Detection of blasts using flags and cell population data rules on Beckman Coulter DxH 900 hematology analyzer in patients with hematologic diseases

Abstract

Objectives: White blood cell (WBC)-related flags are essential for detecting abnormal cells including blasts in automated hematology analyzers (AHAs). Cell population data (CPD) may characterize each WBC population, and customized CPD rules can be also useful for detecting blasts. We evaluated the performance of WBC-related flags, customized CPD rules, and their combination for detecting blasts on the Beckman Coulter DxH 900 AHA (DxH 900, Beckman Coulter, Miami, Florida, USA).

Methods: In a total of 239 samples from patients with hematologic diseases, complete blood count on DxH 900 and manual slide review (MSR) were conducted. The sensitivity, specificity, and efficiency of the five WBC-related flags, nine customized CPD rules, and their combination were evaluated for detecting blasts, in comparison with MSR.

Results: Blasts were detected by MSR in 40 out of 239 (16.7 %) samples. The combination of flags and CPD rules showed the highest sensitivity compared with each of flags and CPD rules for detecting blasts (97.5 vs. 72.5 % vs. 92.5 %). Compared with any flag, the combination of flags and CPD rules significantly reduced false-negative samples from 11 to one for detecting blasts (27.5 vs. 2.5 %, p=0.002).

Conclusions: This is the first study that evaluated the performance of both flags and CPD rules on DxH 900. The customized CPD rules as well as the combination of flags and CPD rules outperformed WBC-related flags for detecting blasts on DxH 900. The customized CPD rules can play a complementary role for improving the capability of blast detection on DxH 900.

Keywords: automated hematology analyzer; DxH 900; blasts; flag; cell population data; rule

Introduction

Automated hematology analyzers (AHAs) generate flags, if there are various quantitative abnormalities or qualitative alterations during complete blood count (CBC). Accordingly, white blood cell (WBC)-related flags are essential features of AHAs that help detect abnormal cells including blasts [1–3]. In spite of reliable performances of state-of-the-art AHAs, manual slide review (MSR) is still mandatory in clinical hematology laboratories to confirm the presence of any abnormalities that are flagged by AHAs [4, 5]. Each laboratory has its own decision-making rules for MSR, and such rules can be highly affected by flagging performances of AHAs being used [1, 5–8]. Each AHA may have a different flagging performance depending on its own characteristics and technologies, even from the same manufacturer [9].

Cell population data (CPD) are the raw measurement signals produced by AHAs that reflect specific morphological and functional characteristics of each WBC population [10–12]. CPD are readily available and generated automatically during CBC and could be changed when new technologies or algorithms are introduced into AHAs [12, 13]. The newest Beckman Coulter DxH 900 (DxH 900, Beckman Coulter, Miami, Florida, USA) adopted improved technologies, compared with its previous version DxH 800 [8]. The DxH 900 provides five flags and 70 CPD that are related to
WBC, and users can construct or customize their own CPD rules using the CPD and other CBC parameters; in addition to the WBC-related flags, such customized CPD rules could also play a role as qualitative alarms that may trigger MSR [2, 8].

As a first-line gatekeeper for triggering MSR, the performance of WBC-related flags may affect blast detection significantly [1, 4, 5, 9, 14]. A few studies have evaluated the flagging performance of DxH 900 for detecting blasts, in comparison with DxH 800 or other AHAs [2, 8]. Regarding CPD, several studies have evaluated the CPD performances of DxH 800 in various clinical settings, including hematologic and non-hematologic diseases [15–19]. However, no study has evaluated the performance of DxH 900 for detecting blasts using CPD and/or customized CPD rules. In this study, we aimed to evaluate the performance of DxH 900 in terms of WBC-related flags, customized CPD rules, and their combination for detecting blasts. We hypothesized that customized CPD rules would have an added value to the WBC-related flags for detecting blasts, and their combination would be more useful to optimize the laboratory’s policy for MSR.

