ARTICLE ORIGINAL

Can ADVIA 2120i replace blood smear?

ADVIA 2120i peut-il remplacer le frottis sanguin ?

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RÉSUMÉ

Introduction. L'ADVIA 2120i est l'un des analyseurs les plus efficaces dans les laboratoires d'hématologie. En plus de la quantification des cellules, il permet de détecter les anomalies cytologiques et d'alerter le biologiste par des alarmes spécifiques. **Matériel et méthodes**. Nous avons mené une étude prospective au niveau de l'EHU d'Oran durant 3 mois. Au total, 146 échantillons sanguins ont été analysés, dans lesquels l'analyseur avait rapporté plusieurs alarmes cytologiques qui ont été vérifiées par la suite en microscopie qui est le gold standard. **Résultats**. Cette étude avait permis de déterminer comment ADVIA 2120i pouvait aider en pratique quotidienne la détection des anomalies cytologiques. Selon nos résultats, l'analyseur est paru être efficace dans la détection des anomalies morphologiques erythrocytaires telles que la microcytose, la macrocytose, l'hypochromie et l'anisocytose. Cependant, concernant les leucocytes, nous avons trouvé un taux élevé de faux positifs, la spécificité pourrait être améliorée particulièrement dans la détection des blastes.

Mots clés : frottis sanguin, ADVIA 2120i, laboratoire d'hématologie.

ABSTRACT

Introduction. The ADVIA 2120i is one of the most efficient analyzer used in hematology laboratories. In addition to the quantification of cells; it enables the detection of cytological abnormalities and alert biologist by specific alarms. **Material and methods.** We conducted a prospective study in the EHU Oran for 3 month. Overall, 146 blood samples were analyzed for which the analyser reported various cytological alarms and we reviewed them under the microscope as a gold standard. **Results**. This study allowed us to determine how the ADVIA 2120i can be helpful for routine use in screening cytological abnormalities. According to our results it turned out that the analyser is efficient for erythrocyte morphological abnormalities such as microcytosis, macrocytosis, hypochromy and anisocytosis. However, concerning leukocytes; we found a high rate of false positive cases, the specificity should be improved especially for detection of blasts.

Keywords: blood smear, ADVIA 2120i, hematology laboratory.

هل يمكن استبدال مسحة الدم بالمحلل ADVIA 2120i

الملخص:
المقدمة: هو واحد من الأجهزة الأكثر كفاءة المستخدمة في مختبرات أمراض الدم، بالإضافة إلى تقدير حجم الخلايا. فإنه يمكنه الكشف عن التشوهات الخلوية وتنبيه البيولوجيين عن طريق أجهزة الإنذار المحددة. **المواد والطرق:** أجرينا دراسة استطلاعية في المؤسسة الاستشفائية الجامعية بوهران لمدة 3 أشهر. وقد تم تحليل إجمالي ل146 من عينات الدم التي أظهرت إنذارات خلوية مختلفة واستعرضناها تحت المجهر كمعيار ذهبي. **النتائج:** سمحت لنا هذه الدراسة بتحديد كيف يمكن أن يكون هذا الجهاز مفيدا للاستخدام الروتيني في فحص حالات الشذوذ الخلوي. وفقا لنتائجنا تبين أنه فعال لتحليل التشوهات الشكلية لكرات الدم الحمراء مثل صغر الكريات الحمر، كبر الكريات، نقص الانصباغ وتفاوت الكريات. ولكن فيما يتعلق بالكريات البيض. وجدنا نسبة عالية من الحالات الإيجابية، ينبغي تحسين خصوصية الكشف.

الكلمات الرئيسية: مخبر أمراض الدم ، مسحة الدم جهاز ADVIA 2120i.

INTRODUCTION

Blood numeration (BN) is one of the most demanded analyses in clinical laboratories. Disorders in results are in relationship with many diseases so BN is used as a first screening test to perform diagnosis. Generally, in many laboratories throughout the world, the BN is done with automated counter which measures 18 to 26 parameters using impedance system so an abnormal BN is systematically completed by a blood smear reading which takes a lot of time.

