Journal of Laboratory Automation
1–6

DOI: 10.1177/2211068215584278
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Interlaboratory Reproducibility of Blood Morphology Using the Digital Microscope

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Abstract

Differential counting of peripheral blood cells is an important diagnostic tool. However, manual morphological analysis using the microscope is time-consuming and requires highly trained personnel. The digital microscope is capable of performing an automated peripheral blood cell differential, which is as reliable as manual classification by experienced laboratory technicians. To date, information concerning the interlaboratory variation and quality of cell classification by independently operated digital microscopy systems is limited. We compared four independently operated digital microscope systems for their ability in classifying the five main peripheral blood cell classes and detection of blast cells in 200 randomly selected samples. Set against the averaged results, the R^2 values for neutrophils ranged between 0.90 and 0.96, for lymphocytes between 0.83 and 0.94, for monocytes between 0.77 and 0.82, for eosinophils between 0.70 and 0.78, and for blast cells between 0.94 and 0.99. The R^2 values for the basophils were between 0.28 and 0.34. This study shows that independently operated digital microscopy systems yield reproducible preclassification results when determining the percentages of neutrophils, eosinophils, lymphocytes, monocytes, and blast cells in a peripheral blood smear. Detection of basophils was hampered by the low incidence of this cell class in the samples.

Keywords

blood differential, hematology, digital microscopy, preclassification

Introduction

Morphological analysis of blood cells is invaluable to patient management by the clinician. Until now, manual morphological assessment using the microscope has been set as the gold standard. However, manual assessment of a blood smear is subject to individual interpretation of images, resulting in significant interobserver variability.[1–3] In addition, correct morphological classification is labor-intensive and requires continuous training of laboratory personnel. Automated digital morphological assessment of blood cells is therefore considered a valuable development, as it can overcome these drawbacks. The digital microscope (DM) offers several advantages. First, the DM ensures the constant presence of a morphological expert system in the routine laboratory. Second, the system stores an image of every analyzed cell, thereby offering the ability to re-evaluate cell types with colleagues and other pathology experts, either directly or by using telehematology.[3–6] Finally, the system enables us to digitally archive blood smear and body fluid samples indefinitely.

Since the 1970s, several automated image processing devices have been developed by various manufacturers.[7] It was previously shown that a DM system, using several advanced mathematical algorithms, is capable of correct classification of leukocytes in peripheral blood and body fluid samples in relation to manual microscopic assessment of the five main peripheral blood cell categories.[3–5,8–10] An overall accuracy of 92.0% was found when the preclassification results of the DM96 (Cellavision, Lund, Sweden) were compared to those of manual assessment.[3,11] It has been shown that the classification performance of this particular system is as reliable as manual classification by

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Received Sep 9, 2014.

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Table 1. Ranges for Each Cell Class Found at the Different Locations (ASz, Centrum, Daniel, and Vlietland).

	ASz		Centrum		Daniel		Vlietland	
	#	%	#	%	#	%	#	%
Neutrophils	2–189	1.1–96.9	0–192	0.0–97.0	0–191	0.0–97.4	0–190	0.0–96.9
Lymphocytes	0–184	0.0–92.4	1–179	0.5–92.7	2–187	1.0–93.5	1–178	0.5–90.8
Monocytes	1–73	0.5–38.2	1–65	0.5–35.7	0–75	0.0–39.3	0–75	0.0–40.8
Eosinophils	0–32	0.0–16.0	0–26	0.0–20.7	0–40	0.0–21.3	0–56	0.0–28.4
Basophils	0–12	0.0–7.7	0–11	0.0–6.0	0–10	0.0–6.5	0–10	0.0–5.4
Blast cells	0–151	0.0–82.1	0–137	0.0–86.2	0–167	0.0–88.4	0–123	0.0–73.7

experienced laboratory technicians in classifying the five main peripheral blood cell categories.[3]

Only limited information is currently available regarding interlaboratory variation and quality in cell classification by independently operated digital microscopy systems. As part of the continuing validation of DM systems, it is important to assess the interlaboratory variation between systems operated at different locations and determine whether they can produce comparable results. To achieve this, we compared four independently operated DM systems when analyzing randomly selected samples.

