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White blood cell evaluation in haematological malignancies using a web-based digital microscopy platform

Karan Makhija[1] | Lisa F Lincz[1,2] | Khaled Attalla[3] | Fiona E Scorgie[1] | Anoop K Enjeti[1,2,3] | Ritam Prasad[3]

[1]Haematology Department, Waratah, NSW, Australia

[2]University of Newcastle. University Drive, Callaghan, NSW, Australia

[3]NSW Health Pathology. Lookout road, New Lambton, NSW, Australia

Correspondence
Karan Makhija, Haematology Department, Calvary Mater Newcastle, Edith and Platt Street Waratah NSW Australia.
Email: karan.makhija88@gmail.com

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Abstract

Introduction: Digital microscopy systems are beginning to replace traditional light microscopes for morphologic analysis of blood films, but these are geographically restricted to individual computers and technically limited by manufacturer's constraints. We explored the use of a scanner-agnostic web-based artificial intelligence (AI) system to assess the accuracy of white blood cell (WBC) differentials and blast identification in haematological malignancies.

Methods: Digitized images of 20 normal and 124 abnormal peripheral blood films were uploaded to the web-based platform (Techcyte©) and WBC differentials performed using the online AI software. Digital images were viewed for accuracy and manual cell reassignment was performed where necessary. Results were correlated to the 'gold standard' of manual microscopy for each WBC class, and sensitivity and specificity of blast identification were calculated.

Results: The AI digital differential was very strongly correlated to microscopy ($r > .8$) for most normal cell types and did not require any manual reassignment. The AI digital differential was less reliable for abnormal blood films ($r = .50$-$.87$), but could be greatly improved by manual assessment of digital images for most cell types ($r > .95$) with the exception of immature granulocytes ($r = .62$). For blast identification, initial AI digital differentials showed 96% sensitivity and 25% specificity, which was improved to 99% and 84%, respectively, after manual digital review.

Conclusions: The Techcyte platform allowed remote viewing and manual analysis of digitized slides that was comparable to microscopy. The AI software produced adequate WBC differentials for normal films and had high sensitivity for blast identification in malignant films.

KEYWORDS

artificial intelligence, digital, haematologic, malignancy, morphology

1 | INTRODUCTION

Morphological analysis of a blood film is essential for the accurate identification of cell abnormalities leading to haematological diagnosis. Traditionally, and most commonly, this analysis is performed manually by light microscopy. However, conventional morphology has the disadvantages of being labour intensive, having a high degree of inter-observer variability and the difficulty of obtaining a second opinion as required.[1,2] Furthermore, there are constraints on the long-term storage of large volumes of slides with slide quality fading and utility diminishing over time. It is also difficult to standardize slide quality and images for education and

examination purposes. Therefore, there has been much interest in utilizing digital systems.

Digital microscopy systems are currently being used in some diagnostic laboratories, with the most peer-reviewed systems being CellaVision (Sysmex America Inc) and Sysmex (Sysmex, America).[3-7] These instruments use a digital camera coupled to a computer system. The digital images of individual cells are then classified by a computer algorithm based on parameters such as size, shape, colour and texture.[8] However, these devices have a large footprint, are relatively expensive and have restricted slide parameters that need to be adhered to in order to generate accurate differentials. Assessment of the more adaptable scanner-agnostic web-based platforms for morphological analysis has not been widely reported in the literature.

Despite their limitations, digital microscopy platforms have demonstrated excellent accuracy compared to manual microscopy.[1,3,5,6,7,9] While many studies include abnormal blood films, they do not elaborate on the number of films with haematological malignancies and do not account for the wide range of morphologic features possible within each pathology.[3,4] Cornet et al specifically assessed digital morphology in patients with malignant haematological diseases; however, a majority of these cases were lymphoproliferative disorders rather than pathologies containing blasts.[10]

The aim of the current study was to evaluate the use of a scanner-agnostic web-based artificial intelligence (AI) system to produce a white blood cell (WBC) differential from digitized slides. We assessed the method as an alternative viewing platform to manual microscopy, as well as the capacity of the AI to correctly identify blast cells in the setting of various haematological pathologies.

