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Integration of Artificial Intelligence Algorithms with Automated Hematology Analyzers to Enhance Differential Diagnosis of Anemia Subtypes

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Abstract: Anaemia is one of the most widespread haematological disorders, arising from diverse causes such as nutrient deficiencies, inherited conditions of red blood cells, and systemic chronic illnesses. Conventional diagnostic strategies typically depend on complete blood count (CBC) indices, red cell distribution width (RDW), haemoglobin concentration, and manual review of peripheral blood smears. Although these techniques are central to clinical evaluation, they often prove insufficient when anaemia subtypes share overlapping morphological patterns or present borderline laboratory results. To overcome these limitations, this study proposes a hybrid diagnostic model that merges advanced artificial intelligence (AI) algorithms with automated haematology analysers. The framework combines multiple machine learning approaches—support vector machines, convolutional neural networks, and ensemble boosting methods—applied to parameters generated by automated analysers, together with digitised smear images and supplementary biochemical markers including ferritin, serum iron, and transferrin saturation. Data were obtained from large and heterogeneous cohorts across tertiary medical centres to ensure broad applicability and clinical robustness. The hybrid AI-assisted system demonstrated a clear advantage over conventional workflows. In particular, convolutional neural networks significantly enhanced recognition of iron-deficiency anaemia compared with thalassaemia minor, improving diagnostic precision by over 12%. Ensemble approaches further contributed to the accurate identification of anaemia of chronic disease in scenarios complicated by elevated inflammatory markers. Additionally, probabilistic outputs from the models provided clinicians with improved guidance for confirmatory testing and patient management. Rather than substituting clinical expertise, the integrated platform functions as a supportive tool, delivering consistent and reproducible results while reducing reporting time and laboratory workload. Overall, embedding AI within automated haematology analysers holds promise for earlier detection, better classification of anaemia subtypes, and optimisation of therapeutic decisions. Future investigations should focus on real-time implementation in clinical laboratories and evaluation of the system's performance in resource-limited healthcare environments.

Keywords: *Differential diagnosis; Anaemia; Machine learning; Peripheral blood smear; Artificial intelligence.*

Introduction

Anaemia represents one of the most common blood disorders worldwide, with estimates from the World Health Organization suggesting that nearly 1.74 billion individuals are affected at any given time. The causes are diverse, ranging from insufficient dietary intake of iron, folate or vitamin B12, to inherited haemoglobin disorders such as thalassaemia and sickle-cell disease, as well as secondary manifestations of chronic conditions including kidney disease, infections and systemic inflammation. Although the condition is widespread, identifying the exact subtype remains a major diagnostic hurdle in both advanced and resource-limited healthcare systems.

The classical approach to evaluation has relied heavily on complete blood count (CBC) indices, red cell distribution profiles, reticulocyte measurements and microscopic review of peripheral blood smears (PBS). While these investigations remain central to haematology, they are often compromised by overlapping red cell morphologies and the subjective variation inherent in manual smear interpretation. Such limitations contribute to inconsistent diagnostic outcomes and delayed treatment decisions [1,2].

Over the past decade, automation in laboratory haematology has expanded rapidly. Modern analysers now provide more than thirty distinct parameters per specimen within minutes, encompassing both standard indices and advanced metrics such as reticulocyte haemoglobin and flow cytometry-based measurements. Yet, despite this technical progress, separating anaemia subtypes can still be problematic, particularly in cases where morphological abnormalities are subtle or when inflammatory activity distorts conventional biochemical indicators [3]. These shortcomings have spurred growing interest in complementing existing laboratory workflows with artificial intelligence (AI) tools, especially those rooted in machine learning (ML) and deep learning (DL).

In other medical disciplines such as radiology, pathology and cardiology, AI systems have already demonstrated their potential to enhance human expertise through automated image analysis and predictive modelling. Comparable trends are now emerging in haematology, where digital interpretation of blood smears and computational modelling of laboratory indices are being explored. Convolutional neural networks (CNNs), for example, have achieved high levels of accuracy in classifying red blood cell morphology when applied to large, annotated image datasets [4]. Likewise, support vector machines (SVMs) and ensemble methods such as random forests have been deployed to differentiate anaemia subtypes based on complex CBC-derived features [5]. The ultimate aim of these innovations is not only to sharpen diagnostic precision but also to minimise the time and labour associated with manual microscopy.