Materials and methods

Study samples

This retrospective evaluation study was conducted at Konkuk University Medical Center (KUMC), Seoul, Korea, from June to July 2021. The study protocol was designed following the criteria of the Declaration of Helsinki and approved by the Institutional Review Board of KUMC (KUMC 2019-11-021) before collecting the first sample. This study used anonymized clinical samples and required neither study-specific intervention nor additional sampling. Therefore, getting written informed consent from the enrolled patients was waived.

A total of 239 peripheral blood (PB) samples were collected from the patients who visited the hemato-oncology department of KUMC. The characteristics of the study population are described in Table 1. In our laboratory, we had the policy to conduct MSR for all samples that were requested from the hemato-oncology department. The PB samples (3 mL) were collected directly into VACUETTE K3-EDTA tubes (Greiner Bio-One GmbH, Kremsmünster, Austria) and were used for CBC within 4 h after collection. All the samples were well mixed immediately prior to be run on the DxH 900. No samples showed hemolysis, clotting, or inadequate volume. The PB slides were prepared and stained by Sysmex SP-50 (Sysmex Co., Kobe, Japan), and the MSR was conducted according to the Clinical Laboratory Standards Institute (CLSI) guidelines, H20-A2 [20]. Two hematology experts scanned the slides at low magnification using light microscopy and counted 200 cells each on each slide at 200× magnification; an additional slide was processed, if the counted cells on each slide were less than 200 cells. The average values of the results obtained by the two experts were used for the evaluation; both of them hadn’t big differences in their manual counting [20]. During the study period, the overall quality assurance or quality control procedures were followed.

The accuracy for the WBC differential parameters in DxH 900 was assessed by comparison with 400-cell manual differential and met the accuracy specification (within the bias ±10 %) [21].

DxH 900 and its flags and CPD rules

The DxH 900 is the Beckman Coulter’s smallest high-volume solution, and its capacity is 20 separate five-tube cassettes with a maximal automated throughput of 100 samples per hour [2, 8, 22]. The DxH 900 offers VCS 360 technology with the multiple transducer module and multiple angle of light scatter, the ‘Snake-eye’ analysis, the DataFusion algorithm, and enhanced Coulter Principle, which helps guide MSR using flagging [23]. The DxH 900 demonstrates five WBC-related flags as suspect messages for triggering MSR. For blasts (≥1 %), three suspect messages are displayed: ‘lymphocytic (LY) Blast’, ‘monocytic (MO) Blast’, and ‘neutrophilic (NE) Blast’. For myelocytes and/or promyelocytes (≥1 %), ‘immature granulocytes (Imm Grans) high sensitivity (HS)’ is displayed. For metamyelocytes (≥2 %), ‘left shift_low sensitivity (LS)’ is displayed. For atypical lymphocytes (≥5 %), ‘variant LY_HS’ is displayed. For NRBCs (≥1 %), ‘nucleated red blood cells (NRBC count)’ is displayed (Table 2).

Table 1: Characteristics of the study population.

<table>
<thead>
<tr>
<th>Age, year (IQR)</th>
<th>Total (n=239)</th>
<th>Abnormal cells (n=51)</th>
<th>Blasts (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>147 (61.5)</td>
<td>40 (78.4)</td>
<td>33 (82.5)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>90 (38.5)</td>
<td>11 (21.6)</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>Complete blood count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal, n (%)</td>
<td>36 (15.1)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Abnormal</td>
<td>203 (84.9)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anemia, n (%)</td>
<td>175 (73.2)</td>
<td>50 (98.0)</td>
<td>40 (100.0)</td>
</tr>
<tr>
<td>Polycythemia, n (%)</td>
<td>2 (0.8)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Leukopenia, n (%)</td>
<td>86 (36.0)</td>
<td>37 (72.5)</td>
<td>32 (80.0)</td>
</tr>
<tr>
<td>Leukocytosis, n (%)</td>
<td>19 (7.9)</td>
<td>7 (13.7)</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>Thrombocytopenia, n (%)</td>
<td>100 (41.8)</td>
<td>48 (94.1)</td>
<td>38 (95.0)</td>
</tr>
<tr>
<td>Thrombocytosis, n (%)</td>
<td>10 (4.2)</td>
<td>1 (2.0)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Disease categories</td>
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<tr>
<td>Benign cytopenia, n (%)</td>
<td>57 (23.8)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Benign cytosis, n (%)</td>
<td>14 (5.9)</td>
<td>1 (2.0)</td>
<td>–</td>
</tr>
<tr>
<td>Coagulopathy, n (%)</td>
<td>3 (1.3)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Myeloproliferative neoplasm, n (%)</td>
<td>19 (7.9)</td>
<td>2 (3.9)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Myelodysplastic syndrome, n (%)</td>
<td>20 (8.4)</td>
<td>5 (9.8)</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>Acute myeloid leukemia, n (%)</td>
<td>30 (12.6)</td>
<td>21 (41.2)</td>
<td>19 (47.5)</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia, n (%)</td>
<td>24 (10.0)</td>
<td>15 (29.4)</td>
<td>15 (37.5)</td>
</tr>
<tr>
<td>Lymphoma, n (%)</td>
<td>72 (30.1)</td>
<td>7 (13.7)</td>
<td>1 (2.5)</td>
</tr>
</tbody>
</table>