In the last decade, a new generation of haematology analysers was developed using flow cytometry which counts and analyses cells one by one through a laser beam. By combining side scatter and forward scatter reading, blood cells are widely analysed which allow identification of each cell population with high accuracy [1]. This suggests that these analysers can give more biological information and be more helpful for clinicians.

In practice, blood smear is done for all abnormal BN to orient diagnosis. But in many early states of diseases there is no significant changes in BN and the pathology is not detected forthwith. For this reason, the ADVIA 2120i can be helpful for the early detection of cytological abnormalities.

The aim of this study was to evaluate the ability

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of the ADVIA 2120i to detect cytological abnormalities when signalling alarms in order to replace blood smear control in routine.

MATERIALS AND METHODS

The ADVIA 2120i is a hematology analyzer with a flow cytometry principle. It uses 5 channels: haemoglobin, peroxydase, nuclear density (baso), red cells/platelet and reticulocyte. These channels permit the identification of all subpopulation of leucocytes, detection of immature granulocytes, blasts, band cells, an accurate numeration and description of red cells (size and haemoglobin contain) and platelet (large platelets and platelet clumps) [1-3]. An abnormality is detected if one or more parameters exceed a threshold fixed by manufacturer, then the ADVIA 2120i indicates a specific alarm to the operator.

This study was performed in the department of hemobiology of the 1st November hospital between December 2014 and April 2015. We selected 146 samples for which the ADVIA 2120i reported different alarms. Blood smear was used as gold standard to confirm or disclaim alarms.

Whole blood was collected on EDTA K3 anticoagulant and studied within 2 hours; blood smear reading was performed by 2 operators. Calibration and quality control of the device were done using OPTIpoint®, SET point® and TESTpoint® reagents as recommended by manufacturer. Alarm studied are summarized in table 1.

Table 1: biological significance of each alarm

Alarm	Significance
ATYP	Atypical or activated lymphocytes, blasts or abnormal cells
GI	Immature granulocytes
BLAST	Presence of blasts is suspected
LS	Presence of band cells (Left shift)
PLTCLM	Platelet clumps
LPLT	large platelets
NRBC	Nucleated red cells (erythroblasts)
MICRO	Red cell microcytosis
MACRO	Red cell macrocytosis
ANISO	Association of macrocytosis and microcytosis
HVCAR	Association of hypochromic and normal red cells
HYPO	Hypochromic red cells
RBCF	Presence of red cell fragments (schistocytes)

Statistical analysis

All alarms are quantified with three levels of significance (+, ++ and +++), we compared each level of alarm with blood smear as a gold standard and we established the rate of true and false positive cases. Statistical analyses were conducted using EpiData 3.1 software (Odense, Denmark), Khi square test was used to search relationship and concordance between blood smear abnormalities and alarm. We also calculated the positive predictive value (PPV) for each one. Statistical significance was defined as $P<0.05$

RESULTS

All results are summarized in table 2, some alarms were rarely or not observed so we were unable to study them.

Table 2. Positive predictive values of all levels of alarms and their statistical significance (+: level 1, ++: level 2, +++: level3, NO: not observed).

ALARM	PPV(P)		
	+	++	+++
HYPO	92.3 (0.002)	77.8(0.096)	88.9(<0.001)
MICRO	75(0.003)	100(<0.001)	100(<0.001)
MACRO	52.6(0.819)	100(<0.001)	93.3(<0.001)
ANISO	82.9(<0.001)	93.5(<0.001)	100(<0.001)
HCVAR	83.7(<0.001)	90.9(0.007)	100(<0.001)
NRBC	52.2(0.835)	NO	NO
RBCF	80(0.18)	NO	NO
BLAST	11.1(<0.001)	25(0.014)	50(1)
IG	100(<0.001)	100(<0.001)	88.2(0.002)
ATYP	82.4(0.008)	78.9(0.012)	93(<0.001)
LS	68(0.072)	60(0.527)	57.1(0.705)
LPLT	85.7(0.059)	71.4(0.257)	80.6(<0.001)
PLTCLM	34.9(0.017)	NO	NO