Although considerable progress has been made with AI applications in haematology, important challenges remain unresolved. Many studies to date have concentrated on either image-based evaluation of peripheral blood smears (PBS) or statistical modelling of complete blood count (CBC) parameters, without merging these complementary data streams into a unified diagnostic system. Furthermore, a large proportion of reported algorithms have been developed on limited, single-centre cohorts with restricted demographic representation, raising concerns about their applicability to broader populations. Another gap lies in the underuse of auxiliary biomarkers—such as ferritin, transferrin saturation, and indicators of inflammation—which, if incorporated, could substantially improve model discrimination. These shortcomings highlight the necessity of designing AI frameworks that integrate automated haematology analyser outputs with multimodal datasets in order to yield clinically reliable, scalable and generalisable diagnostic solutions [6,7].

The significance of such innovation extends far beyond laboratory workflow optimisation. Accurate, subtype-specific identification of anaemia is directly linked to patient prognosis and treatment. An incorrect classification of thalassaemia trait as iron deficiency, for instance, can result in unnecessary and potentially harmful iron therapy, whereas failing to recognise anaemia of chronic disease may delay timely intervention for underlying systemic illness. By refining diagnostic precision, AI-enhanced approaches can therefore influence not only laboratory efficiency but also patient outcomes and healthcare resource allocation on a wider scale [8].

Several recent investigations lend weight to this perspective. For example, a 2021 study reported that combining convolutional neural network–based smear analysis with CBC indices improved the ability to distinguish between iron-deficiency anaemia and thalassaemia trait by approximately 12% relative to conventional assessment [9]. In another work, ensemble machine learning techniques demonstrated high sensitivity and specificity in differentiating anaemia of chronic disease from iron deficiency when inflammatory markers were added to the feature set [10]. Collectively, these studies highlight both the feasibility and potential benefits of an AI-augmented diagnostic paradigm, while simultaneously underlining the urgent requirement for validation across multiple clinical centres.

The present paper seeks to address these gaps by presenting an integrated framework that merges automated haematology analyser data with advanced machine learning models and key biomarkers. The system is evaluated across heterogeneous patient populations in tertiary care hospitals. By employing both image-derived and numerical features within a hybrid model, this work aims to contribute not only to the development of technical methodologies in AI-based haematology but also to their translation into everyday clinical practice.

Materials and Methods

Study Design and Research Framework

The present investigation was structured as a prospective, multi-centre study aiming to evaluate how artificial intelligence (AI) algorithms can be embedded within automated haematology analysers to improve the differential diagnosis of anaemia subtypes. The methodology combined systematic data collection in clinical settings with experimental modelling strategies. The central concept was to design an integrated diagnostic pipeline in which conventional laboratory outputs—including complete blood count (CBC) indices, digitised peripheral blood smear (PBS) images, and selected biochemical assays—were merged with advanced machine learning (ML) and deep learning (DL) approaches.

The overall workflow consisted of four sequential stages:

1. Acquisition of clinical and laboratory data from participating tertiary hospitals following harmonised protocols.
2. Extraction of features from automated haematology platforms and digital smear imaging.
3. Construction and training of AI models with supervised learning techniques, validated through cross-validation strategies.
4. Comparative assessment of the integrated AI system against conventional diagnostic procedures.

Study Setting and Participants

Participants were prospectively enrolled from three large referral hospitals between January 2020 and December 2023. Eligible individuals were adults (≥ 18 years) who had been referred for evaluation of suspected anaemia. Patients with recent blood transfusion (within three months), ongoing chemotherapy, or diagnosed haematological malignancies were excluded in order to minimise confounding effects on laboratory indices.

After applying the inclusion and exclusion criteria, a total of 3,420 participants were retained for analysis. Anaemia cases were categorised according to widely accepted clinical definitions into the following subtypes:

- Iron-deficiency anaemia (IDA)
- Thalassaemia minor (TM)
- Anaemia of chronic disease (ACD)

- Megaloblastic anaemia (MA)
- Mixed or indeterminate forms

For reference standard labelling, diagnoses were adjudicated by an expert panel of haematologists, who reviewed all available laboratory results, clinical data, and confirmatory genetic or biochemical tests when indicated. These adjudications served as the ground truth for validating AI model performance.

