PRACTICE CHANGING UPDATES IN HEMATOLOGY TREATMENT SEQUENCING INSIGHTS

European Haemaology Association (EHA), American Society of Cancer and Oncology (ASCO) and European Bone Marrow Transplant Association (EBMT)

From 15 - 16 November 2024

Armed Forces Bone Marrow Transplant Centre (AFBMTC), Rawalpindi – Pakistan

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This event was organized to present highlights of European Haemaology Association (EHA),American Society of Cancer and Oncology (ASCO) and European Bone Marrow Transplant Association (EBMT) This scientific event was organized to offer a selection of the latest advances in haematology and cutting- edge educational sessions with world experts . It provided a unique opportunity to share local data , delve into world experiences and to have a look into challenges which developing countries are facing in this ever evolving field.

The event was organized by Armed Forces Bone Marrow Transplant Centre (AFBMTC). located in Rawalpindi, Pakistan. AFBMTC was established in 2001. It is the largest transplant centre of the country, with the capacity of performing more than 200 transplants annually.Uptil now more than 1700 transplants have been done. AFBMTC is also the only centre of Pakistan to have a cellular therapy programme and in-house mesenchymal stem cell culture facility since 2015.

This event, i.e., “Practice Changing Updates in Haematology Treatment Sequences Insights” turned out as an excellent opportunity to interact with the experts in the field and for the trainees to get an insight into latest avenues and treatment models, across the globe. Hopefully it will go a long way in haematology practice to be specific and for the patients’ care in general. A few of the available abstracts form the conference are shared below

1 Hematopoietic Stem Cell Transplant Outcomes in Acute Myeloid Leukemia, Insights from Single Center Operating in Low- and Middle-Income Country

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Introduction: Acute Myeloid Leukemia (AML) is a distinct group of genetically heterogeneous disorders requiring intensive chemotherapy andHematopoietic Stem Cell Transplant (HSCT) as the mainstay of management. Despite lack of advanced genetic testing and targeted agents, HSCT is offered frequently in resource constrained countries as the only curative treatment for AML patients . This retrospective observational study aims to analyze the transplant outcomes in patients of AML who have undergone HSCT at AF Bone Marrow Transplant Center (AFBMTC).

Methodology: After obtaining IRB approval,data was collected from the medical records of patients who had undergone HSCT for AML from the year 2006 to September 2024. The data was entered and analyzed on SPSS (Statistical Package for the Social Sciences). Frequency and percentages were calculated. Mean and Standard Deviation were computed for descriptive variables while categorical variables were analyzed by Chi-Square test.

Results: Our current study included 83 patients who had undergone HSCT for AML. The majority of patients were male, 53 (63.9 %) while there were 30 (36.1%) females. The median age at HSCT was 22 years. Nine (10.8 %) patients had a favourable risk disease, 35 (42.2 %) had intermediate-risk disease, 7 (8.4 %) patients had adverse risk disease and risk stratification data was missing for 32 (38.6 %) cases. Five (6.0 %) patients were Minimal Residual Disease (MRD) positive, 18 (21.7 %) were MRD negative and MRD data was not available for 60 (72.3%) patients. Eighteen (22.5 %) patients were in Complete remission 1 (CR1), 28 (35.0 %) in CR2 and 3 (3.8 %) patients were in CR3 before HSCT. Twenty-nine (36.3 %) patients were in remission but could not be sub-categorized to CR1, CR2 or CR3. Two (2.5 %) were not in remission, while data was missing for 3 (3.6%) patients. Sixty (74.0 %) patients underwent Allogenic transplants, 14 (17.4%) underwent Haplo-identical transplants and 7 (8.6%) patients underwent Autologous stem cell transplants. The median days from diagnosis to HSCT were 270 days. The majority of the donors were siblings, 41 (49.4 %) were brothers while 29 (34.9 %) were sisters. One (1.2%) donor was a father and 1 (1.2%) was a mother. Donor's relation data was missing for 4 (4.8 %) patients. Seventy-two (90.0 %) patients were Cytomegalovirus (CMV) IgGpositive, 2 (4.0 %) were negative, CMV testing was not done for 7 (5.8 %) patients and the record was missing for 3 (3.6 %) patients. Sixty-six (86.0 %) out of 76 donors were IgG CMV positive. Forty-six (55.4 %) cases did not have an ABO mismatch, whereas 8 (9.6 %) had a major, 6 (7.2 %) had a minor, and 12 (14.5 %) had a bidirectional ABO mismatch. ABO mismatch data was missing for 3 cases. Cyclosporine and Methotrexate (CSA+MTX) were used for Graft Verses Host Disease (GVHD) prophylaxis in 59 (79.7 %) patients and post-transplant cyclophosphamide (PTCy) was given to 14 (16.9 %), whereas data was missing for 3 cases. Twenty-nine (38.1 %) cases had Anti-thymocyte Globulin (ATG)exposure whereas 43 (56.5 %) cases did not receive ATG and data were missing for 4 (5.2 %) cases. Seventy-seven (98.7 %) cases received Myeloablative conditioning (MAC), 1 (1.3 %) patient received reduced intensity conditioning (RIC) and conditioning data for rest was missing. Bone Marrow Harvest (BMH) was the preferred source of stem cells in 44 (55.7 %) patients, while BMH and Peripheral Blood Stem Cells (PBSC) were used in 11 (13.9 %) cases and PBSC alone in 17 (21.5 %) cases. Seven (8.9 %) patients received PBSC for Autologous HSCT. Stem cell source data was missing for 4 patients. Transplant complications includedFebrile Neutropenia in 43 (51.8 %) cases, Gut toxicity in 24 (28.9 %) cases, Mucositis in 58 (69.9 %) cases with 16 (19.3 %) having Grade III and 7 (8.4 %) having Grade IV mucositis. Thirty (48.4 %) patients developed acute GVHD (aGVHD) with 6 (20 %) cases having Grade III-IV aGVHD and was seen more in patients having BMH as a stem cell source (p-value=0.00). Thirty-two (51.6 %) patients remained aGVHD-free. Cytomegalovirus (CMV) reactivation was encountered

