Proceedings of workshop and CME organized by Department of Pathology at Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun.

Title: Hematology Insights: From Coagulation to Flow Cytometry

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On August 10, 2024, a comprehensive coagulation workshop was held at Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand, led by Dr. Sukesh C. Nair Professor. Department of Laboratory Medicine and Transfusion Medicine and Immunohematology, CMC Vellore. The workshop brought together 40 participants from across Uttarakhand for an in-depth exploration of coagulation diagnostics and management. The workshop commenced with a warm welcome address by Dr. Smita Chandra, Head of the Department of Pathology at Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, Uttarakhand. Dr. Vikas Shrivastava (AIIMS Gorakhpur), delivered the opening lecture on the intricacies of the coagulation cascade, discussing major issues and recent advancements in the field. Dr. Avriti Baveja (HIMS, Dehradun) provided a systematic approach to identifying and evaluating patients with bleeding symptoms, offering insights into underlying causes and management strategies. Dr. Mansi Kala (HIMS, Dehradun) covered essential screening tests, including Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), and Specific Bleeding Time (SBT). She also discussed reagent selection and patient considerations to ensure accurate results. Participants engaged in practical sessions to perform PT, APTT, and SBT tests, reinforcing the theoretical knowledge with hands-on experience.

Dr. Priyavadhana (AIIMS, Rishikesh) explained the significance of mixing studies for diagnosing bleeding and thrombotic disorders, including lupus anticoagulants. She also discussed factor assays for identifying specific coagulation factor deficiencies. Dr. Mansi Kala covered inhibitor screening tests, focusing on Hemophilia A patients and Bethesda Assays. Dr. Sukesh C. Nair provided an overview of global haemostasis testing techniques, including ROTEM/TEG, Thromboelastography (TGT/TGA), and Clot Waveform Analysis (CWA). These tests offer comprehensive insights into the hemostatic process and are crucial for advanced coagulation

assessments. Under the guidance of Dr. Manish Raturi, Dr. Sumit Garg, Dr. Yashaswi Dhiman, and Mr. Manmohan Singh Bisht from HIMS, Dehradun, participants practiced mixing studies, inhibitor screening, and factor assays, applying the concepts discussed in earlier sessions. Dr. Sukesh C. Nair concluded the workshop by discussing the results from the hands-on sessions. He addressed participant questions, clarified key concepts, and offered strategies for maintaining high-quality coagulation testing in laboratories, especially when patient referrals are infrequent. The workshop was a valuable learning experience for all attendees, providing them with practical skills and insights into advanced coagulation diagnostics and management.

The hematology-focused CME was held on 11th August 2024 covered a range of topics including hypercoagulability, lymphoma diagnosis, management strategies of abnormal bleeding and discussion on unique medical cases. The CME commenced with the first session by Dr. (Col) Jyoti Kotwal, Professor Department of Hematology, Sir Ganga Ram Hospital, New Delhi. She highlighted the significant risk of thrombosis at high altitudes, particularly among Indian soldiers, noting increased hemoglobin, platelet activation, and PAI-1 levels which suggest a hypercoagulable state. She emphasized the importance of personalized anticoagulation therapy as standard approaches may not prevent thrombosis or bleeding in all patients. D-dimer testing, crucial for assessing fibrin degradation, plays an emerging role in guiding anticoagulation therapy duration, especially after unprovoked venous thromboembolism (VTE). She also advised careful timing of thrombophilia testing to ensure accuracy and stressed the need for comprehensive panels and appropriate diagnostic assays for conditions like antiphospholipid syndrome. The session was followed by question and answer sessions by hematologist cardiologist, neurologist, physicians and pathologist.

