Parasitised red blood cells misclassified as giant platelets by an automated digital morphology analyser (Sysmex DI-60/ CellaVision): a case report and a retrospective EQA analysis

In recent decades many countries have undergone consolidation of their pathology services in an attempt to reduce costs, meet increasing demand and achieve efficiency savings while modernising laboratory infrastructure with new technologies.[1] Satta *et al.* recently concluded that consolidated units have, on average, achieved cost savings but their impact on the quality of the services should be further analysed and assessed in the different economic and social environments.[2] While the processing of a large numbers of tests in a single laboratory increases the likelihood of detecting rare cases not usually observed in small laboratories, the hub-and-spoke model hinders the communication between clinicians and laboratorians. The patient's clinical information is either not transmitted to the laboratory or is partially transmitted, as shown in the case reported in this article. Major manufacturers of haematology analysers are investing in the digitalisation of blood film and in software capable of autonomously classifying morphological alterations.[3,4] The Greater Romagna Area laboratories network in Italy includes eight laboratories (one hub and seven spokes), each of them equipped with a digital morphology analyser (DI-60 Sysmex, Kobe, Japan) employing CellaVision software (CellaVision AB, Lund, Sweden), which allows remote assessment of blood films.[5] The DI-60/CellaVision was set in the automatic pre-classification mode in order to acquire 200 white blood cells (WBC), to classify them into 14 subtypes of 'leucocytes' (unidentified, neutrophil, lymphocyte, monocyte, eosinophil, basophil, promyelocyte, myelocyte, metamyelocyte, promonocyte, prolymphocyte, blast, plasma cell and hairy cell), and five subtypes of 'non-leucocytes' (erythroblast, giant platelet, thrombocyte aggregation, smudge and artefact). A 35-year-old man was admitted to the Ravenna Hospital emergency room in July 2020, giving 'fever without pharyngodynia or lymphadenopathy' as the specific clinical information in the Order Entry software. The patient was admitted late at night, and the data was therefore remotely evaluated by a

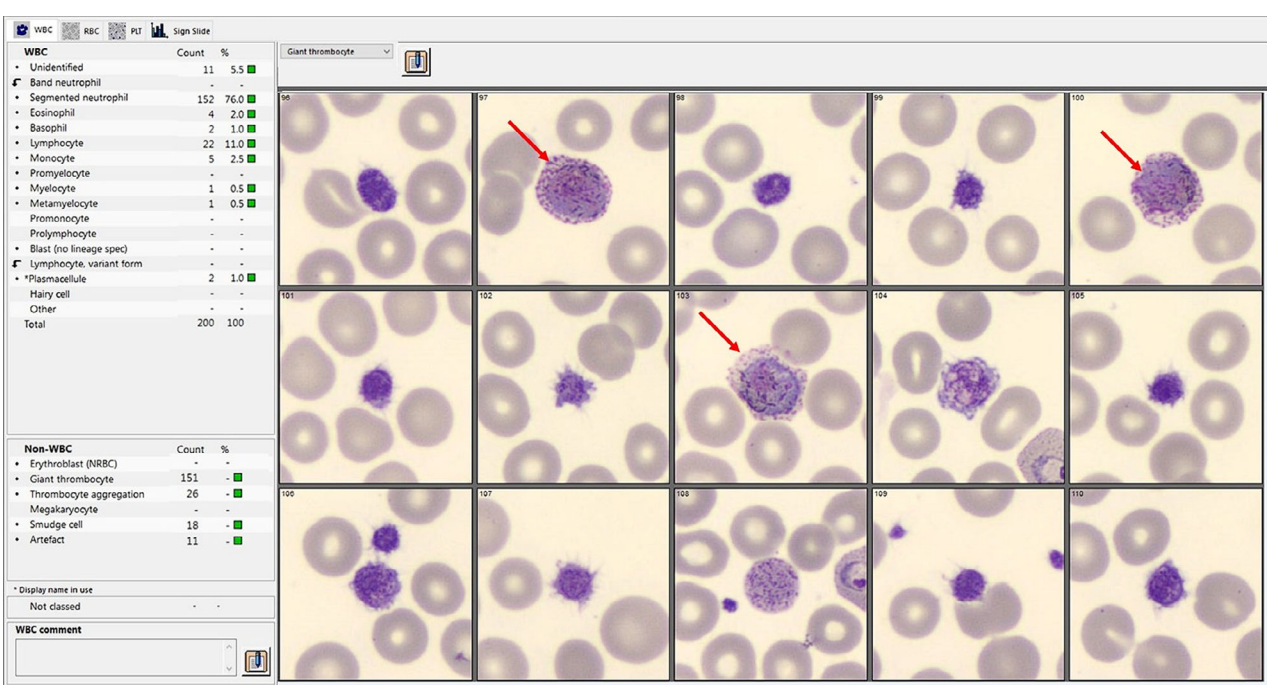

Fig 1. DI-60/Cellavision validation screen of the giant thrombocyte box of the patient. Red arrows show the schizonts or gametocytes. [Colour figure can be viewed at wileyonlinelibrary.com]