2 | MATERIALS AND METHOD

2.1 | Study protocol

Manual microscopic WBC differentials were established on a selection of 20 normal and 124 abnormal blood films after exclusion of eighteen abnormal peripheral blood samples due to incomplete data. These were then digitized and uploaded to the online morphology analysis platform, and cells were initially classified using the pretested and predetermined manufacturer's AI algorithm. These digital films were then reviewed remotely by a morphologist using the same online platform, and cells were reassigned as appropriate for each blood film individually. A total of approximately 80 000 blood cells were reviewed with the results compared to those recorded through previous manual microscopy. A schematic of the study procedure is shown in Figure 1 with further details provided below.

2.2 | Blood Samples

Normal blood films were obtained from the Royal College of Pathologists of Australasia Quality Assurance Program (RCPA QAP). These blood films were stained with May Grunwald Giemsa and were cover slipped. Abnormal blood films were obtained from our local haematology pathology service (New South Wales Health, New South Wales, Australia) where the patient population consisted primarily of chronic lymphocytic leukaemia (CLL), acute leukaemia (AL), acute myeloid leukaemia (AML), acute lymphoblastic (ALL) and acute promyelocytic leukaemia (APML). The slides were randomly chosen across these pathologies. Slides had been prepared in the last 12 months and were made based on routine laboratory criteria (quantitative abnormality, qualitative flags from Beckman Coulter DXH 800 and 900, Beckman Coulter Australia, Lane Cove West, Australia) that required a manual review. The abnormal blood films were stained with Wright's stain and cover slipped.

For abnormal blood films, our laboratory requires criteria-led flagged abnormal blood films to be manually reviewed by trained morphology laboratory personnel, and a haematology trainee and/or consultant before the automated differential is validated. These WBC differentials were performed at the time the slide was first made and are based on a 100-cell count by light microscopy.

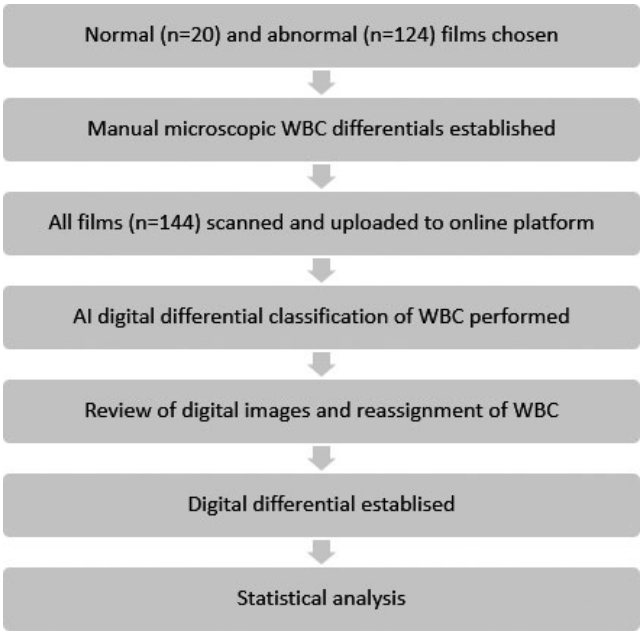

FIGURE 1 Flow diagram of method

2.3 | Blood film digitization

The blood films were scanned by a commercial scanner, Motic Easy Scan (MOTIC, www.motic.com, British Columbia, Canada). The scanner takes 6 minutes to scan one slide up to 40x magnification. The images were stored in svs format on a local hard drive and then uploaded to the online morphology platform via an account that requires a secure login and password. Each blood film image had a unique de-identified code.