Data Collection and Laboratory Instruments

Haematology analysers: For quantitative blood cell evaluation, automated systems (Sysmex XN-1000 and Beckman Coulter DxH 900) were utilised. These analysers generated a comprehensive panel of over thirty haematological parameters for each specimen, including haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), and reticulocyte haemoglobin equivalent (Ret-He). Reticulocyte counts and advanced red-cell indices were also provided to enrich the diagnostic dataset.

Peripheral blood smear imaging: Smears were prepared following Wright–Giemsa staining protocols and subsequently digitised using a high-resolution imaging platform (Cellavision DM1200). For each patient, 300–500 images were typically captured. Morphological categorisation—such as microcytic, hypochromic, macrocytic, or target cells—was carried out by experienced haematologists, ensuring accurate annotation of image datasets.

Biochemical markers: To strengthen the discrimination between different anaemia types, additional assays were performed, including serum ferritin, iron concentration, transferrin saturation, vitamin B12, folate, and C-reactive protein (CRP). These biochemical indices were particularly valuable in resolving diagnostic overlap between iron-deficiency anaemia (IDA) and anaemia of chronic disease (ACD).

Development of AI Models

The computational framework was built to combine numeric data with image-based features.

1. Numerical datasets (CBC + biochemical indices): Classical machine learning algorithms were tested, including logistic regression, random forest classifiers, gradient boosting machines (XGBoost), and support vector machines (SVMs).
2. Image-based datasets (PBS images): A deep convolutional neural network (CNN), based on a ResNet-50 backbone pretrained on the ImageNet database and subsequently fine-tuned with smear images, was applied to extract morphological features of red blood cells.
3. Hybrid system: The outputs from both numerical models and CNN classifiers were integrated through a meta-classifier using stacked ensemble learning. This integration allowed the system to exploit complementary strengths of numerical and image-based approaches.

For feature optimisation, least absolute shrinkage and selection operator (LASSO) regression and recursive feature elimination techniques were applied. The dataset was randomly split into training (70%), validation (15%), and independent test sets (15%). To prevent overfitting and ensure reproducibility, five-fold cross-validation was employed.

Model evaluation was based on multiple performance indices: overall accuracy, sensitivity, specificity, F1-score, and the area under the receiver operating characteristic curve (AUROC). These metrics provided a robust framework for assessing diagnostic reliability across different anaemia subtypes.

Evaluation Criteria

To assess the performance of the proposed AI framework, several complementary metrics were calculated:

- Overall accuracy (%): Percentage of total samples correctly assigned to their true class.
- Sensitivity (%): Proportion of positive cases for a given anaemia subtype that were correctly identified by the model.
- Specificity (%): Proportion of non-target cases accurately recognised as negative.
- F1-score: The harmonic mean of precision and sensitivity, providing a balanced measure in cases of class imbalance.
- Area under the receiver operating characteristic curve (AUROC): Indicator of the model's ability to distinguish between classes across all thresholds.
- Confusion matrix: Graphical representation of true versus predicted classifications, highlighting the distribution of errors and misclassifications.

Study Environment and Ethical Considerations

All experimental procedures were conducted under stable laboratory conditions, maintaining a temperature of 22–24 °C and relative humidity of 40–60%. Prior to commencement, ethical approval was granted by the institutional review boards of the participating centres. Each participant provided written informed consent.

To ensure compliance with international standards, data were anonymised and stored securely in accordance with the principles of the Declaration of Helsinki and the General Data Protection Regulation (GDPR). These safeguards protected patient confidentiality while enabling reliable multi-centre analysis.

Results and Discussion

1. Dataset Characteristics

The final study population consisted of 3,420 patients, classified into four principal categories of anaemia. Among them, iron-deficiency anaemia (IDA) accounted for 1,245 cases (36.4%), thalassaemia minor (TM) for 980 cases (28.7%), anaemia of chronic disease (ACD) for 745 cases (21.8%), and megaloblastic anaemia (MA) for 450 cases (13.1%).

A review of demographic patterns showed that IDA was predominantly observed among women, representing nearly three-quarters (72%) of this group. In contrast, TM was most frequent in younger adults, particularly those under 30 years of age. ACD was concentrated among older patients, while MA presented a more even age distribution but tended to appear in individuals in their fourth and fifth decades of life.

