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CASE REPORT HAEMATOLOGY & LABORATORY MEDICINE

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The clinical significance of manual microscopy in resolving automated haematology discrepancies: a case study of familial Pelger-Hüet anomaly

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Abstract

Background Automated haematology analysers classify leucocytes based on quantitative parameters of volume, conductivity, and light scatter. However, this design cannot interpret certain benign hereditary nuclear variants. Pelger-Hüet anomaly (PHA), a hereditary, autosomal-dominant condition arising from mutations in the lamin B receptor (LBR) gene, is frequently misclassified by such platforms as a pathological left shift, since its hyposegmented and densely condensed nuclei present a reduced scatter complexity resembling that of immature band forms.

Case presentation This case study documents a familial cluster of PHA in two siblings evaluated in Tbilisi, Georgia, with a reported paternal history of the same trait consistent with autosomal-dominant transmission. Peripheral blood smears from both patients were prepared using the standard wedge technique, stained with Wright-Giemsa, and evaluated under oil-immersion microscopy. One hundred neutrophils per patient were classified by nuclear lobe number and chromatin architecture.

Results Patient 1 showed 94% hyposegmented neutrophils (87% bilobed, 7% monolobed) and Patient 2 showed 96% (91% bilobed, 5% monolobed). The automated five-part differential did not report an independent hyposegmented neutrophil or PHA category for either sample; instead, affected cells were distributed across the neutrophil and band-form channels without a distinguishing flag.

Conclusion Manual smear evaluation, through assessment of chromatin condensation, nuclear architecture, and cytoplasmic maturity, remains necessary to differentiate benign hereditary PHA from false left-shift misclassification and from the acquired pseudo-Pelger-Hüet anomaly associated with myelodysplastic and malignant myeloid processes. Manual smear review following an automated left-shift flag should be regarded as a primary diagnostic procedure, not a supplementary step.

Keywords Pelger-Hüet anomaly; lamin B receptor; neutrophil morphology; automated haematology analyser; left shift; peripheral blood smear; Wright-Giemsa; manual microscopy; band form; hyposegmentation

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1. Introduction

Automated haematology platforms — such as the Beckman Coulter DxH series and the Sysmex XN series — process thousands of complete blood counts (CBCs) per shift using Volume-Conductivity-Scatter (VCS) technology and multi-angle light scatter [1]. These instruments classify leucocytes at speeds no manual technologist could match and are indispensable for detecting anaemia, malignancy, infection, and immunodeficiency. Yet they excel at quantitative cell counting while remaining fundamentally limited in qualitative assessment of individual cell morphology — a distinction that becomes clinically critical in the presence of hereditary nuclear variants.

Pelger-Hüet anomaly (PHA), first described by Pelger in 1928 and characterised by Huët in 1931 [2,3], is a benign, autosomal-dominant condition caused by mutations in the lamin B receptor (LBR) gene at chromosome 1q41–43 [4]. Affecting approximately one in 6,000 individuals [5], heterozygous carriers produce neutrophils with characteristic bilobed "pince-nez" nuclei rather than the normal three-to-five-lobed architecture [6]. Automated analysers consistently misclassify these as band forms or immature granulocytes, generating a spurious left shift that can trigger unnecessary infectious workups [7]. Equally, automated indices cannot distinguish benign hereditary PHA from pseudo-Pelger-Hüet anomaly (PPHA) — which may signal myelodysplastic syndrome, chronic myeloid leukaemia, or drug toxicity — making clinical context, family history, and trained morphological assessment indispensable. This case study documents familial PHA in two siblings through paired quantitative morphometric analysis of manual smear review and automated differential counts, demonstrating why peripheral blood smear review remains the diagnostic gold standard for leucocyte nuclear anomalies.