The next session was taken by Dr. Prashant Tembhare, Professor Department of Hematopathology, Advanced Centre for Treatment Research & Education in Cancer, TMH Mumbai. This session highlighted the use of flow cytometry, a powerful tool in diagnosing different types of lymphoma. Dr. Prashant Tembhare delivered an in-depth discussion on the processing and analysis of samples using flow cytometry with a particular focus on B-cell and T-cell Non-Hodgkin Lymphomas (NHL). He began by explaining the preparation of single-cell suspensions from various specimen types, such as peripheral blood, bone marrow aspirate, cerebrospinal fluid, pleural fluid, and bronchoalveolar lavage which naturally contain cells in suspension. For solid tissues special techniques like slicing, mincing, and teasing, followed by filtering are necessary to create single-cell suspensions. Dr. Tembhare also stressed the importance of sample stabilization, highlighting the use of anticoagulants like EDTA or heparin to prevent clotting in blood samples and media to preserve cells in fluid samples and fine needle aspiration cytology. On his analysis of B-cell NHL, Dr. Tembhare described a systematic approach that begins with identifying CD19-positive, CD45-bright abnormal lymphoid cells. If CD200 expression is positive, the next step is to assess CD20. A bright CD20 suggests an atypical Chronic Lymphocytic Leukemia (CLL), while a dim CD20 indicates typical CLL. If CD200 is negative and CD20 is moderate, Mantle Cell Lymphoma is considered. He also noted that approximately 5% of other B-NHL cases may express CD5, making it crucial to check for CD10 expression. Tumor cells that are CD10 positive with dim to negative CD38 expression and low forward scatter typically indicate follicular lymphoma whereas bright expressions of CD38 and CD81 supported by morphological findings suggest Burkitts Lymphoma.

For T-cell NHL, Dr. Tembhare's approach begins with assessing CD3-positive cells. If these cells exhibit aberrant expression of TCR gamma delta along with CD8, CD5, CD16, and CD56, a

diagnosis of gamma delta T-cell NHL is made. If TCR gamma delta is negative abnormalities in other T/NK cell markers like CD2, CD5, CD7, CD16, and CD56, combined with TRBC1 (a T-cell clonality marker) indicate a neoplastic process further classified based on CD4 and CD8 expression. If two or more NK markers are positive along with HLADR and CD94 a diagnosis of NK-cell NHL is made. This comprehensive framework underscores the critical role of specific marker expressions in accurately diagnosing various types of lymphomas using flow cytometry.

The next session was by Dr. Sukesh C. Nair (CMC, Vellore) who addressed the critical issue of abnormal bleeding, focusing on understanding its causes (why it occurs), its location (where it occurs), the mechanisms involved (how it happens), and the timing (when it occurs) to effectively manage and stop bleeding episodes. This discussion was crucial for clinicians managing patients with bleeding disorders or those at risk of bleeding due to surgery, trauma, or other medical conditions. He emphasized that high hematocrit levels can increase the likelihood of platelets interacting with activated endothelium, which can lead to hemostasis or thrombosis.

Dr. Prashant Tembhare (TMH, Mumbai) in next session presented a series of unique and challenging clinical cases offering valuable insights into complex diagnostic processes, unusual disease presentations, and innovative treatment approaches. Dr. Tembhare engaged the audience with intriguing cases primarily focused on B and T cell Non-Hodgkin lymphoma, Hodgkin lymphoma, and leukemias.

One of the most engaging sessions conducted by Dr. Sukesh C. Nair (CMC, Vellore) focused on the principles of hematology analyzers and their impact on modern diagnostics, virtually replacing traditional microscopy. He highlighted how additional data along with dot plots can provide valuable insights into pathology without the need for detailed peripheral smear analysis. He emphasized the reliability of platelet counts when all flags and histograms are carefully considered. Furthermore, Dr. Nair discussed how advanced parameters such as fluorescence, volume, conductivity and scatter can be utilized in research to better understand and differentiate various disease processes.

The last session was taken by Dr. Suchitra Jain (Delhi) who compared automated urine analyzers with traditional manual methods for detecting dysmorphic red cells in urine which is a key indicator of kidney-related conditions. The discussion focused on the accuracy, efficiency, and practical implications of using automated systems versus manual examination particularly in clinical settings where timely and accurate diagnosis is important.

A concurrent oral and poster presentation session was held where researchers showcased their work. These sessions offered attendees the chance to engage directly with researchers, discuss findings, ask questions, and explore new ideas in an informal setting.

The CME ended with vote of thanks by Dr Mansi Kala, HIMS, Dehradun.