in 19 (22.9 %) cases, having a statistically significant correlation with ATG exposure (p-value =0.049). Forty-five (54.2 %) did not have a CMV reactivation and data was missing for 19 (22.9%) cases. Chronic GVHD (cGVHD) was documented in 22 (28.9 %) patients, while 37 (48.6 %) patients remained cGVHD-free. cGVHD was observed more in patients receiving a mean CD3 dose of 23.42 x 107/kg (p-value = 0.00). cGVHD Data was missing for 17 (22.3 %) cases. Out of the entire cohort, 16 (19.3%) cases relapsed while 46 (55.4%) had sustained remission and data was missing for 21 (25.3%) cases. Disease relapse had no statistically significant correlation with disease risk class or stem cell dosage. Currently, 45 (54.2%) cases are alive while there have been 34 (41 %) deaths and 4 (4.8%) cases are lost to follow-up. The Overall Survival (OS) was 68 % at a median follow-up of 288 Days.Post-HSCTOS was superior in AML patients having a favourable risk disease at the outset (p-value 0.001). Although the OS was better in patients taken to transplant in CR1, the current study could not establish any statistical significance in this finding. MRD Status also had no statistically significant correlation with the OS and this finding needs to be reassessed with a larger cohort. OS was found to be inferior in patients having CMV Reactivation (p-value 0.00). Disease-free survival (DFS) was 62 % at a median follow-up of 217 days.

Conclusion: In resource constrained setting lacking access to advanced genetic testing and therapeutics , HSCT remains a powerful treatment modality to cure the patients of this fatal disease. Further improvements in transplant strategies are needed to improve outcomes.

2 Impact of Bone Marrow Fibrosis on the Outcome of Chronic Myeloid Leukemia Treated With Second-Generation Tyrosine Kinase Inhibitors

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Objective: To evaluate the treatment response to second-generation tyrosine kinase inhibitors in patients with chronic myeloid leukemia, stratified by different grades of fibrosis at the time of diagnosis.

Methods: A longitudinal study was conducted at the NIBD and BMT, Karachi, from October 2022 to October 2023. Total of 150 CML patients receiving second-generation TKIs were included. BM fibrosis was graded according to WHO criteria. Patient having age 18 to 80 years of either gender diagnosed with CML on treatment with second generation TKIs were included in the study. Patients with other hematological malignancies were excluded from the study.

Results: The median age of patients was 43.5 years, with male predominance (60.7%). BM fibrosis was present in 69.3% of patients at diagnosis. Patients with lower fibrosis grades (MF 0-1) had significantly higher rates of CHR at 3 months (73.5%) and MMR at 6 months (64.7%) compared to those with higher grades (MF 2-3) who had CHR of 66.7% and MMR of 50%.

