THE HEMATOLOGY INDICA

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INDEPENDENCE DAY ISSUE

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NEWSLETTER

Theme: Augmenting Human Capabilities - The Role of Machine Intelligence

Message from President, ISHBT

Dr. Sarmila Chandra

The Indian Society of Haematology and Blood Transfusion, founded in 1973 by our very learned and respected predecessors is now 52 years old with a long tradition of nurturing the subject of haematology all over the country. The main aim was to promote excellence in patient care, research and education in the fields of clinical, laboratory haematology and transfusion medicine until the latter decided to form their own body and separated themselves from the parent body. At the same time the subject of haematology underwent a tremendous expansion with the inclusion of genetic medicine and molecular and immunological branches of medicine in relation to haematological disorders. The treatment of haematological disorders was also gradually becoming more precise and target oriented with the inclusion of newer diagnostic tools. More technology oriented procedures like Stem Cell Transplantation, CAR-T Cell Therapy, Gene therapy and editing were becoming more commonly available to the public. Besides helping in the building of institutions of excellence, another important service rendered by ISHBT is dissemination of knowledge for doctors by holding regular Conferences, updates, workshops and symposia all over the country to make it available to all doctors far and near. The international collaborations set up between ISHBT and American Society of Haematology and European Society of Haematology also provide a platform to discuss the latest of research and information directly to our doctors in India. The Indian College of Haematology, the academic wing of ISHBT also helps the government to formulate guidelines, give fellowships to the esteemed members with good contribution in the field of haematology.

We know that India is a big country and in spite of the good work done by our predecessors and ISHBT, the subject of haematology is mainly restricted to the large and metro cities. It is difficult to get trained haematologists for teaching in medical colleges which are coming up in tier two and three cities. Similarly, there is a dearth in the availability of practising haematologists in the private sector to look after the general public. It is difficult to get good infrastructure for treating haematological patients in hospitals situated in smaller cities and towns. Availability of good blood banks catering to the needs of these patients is another problem area which is not only due to lack of willingness to donate blood in the general public, but also due to the lack of trained manpower. Considering all these problems, we decided to spread the message of haematology to the smaller cities and peripheral areas, whether be it medical colleges or general practitioners practising in the area. In other words, if we can think of the spread of haematology in big cities in the form of a pyramid then, now we have to spread farther laterally away from these pyramidal growth areas in order to bring uniformity of knowledge dispersion throughout the country.

Keeping this in mind, we decided to formulate our future plans for this and future years. The plan has been well elucidated by our secretary Sri T. K. Dolai in our last newsletter. In short, this would entail i) carrying out all past activities including Conferences, masterclasses, CMEs and updates, publication of the prestigious Journal etc which were carried out earlier. Apart from that, ii) regular updates and Seminars in smaller cities and towns involving local haematology organisations, iii) preparing a teaching module for undergraduate students where teachers trained in haematology are not available locally, iv) completion of guidelines for the treatment of haematological diseases, v) opening a central library facility where journal & articles on haematology can be easily procured by doctors living in far flung areas where library facilities are not easily available, vi) better utilization of the ISHBT office and continue with the outreach programmes as many as we can.

I would like to end my message by thanking you sincerely for electing me to the office. I hope that my contribution, however small, will help to make ISHBT a democratic and more member-friendly organization.

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Message from President - Elect, ISHBT

Brig. Tathagata Chatterjee

Indian Society of Hematology and Blood Transfusion has now completed more than fifty years of its challenging journey as the sole national scientific society in hematology. It is a society with a difference inculcating both benign and malignant hematology, adult and paediatric, from both the laboratory and clinical aspects as well as Transfusion medicine. It is a unique society having clinicians and pathologists who have adequate expertise/training in hematology working for a common cause and speaking the same language for the betterment of diagnosis and management of patients.

The annual meeting of the society provides a platform for all such professionals to come together and discuss recent advances in diagnosis and management of haematological disorders. The past twenty-five years have witnessed tremendous advancement in the training and fellowship programmes including DM Clinical Hematology/Hematopathology as well as Dr NB Doctorate programmes of NBE in Hematology. This has increased the specialist cadre in hematology and improved the prospects of this subject nationally catering to the professional needs of patients with haematological diseases. However, we need more of such specialists in our country and for this, robust awareness programmes are the need of the hour. A strategic plan must be developed so that more and more CMEs and community programmes are implemented in the lesser known states and cities of our country. This is the only realistic way to increase awareness in the country on all aspects of haematological disorders.

Research in hematology has to be taken more seriously now. Collaborative projects with scientific institutes of excellence is the need of the hour. We must have MoUs with top institutes like IITs, NCCS(Pune), DIPAS (Delhi), IISc (Bangalore) in the fields of nanotechnology, cell sciences and molecular biology, proteomics and artificial intelligence. Many of our young DMs and Fellows must be encouraged to collaborate with scientists in such institutes for the same. I request the executive body to look at this aspect and develop MoUs with such institutes where our young specialists can train in various technologies and they are not required to go abroad when most advanced facilities exist in our country. Liaison with Health ministry officials of centre and state can also be addressed to implement community research programmes like the one already being done for Sickle cell anemia.

Bone marrow transplant programmes should no longer be considered the sole property of top corporate hospitals and COEs. A strategy to implement such programmes in govt hospitals and in resource limited settings must be addressed. ISHBT can form a committee to look into this aspect.

A strong committee must be formed to implement DM Hematopathology in NEET-SS exams so that appointments of Hematopathologists can be created in the departments of Pathology in various medical colleges. I request the Executive body to once again re-look at this important aspect since oncopathology is now included in NEET-SS, so why not Hematopathology? This will help many Pathologists with DM Hematopathology to earn their recognition which they rightly deserve.

We must also go all out to increase the number of life members of our society to at least 5000 in the next two years. This is a realistic target and all of us must share this responsibility. We all must encourage our colleagues in medical colleges and hospitals to become members of ISHBT. Incidentally the fees for life member of our society is probably the lowest for any professional body in India

I am delighted to see the energetic secretary and executive body of ISHBT managing the society so well. Professional management and a permanent dedicated address of our society has all been achieved through their hard work and I congratulate them for the same.

I take this opportunity to thank all the respected members of our society for giving me the privilege to be Executive body member in the past and now the President Elect of ISHBT this year. I assure you of my hard work and dedication that will bring laurels to the society.

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Long live ISHBT. Jai Hind

"Diagnosis is not the end, but the beginning of the practice of medicine." — Martin H. Fische

INSIDE

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Newer Parameters in Hematology Analyzers: Principles, Technologies, Interpretation & Clinical Utilities

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Introduction

Blood cell analysis has undergone a remarkable transformation since the early 20th century. What began as manual counting under microscopes has evolved into sophisticated automated systems capable of analyzing multiple parameters within seconds. The journey of hematology analyzers represents one of the most significant technological advances in laboratory medicine, revolutionizing both the speed and accuracy of blood diagnostics.

The first automated blood cell counter was developed by Wallace Coulter in the 1950s based on the principle of impedance, which detected changes in electrical conductance as cells passed through a small aperture. This innovation, known as the Coulter Principle, laid the foundation for modern hematology analysis. By the 1970s, multi-parameter analyzers emerged that could measure cell volume along with count, and by the 1980s, flow cytometry techniques were incorporated to enhance cellular differentiation capabilities.