2. Materials and methods

2.1 Patients and clinical context

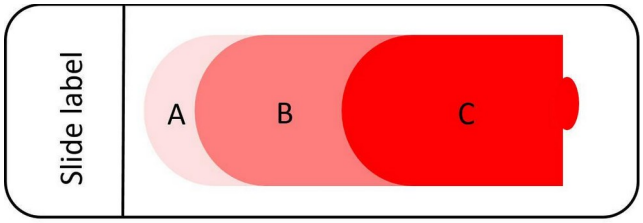
Two siblings — Patient 1 (female) and Patient 2 (male) — presented for routine blood analysis at Zurich Medical Research Centre, a laboratory unit of JSC Curatio, Tbilisi, Georgia. Both were clinically asymptomatic with no prior history of myeloproliferative or myelodysplastic disorders and no exposure to cytotoxic medications. The nuclear anomaly was identified incidentally during a routine complete blood count. A paternal history of the same hereditary trait was reported during clinical interview, consistent with autosomal-dominant transmission across two generations, although laboratory documentation of the father's peripheral smear was not available for direct inclusion. Because both patients were minors at the time of evaluation, written informed consent was obtained from their legal guardians before any research use of clinical data.

2.2 Automated haematological profiling

Complete blood counts (CBC) and automated five-part white blood cell differentials were performed on the Beckman Coulter DxH 650 automated haematology analyser (Beckman Coulter, Brea, CA, USA). The instrument uses volume, conductivity, and scatter (VCS) technology, incorporating low-frequency direct current impedance for cellular volume measurement and multi-angle laser light scatter to determine surface topography and nuclear configuration. Samples were collected via standard venipuncture into EDTA-anticoagulated tubes and processed within two hours of withdrawal to minimise storage-induced morphological artefacts.

2.3 Smear preparation

Peripheral blood smears were prepared using the standard wedge technique, as illustrated in Figure 1. A small drop of fresh EDTA-anticoagulated whole blood was placed near one end of a clean glass slide; a spreader slide held at 30–45° was drawn back into contact with the drop, allowing it to spread by capillary action, and then advanced smoothly across the base slide at constant speed. This produced a smear with a graded thickness — a thick body, a thin body, and a feathered edge — where individual leucocytes lay in a single, non-overlapping layer suitable for morphological evaluation. Smears were air-dried completely at room temperature before fixation.



A: Feathered edge- Platelet clump evaluation
B: Monolayer- Cell evaluation
C: Body- Multiple cells thick- do not use

Figure 1. Schematic of the wedge-smear technique: Zone A — feathered edge (platelet assessment); Zone B — monolayer (cell evaluation); Zone C — thick body (not used). Source: WVS Academy, 2026 [8].

2.4 Staining methodology

Air-dried smears were fixed in absolute methanol for 30 seconds to stabilise cellular membranes and preserve nuclear morphology, then air-dried a second time. Fixed smears were stained with Wright-Giemsa solution for 30 seconds — the basic component (methylene blue) binding acidic nuclear and cytoplasmic structures, and the acidic component (eosin) binding basic cytoplasmic constituents. Slides were rinsed twice in phosphate-buffered saline (PBS) to remove unbound stain and promote differentiation, air-dried a final time, and mounted for microscopic evaluation.

2.5 Microscopic analysis and neutrophil classification

All morphological evaluation was performed within the monolayer region using a compound light microscope under a 100× objective with oil-immersion for detailed nuclear assessment. Neutrophils were identified by their pale eosinophilic cytoplasm containing fine neutral-staining granules and distinguished from other white blood cell types by standard cytological criteria. For each patient, 100 neutrophils were evaluated across multiple non-overlapping fields. Each nucleus was classified into one of four morphological categories: (1) normally segmented — three or more distinct nuclear lobes connected by thin chromatin filaments; (2) bilobed — two rounded lobes connected by a thin chromatin strand with grainy, densely clumped chromatin; (3) monolobed — a single, non-segmented, densely condensed nucleus without lobulation; and (4) band form — an elongated, unsegmented nucleus of relatively uniform width, distinguished from monolobed PHA morphology by finer, less condensed chromatin. Figures 2 and 3 illustrate representative neutrophil morphology in each patient.