Discussion: Majority of patients in our study also present with higher grades of bone marrow fibrosis, as published in multiple international studies. Previous studies have also suggested that BM fibrosis grades are associated with outcomes of treatment in CML patients.[

Conclusion: BM fibrosis is a critical factor influencing the response to second-generation TKIs in CML patients. Lower grades of fibrosis correlate with higher response rates and better clinical outcomes, suggesting the importance of early intervention and tailored treatment approaches based on fibrosis grading.

3 Measurement of Telomere Length: A Prospective Biomarker for Disease Diagnosis

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Background: Telomeres are repetitive sequences of DNA (TTAGGG) found at the end of linear chromosomes. They protect gene loss during cell division and regulate somatic tissue replication, acting as a mitotic clock in the ageing process, and gradually shorten by 20-200 bp during each cycle of cell division. Telomere length (TL) shortening influenced by genetic mutations and environmental factors has been associated with worsened prognosis of diseases, and is believed to be a potential biomarker for certain conditions including aplastic anemia, dyskeratosis congenita, ulcerative colitis, myocardial infarction (MI), infertility, and diabetes mellitus (DM). Certain studies suggest a significant link between TL shortening and an increased risk of diseases.

Objective: This study aimed to explore the association between TL shortening and MI, infertility, and DM in Pakistani patients, to evaluate its potential as a diagnostic and prognostic biomarker for these diseases.

Methodology: This study included 50 patients of each MI, infertility, and DM, and 150 healthy volunteers as controls, along with fanconi anemia families. Peripheral blood samples were obtained from the participants after signing written informed consent. DNA was extracted followed by relative TL (rTL) measurement through Real Time-quantitative PCR using 36B4 as housekeeping genes, based on the Cawthon method (Cawthon, 2002). SPSS software was used to calculate the rTL to single copy gene ratio through the $2^{-\Delta\Delta Ct}$ method, along with T/S ratio.

Results: The findings of this study suggested a significant link between the T/S ratio and increasing age of patients in MI (P-value = 0.00018), infertility (P-value < 0.001), and DM (P-value < 0.001) as compared to healthy controls. The $2^{-\Delta\Delta Ct}$ was found to be non-significant in MI patients (P-value = 0.053). However, it was more significant in DM (P-value < 0.001) and infertility patients (P-value < 0.001) than healthy controls. A statistically significant (P-value = 0.031) positive correlation was noted between the TL and high-density lipoproteins of MI patients; while stress levels and TL had a significant negative correlation (P-value = 0.001). Conversely, MI patients who smoked had considerably shorter TL compared to non-smoking MI patients, but their correlation was statistically non-significant (P-value = 0.451). The fanconi anemia patients exhibited decreased TL as compared to healthy controls. Hence, our findings highlight a complex relationship between TL shortening and various clinical parameters, depicting that TL can be affected by multiple factors in different diseases.

Conclusions and Recommendations: The TL in MI, infertility, and DM in Pakistani patients was shorter than that of healthy controls, thus establishing a significant connection between TL shortening and onset and progression of disease. The aim of this study was to establish the TL testing protocol as easily accessible and adaptable for laboratories in Pakistan to support early detection and monitoring of various diseases. Longitudinal studies can be conducted in the future to better understand the correlation between progression of disease and TL shortening, and the role of TL as a prognostic biomarker.

4 Achieving Outcomes in Acute Promyelocytic Leukemia Comparable to Developed Countries by Implementing Locally Adapted European LeukemiaNet Guidelines

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Objective: This study aims to mitigate early mortality and improve APL patient outcomes in developing countries by implementing stringent standard operating procedures based on locally adapting European LeukemiaNet (ELN) guidelines.

Methodology: This longitudinal-observational study was conducted in the Section of Acute Leukemia at the National Institute of Blood Diseases & Bone Marrow Transplantation from April 2019 to December 2023.Diagnosis was established through the detection of PML-RARA by PCR with the average reporting time of not more than 48-hours. All patients were managed according to institutional standard operating procedures based on ELN guidelines. Statistical Package for the Social Sciences version 24.0 (SPSS Inc., Chicago, IL, USA) was used to analyze patients' data, and Kaplan-Meier method was employed to compute survival curves.

Selection & Description of Participants: Adult patients >15 years of age were enrolled after the diagnostic confirmation of APL. Other inclusion criteria included an ejection fraction of 40% or higher and an ECOG score of three or less. Patients with secondary APL, relapsed APL, and those with significant cardiac dysfunction (EF <40%) or renal impairment requiring dialysis were excluded from the study. A total of twenty-seven patients with de novo APL were enrolled over the aforementioned period.