The 1990s witnessed rapid advancement with the introduction of multi-angle light scatter technology, allowing for more precise cell classification. The early 2000s brought the integration of digital imaging and artificial intelligence, enabling analyzers to provide more detailed cellular morphology information. Today's advanced hematology analyzers combine multiple technologies—impedance, flow cytometry, fluorescence, and digital imaging—to deliver comprehensive blood cell analysis with remarkable precision and efficiency. These technological advances have expanded the range of measurable parameters far beyond the traditional complete blood count (CBC), introducing newer parameters that provide deeper insights into blood cell characteristics, maturation, and function.

A.Reticulocyte Parameters

1. Reticulocyte Hemoglobin Content (RET-He)

Reticulocyte Hemoglobin Content (RET-He) is a parameter that measures the hemoglobin content in reticulocytes, the youngest red blood cells (RBCs). This parameter is derived using flow cytometry combined with fluorescence or optical scattering. Reticulocyte hemoglobin content provides an indirect measure of available functional iron availability for erythropoiesis and normal hemoglobinization. A low content suggests functional iron deficiency, early depletion or inadequate erythropoiesis. A high value may be found in hemoglobinopathies or excessive iron therapy. The normal range used in most of the analyzers is 28 to 35 picograms.

This parameter helps in the early detection of iron deficiency before anemia develops. Useful in monitoring response to iron or erythropoiesis-stimulating therapy. It is a useful marker to differentiate between iron deficiency anemia and anemia of chronic disease.

2. Immature Reticulocyte Fraction (IRF)

Reticulocytes are immature RBCs containing residual RNA that can be detected by supravital staining. Modern hematology analyzers use flowcytometry with the help of fluorescent dyes like thiazole orange or polymethine dyes, that specifically stain the reticulocyte RNA. Based on the RNA content, reticulocytes are classified into low fluorescence reticulocyte (LFR-most mature), medium fluorescence reticulocyte (MFR – intermediate maturity) and high fluorescence reticulocyte (HFR – most immature). Immature reticulocyte fraction is the proportion of young, newly released reticulocytes which includes MFR and HFR. (Figure 1). Its clinical utility is manifold. These includes

1. Early detection of functional iron deficiency in chronic kidney disease
2. Monitoring response to erythropoiesis-stimulating agents
3. Assessment of bone marrow recovery post-chemotherapy
4. Differentiation of causes of anemia
5. Evaluation of erythropoietic activity in hemolytic conditions
6. Optimizing the timing of stem cell harvesting

Certain advanced analyzers also measure reticulocyte parameters including reticulocyte maturation index and mean reticulocyte volume.

B.Platelet Parameters

Modern analyzers evaluate platelets using multiple technologies simultaneously:

1. Impedance method - based on cell volume
2. Optical method - based on light scatter properties
3. Fluorescence method - using platelet-specific markers

These techniques allow for accurate assessment of platelet count and various qualitative parameters.

1. Mean Platelet Volume (MPV)

- Normal range: 7.5-11.5 fL
- Elevated in:
  - Immune thrombocytopenia (ITP)
  - Bernard-Soulier syndrome
  - May-Hegglin anomaly
  - Myeloproliferative disorders
  - Recovery phase of thrombocytopenia
- Decreased in:
  - Wiskott-Aldrich syndrome
  - Chemotherapy-induced thrombocytopenia

2. Platelet Distribution Width (PDW)

- Percentage range: (9-17%)
- Reflects the heterogeneity in platelet size
- Increased in active platelet production and ITP
- Useful in differentiating reactive thrombocytosis from essential thrombocythemia

3. Immature Platelet Fraction (IPF)

- Normal range: 1-7%
- Represents newly released platelets containing residual RNA
- Elevated values indicate enhanced thrombopoiesis
- Useful in:
  - Differentiating hyperdestructive/consumptive from hypoproductive thrombocytopenia
  - Predicting platelet recovery after chemotherapy
  - Assessing bleeding risk in thrombocytopenic patients

4. Platelet-Large Cell Ratio (P-LCR)

- Normal range: 15-35%
- Percentage of platelets larger than 12 fL
- Elevated in conditions with increased platelet turnover

5. Reticulated Platelets

- Normal range: 1-12%

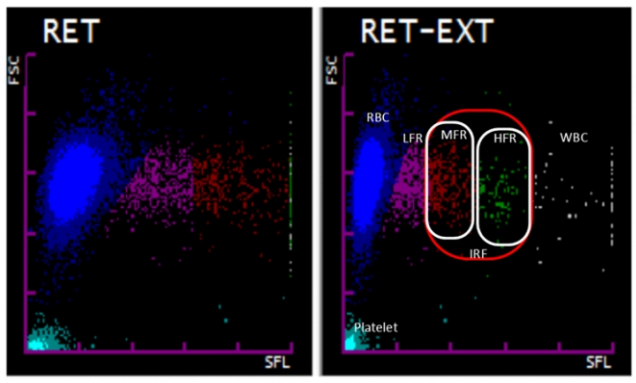

Figure 1: Immature reticulocyte fraction is the proportion of young, newly released reticulocytes which includes MFR and HFR.

- Represents newly released platelets containing residual RNA
- Measured using flow cytometry with RNA-binding fluorescent dyes
- Correlates with platelet production rate

Clinical Applications:

1. Differentiation of causes of thrombocytopenia (hypoproductive vs. hyperdestructive)
2. Monitoring thrombopoietic activity and predicting platelet recovery
3. Identifying patients at risk of bleeding despite normal platelet counts
4. Monitoring antiplatelet therapy
5. Assessment of platelet activation status
6. Early detection of sepsis-associated coagulopathy

C. White Blood Cell Advanced Parameters

Principle and Technology

Modern analyzers characterize white blood cells using volume/impedance measurement, light scatter properties at multiple angles, fluorescence intensity after specific staining, cell peroxidase activity and nuclear complexity assessment. These technologies enable the differentiation of standard five-part differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), Immature granulocytes (IG), Atypical lymphocytes, Blasts and Nucleated red blood cells (NRBC).

1. Immature Granulocyte Fraction (IG%)

- Normal range: Usually no immature granulocyte is seen in adults
- Includes promyelocytes, myelocytes and meta-myelocytes
- Elevated in:
  - Bacterial infections and sepsis
  - Inflammatory conditions
  - Myeloproliferative disorders
  - Chemotherapy recovery
  - Pregnancy (third trimester)

2. Neutrophil Reactivity Index (NEUT-RI) or NEUT-X

- Reflects the cellular activation and metabolic activity of neutrophils
- Elevated in:
  - Bacterial infections and sepsis
  - Inflammatory disorders
  - Post-surgical inflammation

3. High Fluorescence Lymphocyte Cells (HFLC)

- Represents activated lymphocytes with high nucleic acid content
- Elevated in:
  - Viral infections (e.g., EBV, CMV, HIV)
  - Lymphoproliferative disorders
  - Autoimmune conditions

4. Nucleated Red Blood Cells (NRBC)

- Normal range: 0/100 WBC in peripheral blood
- Automated quantification eliminates manual counting errors
- Clinical significance:

"The greatest enemy of knowledge is not ignorance, it is the illusion of knowledge." Stephen Hawking

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Microcytic RBC Percentage (%MICRO-R)	Macrocytic RBC Percentage (%MACRO-R)
• Normal range: <5% • Elevated in: • Iron deficiency anemia • Thalassemia • Lead poisoning • Sideroblastic anemia	• Normal range: <5% • Elevated in: • Vitamin B12/folate deficiency • Liver disease • Alcoholism • Myelodysplastic syndromes • Reticulocytosis