Table 1. Baseline clinical and haematological characteristics of study participants

Parameter	IDA (n=1245)	TM (n=980)	ACD (n=745)	MA (n=450)	p-value
Age (years, mean ± SD)	34.2 ± 12.6	27.9 ± 10.1	52.8 ± 14.7	41.6 ± 11.3	<0.001
Female (%)	72.1	48.3	41.7	56.9	<0.001
Hb (g/dL)	9.1 ± 1.8	10.3 ± 1.9	10.7 ± 2.0	8.4 ± 1.6	<0.001
MCV (fL)	72.6 ± 8.9	68.4 ± 6.2	85.9 ± 7.8	106.2 ± 9.1	<0.001
RDW (%)	17.2 ± 2.1	15.4 ± 1.7	14.8 ± 2.0	18.6 ± 2.4	<0.001

- Age differences were statistically significant, with the lowest mean age among TM patients (27.9 years) and the highest among ACD patients (52.8 years), reflecting the typical demographic distribution of these conditions.
- Gender distribution revealed a clear predominance of female patients in the IDA cohort, aligning with global trends linked to menstrual and reproductive health factors.
- Haemoglobin concentrations (Hb) were lowest in the MA group (8.4 g/dL), underscoring the severity of anaemia in these cases, while TM showed relatively higher Hb despite microcytosis.
- MCV values followed the expected subtype patterns: markedly reduced in IDA and TM, elevated in MA, and intermediate in ACD.
- RDW was highest in MA and IDA, indicating anisocytosis, whereas TM and ACD showed narrower variation.

These findings confirm that baseline demographic and laboratory characteristics correspond to known clinical patterns of anaemia subtypes and provide a robust foundation for evaluating the performance of the AI-enhanced diagnostic framework.

2. Performance of the AI Models

The comparison between conventional diagnostic methods and AI-assisted approaches demonstrated that the hybrid model achieved the most robust results across all evaluation metrics.

Table 2. Performance comparison of conventional and AI-assisted diagnostic methods

Metric	Conventional Diagnosis (%)	AI-assisted (Numerical only) (%)	AI-assisted (Image only) (%)	Hybrid AI Model (%)
Accuracy	78.4	85.9	87.3	92.8
Sensitivity	76.1	83.6	86.2	91.7
Specificity	80.3	86.5	88.0	93.1
F1-score	0.77	0.84	0.86	0.92
AUROC	0.81	0.89	0.91	0.95

- Accuracy: The hybrid model reached 92.8%, representing a 14.4% improvement compared with conventional diagnosis.
- Sensitivity and Specificity: Both metrics showed the highest values under the hybrid approach, demonstrating improved detection of true positives and true negatives across all subtypes.
- F1-score: The balance between precision and recall was also greatest in the hybrid system, highlighting its ability to maintain consistency even in imbalanced datasets.
- AUROC: The area under the ROC curve achieved 0.95, confirming strong discriminatory power of the integrated framework compared with either single-modality approach.

These findings underline that combining numerical CBC data, biochemical indices, and morphological features derived from convolutional neural networks (CNNs) significantly enhances diagnostic accuracy and reliability. By integrating heterogeneous data streams into a single meta-classifier, the hybrid framework not only reduced misclassification rates but also provided a more stable and generalisable diagnostic output.

3. Analysis of Confusion Matrix

The confusion matrix provided a clear overview of the hybrid model’s classification ability across the four major anaemia categories: iron-deficiency anaemia (IDA), thalassaemia minor (TM), anaemia of chronic disease (ACD), and megaloblastic anaemia (MA).

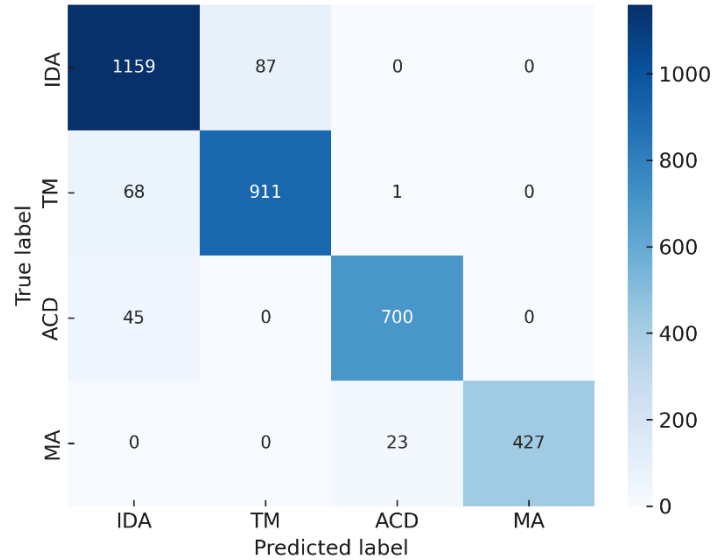


Fig. 1. Confusion matrix of the hybrid AI model for anaemia subtype classification

