics in South Indian patients with T-cell acute lymphoblastic leukemia. 221: 215-221.

43 Patkar N (2012) Standardizing minimal residual disease by flow cytometry for precursor B lineage acute lymphoblastic leukemia in a developing country. 258: 252-258.

44 Issues P (2019) Role of mid-induction peripheral blood minimal residual disease detection in pediatric B-lineage acute lymphoblastic leukemia role of mid-induction peripheral blood minimal residual disease detection in pediatric B-lineage acute lymphoblastic leukemia. 6: 21-23.

45 Kumar K (2017) Minimal Residual Disease (MRD) assessment after induction chemotherapy in children and adults with acute lymphoblastic leukemia (ALL): A valuable strategy in resource constraint settings in the Indian Subcontinent. Blood 130.

46 Tathagata B, Col L, Somasundaram V (2017) Flow cytometric detection of minimal residual disease in B-lineage acute lymphoblastic leukemia by using MRD lite panel. Med J Armed Forces India 73: 54–57.

47 Dinesh CS (2002) Standard protocol helps improve ALL survival rates in India. Newsdesk 3: pp: 710.

48 Magrath I (2005) Treatment of acute lymphoblastic leukaemia in countries with limited resources; lessons from use of a single protocol in India over a twenty year period. Eur J Cancer 41: 1570-1583.