Hypochromic RBC Percentage (%HYPO-He)	Hyperchromic RBC Percentage (%HYPER-He)
• Normal range: <5% • Elevated in: • Iron deficiency anemia • Thalassemia • Anemia of chronic disease Lead toxicity	• Normal range: <2.5% • Elevated in: • Hereditary spherocytosis • Hemolytic anemias • Burn patients

- o Severe hypoxia
- o Hemolysis
- o Myelofibrosis
- o Extramedullary hematopoiesis
- o Infiltrative bone marrow disease
- o Post-splenectomy state

5. Hematopoietic Progenitor Cells (HPC)

- Normal range: <0.1%
- Elevated in:
  - o Recovery phase post-chemotherapy
  - o After stem cell mobilization procedures
  - o Myeloproliferative disorders
  - o Acute leukemias
- Clinical applications:
  - o Optimizing timing for stem cell collection
  - o Early detection of bone marrow recovery
  - o Monitoring stem cell mobilization treatments
  - o Screening for hematological malignancies

6. Neutrophil Granularity Index (NEUT-GI)

- Reflects the internal complexity and granularity of neutrophils
- Decreased in:
  - o Sepsis (early sign often preceding other markers)
  - o Systemic inflammatory response syndrome (SIRS)
  - o Toxic granulation
- Clinical utility:
  - o Early recognition of sepsis (changes within hours)
  - o Monitoring response to antibiotics
  - o Distinguishing bacterial from viral infections

Clinical Applications of Advanced WBC Parameters:

1. Early detection of sepsis and infection (24-48 hours before conventional markers)
2. Differentiation between bacterial and viral infections
3. Monitoring of neutrophil activation states in critically ill patients
4. Screening and monitoring of hematological malignancies
5. Reducing manual differential counts and slide reviews
6. Optimization of stem cell mobilization and collection
7. Monitoring bone marrow recovery post-chemotherapy or transplantation

D. Cell Population Data (CPD)

Principle and Technology

Cell Population Data (CPD) represents a significant advancement in hematological analytics, extending beyond traditional CBC parameters to provide detailed morphological and functional information about blood cells. These parameters are derived from high-resolution analysis of light scatter, fluorescence and impedance measurements. For neutrophils, these typically include:

Volume/Size Parameters:

- NEUT-Z: Mean neutrophil volume
- NEUT-WZ: Neutrophil volume distribution width

Complexity/Granularity Parameters:

- NEUT-X: Mean neutrophil internal complexity
- NEUT-WX: Neutrophil complexity distribution width

Nuclear Structure Parameters:

- NEUT-Y: Mean neutrophil nuclear complexity
- NEUT-WY: Neutrophil nuclear complexity distribution width

Similar parameters exist for other cell types (lymphocytes, monocytes, etc.)

Clinical Applications:

1. Early Sepsis Detection:

- o Neutrophil volume and complexity parameters change within hours of infection
- o Can precede clinical signs by 24-48 hours
- o Demonstrated sensitivity of 90-95% and specificity of 80-85% for early sepsis

2. Inflammatory Response Monitoring:

- o Distinguishing bacterial vs. viral infections
- o Tracking resolution of inflammation
- o Predicting clinical deterioration

3. Hematological Malignancies:

- o Early detection of dysplastic changes
- o Monitoring of treatment response
- o Minimal residual disease detection

4. Systemic Disorders:

- o Autoimmune diseases
- o Metabolic disorders affecting cell morphology
- o Toxic exposures

E.RBC Cytogram Analysis

Principle and Technology

RBC cytogram analysis employs multi-dimensional assessment of red blood cell characteristics using a combination of Volume measurement (impedance), Hemoglobin concentration (spectrophotometry), Cell shape assessment (light scatter analysis) and Membrane deformability analysis (flow dynamics)

The data is typically presented as a 2D or 3D scatter plot, allowing visualization of RBC subpopulations.

Extended RBC Parameters

Advanced hematology analyzers can now provide detailed information about RBC membrane properties and hemoglobin content using techniques such as:

- Multi-angle light scatter analysis
- Hemoglobin absorbance measurement
- Deformability assessment
- Advanced algorithmic analysis of RBC populations

These technologies enable the evaluation of RBC fragility, density, and functional characteristics beyond traditional morphological assessment.

1. RBC Fragmentation Score (FRC)

- Normal range: <1%
- Elevated in:
  - o Thrombotic microangiopathies (TTP, HUS, DIC)
  - o Mechanical heart valves
  - o Severe burns
  - o Malignant hypertension
  - o HELLP syndrome

2. Reticulocyte Production Index (RPI)

- Corrects reticulocyte count for the degree of anemia and bone marrow response
- When hemoglobin is normal : ranges from 1.0-2.0
- In anemia <2.0 in hypoproliferative states (marrow failure, nutritional deficiencies), >3.0 in hyperproliferative states (hemolysis, hemorrhage)

3. Delta-He

- The difference between reticulocyte and mature RBC hemoglobin content
- Reflects the trend in iron availability for erythropoiesis
- Negative values indicate worsening iron status
- Positive values suggest improving iron availability

4. RBC Lifespan Estimation

- Indirect measurement of RBC survival based on reticulocyte dynamics
- Shortened in hemolytic conditions, extended in certain pathological states

Clinical Applications:

1. Early detection of microangiopathic hemolytic anemias
2. Monitoring for fragmentation in patients with mechanical heart valves
3. Classification of anemia as hypoproliferative vs. hyperproliferative
4. Real-time assessment of iron therapy effectiveness
5. Early detection of erythropoietic dysfunction in critically ill patients
6. Monitoring of transfusion requirements in chronic conditions

Conclusion

The evolution of hematology analyzers from simple cell counters to sophisticated diagnostic platforms has revolutionized laboratory medicine. Newer parameters provide insights into cellular morphology, function, and pathophysiology, enabling earlier detection of disease, more precise therapeutic monitoring, and improved patient outcome. The integration of these advanced parameters into clinical practice requires understanding their principles, technology, interpretation, and limitations. As these technologies continue to evolve, the collaboration between laboratory scientists, clinicians, and manufacturers will be crucial in optimizing their utility and advancing patient care.

■■

"It is much more important to know what sort of patient has a disease than what sort of disease a patient has." — William Osler

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Automated Hematology Analyzers: Evolution and Novel Parameters

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History and evolution of automated hematology analyzers

The discovery of the "Coulter principle" by Wallace H. Coulter in the 1950s revolutionized the estimation of the complete blood count (CBC). Compared to the cumbersome and imprecise manual method, automation provides much more rapid, precise and accurate CBC results. From the traditional 3-part hematology analyzers, now 5-part, 6-part, and still more advanced analyzers are available with added ability to refine the WBC differential to include atypical lymphocytes, blasts and immature granulocytes. Some analyzers also enumerate nucleated RBCs and fragmented RBCs. Other advancements include more accurate platelet, reticulocyte counts and newer parameters with clinical utility in various conditions.

The following section gives general salient features of commonly used automated hematology analyzers which are not model specific:

Beckman Coulter analyzers:

- WBCs segregation by simultaneous measurements of cells based on the Volume, Conductivity and Scatter (VCS) properties. Cell populations are discriminated by 3-dimensional cluster analysis.
- Cell population data for all white cells can be generated which have a role in infections, inflammatory conditions and hematological malignancies.
- Precision is enhanced by counting cells in triplicate and by extending the counting time in cases of leukopenia or thrombocytopenia.
- NRBC correction is also available.

Sysmex analyzers

- Cyanide free methodology (lauryl sulphate) for hemoglobin estimation.
- Hb channel is distinct from the WBC channel. Hence a strong lytic agent can be used without interfering in hemoglobin estimation.
- Fluorescence flow cytometry is used for WBC enumeration: laser light and direct current (impedance measurements) and radiofrequency current (internal structure of WBCs).
- Immature myeloid information (IMI) channel can flag abnormalities as left shift, immature granulocytes or blasts.
- Optical platelet count can be done.