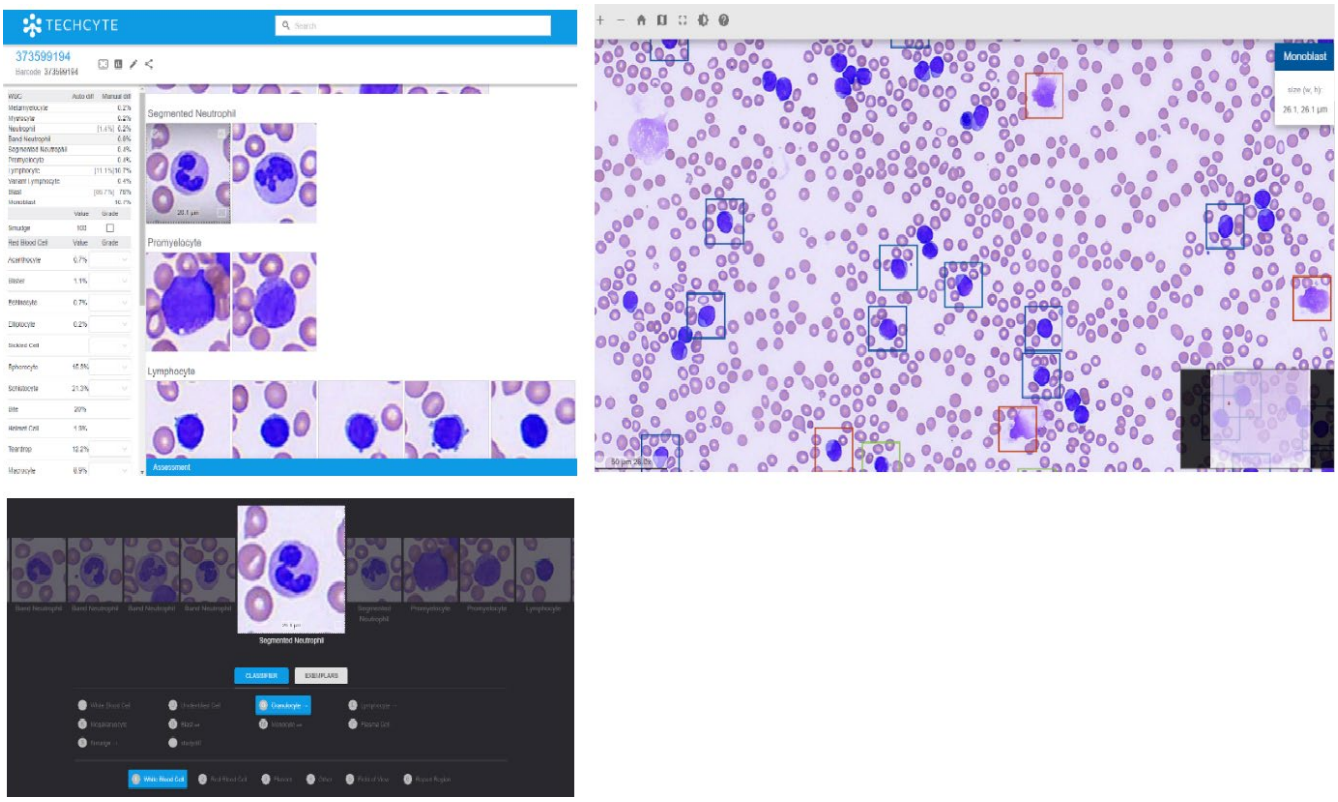

FIGURE 2 Techcyte Interface

2.4 | Web-based AI system

The digital morphology analysis platform, Techcyte (Lindon, USA; www.techcyte.com), was used to evaluate all digitized slides. The software is scanner-agnostic which means any commercially available slide scanner capable of producing svs files may be used.[11] There are no restrictions on slide preparation such as manual or automated slide smears, cover slipping or stain used. Once the digitized slides are uploaded, individual cells are automatically identified and classified using AI. The cells can then be viewed in context on the slide or in an image library which categorizes them by cell type (Figure 2). The software uses deep machine learning and image analysis technology for WBC identification, and has the capacity to incorporate expert feedback to further refine its algorithms and improve image analysis over time.[11]

2.5 | AI digital differential

The Techcyte software analysed approximately 500 WBCs per slide to generate an AI informed digital differential. The cell types analysed included basophils, eosinophils, blasts, promyelocytes, metamyelocytes, myelocytes, neutrophils, segmented or band forms, promonocytes, monocytes, lymphocytes and smudge cells. Techcyte automatically classifies neutrophils into segmented or band forms; however, we chose to analyse these together as neutrophils because this is clinically acceptable. Because of the limited numbers of promyelocytes, metamyelocytes and myelocytes, these cells were grouped together as immature granulocytes. There is significant inter-observer subjectivity in classifying granulocytes into early or late maturity class, so it is for this reason we chose to analyse total granulocytes (promyelocytes, metamyelocytes, myelocytes and neutrophils) rather than separate them into early or late stages.[2,12] This initial AI differential count was then downloaded for comparison to the manual microscopy film examination results.

2.6 | Cell reassignment and manual digital differential

The WBC images of each slide were presented in an online gallery by cell class. A second morphologist (Haematology senior registrar or consultant) reviewed each case remotely to confirm or reassign each cell individually. This manual digital differential was compared to the microscopic WBC differential results in order to assess the ability of Techcyte to act as an alternative viewing platform to microscopy.