Results: Of the total 27 evaluable APL patients, 19 (73.1%) were male. The median age at diagnosis was 31 years (range 18-53). Fever (n=19, 73.1%) and muco-cutaneous bleeding (n=9, 34.6%) were the most common findings at presentation. Sixteen (59%) patients were classified as high-risk. All twenty-seven patients underwent remission-induction chemotherapy. Two patients abandoned treatment before assessment of complete remission and were subsequently excluded from the analysis. Among the evaluable patients (n=25), 22 patients (88%) achieved CR. The diagnosis of differentiation syndrome (DS) was established in 8 (32%) patients. There were 3 (12%) mortalities during induction chemotherapy, all due to hemorrhage: one patient succumbed to a massive intracranial bleed, and two to diffuse alveolar hemorrhage. Another mortality was reported during the consolidation phase of chemotherapy.

The median duration of follow-up was 27.5 months. A single relapse was reported in the high-risk group during the study period. Overall, 2-year OS and DFS were 84.7% and 95.2%, respectively (Figure 1a). OS and DFS for the non-high risk and high-risk groups were 90% & 100%, and 81.3% & 90.9%, respectively. The 2-year cumulative incidence of relapse and non-relapse mortality was 4.8%(Figure 1b). No relapse mortality was reported during the study period.

Discussion: Despite APL being highly curable, early mortality in developing countries often remains high resulting is dismal outcomes. Our findings demonstrate that implementing a structured approach, guided by standard operating procedures based on locally adapted guidelines, provided critical clarity in managing APL care. This clarity ensured that all team members knew precisely what actions to take, when to take them, and how to execute them

effectively. By reducing reliance on ad hoc decisions, this systematic approach substantially improved early survival rates and long-term outcomes in our cohort.

Conclusion: Structured, guideline-driven management effectively reduces early mortality and improves survival outcomes in APL patients in developing countries, supporting broader implementation of these approaches.

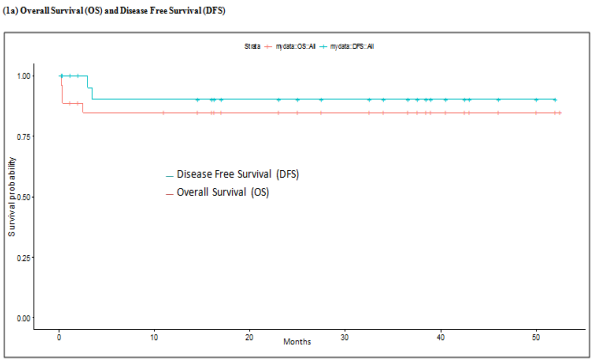

Figure 1a

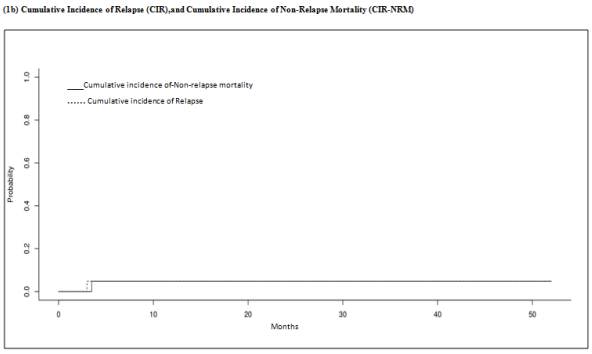

Figure 1b

5 Peripheral Hematological Predictors of Morphological Remission/Hematopoietic Recovery in Plasma Cell Myeloma After Induction Chemotherapy

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Objectives: to determine the predictive values of peripheral hematological markers for remission in cases of Plasma cell disorders/Multiple myeloma after induction therapy.

Methodology: This prospective study was conducted in the department of Hematology, MTI Hayatabad Medical Complex, Peshawar from 1st June 2021 to 30th June 2022. All cases of Plasma cell disorders referred to department for remission after taking induction therapy, irrespective of age and gender were included. Relevant information's were collected on a predesigned proforma prepared in accordance with the objectives of the study.