Advia (Siemens) analyzers

- Principle: Light scatter as cells pass through a laser beam.
- RBCs are sphered isovolumetrically ensuring that cell shape does not affect volume measurement.
- Scattered light has two measurements: low angle (2–3°) and high angle (5–15°).
- Volume-hemoglobin cytogram is generated.
- Hemoglobin is measured spectrophotometrically. Sodium lauryl sulphate method is also available.
- WBC differential is obtained from the peroxidase channel which uses white light and incorporates a cytochemical reaction. The subsequent light scatter is proportional to the intensity of the peroxidase reaction. Peroxidase-negative cells are larger than most lymphocytes and are designated large unstained cells (LUC).
- Mean peroxidase index (MPXI) is also generated.

Abbott (Cell-Dyn) analyzers

- WBCs are estimated in a light-scattering (laser) channel. The red cells are rendered transparent as their refractive index is similar to that of the sheath reagent.
- Light scattering parameters measured are: Forward scatter (1–3°), narrow-angle scatter (7–11°), total polarised orthogonal scatter (70–100°) and depolarised orthogonal scatter (70–100°).
- Cell clusters with anomalous characteristics (blasts, atypical lymphocytes, NRBC and immature granulocytes) are flagged.

Horiba ABX instruments

- Hemoglobin measurement by cyanmethaemoglobin method or by oxidation of heme iron followed by stabilization to produce quantifiable chromogenic substances.
- 5-part differential count in two channels by differential staining of white cells. White cell populations are represented on a plot of light absorbance against impedance and enumerated by cluster analysis with moving thresholds.
- Atypical lymphocytes are counted separately and also included in the total lymphocyte count.
- NRBC count is available after nuclear staining by thiazole orange, a fluorescent nucleic acid stain.

Mindray analyzers

- Laser light scatter at two angles along with fluorescence signals.
- Generates five-part differential count, reticulocyte count, IRF, NRBC count, immature granulocyte count, high fluorescent cell (HFC) count (atypical lymphocytes and blast cells) and two flags, 'infected RBC?' and 'InR#' count which indicate possible presence of malaria parasites.

Novel Automated Blood Cell Parameters

The advent of new generation automated hematology analyzers has also introduced many novel automated CBC parameters. While many of these are clinically useful, there are still some parameters which are redundant and remain only a marketing tool. Some general principles in evaluation and application of novel automated parameters are:

- The hype regarding any newly introduced parameter should be carefully tested across laboratories and validated by robust studies. This also applies to 'For research use only' parameters.
- Quality control material, both internal and external, is not available easily established for most novel parameters.
- Similar parameters may behave differently across analyzers due to their varied measurement principles.
- Any novel parameter that becomes part of a clinical guideline or recommendation has definite utility in the analyzer tested for the same. Its use across different analyzer platforms must be interpreted with caution.
- Pre-analytic variables significantly affect many novel parameter measurements.

The following text will refer to novel automated parameters. Some of these are instrument specific and may not be available in all platforms. The discussion is primarily on their possible clinical utility and not on specific analyzer related technical aspects.

Red blood cell related parameters:

1. **Fragmented red blood cell percentage:** Fragmented RBCs (schistocytes) are seen in thrombotic microangiopathies (TMA), DIC, HUS and TTP. Automated enumeration is more precise and accurate. The FRC flag, though having a low specificity has an advantage in its high negative predictive value.
2. **Percentage of hypochromic cells:** This provides a quantitative measure of hypochromic red blood cells, a valuable tool in iron deficiency anemia. Reducing percentage of hypochromic cells indicates response to hematinic therapy.
3. **Percentage of hyperchromic cells:** This provides a fairly accurate estimation of RBCs with a low surface area to volume ratio as seen in hereditary spherocytosis and can be used as a screening tool.
4. **Unghosted cells:** This parameter is available in select analyzers and is a potential surrogate for the presence of frequent target cells, a vital clue in patients with suspected thalassemia. Target cell though, can also be seen in iron deficiency anemia, hemoglobinopathies, alcoholism and chronic liver disease.
5. **Hemoglobin distribution width (HDW):** It is a measure of anisochromia (variation in hemoglobin content of red blood cells). Increased HDW is seen in iron deficiency anemia while in thalassemia trait, the HDW is normal or only mildly elevated.
6. **Nucleated red blood cell count:** NRBCs interfere in the WBC count as they are nucleated. The WBC count in cases where significant numbers of NRBCs are present needs a correction to obtain the true TLC. Many analyzers can provide a fairly accurate NRBC count and also generated a corrected WBC count.
7. **Immature reticulocyte fraction (IRF):** Some analysers can provide the immature reticulocyte fraction. This is derived from the sum of the high fluorescence and middle fluorescence reticulocytes. IRF is an indicator of erythropoiesis in the bone marrow.
8. **Reticulocyte hemoglobin content (Ret-He):** It represents the hemoglobin content in

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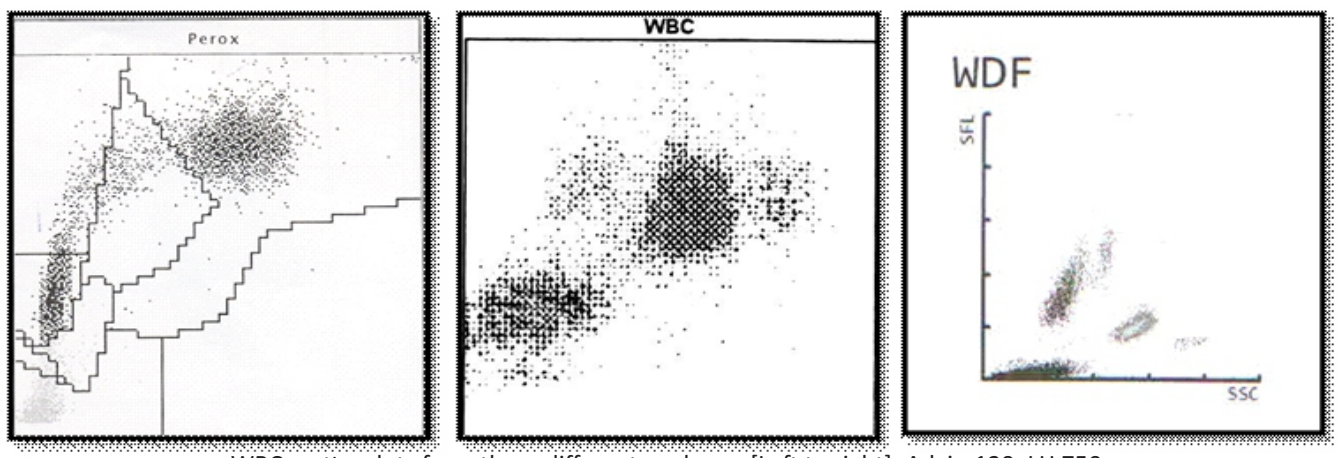

WBC scatterplots from three different analyzers [Left to right]: Advia-120, LH 750 (Beckman Coulter) and Sysmex XN-550.