Materials and Methods

Digital Microscope Systems and Locations

In this study we set out to compare four independently operated digital microscope systems (DM96) for their ability to classify the five main peripheral blood cell classes (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) and blast cells in 200 samples. The DM machines were located at four different clinical chemistry laboratories in the Netherlands: the Albert Schweitzer Hospital (ASz), the Vlietland Hospital (Vlietland), the Erasmus Medical Centre, central location (Centrum), and the Erasmus Medical Centre Daniel den Hoed Cancer Clinic (Daniel).

Blood Sample Collection and Analysis

Using standardized protocols, each laboratory collected peripheral blood samples from 50 randomly selected patients and generated four blood smear specimens per sample. Each hospital location received one smear specimen from each patient. A total of 200 specimens were analyzed at each location. Prior to sample analysis, all four DM systems were calibrated using a calibration slide. In addition, each system was set to analyze and classify 200 leukocytes per sample. At each location the samples were processed on the DM by two local technicians following standardized procedures. This study focused on the preclassification results obtained from the four different DM systems. Preclassification is defined as the initial classification performed by the DM, without intervention or correction by the local operator. Therefore, results could not be influenced by manual interference.

Interlaboratory Variation

The number of each cell type found (neutrophils, lymphocytes, monocytes, eosinophils, basophils, and blast cells) was expressed as a percentage of the total amount of cells classified. For each location, the individual preclassification result per cell class was compared to the averaged percentage of the other three locations in order to determine the interlaboratory variation.