Results: A total of 36 cases referred for remission of plasma cell disorders were included, with 21(58.33%) were males and 15 (41.66%) females. The mean with standard deviation of numerical variables were; Age (56+ 8 years), Hb% (11+ 2.3 g/dl), Reticulocyte count (0.9+ 0.5%) and plasma cells in the remission cases as 16+ 2.76%. Median of the TLC and Platelets in remission cases were 6275/cmm3 and 198000/cmm3 respectively. There was a significant association of remission with female gender as compared to male gender (p=0.05) while no such association was seen in age groups (p=0.57). We observed a statistically significant an inverse correlation of remission (in term of lower percentage of plasma cell in the bone marrow aspirations) with an increase in retic count (rs = -0.397, p = 0.053). A similar inverse correlation was seen between remission (plasma cell percentage) with TLC and platelet count that was not statistically significant (p >0.05). Reticulocyte count showed a higher clinical sensitivity for remission with an Area Under Curve (AUC) of 0.733 on Receiver Operating Curve (ROC).

Conclusion: In cases of Plasma cell disorder (Multiple myeloma) with post-induction therapy, the peripheral blood values for an increased in Reticulocyte count predict the remission with 95% confidence.

Remission favors Female gender more as compared to male gender in all age categories. No significant relationship was noted for TLC/ANC and platelet count to predict remission in plasma cell disorders. These values, if observed with vigilance, can reduce the frequency of invasive procedures like bone marrow aspiration.

Key words: Multiple Myeloma, Remission, Reticulocyte count, Hematological markers

6 Insights into Rare Hematological Disorders (RHDs) in Pakistan: (Variants analysis, Epidemiology, and Database Establishment)

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Rare hematological disorders (RHDs) are uncommon and life-threatening blood disorders, affecting the lymphoid tissues, causing bone marrow failure, and forming abnormalities in blood cell production and function. RHDs have heterogenous presentation; they vary in symptoms, severity, and progression. Some of the main RHDs include; Thalassemia, Fanconi Anemia (FA), Aplastic Anemia, Paroxysmal Nocturnal Hemoglobinuria (PNH) and Autoimmune Hemolytic Anemia (AIHA). RHDs can be categorized on the basis of a) Cell lineage “like Red Blood Cell Disorders, White Blood Cell Disorders, Platelet Disorders”, b) Disease mechanism “Immune Mediated, Genetic, and other Environmental factors”, and c) Severity “ mild, moderate, and severe”. Diagnosis of RHDs often requires; clinical evaluation, laboratory tests, imaging studies, molecular analysis, and immunophenotyping. RHDs can be treated with immunotherapy, chemotherapy, targeted gene therapy, stem cell or bone marrow transplantation and palliative care. The management of (RHDs) in Pakistan faces numerous challenges. Despite their profound impact on public health, RHDs remain vastly understudied in Pakistan, resulting in a significant knowledge gap. The lack of robust epidemiological data and centralized registries hinder accurate estimation of RHDs prevalence, incidence, and mortality rates. Furthermore, limited access to genetic testing and counseling services complicates the diagnosis and management of genetically linked RHDs. These disorders are rare but have profound implications on patients’ lifestyle and treatment strategies. The prevalence of RHDS at Global and Pakistani level is as follows: DISORDER GLOBAL PAKISTAN Aplastic Anemia 2-3 cases per million 5-6 cases per million Methods: The methodology of this study involves following steps: [1.] Collecting epidemiological data alongside consent form, laboratory test reports, including CBC, BM, immunophenotyping, karyotyping, etc. [2]. Multi-faceted diagnostic approaches including Next Generation Sequencing (NGS) for variant(s) detection and Sanger Sequencing for the segregation of variant(s). [3]. To determine protein function and variant(s) effect on signaling pathways through multiple In Silico Tools. Expected outcomes [1]. To establish Pakistan’s first national RHD database to: o Develop regional cost-effective diagnostic networks o Standardized disease classification and genetic variant (s) documentation o Treatment strategies and outcomes [2]. Enhance the understanding of RHD patterns in Pakistani population [3]. Improve research collaboration opportunities [4.] Evidence-based healthcare treatment framework [5]. Formulate regional policies Conclusions: This study emphasizes on the importance of genetic screening in Pakistan to better understand disease mechanisms, prevalence, management, and

segregation of RHDs in Pakistani population. It also aims to establish data registry to collect and organize RHDs data in Pakistan to facilitate the affected individuals by providing timely diagnosis and targeted therapies. We are open to collaborating to form a multidisciplinary team to achieve these targets. Keywords: Rare hematological disorders, Pakistan, genetic testing, consanguinity, thalassemia. Falconi Anemia 1 in 130,000 4-5 cases per million Paroxysmal Nocturnal Hemoglobinuria 1-1.5 cases per million Exact figures are not available Beta Thalassemia 3% Carrier Frequency 5-8% Carrier Frequency Autoimmune Hemolytic Anemia 1-3 cases per 100,000 Exact figures are not available

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