2.7 | Statistics

Raw counts for individual cell types were converted to percentage of total cells counted, and summary data are presented as mean ± standard deviation (stdev). Statistical analysis was performed using GraphPad Prism 8.0.2 (GraphPad Software, Inc, San

Diego, USA). For correlation analyses, regression slopes were plotted and the calculated Pearson's correlation coefficients (r) were interpreted as 'very strong' (r = .8-.9), 'moderate' (r = .6-.7), 'fair' (r = .3-.5) or 'poor' (r = .1-.2) after values were rounded to the nearest decimal point.[13] Lin's concordance correlation coefficient (CCC) was calculated to assess the level of agreement, while Bland-Altman analysis with a 95% confidence interval was used to quantify the amount and direction of bias between digital and microscopic differential results for cell populations of interest. Clinical sensitivity and specificity of blast detection on the digital images were defined by the ability to obtain positive and negative results concordant with manual microscopy.

3 | RESULTS

WBC digital differentials were performed on normal (n = 20) and abnormal (n = 124) peripheral blood films and compared to results obtained by microscopy. The blood film pathologies included AML (n = 39), ALL (n = 3), APML (n = 22), CLL (n = 22) as well as other pathologies described in Table S1.

3.1 | Normal blood films

In the normal blood films, there was very strong correlation between AI digital differential and manual microscopy for neutrophils (r = .94), lymphocytes (r = .89), eosinophils (r = .81) and total granulocytes (r = .91). There was fair and poor correlation for monocytes (r = .32) and basophils (r = .21). Concordance assessments were similar, but overall mean bias was nominal, ranging from AI underestimation of neutrophils by −2.25% and overestimation of lymphocytes by.85% (Table S2). No manual reassignment was performed on any of the normal blood films.

3.2 | Abnormal blood films

There was manual reassignment performed on all abnormal blood films. The regression slopes are illustrated in Figure 3, with a detailed analysis in Table S3. Initial AI digital differential analysis of abnormal blood films by the Techcyte algorithms showed fair to very strong correlation with manual microscopy results, with correlation coefficients ranging from r = .50 (CCC = .38) for immature granulocytes to r = .87 (CCC = .77) for blasts. Upon manual review and reassignment, these correlations became very strong for neutrophils, lymphocytes, total granulocytes and blasts (r = .96-.97; CCC = .95-.97), but only moderate for immature granulocytes (r = .62; CCC = .57). Although the average biases were similar for both initial AI and reassignment digital differential counts (range:−8.84-6.25 vs −4.13-4.60, respectively), the latter showed less variation from the microscopy results, as illustrated by the tighter confidence intervals (shaded areas) on the Bland-Altman plots in Figure 3 (right panel).

3.3 | WBC differential by disease

When the results were analysed by disease and focused on cells of clinical importance (Figure 4), it became clear that the AI differential was least accurate in the settings of CLL and AL, with only moderate correlations to microscopy for lymphocytes in CLL (r = .63; CCC = .57) and only slightly better correlations for neutrophils (r = .71; CCC = .70), granulocytes (r = .68; CCC = .66) and blasts (r = .78; CCC = .52) in AL. In contrast, the AI was much better at classifying blasts (r = .92; CCC = .84) and combined promyelocytes and blasts (r = .92; C = .89) in APML. Full details of all cells analysed are available in Table S3.

Manual review of the digital images improved all of the AI generated disease specific differentials, resulting in higher correlations to manual microscopy (r>.90, CCC>.85) for the clinically relevant cell types.

3.4 | Blast analysis

For all abnormal blood films, blasts were correctly identified by the AI software for 63/65 slides with blasts on manual microscopy (sensitivity 97%), and correctly not identified in 14/58 slides without blasts on manual microscopy (specificity 24%, see Table 1). After manual review, sensitivity and specificity improved to 100% and 88%, respectively. In blood films of AL and APML, sensitivity of reassignment blast identification on the digitized images was equivalent to that of manual microscopy. For blood films where there was discordant reporting between microscopic and digital methods, the reasons for discrepancy are presented in Table S3. One blood film was omitted from analysis, and the reason is detailed in the discussion.