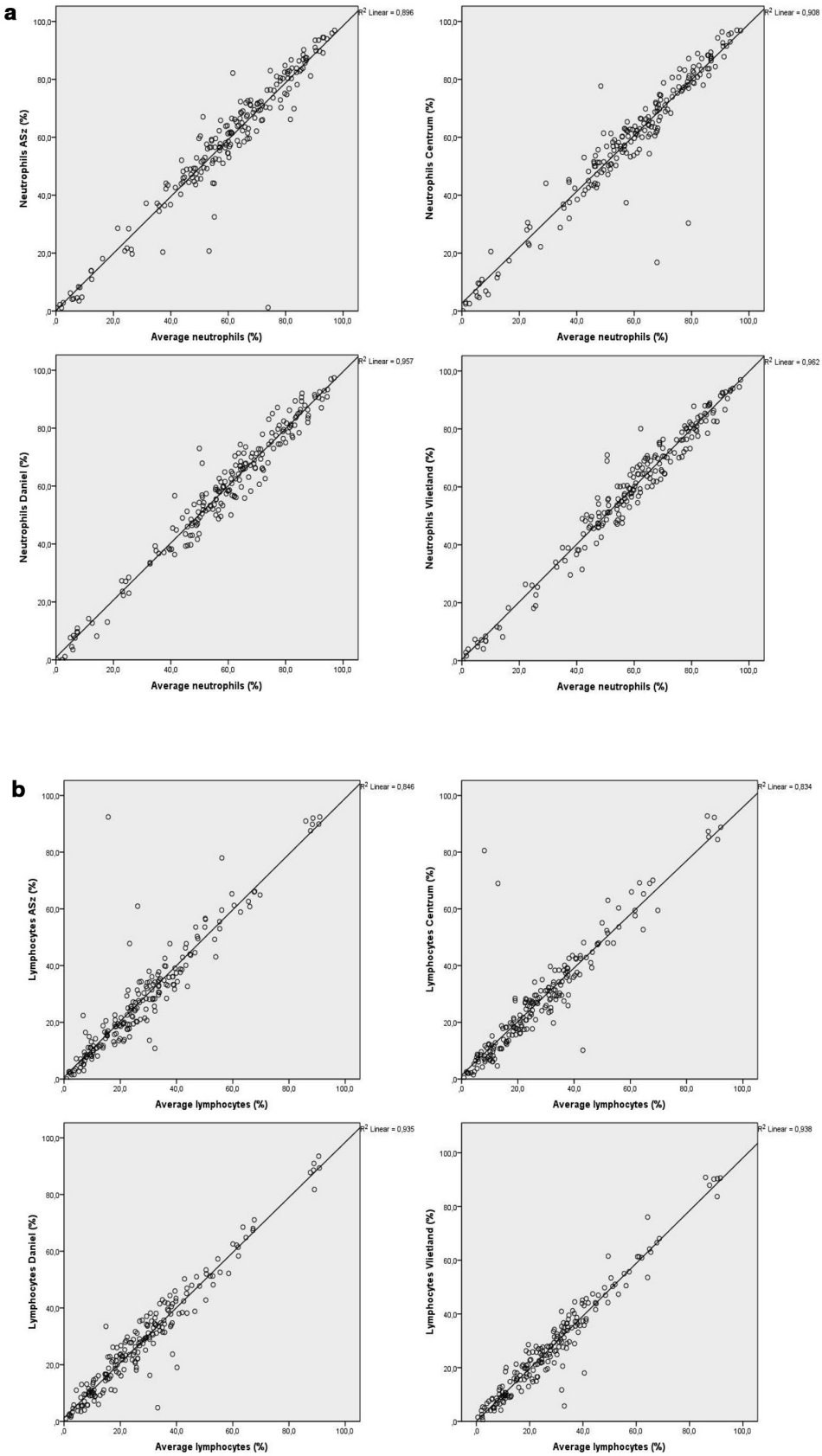
Statistics

The coefficient of determination (R^2) was calculated for each comparison in order to determine the interlaboratory variation, using Statistical Package for the Social Sciences (SPSS) version 18 for Windows.

Results and Discussion

The total number of classified leukocytes did not reach 200 cells in all samples. The range in numbers and percentages per cell class per location is shown in **Table 1**. **Figure 1a–f** shows scatter plots and the associated R^2 value for each comparison per cell class. Overall, small interlaboratory variation was found for neutrophils (R^2 = 0.90–0.96), lymphocytes (R^2 = 0.83–0.94), monocytes (R^2 = 0.77–0.82), eosinophils (R^2 = 0.70–0.78), and blast cells (R^2 = 0.94–0.99). Only basophils showed a large variation (R^2 = 0.28–0.34).

As part of the continuing validation of DM systems for morphological analysis of a peripheral blood smear, the interlaboratory variation for the five main blood cell classes and blast cells was determined. This is the first published study that considers this variation between independently operated digital microscopy systems. The preclassification results show small interlaboratory variation for four of the five main peripheral blood cell classes. This is comparable to R^2 values found when comparing two manual differential counts, as done by Ceelie et al.[3] The DM showed even less variation between several machines than between the two



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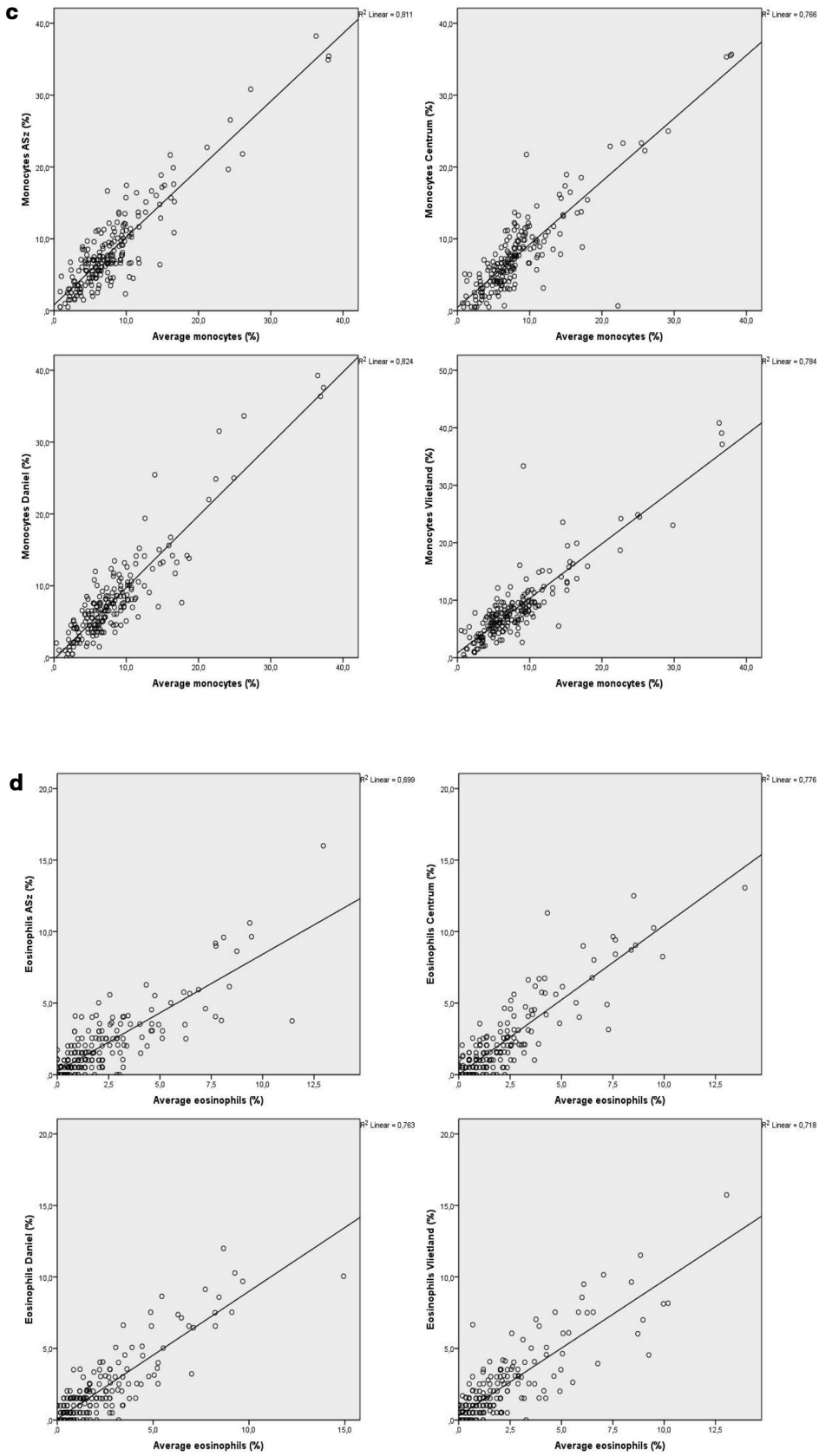