"The microscope doesn't lie — it reflects only what we're trained to see." — Anonymous

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RDW-SD Better Than RDW-CV Still Underreported! Time for a Change

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ments and educate practitioners on its implications for conditions such as anaemia, cardiovascular diseases and inflammatory states. RDW-SD offers a more precise assessment of RBC variability compared to RDW-CV, yet it remains underreported in clinical settings. This under-utilization limits its potential as a diagnostic tool and reduces comprehensive patient evaluations. It's time for healthcare providers to recognize the clinical significance of RDW-SD, educate themselves on its benefits and incorporate it into

Introduction

Red blood cells (RBCs) account for the most numerous cell type in the bloodstream and serve various physiological functions.[1] RBCs help in oxygen and carbon dioxide transport, nitric oxide metabolism, redox balance and inflammatory response regulation. So, changes in their size and shape is linked to various diseases. Red cell distribution width(RDW) measures the variation of RBC volume and size, a part of complete blood count. The "width" of RDW is a misnomer as it is a measure of the deviation of the volume and size of RBC and not directly the width or diameter of the RBCs. A high RDW indicates that RBCs vary more in size than normal and low RDW means that RBCs are mostly similar in size. RDW is one of the RBC indices, easily measured by the haematology autoanalyser. It is of two types - RDW-CV (Red Cell Distribution Width - Coefficient of Variation) and RDW-SD (Red Cell Distribution Width - Standard Deviation).

RDW-CV (Red Cell Distribution Width-Coefficient of Variation)

It measures the variation in the size of RBC. It is calculated from a RBC histogram. It is expressed as a percentage. A normal RDW-CV indicates that RBCs are relatively uniform in size. An increased RDW-CV indicates a greater variation of RBC sizes. It is derived with the following formula -

RDW-CV = (1 standard deviation of RBC volume ÷ MCV) X 100%.[2]

As RDW-CV is mathematically derived from mean corpuscular volume (MCV), it is affected by the average RBC volume (MCV). It is inversely proportional to the MCV. So, if the majority of RBCs in the blood are small, it will remain within the normal range. It is less sensitive to the presence of a small population of microcytes, macrocytes and reticulocytes. But, it is better to express the overall changes in the size of RBCs in macrocytic or microcytic anaemia . So, a higher or lower RDW-CV value is not an indication of how much bigger or smaller the RBCs are, rather it indicates how big the variation in RBC's size and volume is. Normal value of RDW-CV is 11.6-14%.[2]

RDW-SD (Red Cell Distribution Width- Standard Deviation)

It is the standard deviation (SD) of the volume of RBCs. It is measured in femtolitres(fl). It does not depend on the MCV. It is a direct measurement of the width of RBC histogram at 20% of the height of the curve,[2] where the height of the RBC histogram is taken as 100%. It reflects the maximum and minimum volume of RBCs in the blood. It is a more sensitive indicator when a small number of macrocytes and microcytes are present in the blood as it measures the lower part of the RBC distribution by volume. It will change more rapidly with reticulocytosis as there will be a widening of the base of RBC histogram. So, it more accurately measures the variation of RBC's size and shape. The normal range of RDW-SD in adults is 39-46 fl.[2]

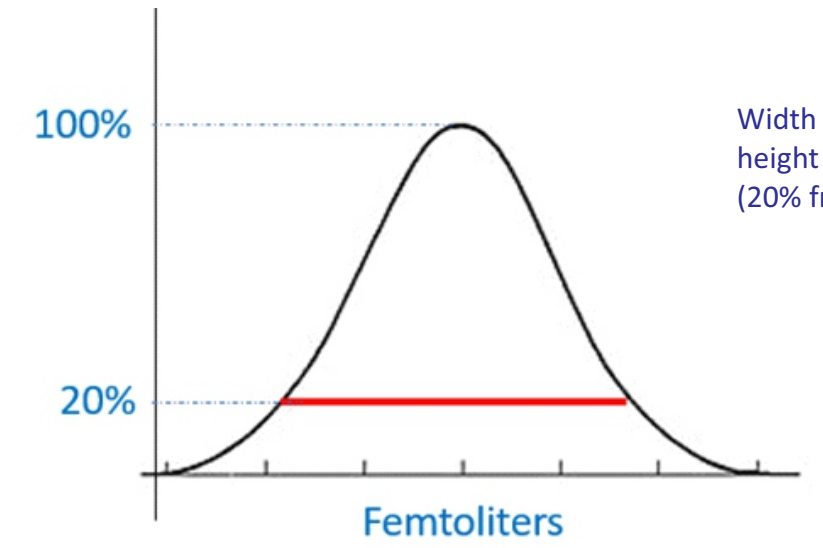

Image - Measurement of RDW-SD

Discussion

RDW-CV may be elevated if homogenous RBC population has narrow distribution curve with low MCV and RDW-CV may be normal if heterogenous RBC population has a broad distribution curve with high MCV. So, RDW-SD is more accurate in diagnosis in the variation of size and shape of RBC's due to direct measurement of the width of RBCs distribution. Thus, RDW-SD is a better representative of RBCs volume heterogenesity. Studies have suggested that RDW-SD may provide additional diagnostic value over RDW-CV.[3] When RBCs agglutinate or clump together, cell counter may count the clumps or aggregates of RBCs as larger cells, or single cell and then it gives a falsely elevated MCV value. In case of rouleaux formation also when RBCs stick together in stacks, cell counter machine may give a falsely raised MCV. So in these cases, RDW-CV value is falsely decreased. But as MCV has no role in calculation of RDW-SD, it is not affected during RBC agglutination or clumping. Cold-reactive (i.e., reactive at temperatures <37^{0}C) IgM antibodies are typically associated with cytomegalovirus infection, mycoplasma pneumonia, and cold agglutinin disease. These antibodies generally cause RBC autoagglutination (RBC clumping), which interferes with the measurement of RBC- associated CBC parameters and yields falsely lower RBC count and Hct and falsely higher MCV, MCH, and MCHC.[4]

Conclusion

Indeed, the RDW is a key haematological marker, often overlooked in clinical practice despite its potential to provide valuable insights into various health conditions. Increasing awareness among healthcare professionals about the significance of RDW could lead to improved diagnostics and better patient outcomes. It's time to advocate for its more prominent role in routine assessments and routine practices, ensuring better patient manangement and improved outcomes across various conditions.

Difference between RDW-CV and RDW-SD

RDW-CV	RDW-SD
It is a ratio	It is a direct measurement of the width of the distribution curve of RBC's volume at a level 20% above the baseline
It depends on MCV RDW-CV= (1 standard deviation of RBC volume/ MCV)X 100%	It does not depend on MCV
It is limited to measure RBC distribution within one standard deviation, though there may be other abnormally large or small RBCs beyond this one standard deviation.	It is an absolute value and it measures the wide range of RBC volume distribution that may extend beyond one standard deviation.

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"Diagnosis is not the end, but the beginning of the practice of medicine." — Martin H. Fischer

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NEWSLETTER
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Artificial Intelligence & Total Lab Automation in Haematology

Dr. Anjali J. Kelkar
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There are proven values and benefits of diagnostics such as:

- Early detection leading to better prognosis
- Targeted treatments possible due to accurate diagnosis
- Disease monitoring
- Risk stratification
- Improved patient management
- Cost benefits and Public health benefits

Artificial intelligence (AI) has influenced our world at multiple levels. There are examples of Neurolink - The initial goal of technology is to help people with paralysis regain independence through the control of computers and mobile devices, AI brain technology with pig Gertrude - wireless device implanted in her brain that was able to monitor a thousand neurons at a time instead of 300, Mind - Pong - A Macaque monkey demonstrated its ability to play a video game called Mind- Pong using only brain power - N1 device and pager.