4 | DISCUSSION

Many studies have shown good agreement between manual and digital microscopy for abnormal films. However, they did not stratify results by disease pathology or include many malignant haematologic films in their analyses. Both CellaVision DM96 and Sysmex DI-60 have shown good correlation coefficients for neutrophils (>.95), lymphocytes (>.8) and blasts (>.8), moderate correlation coefficients for eosinophils (>.7) and monocytes (>.6).[3,4,7] Similar to our results, other studies have reported low concordance for infrequently encountered cells: basophils; eosinophils; metamyelocytes and promyelocytes.[1,3,6,14]

Koepke et al evaluated the performance of manual WBC differential by 73 different morphologists and reported correlation coefficients for the major white cell classes: neutrophils (.87), lymphocytes (.73), variant lymphocytes (.3), monocytes (.41), eosinophils (.83) and basophils (.32).[2] The correlation coefficients using Techcyte compare favourably with these results. Koepke et al found there was only 34% agreement when evaluating left-shift, highlighting the high degree of inter-observer variability when categorizing granulocytic maturation.[2]

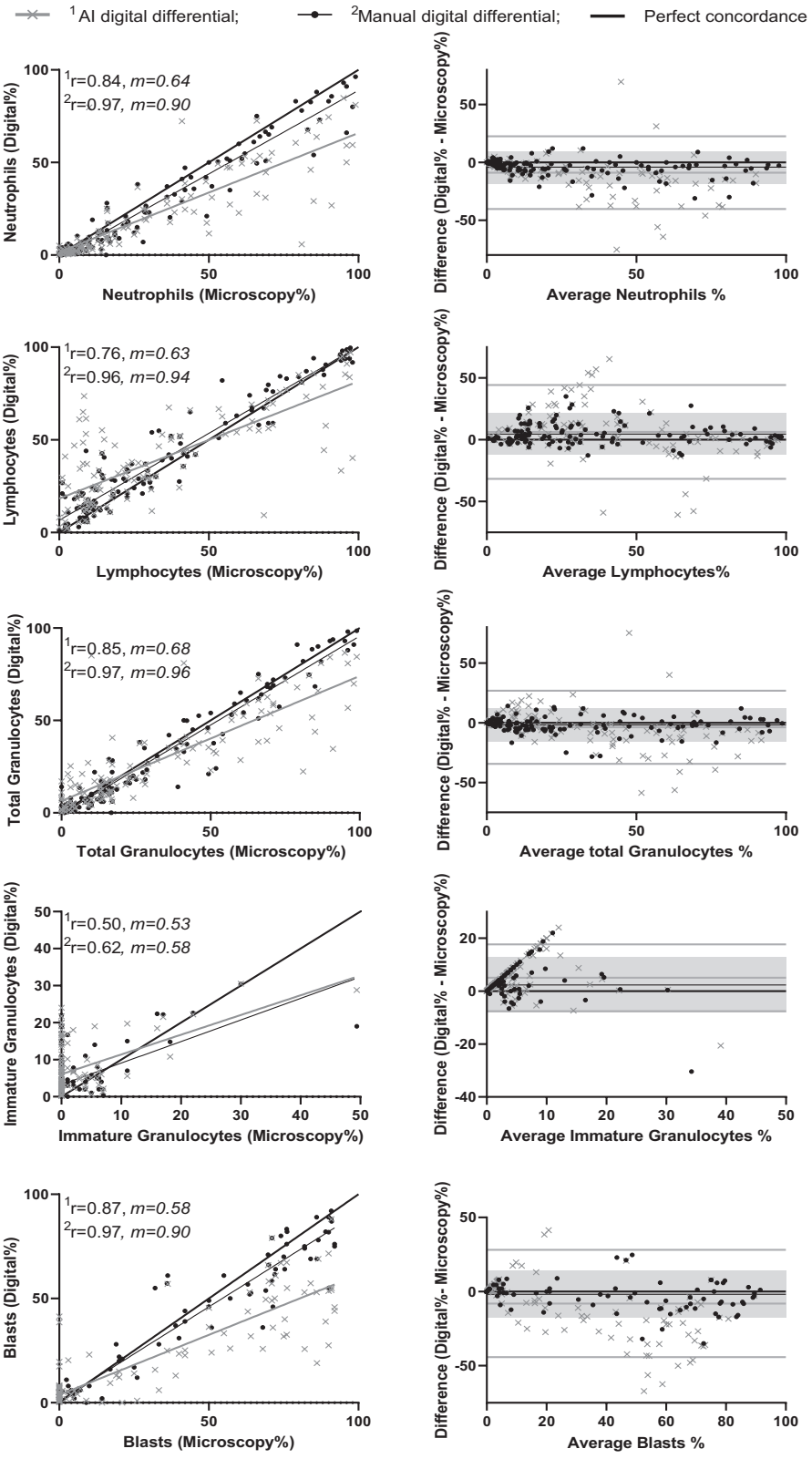

FIGURE 3 Digital differentials performed by AI or manually compared to microscopy for abnormal blood films