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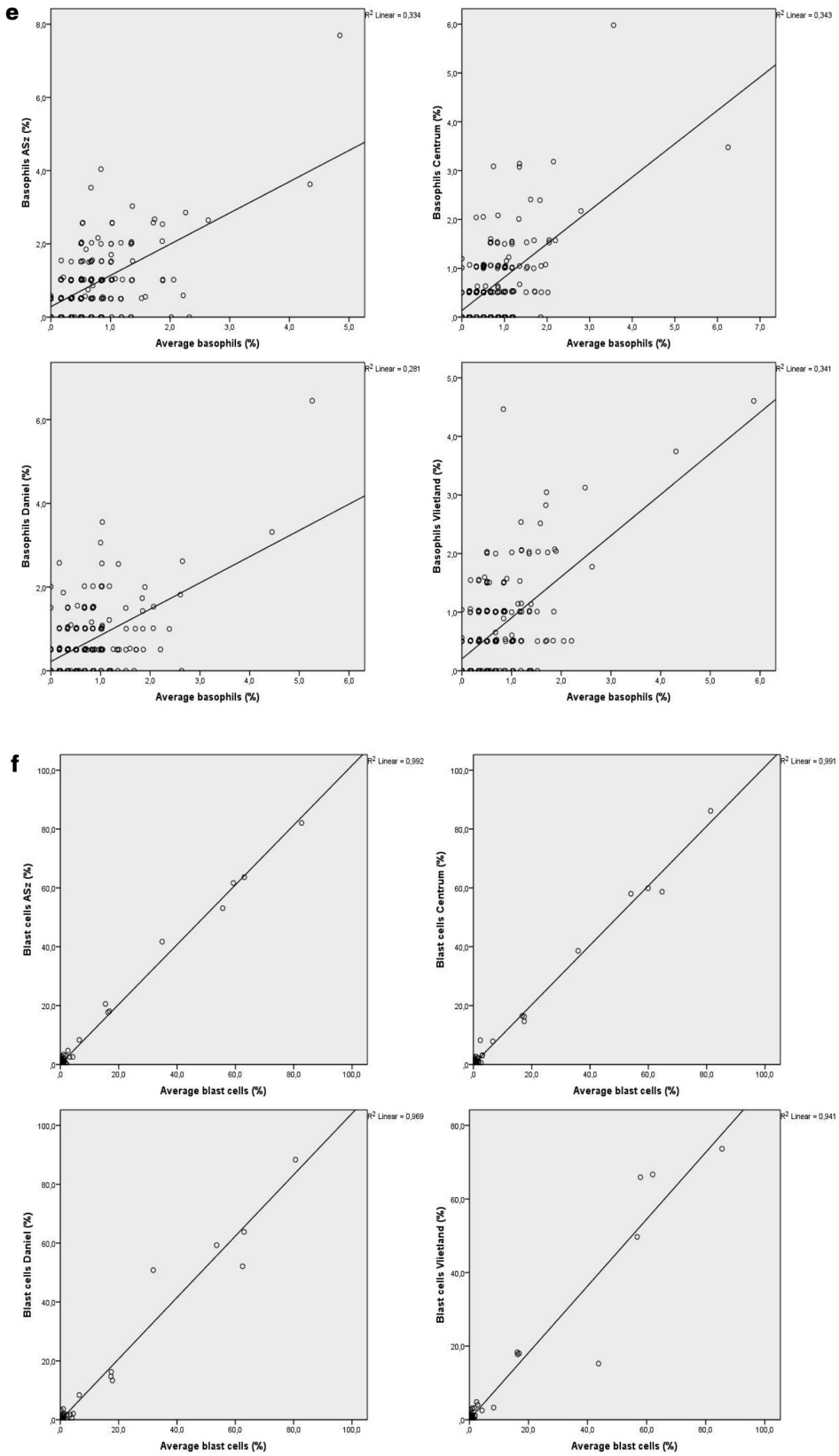