Phenotypic features are important for diagnosis despite increasing genetic context. Diagnostic ambiguity is a regular occurrence in clinical practice. Expertise and skills are necessary in many situations demanding personal presence. Considering these limitations, the efforts to improve the interpretation of data by application of AI are ongoing. Increasing the consistency of data interpretation, the data needs to be generated in standardized and structured manner, which can support various downstream AI leading to development of reliable and trustworthy AI models.

AI and ML in Clinical Laboratories:

Following are the examples of pattern recognitions in images:

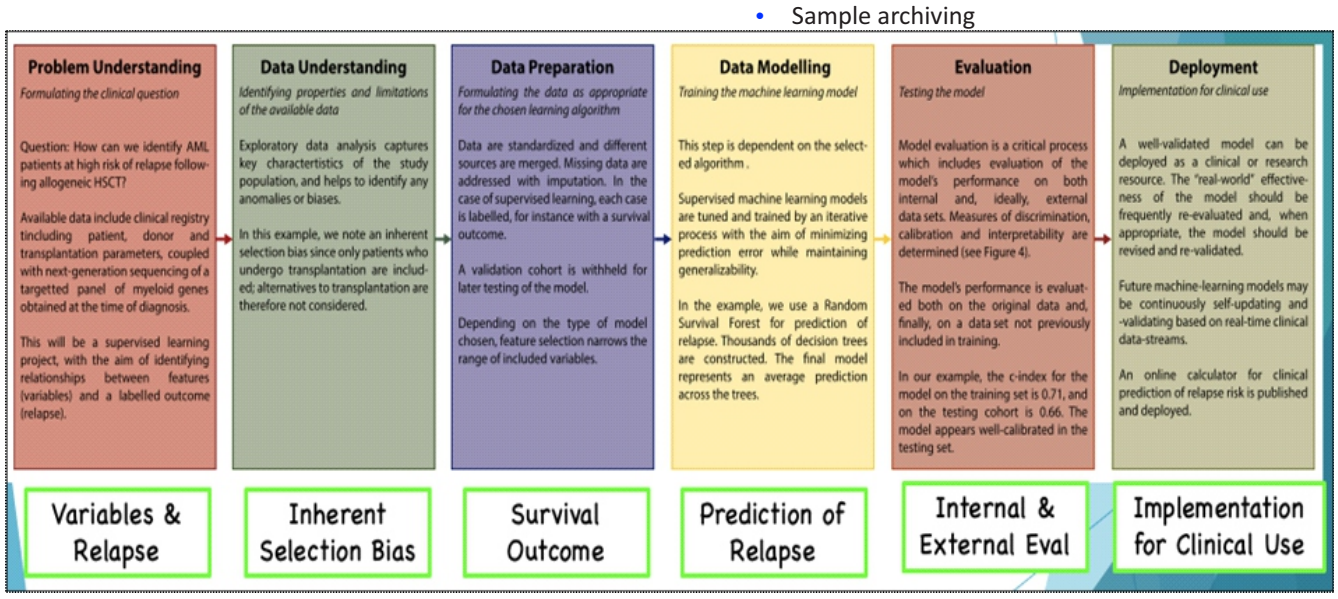
- Whole slide pathology imaging
- Breast cancer screening
- Cell based screening

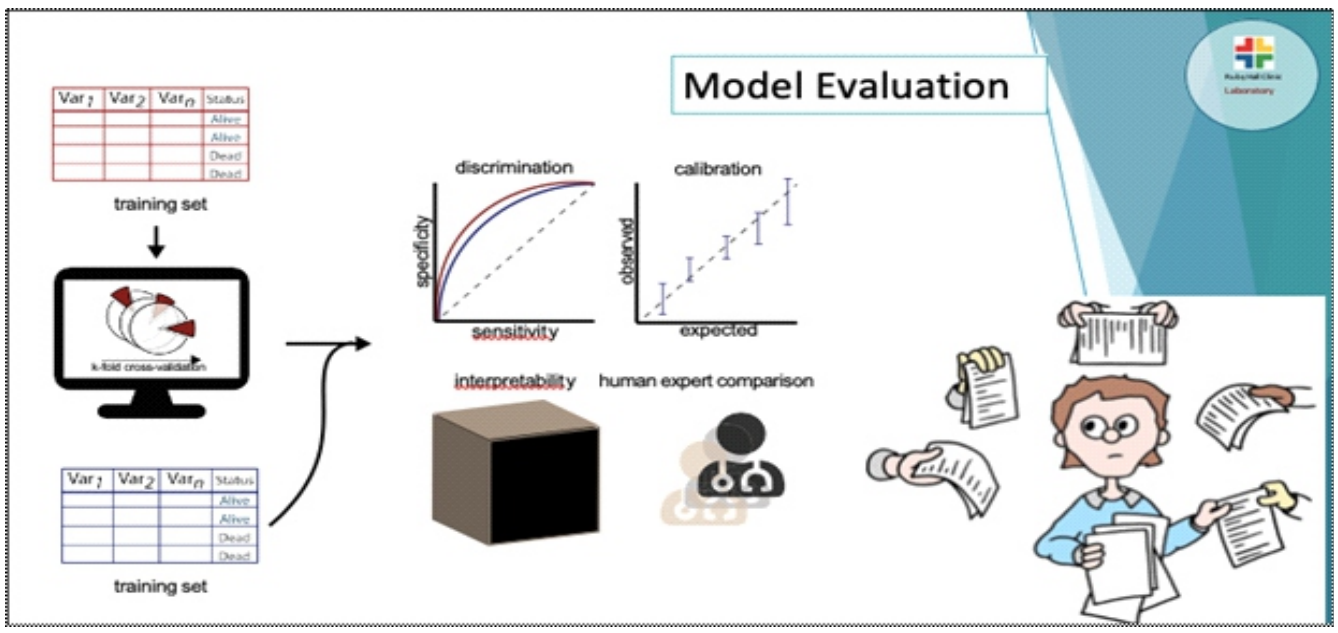
These systems do not suffer from normal human frailties such as need for sleep, rest periods, distractions etc. They can deliver high throughput and quality of detection above and beyond their human counterparts. Substantial uncertainty exists about accuracy of AI and it cannot outperform their human counterparts. The technology however, is continually advancing.