Cornet et al reported on the performance of the Cellavision DM96 (Sysmex America Inc) in 84 patients with malignant haematologic pathology. After re-classification, they were able to detect blasts on digital analysis in 100% of patients with blasts detected on manual microscopy with a correlation coefficient of.9.[10] Similarly, Kratz et al and Stouten et al also using the CellaVision DM96 have reported sensitivity: specificity for blast identification of 100%: 94% and 100%: 67%, respectively.[3] Similarly, they suggest digital tools may be of use for initial screening to detect blasts but there is still a need for the operator to routinely check the exact percentages. Both studies did not state how many slides with blasts were included in the analysis, nor what the underlying pathologies in the slides were.

There was reassignment for all abnormal blood films, implying that at this stage Techcyte served as a remote digital image

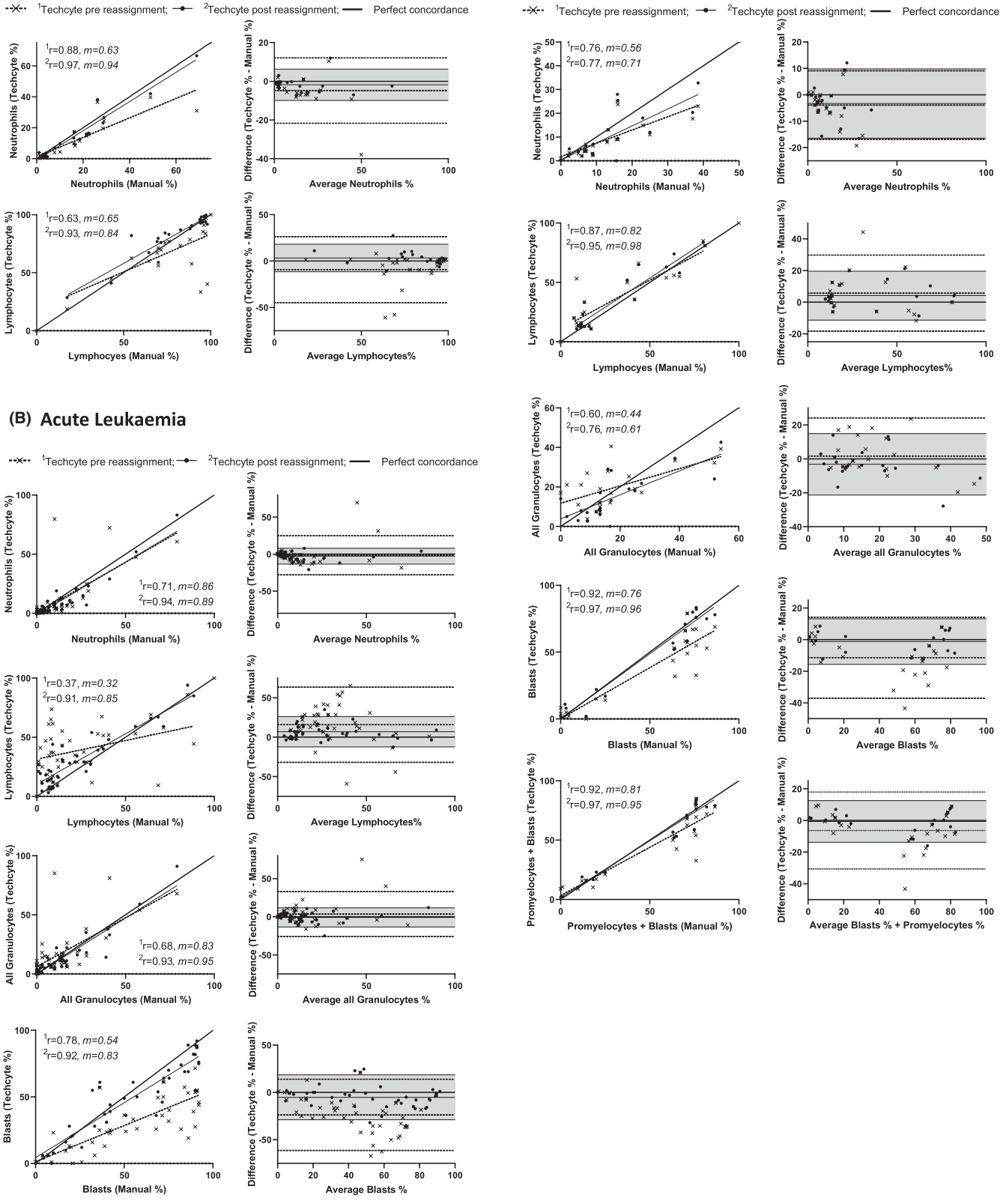

FIGURE 4 Digital differentials performed by AI or manually compared to microscopy for relevant cells grouped by selected pathologies. The AI digital differential was produced bythe Techcyte proprietary algorithm on a 500-cell count. The manual digitaldifferential was produced after manual reassignment of cells by ahaematologist. A, Chronic lymphocytic leukaemia. B, Acute leukaemia. C,Acute promyelocytic leukaemia. Left panels: Regression slopes (*m*) with Pearson's correlationcoefficients (r) for AI digital differential and manual digital differential. Right panels: Bland-Altman plotsshowing average bias and 95% confidence intervals indicated by grey horizontallines for AI digital differentials and shaded areas for manual digitaldifferentials

TABLE 1 Blast analysis for abnormal blood films

Films with blasts on manual microscopy	Number	Films correctly identified as having blasts by AI	AI Sensitivity	Films correctly identified as having blasts after manual review of digital images	Digital review Sensitivity
Total	65*	63	97%	65	100%
AL	40	39	98%	40	100%
APML	20	20	95%	20	100%
Other	5	5	100%	5	100%
Films with NO blasts on manual microscopy	Number	Films correctly identified as having NO blasts by AI	AI Specificity	Films correctly identified as having NO blasts after manual review of digital images	Digital review Specificity
Total	58	14	24%	51	88%

Note: *One blood film was excluded from this analysis due to incorrectly identified blast on manual microscopy. See Discussion; Blasts analysis and appendix.

interface rather than for its AI identification. There is limited published data on the correlation between manual microscopy and digital differentials before reassignment, making our study unique in this respect. Other studies focus on only reassigned cell morphology rather than the raw image preclassified results.[1] Although the preclassification or AI sensitivity for blast identification was 97%, which is promising, it was not satisfactory for clinical use. The preclassification specificity for Techcyte was 24%, suggesting there is still much work needed in the AI algorithm in order to reduce the need for any manual microscopy.