doi: 10.1111/bjh.17276

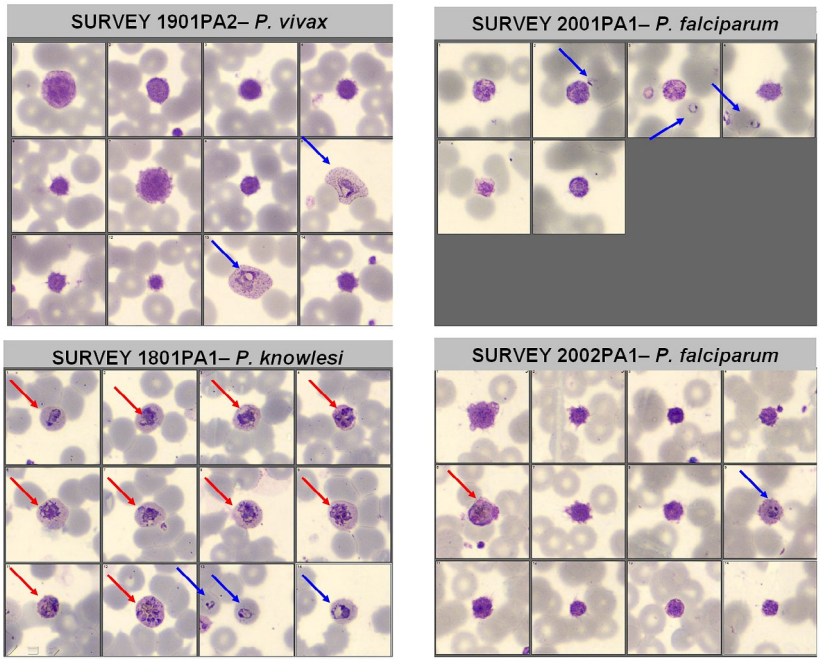

Fig 2. DI-60/Cellavision validation screen of the giant thrombocyte box of the four UKNEQAS surveys tested. Red arrows show the schizonts or gametocytes and blue arrows show the trophozoites. [Colour figure can be viewed at wileyonlinelibrary.com]

laboratory specialist using a personal computer linked to the laboratory information system. The full blood count showed a mild thrombocytopenia (platelets = 74 × 10^9/l) with WBC = 5·45 × 10^9/l and haemoglobin = 142 g/l, both within the reference interval. A blood film was observed with CellaVision to exclude firstly a pseudo-thrombocytopenia caused by PLT-clumps or clots. Next, all the leucocyte and non-leucocyte boxes were evaluated to investigate the cause of the thrombocytopenia and, as shown in Fig 1, many elements which could have been referenced as malaria parasites were found to be incorrectly placed in the giant platelet subtype box. The suspicion of malaria was communicated to the clinician who added the information that the patient had travelled to New Guinea six months before. A blood sample was therefore sent to the microbiology laboratory where *Plasmodium ovale* was identified with a microscopy examination and confirmed by real-time polymerase chain reaction (PCR) (Clonit Srl, Milan, Italy). We recently reported the validity of EQA UKNEQAS surveys to verify the performance of the DI-60 digital analyser.[6] Therefore, in order to confirm our observation we processed some EQA UKNEQAS malaria samples using DI-60/CellaVision: survey 1901PA2 positive for *Plasmodium vivax*, survey 1801PA1 positive for *Plasmodium knowlesi*, and surveys 2001PA1 and 2002PA1 positive for *Plasmodium falciparum*. Some parasitised red blood cells (RBCs) were also observed in the giant platelet box (Fig 2) in all three of those surveys. This data not only highlights the usefulness of the remote observation of haematological digital morphology, but also underlines the importance of carefully evaluating all the CellaVision boxes ('leucocytes' and 'non-leucocytes'), since useful information could be provided by misclassification. Other authors previously reported the effectiveness of digital morphology in assessing a *Plasmodium* infection with conflicting results,[7,8] but they focused on the capability of the software to correctly enumerate parasites in patients known to be infected or they evaluated the performance of a specialised CellaVision software (Advanced RBC Application, Lund, Sweden).[9] Despite the fact that malaria is easily preventable and treatable, it continues to have a devastating impact on people's health and livelihoods around the world, and a rapid and accurate diagnosis is critical for appropriate treatment.[10] To our knowledge, we here present for the first time an aspect of the CellaVision software that can help to detect unexpected malarial infections and speed up a diagnosis: the giant platelet section can include images of elements which can be attributed to malarial infection. Our group and others previously reported in the past that analytical errors can yield useful information.[11,12] Once again, 'fortune favours the prepared mind' another instrumental misclassification linked the correct diagnosis to a laboratorian working in a wide hub-and-spoke network in the middle of the night.