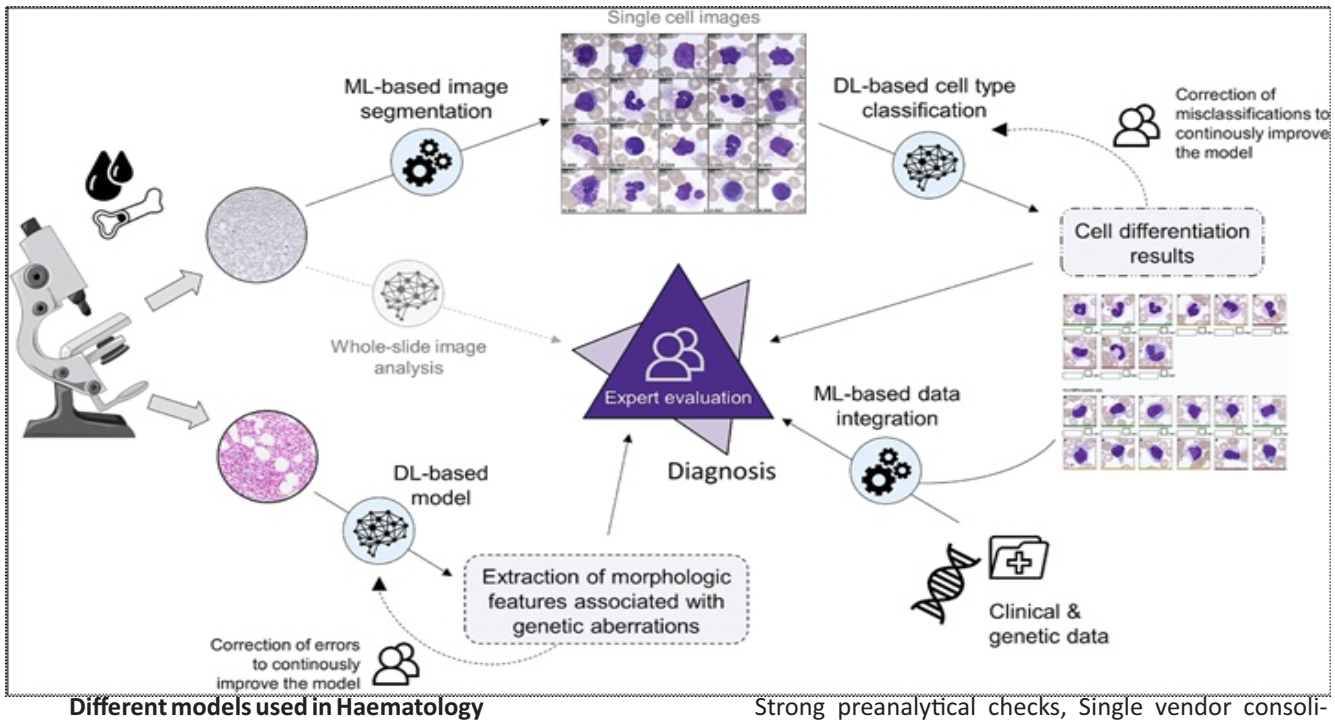
ML in a Nutshell for Haematologists

Machine learning is a subdomain of Artificial intelligence that attempts to computationally extract meaningful insights from complex data sets. In order to solve a problem computationally, the input (e.g. microscopic images, mutation profiles) is transformed to the desired output (e.g. cell type classification, prognostic score) by following a sequence of instructions (i.e. algorithm). Following are few examples:

- Digital microscopy – Single cell/ Patterns analysis
- Support the evaluation of flow cytometric data
- Aid cytogeneticists in karyotyping
- Develop personalized models integrating multiple data sources
- Estimate the response to treatment.
- Accurately predict the prognosis of various leukemias







Different models used in Haematology

Total Lab Automation

Automation Drives Operational Excellence: What goals can be achieved in a clinical/ haematology laboratory?

- Reduces the sample draw volume
- Reduces number of tubes/ aliquots
- Reduces waste
- Reduces error rate
- Increases productivity
- Increases efficiency
- Increases quality

Components of Total Lab Automation

- Sample identification & sorting
- Centrifugation
- Transfer to analyser
- Testing
- Sample archiving

Strong preanalytical checks, Single vendor consolidation, Haematology automation with Digital morphology, Strong middleware capabilities, Positive user experience, Positive team interactions with resolution of queries were the points considered for finalizing on the vendor. After installation of the complete system, 100% STAT samples were handled withing TAT, overall, TAT improved by 44%, process efficiency improved by 66% and space utilization improved by 68%.

The clinical laboratories need to ensure that following are in place for implementation of AI and ML systems:

- Consent for personal data usage & safeguarding

"Clinical hematology isn't just numbers — it's narratives written in blood." — Anonymous

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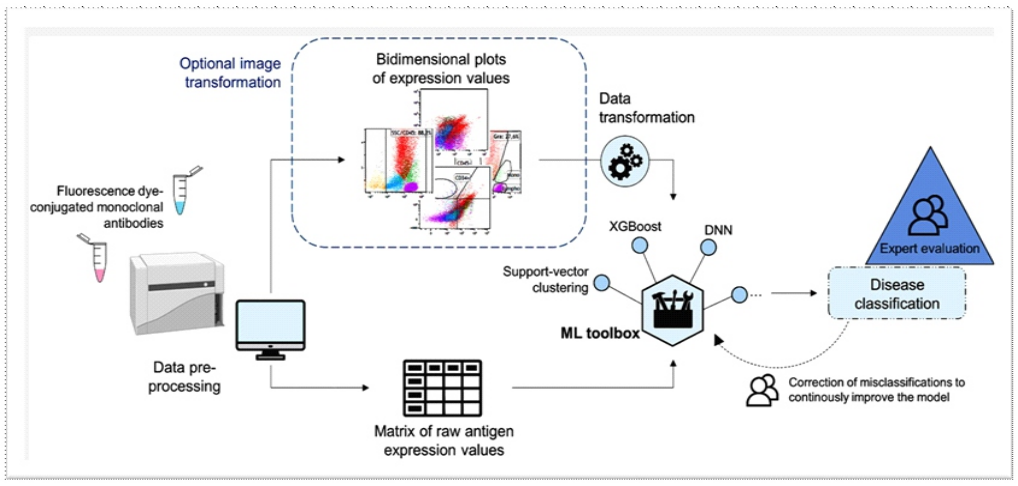

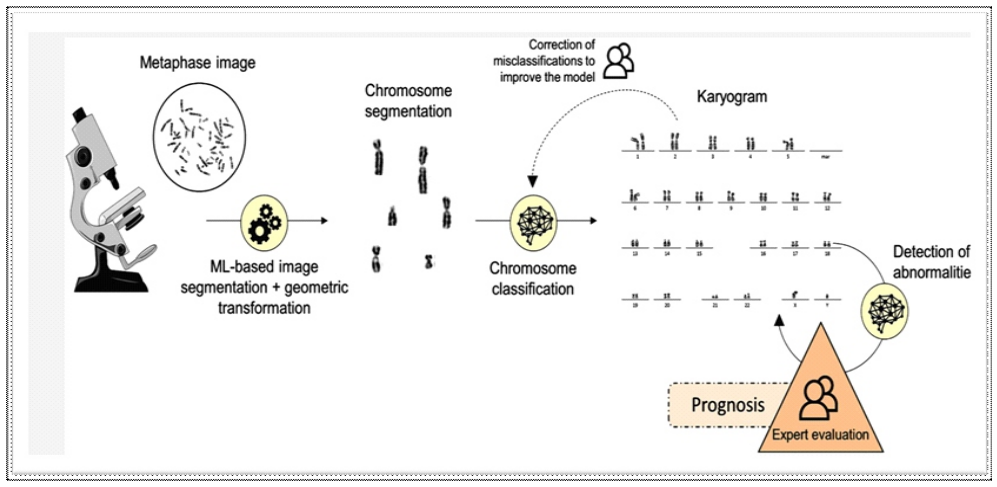

- privacy
- Diverse sources of medical records and diagnostic tests results
- Automatic data transfer from the instruments, which is clean, of good quality, well structured, and consistently named.
- Seamless integration between systems
- Infrastructure for large data volumes
- Strict data governance
- Algorithm on data containing misdiagnosis, erroneous treatments, wrong scenarios
- Ethical and Legal frameworks for research

Conclusion:

AI in hematology has the potential to revolutionize diagnostics, treatment, planning and patient care leading to more accurate, efficient and personalized healthcare solutions.