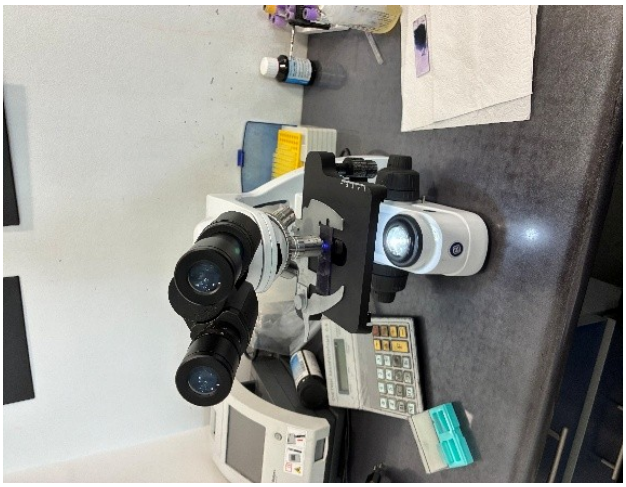


Figure 2. Oil-immersion photomicrograph (×100) of Patient 1 peripheral blood smear (Wright-Giemsa). Bilobed "pince-nez" neutrophils with densely condensed chromatin characteristic of Pelger-Hüet anomaly are visible.

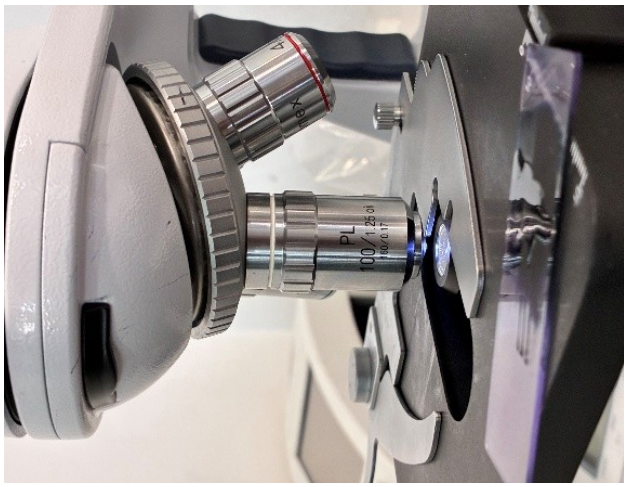


Figure 3. Oil-immersion photomicrograph (×100) of Patient 2 peripheral blood smear (Wright-Giemsa). Bilobed and monolobed hyposegmented neutrophils with coarse chromatin consistent with familial Pelger-Hüet anomaly are demonstrated.

3. Results

3.1 Automated haematological profiling

Automated CBC profiling on the Beckman Coulter DxH 650 showed normal complete blood count parameters for both patients; full results are presented in Table 2 (Appendix B). Patient 1 and Patient 2 presented total leucocyte counts of 5.30 × 10^9/L and 5.65 × 10^9/L, haemoglobin levels of 12.09 g/dL and 15.85 g/dL, and platelet counts of 326.2 × 10^9/L and 300 × 10^9/L, respectively. Neutrophil percentages were 43.16% (Patient 1) and 51.57% (Patient 2), yielding absolute neutrophil counts of 2.29 × 10^9/L and 2.91 × 10^9/L — both within standard physiological reference ranges. Erythrocyte sedimentation rates were 15 mm/h and 10 mm/h, respectively.

The automated five-part differential was unable to report an independent "hyposegmented neutrophil" or "PHA" category for either sample. Instead, affected hyposegmented cells were distributed across the instrument's standard neutrophil and band-form channels without any flag indicating nuclear chromatin condensation.