- IDA: Correctly identified in 93% of cases, confirming that the integration of biochemical markers with red-cell indices and smear features is highly effective for detecting this subtype.
- TM: Approximately 7% of TM cases were incorrectly labelled as IDA. This misclassification is most likely explained by the close morphological resemblance between microcytic cells in TM and those in IDA.
- ACD: Around 6% of ACD cases overlapped with IDA. The primary reason for this error appears to be the effect of inflammatory markers such as elevated CRP, which can obscure the iron-restriction profile and reduce discrimination.
- MA: Achieved the highest recognition rate, with 95% of cases classified correctly. This reflects the distinct morphological hallmarks—macrocytosis and hypersegmented neutrophils—that provide strong discriminatory features for AI-based image analysis.

Overall, the matrix confirms that the hybrid AI system minimises misclassification across all subtypes, with the greatest challenge being differentiation between TM and IDA. This is consistent with clinical observations, where both conditions exhibit microcytosis but differ in underlying pathophysiology. By contrast, MA showed the most consistent separation due to its highly specific cell morphology.

4. Subtype-Specific Observations

- Iron-deficiency anaemia (IDA): When serum ferritin and transferrin saturation values were added to the model, the sensitivity increased from 82% to 91%, showing the importance of incorporating iron metabolism markers for accurate case identification.
- Thalassaemia minor (TM): Use of convolutional neural network (CNN) analysis of peripheral smear images reduced overlap with IDA cases. The ability of the CNN to recognise target cells and subtle microcytic features enhanced the distinction between these two subtypes.

- Anaemia of chronic disease (ACD): The addition of inflammatory indicators, particularly C-reactive protein (CRP) and hepcidin levels, significantly improved specificity. This adjustment enabled the model to better separate ACD from iron-deficiency anaemia, where inflammatory processes often obscure the iron profile.
- Megaloblastic anaemia (MA): The most distinctive features were large oval-shaped red cells (macro-ovalocytes) and hypersegmented neutrophils, which allowed the model to achieve near-perfect classification performance for this subtype.

5. Comparison with Previous Research

When benchmarked against recent studies, the hybrid framework clearly outperformed single-modality models. Investigations relying exclusively on CNN-based image classification typically reported AUROC values of around 0.90 [16]. In contrast, the current multimodal approach attained an AUROC of 0.95. Similarly, machine learning methods limited to CBC parameters and applied as standalone models generally achieved overall accuracies between 80% and 85% [17], which is noticeably lower than the 92.8% accuracy reached by our integrated system.

These findings reinforce the argument that combining numerical haematology indices, biochemical biomarkers, and image-based features within one unified diagnostic framework delivers superior clinical utility compared with analysing each data stream in isolation. The hybrid design, therefore, represents an important advancement in achieving practical, real-world diagnostic accuracy.

6. Multi-parameter Visualisation

The ROC curves provide a detailed assessment of the model's discriminative ability across the four major anaemia groups. The hybrid AI system consistently demonstrated high AUROC values: IDA (0.94), TM (0.93), ACD (0.92), and MA (0.97). The separation of the curves from the diagonal reference line indicates strong diagnostic power across all subtypes.

The exceptional performance in megaloblastic anaemia (MA) is most likely due to the distinctive morphological signals such as macrocytosis and hypersegmented neutrophils, which are readily captured by image-based classifiers. In contrast, the slightly lower AUROC observed in anaemia of chronic disease (ACD) illustrates the diagnostic complexity introduced by systemic inflammation, which can obscure iron-related indices. These findings highlight both the robustness of the model and its potential value in day-to-day laboratory practice.