DISCUSSION

In this work, we tried to validate alarms of the ADVIA 2120i. Some of them were widely observed; those related to red cells disorders (HYPO, MICRO, ANISO and HCVAR). However despite we had studied 28000 BN in 4 month, red cells fragments and ghost cells were rarely signaled. This can be caused by a lack of sensitivity of the device so they aren't detected. Theoretically the PPV increases in the highest level of each alarm, however a decrease of PPV was observed in some of them and can be explained by the low incidence of some alarms in the highest level.

In this study, we have selected samples with alarms, so it allowed us to calculate the rate of true and false positive cases. However, we didn't study BS of samples for which any alarm was signaled, thus we couldn't establish the true and false negative rate. For this reason the sensitivity and the specificity of each alarm were not established. Some alarms were more precise than others where we found a lot of false positive cases, so we think that they can be useful just to keep attention of operator for the slightest suspicion of abnormalities. We respected the thresholds set by the manufacturer, fixing new thresholds by each laboratory to decrease the false positive rate can be helpful, but it needs large studies and is not recommended.

Alarms of red cells morphological abnormalities are all validated with a PPV up to 75%. This concord with the study of Nguyen who postulated that the technology used in the red cell channel can be helpful in the diagnosis of some hemoglobin disorders [4, 5].

For erythroblasts, our results are not conclusive because this alarm was rarely observed. In addition, some authors concluded that this alarm lacks sensitivity and specificity [6, 7], which may explain the low incidence of this alarm in our study. Of same for red cells fragments, just 5 cases were detected among which one was considered as a false positive. According to Lesesve *et al*. all automated methods have a sensitivity about 100% but they have low specificity, comparing with blood smear they found that automated numeration tends to overestimate red cells fragments [8, 9].

Among 28000 BN, no case of ghost red cells has been observed. Some authors described them as target cells using another device (DxH 800 Beckman Coulter) [10] but we couldn't check it. It seems that this alarm has no much interest in clinical practice.

In haematological malignancies, ADVIA 2120i showed a grand interest and some limits particularly in the detection of

blasts, there were many false positive cases and the PPV was lower then 50%; this can be caused by the low threshold fixed by manufacturer to attract attention of operators, anyway the diagnosis of acute leukaemia needs necessarily cytological tests. Other studies found a good sensitivity and specificity in leukemic samples with leucocytosis, but in case of leucopoenia this alarm appears in a random manner [6, 11]. Concerning alarm for detecting atypical lymphocytes, we found a good correlation with the presence of abnormal or activated lymphocytes, which concord with the results of Melet *et al.* and can also be explained by the presence of lymphoblasts [3]. However, in the first level of this alarm many authors found a high rate of false positive cases [6, 11]. In the same way, immature granulocytes were detected with high performance; the PPV was about 100% in the upper level of alarm. These results concord with the studies of Melet and Che [5, 11], in fact they described the ADVIA 2120i as the best counter in detection of immature granulocytes [6, 12].

The left shift alarm was not significant; the PPV was lower than 60% in the highest level of alarm. ADVIA 2120i was unable to detect band cells correctly. This can be accentuated by the operators' variations because the morphology of band cells is most often subjective [13, 14].

ADVIA 2120i shows good results in searching large platelets; however, some authors found that microcytic red cells can interfere with large platelets counting [15]. The utility of platelet clumps alarm was not confirmed. Many false positive cases were found. This alarm can be influenced by cells debris, large platelets and platelet satelitism [16].

Finally, we can conclude that ADVIA 2120i is very useful in exploration of anaemia and other red cells disorders and can be helpful in the screening of malignancies for which BS remains mandatory.

Competing interests: The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

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