3.2 Qualitative morphological assessment

Manual microscopic review of both patients' peripheral smears confirmed a consistent, distinctive nuclear phenotype. The majority of neutrophils in both patients showed a bilobed "pince-nez" nuclear configuration — two rounded lobes joined by a thin, often barely visible chromatin filament — compared with the three-to-five-lobed, thread-connected architecture of normal mature neutrophils. A smaller subset showed a single, non-segmented ovoid nucleus with the same coarse chromatin pattern, consistent with the extreme end of the hyposegmentation spectrum. Cytoplasmic morphology was within normal limits in both patients, with normal pale eosinophilic staining and a normal complement of fine neutral granules, thereby excluding a toxic or reactive process.

The two band-form neutrophils observed in Patient 2 were qualitatively distinguishable from the monolobed PHA morphology: the band forms retained an elongated, sausage-shaped nucleus of relatively uniform width with finer, less condensed chromatin — distinct from the compact, densely clumped oval nucleus typical of PHA. This morphological distinction, which cannot be encoded in a purely

numerical differential count, illustrates precisely why visual assessment by a trained observer is irreplaceable.

3.3 Quantitative nuclear morphology

Table 1 presents the quantitative results of the 100-neutrophil differential count for both patients.

Table 1. Quantitative neutrophil nuclear morphology from manual 100-cell differential count.

Morphological category	Patient 1 — female (n = 100, %)	Patient 2 — male (n = 100, %)
Normally segmented (≥3 lobes)	5	2
Bilobed (pince-nez)	87	91
Monolobed (single, non-segmented)	7	5
Band form	1	2
Total hyposegmented (bilobed + monolobed)	94	96

Bilobed and monolobed neutrophils were classified as hyposegmented on the basis of nuclear lobe number and chromatin condensation pattern. Band forms were distinguished from monolobed PHA nuclei by their elongated shape and finer chromatin texture.

Across both patients, the vast majority of neutrophils demonstrated hyposegmented nuclear morphology consistent with heterozygous Pelger-Hüet anomaly. Patient 1 showed 87% bilobed and 7% monolobed neutrophils (total hyposegmented: 94%). Patient 2 showed 91% bilobed and 5% monolobed neutrophils (total hyposegmented: 96%). The two band-form neutrophils observed in Patient 2, distinguished from monolobed PHA nuclei by their elongated shape and finer chromatin pattern, were present at a low frequency consistent with the absence of an active infectious or inflammatory process.

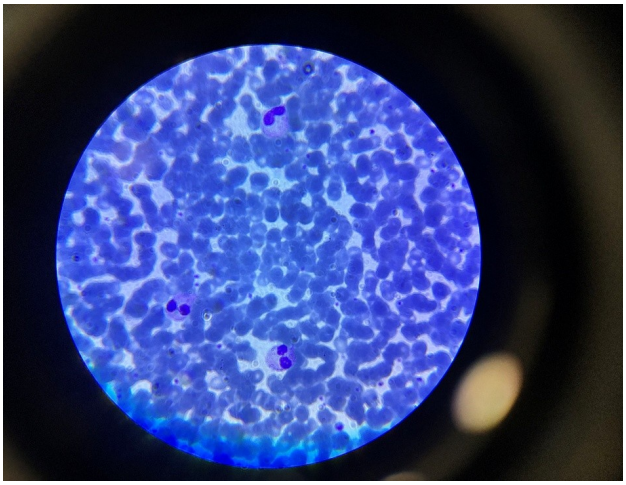


Figure 4. Representative VCS scatterplot from the Beckman Coulter DxH 650 automated analyser for Patient 1. The population distribution shows hyposegmented cells integrated within the standard neutrophil cluster without a distinct morphological flag.