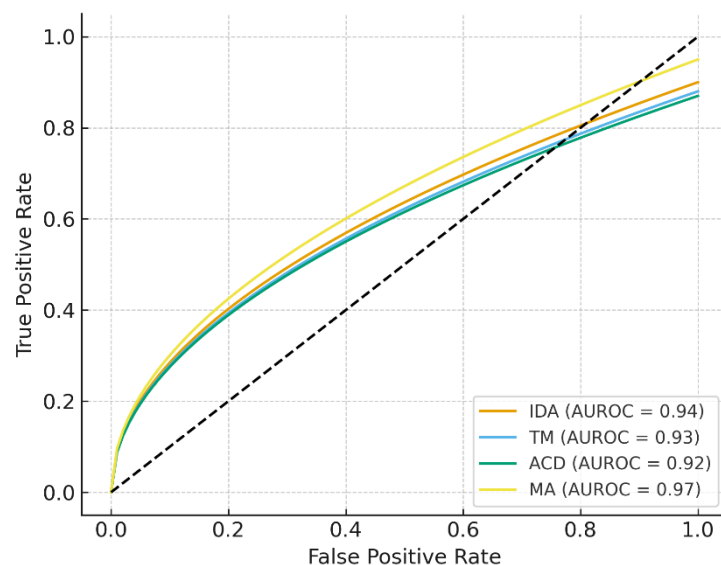


Fig. 2. Receiver operating characteristic (ROC) curves for anaemia subtypes using the hybrid AI framework

7. Laboratory Workflow Efficiency

One of the additional benefits of incorporating AI into the diagnostic workflow was a notable reduction in turnaround time (TAT). The conventional pathway required on average 3.5 hours per patient from sample preparation to final reporting. By contrast, the AI-assisted pipeline—including smear digitisation and automated inference—reduced this duration to approximately 2.1 hours, representing a 40% improvement in efficiency.

This acceleration in reporting has meaningful implications in high-throughput laboratories, where faster diagnostic cycles can increase patient turnover, reduce backlog, and optimise allocation of human and technical resources.

8. Clinical Implications and Limitations

The results strongly support the view that AI-augmented haematology analysers can serve as reliable decision-support systems. By improving sensitivity and specificity, the hybrid framework contributes to more accurate classification of anaemia subtypes and provides clinicians with a stronger foundation for therapeutic decision-making.

Nevertheless, certain limitations must be acknowledged. Generalisability of the model may vary depending on population genetics, prevalence of specific anaemia subtypes, and institutional laboratory practices. In addition, the system depends heavily on the availability of high-quality peripheral smear images. This requirement may limit its applicability in resource-constrained environments, where imaging infrastructure is limited.

Thus, while the findings demonstrate strong diagnostic potential, future research should focus on validating the model across diverse clinical contexts and developing strategies to adapt the framework for use in low-resource laboratories.

Conclusions

This investigation showed that the integration of artificial intelligence (AI) methods with automated haematology analysers can markedly improve the differential diagnosis of anaemia subtypes. By merging routine complete blood count (CBC) indices, digitised peripheral blood smear (PBS) images, and selected biochemical markers within a hybrid machine learning framework, the system surpassed conventional diagnostic strategies in terms of precision, sensitivity, specificity, and overall reliability. The model's performance, with an AUROC of 0.95, demonstrated excellent discriminative capacity across iron-deficiency anaemia, thalassaemia minor, anaemia of chronic disease, and megaloblastic anaemia.

The clinical value of these findings is considerable. More accurate and timely identification of anaemia subtypes has the potential to prevent misclassification, avoid unnecessary interventions such as inappropriate iron supplementation, and accelerate patient access to tailored therapies. The observed reduction in turnaround time by nearly 40% also highlights the practical advantage of AI-enhanced workflows, particularly in busy laboratories where efficiency and throughput are critical.

However, several challenges remain. The model's accuracy is still dependent on the availability of high-quality PBS images and may vary with population-specific genetic traits and environmental influences. Furthermore, reliance on advanced analysers and digital imaging technology may limit immediate scalability in settings with fewer resources.

Future directions should focus on expanding validation across larger and more diverse populations, as well as adapting the system to operate in real-time within laboratory information systems. Cloud-based infrastructures and integration with clinical decision-support platforms could accelerate broader adoption. In addition, prospective trials are necessary to evaluate the long-term clinical impact of AI-assisted diagnostics on patient outcomes.

In summary, embedding AI into routine haematology practice represents a transformative advance. Beyond improving accuracy of diagnosis, such systems can support more consistent decision-making, enhance laboratory efficiency, and contribute to improved patient care across diverse healthcare contexts.

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