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Output (Label)/ Population	Input	Model
Benign v/s malignant adenopathy	Images from contrast-enhanced micrography device	Convolutional neural network
Haematologic diagnosis by likelihood	Clinical & lab data	Random forest
MRD in AML & MDS	Multicolour Flow Analysis	Support vector machine
Leukaemia subtype	Images of PBS	Convolutional neural network
Leukaemia subtype or infection	Images of PBS	Linear discriminant analysis
AML versus non-AML	Gene expression data from PB MNC/ BM	Lasso (L1)-regularized logistic regression
Classification of BM cells type	Images of Wright-stained BMA	Convolutional neural network
Histology-based classification of DLBCL v/s Burkitt	Images of H&E-stained tissue	Convolutional neural network

contd. from page 4

reticulocytes and is the earliest sign of iron deficiency as iron incorporation first happens in reticulocytes. It can also be used to monitor response to iron therapy.

White blood cell related parameters:

1. **Immature granulocytes (IG):** These are granulocytic precursors which can be quantified by some automated hematology analyzers. Increase in IG indicates increased marrow turnover of myeloid cells seen in infections, inflammatory disorders, myeloproliferative neoplasms, acute leukemia and non-hematological malignancies.
2. **Cell Population data (CPD) from VCS parameters:** Volume Conductivity and Scatter (VCS) measurements, in addition to WBC enumeration, provide valuable data on the physical, conductivity, optical and physical properties of WBCs. Mean neutrophil volume (MNV) is a useful tool in the diagnosis of sepsis and even in differentiating between gram-negative and gram-positive sepsis. CPD have also shown utility in the diagnosis of malaria, dengue and hematological malignancies. Monocyte distribution width also has a role in sepsis and Covid-19 infection.
3. **Atypical lymphocytes:** These are denoted by different names on different analyzer platforms (High fluorescent lymphocytes, Atypical lymphocytes, or Large unstained cells). This parameter quantifies the reactive/abnormal lymphocyte population. Rise is seen in infectious mononucleosis and many other viral infections, circulating lymphoma cells and circulating blasts especially of lymphoid origin.
4. **Granularity index:** This parameter is a reflection of neutrophil granularity based on its scatter properties. A high index is seen in sepsis where neutrophils show toxic and large granules. Myelodysplastic syndrome which is associated with neutrophil hypogranularity, shows a low granularity index.
5. **Hematopoietic progenitor cell (HPC) count:** Few analyzers can provide the HPC count which is vital in the pre-transplant setting. This parameter has been found to have good correlation with the flow cytometry based CD34 enumeration.

Platelet related parameters:

1. **Mean Platelet Volume (MPV):** Care must be taken to interpret MPV as it is very sensitive to pre-analytic variables especially blood: anticoagulant ratio, temperature and storage conditions. It is especially useful in macrothrombocytopenias and conditions like Wiskott Aldrich syndrome where microplatelets are seen. At times, some instruments may not generate the MPV. Rather than an aberration, this is a good clue that the analyzer is experiencing interference at the upper discriminator, usually due to large platelets. In this situation, large and/or giant platelets are likely to be seen in the peripheral blood smear and in most cases the automated platelet counts are lower than the manual counts.
2. **Platelet distribution width (PDW):** It is a measure of the variation in platelet volumes. A significant rise is seen in myeloproliferative disorders and other conditions with significant numbers of large platelets. However in analyzers with a limited ability to count large and giant platelets, these can be counted with RBCs leading to an underestimation of the PDW.
3. **Plateletcrit:** This is akin to hematocrit for RBCs and is the volume occupied by platelets in the blood. It is dependent on the platelet numbers. It is low in thrombocytopenia and high in thrombocytosis. The clinical utility is limited.
4. **Platelet/Large cell ratio:** This parameter indicates the percentage of large platelets among the total platelet population. It is raised in conditions where significant numbers of large and/or giant platelets are present. Studies have also shown a possible role in differentiation of destructive thrombocytopenia from hypoproliferative thrombocytopenia.
5. **Immature platelet fraction (IPF):** Platelets which are newly formed are immature (reticulated platelets). This parameter is useful in differentiating destructive from hypoproliferative thrombocytopenia. It also has a role in indicating recovery of platelet counts in patients with dengue fever, following chemotherapy and hematopoietic stem cell transplantation (HSCT).

"In the fields of observation, chance favors only the prepared mind." — Louis Pasteur

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UPDATES ON COAGULOPATHY 2025

Dr. Deepa Rani
Associate Professor,
Department of Pathology,
IMS, BHU

The "Updates on Coagulopathy" Conference was organized by the Kashi Hematology Group and the Center for Genetic Disorders under the aegis of ISHBT and UPHG on February 9th, 2025, at Hotel Hindustan International in the holy city of Varanasi. This one-day Conference was accredited with three credit hours by the Uttar Pradesh Medical Council. The theme of the Conference focussed on coagulation disorders.

Ten renowned national experts, stalwarts in the field of hematology, participated in this Conference and delivered insightful talks on various coagulation disorders. It was our privilege to have Professor Jasmina Ahluwalia from PGI Chandigarh, Professor Tathagata Chatterjee from ESIC Faridabad, Professor Abhishek Sharma from NRS Medical College Kolkata, Professor S. P. Verma from KGMU Lucknow, Professor L. P. Meena from IMS BHU and Professor Akhtar Ali from the Center for Genetic Disorders at the Institute of Science, BHU, Dr. Abhishek Maurya from IMS BHU, Dr. Priyanka Aggrawal from the Department of Pediatrics, IMS BHU, and Dr. Iffat Jamal from IGIMS Patna.

The speakers delivered excellent and enlightening talks on various coagulation disorders, including APLA, Von Willebrand disease, hemophilia, and pediatric coagulation disorders. During the Conference, there was also a poster presentation session in which 12 posters were showcased. A postgraduate quiz was also organized with Dr. Shruti Mishra as quiz master, in which around 25 postgraduate students participated.

The Conference was held at Hotel Hindustan International, a four-star hotel in Varanasi, where the overall ambience and comfort were of exceptional quality. The event was meticulously organized, with every detail well-planned. The media provided excellent coverage of the event.

The inaugural ceremony was the highlight of the Conference, with Professor Tathagata Chatterjee, President-Elect, ISHBT serving as the Chief Guest, and Professor Shampa Anupurba, Acting Director & Dean of IMS BHU, as the Guest of Honour. Along with them, Professor Jasmina Ahluwalia and Professor S. P. Verma were the Special Guests. During the inauguration, Professor VP Singh, former Director of IMS BHU, was felicitated. Professor B. Dube, known as the Father of Hematology in Uttar Pradesh and the former Head of the Department of Pathology, was also honoured at his home in Varanasi. Additionally, Mr. Sumaru Ram, a technical staff member from the Department of Pathology who had worked with Professor B. Dube and had made significant contributions in establishing the coagulation lab at IMS BHU, was also felicitated.

Overall, the Conference was a well-planned and well-executed academic event, with hematology experts sharing their valuable knowledge. More than 100 delegates attended, including many faculty members from Varanasi. This academically oriented one-day Conference was a great success, and the organizing committee deserves credit for their hard work in ensuring its successful completion.

The credit of this successful Conference goes to the organizing committee of updates on coagulopathy-Organizing Chairman Prof Vijai Tilak, Organizing Secretary Dr Deepa Rani, Joint Organizing Secretary Dr Priyanka Aggrawal, Dr Pratishtha Senger, Scientific Secretary Dr Anju Bharti, Inaugural Committee Incharge Dr Mahima Yadav & Treasurer Dr Akhtar Ali.

Members of the Organising Committee with the Guest Speakers & Chairpersons

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With best compliments
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Presidential address by Prof. Vijai Tilak, President, KHG

Felicitation of Prof. V. P. Singh

Felicitation of Mr. Somaru Ram

Group Photograph of Organising Committee members with guest speakers & delegates

"Science and medicine do not rest on authority, but on evidence and verification." — Richard Feynman