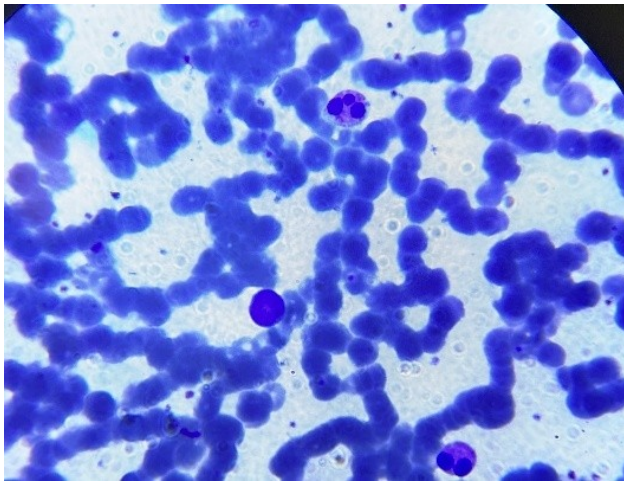


Figure 5. Representative VCS scatterplot from the Beckman Coulter DxH 650 automated analyser for Patient 2. Hyposegmented neutrophils are similarly absorbed into the neutrophil and band-form channels without triggering an independent Pelger-Hüet anomaly category.

4. Discussion

This case report characterises familial PHA in two siblings through paired quantitative morphometry and demonstrates — directly and numerically — the failure mode of automated haematology platforms when encountering a hereditary nuclear variant. The finding that 94% and 96% of evaluated neutrophils were hyposegmented, in the context of otherwise normal CBC parameters and an asymptomatic clinical presentation, is entirely consistent with the established phenotype of heterozygous PHA [4,6].

The familial context strengthens the morphological interpretation. The siblings' father was reported to carry the same trait — consistent with autosomal-dominant inheritance — although laboratory documentation of his peripheral smear was

unavailable. PHA is classically caused by heterozygous mutations in the LBR gene; homozygous individuals show extreme nuclear hyposegmentation and are rare [4]. Because neither sibling underwent molecular LBR sequencing, the diagnosis rests on the concordant characteristic phenotype rather than genotypic confirmation. Future studies should incorporate targeted genomic sequencing to validate phenotypic findings.

The automated system's inability to flag PHA has direct clinical consequences. A right-shifted population of bilobed and monolobed neutrophils absorbed into the band-form channel generates a spurious left shift — an artefact that could prompt an infectious workup, blood cultures, empirical antibiotic therapy, or costly inflammatory panel testing in a patient who is entirely well. The same limitation makes it impossible, by automated count alone, to distinguish benign hereditary PHA from PPHA, in which hyposegmented neutrophils signal myelodysplastic syndrome, chronic myeloid leukaemia, or medication toxicity [7]. Without manual smear evaluation, these two conditions — one innocuous, one potentially life-threatening — are indistinguishable by their instrument output.

A notable technical limitation of this study is the inability to retrieve the raw VCS numerical scatterplots in sufficient resolution for rigorous comparative analysis. Access to these raw scattergrams would have permitted direct visualisation of how the instrument's optical light scatter channels distributed condensed hyposegmented cells relative to mature neutrophils and true band forms. Additionally, single-observer morphology classification, absence of LBR molecular testing, and limited longitudinal follow-up restrict the generalisability of the findings. The results should be interpreted as a descriptive familial case report, not as a diagnostic performance study of the analyser.

The broader implication is clear: manual peripheral blood smear review following an automated left-shift flag should be treated as a primary diagnostic procedure rather than a supplementary confirmatory step. Assessment of chromatin condensation, nuclear lobe architecture, and cytoplasmic maturity permits reliable differentiation between benign hereditary PHA, true infectious left shift, and acquired pseudo-PHA — distinctions that automated platforms, optimised for quantitative throughput rather than qualitative cytological discrimination, are structurally unable to make.

5. Conclusion

Two siblings demonstrated a highly concordant neutrophil hyposegmentation phenotype on Wright-Giemsa-stained peripheral blood smears, with 94% and 96% of assessed neutrophils showing bilobed or monolobed morphology consistent with familial Pelger-Hüet anomaly. Automated CBC profiling on the Beckman Coulter DxH 650 distributed affected cells across the neutrophil and band-form channels without generating an independent PHA flag, illustrating the fundamental limitation of quantitative platforms in the face of qualitative nuclear variants.