4.1 | Blast analysis

Blasts are one of the most important features to identify correctly on peripheral blood film examination. Our study had a reassignment sensitivity of 100% and specificity of 88% for blast identification, which is comparable to other studies.[3,4,10] There was one blood film (Case 13, Table S3) on which manual microscopy reported 9% blasts and digital morphology reassignment did not detect any blasts. The total white cell count was low, $.8 \times 10^{11}$ cells/L and was in a patient with AML who was undergoing induction chemotherapy. The blood film was re-reviewed by two independent blinded haematologists, and no blasts were identified on the original blood film by manual microscopy. The cells initially identified as blasts were lymphocytes. A bone marrow biopsy at the completion of induction chemotherapy confirmed this patient to be in a morphologic remission. As such, we omitted this blood film from our blast analysis.

There were four patient blood films (Cases 1-4, Table S3 and Image 1) with circulating abnormal lymphocytes that were classified as blasts on the reassigned digital differential. These cells represent challenging morphology and were categorized on manual microscopy as reactive, abnormal or unknown cells. The identification of these cells as blasts may be more clinically appropriate as it more urgently flags the clinician to act on the result. So, after excluding these cases, the results were then re-analysed to determine the correlation between manual and digital microscopy.

Misclassification of nonblast cells as blasts on digital morphology was seen on leucoerythroblastic films, and in some CLL films with abnormal lymphocytes or increased prolymphocytes. In clinical practice, a system that overcalls blasts may be acceptable whereas under-calling blasts may have significant clinical consequences. Paediatric lymphocytes are prone to over classification as blasts, and further studies on paediatric samples are needed to evaluate the utility of digital morphology in this setting.[15]

4.2 | Validation of digital morphology

Currently, there is no accepted standard method to validate digital morphology methods to standard manual microscopy; however, the International Council for Standardization in Haematology (ICSH) has

published some recommendations. The ICSH recommend individual laboratories should perform comparison between their current method and digital method with at least 50 slides including abnormal slides. There is no minimum number of slides to be reviewed by disease pathology or correlation values which are considered acceptable for WBC differential concordance and identification of blast sensitivity and specificity.[1]