Acknowledgements

Marco Rosetti designed the study, performed the research, analysed the data, drafted the paper, approved the submitted version of the paper. Giorgia Farneti analysed the digital

patient's data, designed the study. Marta Monti analysed the data, drafted the paper. Arianna Torri contributed with microscopy and PCR analysis. Giovanni Poletti designed the study. Evita Massari analysed the data, contributed with digital EQA analysis. Valentina Polli contributed with digital EQA analysis. Alice Clementoni contributed with digital EQA analysis. Romolo M. Dorizzi drafted the paper and revised it critically, approved the submitted version of the paper.

Marco Rosetti[1]
Giorgia Farneti[1]
Marta Monti[1]
Arianna Torri[2]
Giovanni Poletti[1]
Evita Massari[1]
Valentina Polli[1]
Alice Clementoni[1]
Romolo M. Dorizzi[1]

[1]*Clinical Pathology Unit, Hub Laboratory, AUSL della Romagna, Cesena and* [2]*Microbiology Unit, Hub Laboratory, AUSL della Romagna, Cesena, Italy.*
E-mail: marco.rosetti@auslromagna.it

References

1. Dorizzi RM, Poletti G, Sensi A, Tonelli M, Giannini B. "Factory" laboratories: Blessed are those who have been persecuted for the sake of righteousness, for theirs is the kingdom of heaven. *Clin Biochem*. 2010;**43**(18):1481–3.
2. Satta G, Edmonstone J. Consolidation of pathology services in England: have savings been achieved? *BMC Health Serv Res*. 2018;**18**(1):862.
3. Kratz A, Bengtsson HI, Casey JE, Keefe JM, Beatrice GH, Grzybek DY, et al. Performance Evaluation of the CellaVision DM96 System: WBC differentials by automated digital image analysi supported by an artificial neural network. *Am J Clin Pathol*. 2005;**124**(5):770–81.
4. Kratz A, Lee SH, Zini G, Riedl JA, Hur M, Machin S. Digital morphology analyzers in hematology: ICSH review and recommendations. *Int J Lab Hematol*. 2019;**41**(4):437–47.
5. Pantanowitz L, Wiley CA, Demetris A, Lesniak A, Ahmed I, Cable W, et al. Experience with multimodality telepathology at the University of Pittsburgh Medical Center. *J Pathol Inform*. 2012;**3**:45.
6. Rosetti M, Massari E, Poletti G, Dorizzi RM. Could the UKNEQAS program "Manual Differential Blood Count" be performed by the use of an automated digital morphology analyzer (Sysmex DI-60)? A feasibility study. Clin Chem Lab Med. 2020 Jul 3: Online ahead of print.
7. Racsa LD, Gander RM, Southern PM, McElvania TeKippe E, Doern C, et al. Detection of intracellular parasites by use of the CellaVision DM96 analyzer during routine screening of peripheral blood smears. *J Clin Microbiol*. 2015;**53**(1):167–71.
8. Park M, Hur M, Kim H, Kim HN, Kim SW, Moon HW, et al. Detection of Plasmodium falciparum using automated digital cell morphology analyzer Sysmex DI-60. *Clin Chem Lab Med*. 2018;**56**(12):e284–e287.
9. Yoon J, Kwon JA, Yoon SY, Jang WS, Yang DJ, Nam J, et al. Diagnostic performance of CellaVision DM96 for Plasmodium vivax and Plasmodium falciparum screening in peripheral blood smears. *Acta Trop*. 2019;**193**:7–11.
10. World Health Organization. *World Malaria report 2019*. Geneva: World Health Organization; 2019. 185p.
11. Rosetti M, Poletti G, Ivaldi G, Rondoni M, Baldrati L, Dorizzi RM. Serendipitous detection of Hemoglobin G-Ferrara variant with Sysmex DIFF channel. *Clin Biochem*. 2016;**49**:192–3.
12. Moioli V, Seghezzi M, Previtali G, Baigorria MDC, Dominoni P, Michetti L, et al. Mozhaisk haemoglobin variant effects on leukocyte differential channel using the Sysmex XN series. *Clin Chem Lab Med*. 2019;**57**(2):e324–e327.