Manual smear evaluation — integrating chromatin condensation, lobe number, cytoplasmic maturity, and clinical context — remains the indispensable step by which this discrepancy is resolved. Recognition of the characteristic PHA morphology can prevent misinterpretation of a benign hereditary trait as evidence of infection, dysplasia, or malignancy, and thereby avert unwarranted antibiotic interventions, unnecessary bone marrow evaluations, and the diagnostic and emotional burden that these entail. Automated haematology platforms have transformed clinical laboratory efficiency, but they do not eliminate — and in morphologically complex cases actively necessitate — the trained eye of the microscopist.

Abbreviations

ANC — Absolute neutrophil count
CBC — Complete blood count
EDTA — Ethylenediaminetetraacetic acid
ESR — Erythrocyte sedimentation rate
GMJ — Georgian Medical Journal
LBR — Lamin B receptor
MDS — Myelodysplastic syndrome
ORCID — Open Researcher and Contributor ID
PBS — Phosphate-buffered saline
PHA — Pelger-Hüet anomaly
PHIG — Public Health Institute of Georgia
PPHA — Pseudo-Pelger-Hüet anomaly
VCS — Volume-Conductivity-Scatter
WBC — White blood cell count

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Author's contributions

GT: Conceptualisation; study design; peripheral blood smear preparation, staining, and microscopic evaluation; quantitative morphometric analysis; writing — original draft; writing — review and editing; final approval. The author read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate This case study was a retrospective review of clinical data and peripheral blood smears originally obtained for routine diagnostic evaluation at Zurich Medical Research Centre, a laboratory unit of JSC Curatio, Tbilisi, Georgia. Written informed consent was

obtained from the patients' legal guardians for secondary research use of clinical data. The full informed consent form is provided in Appendix A.

Consent for publication Written informed consent was obtained from the legal guardians of both patients for the publication of anonymous clinical data, laboratory findings, and microscopic images in this manuscript.

Conflict of interest disclosures The author declares no conflicts of interest.

Data sharing The treating institution retains raw automated differential reports and original peripheral blood smear slides. De-identified data are available from the corresponding author upon reasonable request, subject to institutional data-sharing policy.

AI use disclosure During manuscript preparation, generative AI tools (ChatGPT, Gemini, Claude) were used for language editing, manuscript restructuring, and drafting assistance. AI tools were not used to generate patient data, laboratory measurements, microscopy counts, or statistical results. The author independently verified all reported laboratory values, morphology counts, scientific claims, and references, and takes full responsibility for the accuracy, originality, ethical compliance, and final content of this manuscript.

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Appendix B — Automated haematology results

Table 2. Automated complete blood count parameters for both patients (Beckman Coulter DxH 650).

Parameter	Patient 1 (female)	Patient 2 (male)	Reference range
WBC (×10⁹/L)	5.30	5.65	4.0–11.0
Haemoglobin (g/dL)	12.09	15.85	12.0–16.0 (F); 13.5–17.5 (M)
Platelets (×10⁹/L)	326.2	300.0	150–400
Neutrophils (%)	43.16	51.57	40–75
Absolute neutrophils (×10⁹/L)	2.29	2.91	1.8–7.7
ESR (mm/h)	15	10	<20 (F); <15 (M)

ESR — erythrocyte sedimentation rate; WBC — white blood cell count; F — female; M — male. All values were within established reference ranges. The automated differential did not generate a specific Pelger-Hüet anomaly or hyposegmented neutrophil flag for either sample.

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Appendix A — Informed consent form

The full informed consent form for secondary research use of clinical data and biological samples is archived with the corresponding author and is available upon request. The form covers: purpose and nature of the research; scope of participation

(retrospective review only, no new procedures); risks and benefits; confidentiality and data protection; and the right to withdraw. Separate sections address participant declaration, minor assent, and parental/legal guardian consent, as well as investigator attestation. All signatories provided written consent prior to any research use of clinical data or microscopy images.

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