The American College of Pathologists (ACP) has also published guidelines for whole slide imaging; however, this is focused primarily on histopathology. They suggest a review of at least 60 cases and comparison of intra-observer concordance between the two methods at least two weeks apart.[16]

In our methodology, we compared 144 blood films, normal and abnormal, with a wide range of pathologies using manual and digital platforms. This is in excess of both the ICSH and ACP guidelines, and includes more films with haematological malignancy than any other published series. After manual assessment of digital images, we were able to reliably demonstrate the presence of blasts and generate a WBC differential comparable to manual microscopy for most abnormal blood films. The results of our study are promising, bringing us closer to validating the Techcyte platform for the evaluation of WBC pathology.

4.3 | Limitations

A limitation of this study is the potential inter- or intra-observer variation in reviewing slides. We did not control for these and therefore any difference in reporting on the two platforms may in part reflect this variability rather than the inferiority of either method. In order to overcome this, two morphologists could have assessed the same slide by both manual and digital microscopy, independently over a two-week period as suggested by the ACP.[16] Despite this, the agreement in results remain satisfactory. Future studies will need to incorporate a more systematic method to reduce this variability.

Another limitation is the lower 40x magnification of the scanner compared to conventional light manual microscopy that can magnify to 100x under oil. We also noticed that some nuclear details on the digital images were not as refined as compared to traditional light microscopy.

The time taken to re-classify cells on each digital slide was not formally assessed; however, it was likely longer than on manual microscopy. A possible reason for this is that the Techcyte differentials were performed on 500 cells, rather than the standard 100-cell count by manual microscopy. However, Techcyte has an option to process smears with a 100-cell differential, and this may increase the speed and efficiency of analysis.

We had a limited number of blood films with ALL, and often, the morphology of lymphoblasts can be difficult to correctly classify. This was not an intentional omission, but rather due to a lack of adult ALL cases in our institute while blood films were being collected. In future studies, we will aim to collect more blood films of patients with ALL. We did not assess red cell or platelet changes at this time, or the AI learning capacity of the Techcyte instrument. Both of these parameters will need prospective studies with periodic assessment as the system adapts to expert feedback.

4.4 | Strengths

A strength of our study is the high number of malignant blood films with abnormal and difficult to assess morphology, reflecting the real-world pathology laboratory. We had a wide range of blast morphology and counts amongst various pathologies. We also reported the raw AI differential analysis and reassignment values unlike most published studies evaluating digital morphology. The study was independently conducted with no financial assistance from Techcyte.

Potential advantages of the Techcyte online tool are that it can be accessed from any Internet enabled device such as a laptop, tablet or smartphone, thus allowing quick viewing by a haematologist remotely. Also, the image resolution and quality were excellent. The Techcyte instrument then allows for rapid expert evaluation or second opinions for urgent cases due to the flexibility of reporting from any location. The Techcyte technology could also be particularly useful for centralization of haematology reporting services for a laboratory that oversees multiple laboratories over a large geographic area. Education and competency may be more robust and efficiently delivered by assessing accuracy on a cell-by-cell level. These potential applications need to be assessed prospectively. The Techcyte platform is a commercial entity, and there are fees associated with its use via a subscription service.

5 | CONCLUSION

Our study was aimed at assessing the ability of a digital imaging platform, Techcyte, to produce a reliable WBC differential and identify blasts accurately in abnormal films with a focus on malignant haematologic pathology. We report that the AI software produced adequate WBC differentials for normal films and had high sensitivity for blast identification in malignant films. Techcyte. AI assessment with manual reassignment may be used as an acceptable alternative to manual microscopy for abnormal WBC pathology with further evaluation needed for red cell and platelet morphology.

CONFLICT OF INTEREST

There are no conflicts of interest for any authors.

AUTHOR CONTRIBUTIONS

Dr Makhija involved in blood film interpretation, data analysis, manuscript preparation. Associate Professor Lincz involved in data analysis and manuscript preparation. Mr Attalla involved in slide preparation and management of the data library. Ms Scorgie involved in data analysis and manuscript preparation. Associate Professor Enjeti involved in data analysis and manuscript preparation.

Dr Prasad involved in slide preparation, film interpretation and manuscript preparation.

ORCID

Karan Makhija https://orcid.org/0000-0002-7357-7041
Anoop K Enjeti https://orcid.org/0000-0001-8069-090X

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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