Figure 1. Results of preclassification comparisons for segmented neutrophils (a), lymphocytes (b), monocytes (c), eosinophils (d), basophils (e), and blast cells (f). The *Y* axis shows the percentage of the cell classes found in each of the 200 samples per location (Vlietland, ASz, Daniel, and Centrum). The *X* axis shows the average percentage of the difference cell classes found at the four locations excluding the location on the *Y* axis.

manual differentials for neutrophils (R^2 = 0.90 for manual count) and monocytes (R^2 = 0.65 for manual count).[3]

Blast cells were detected with an excellent accuracy, despite the fact that the overall average percentage of blast cells was low. This is probably due to the large spread in counted cells per sample. Not every sample contained blast cells, which lowers the overall average percentage. The same was observed when two manual counts of blast cells were compared, resulting in an R^2 value of 0.97.[3] Again, the DM showed even less variation between systems than between experienced morphologists, since R^2 values between 0.94 and 0.99 were found in this study.

Only the preclassification results of the basophils showed considerable interlaboratory variation. This variation was also seen when comparing manual assessment by an experienced morphologist to a reference differential, as done by Briggs et al.[4] Even an experienced morphologist could not achieve an R^2 value higher than 0.30 when manually classifying basophils. Briggs et al.[4] also compared the manual differentials executed by two experienced morphologists with the DM. This resulted in an R^2 value of 0.00.[4] Similar results were found by Ceelie et al.[3] when comparing two manual differential counts with each other and with the DM. The poor R^2 values for basophils are due to the low number of detected cells of this class per peripheral blood smear, leading to profound relative differences in detected percentages of basophils at different locations.

A database containing approximately 1.4 million leukocytes was set up to compare the preclassification performance of the DM with the manual assessment of peripheral blood smears by experienced morphologists. This database yielded an R^2 value of 0.88 for the basophils (Riedl, data not yet published). The size of that database overcomes the problem encountered in this study, which was hampered by the low number of counted basophils in the various samples. The same was observed in a study by Lee et al.,[12] who did not include normal blood smears and therefore may have had more basophils than is usually observed. Their comparison between the DM96 and a manual count gave an R^2 value of 0.76.[12]

In conclusion, this study shows that independently operated digital microscopy systems, stationed at four different locations, yield reproducible preclassification results when determining percentages of neutrophils, lymphocytes, monocytes, and eosinophils present in a blood smear. In addition, blast cells were also detected correctly and with only minor variation in detected percentages between the different microscopy systems. The classification of basophils was less accurate because of the low number of basophils present in these samples.

Contributorship

JAR, WG, JB, and KS: experimental design setup and writing of the manuscript. HC and MDL: affiliated researchers. All authors read and approved the final manuscript.

Acknowledgement

We thank Rebecca Buis for critical reading of the manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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