Data are presented as number (percentage) or median (IQR). Abnormal cells were defined as left-shifted neutrophils, blasts, atypical or abnormal lymphocytes, and nucleated red blood cells. IQR, interquartile range; n, number; y, years.
Table 2: Definition of flags as suspect message and CPD rules from Beckman Coulter DxH900.

<table>
<thead>
<tr>
<th>Description</th>
<th>Suspect message</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flagging</td>
<td></td>
</tr>
<tr>
<td>#1 Blasts ≥1 %</td>
<td>LY Blast, MO Blast, NE Blast</td>
</tr>
<tr>
<td>#2 Metamyelocytes ≥2 %</td>
<td>Left shift LS</td>
</tr>
<tr>
<td>#3 Myelocytes and/or promyelocytes ≥1 %</td>
<td>Imm grans HS</td>
</tr>
<tr>
<td>#4 Atypical lymphocytes ≥5 %</td>
<td>Variant LY HS</td>
</tr>
<tr>
<td>#5 NRBCs ≥1 %</td>
<td>NRBC count</td>
</tr>
<tr>
<td>CPD rules</td>
<td></td>
</tr>
<tr>
<td>#1 If MN-V-NE&gt;170 and PLT# &lt;80</td>
<td></td>
</tr>
<tr>
<td>#2 If MN-V-NE&gt;163 and PLT# &lt;80 and HGB&lt;105 g/L</td>
<td></td>
</tr>
<tr>
<td>#3 If MN-LMALS-NE&lt;122 and PLT# &lt;80</td>
<td></td>
</tr>
<tr>
<td>#4 If MN-MALS-NE&lt;127 and PLT# &lt;80</td>
<td></td>
</tr>
<tr>
<td>#5 If MN-AL2-NE&gt;140 and PLT# &lt;80 and HGB&lt;105 g/L</td>
<td></td>
</tr>
<tr>
<td>#6 If SD-V-LY&gt;21.5</td>
<td></td>
</tr>
<tr>
<td>#7 If SD-V-LY=19.5 and SD-V-LY ≤21.5</td>
<td></td>
</tr>
<tr>
<td>#8 If SD-V-LY=21 and LY% &gt;2.0</td>
<td></td>
</tr>
<tr>
<td>#9 If SD-V-LY=14.2 and LY% &gt;65 %</td>
<td></td>
</tr>
</tbody>
</table>

The unit of PLT# and LY# is 10^9/L, AL2, axial light loss; CPD, cell population data; HGB, hemoglobin; HS, high sensitivity; Imm grans, immature granulocytes; LMALS, lower median angle light scatter; LS, low sensitivity; LY, lymphocytes; MALS, median angle light scatter; MN, mean; MO, monocytes; NE, neutrophils; NRBC, nucleated red blood cell; PLT, platelet; SD, standard deviation; V, volume; #, number; %, proportion.

The DxH 900’s technology provides detailed cellular analysis for WBC differential with 70 CPD using 256 channels that are currently research-use-only parameters. The CPD consist of the mean and standard deviation of volume, conductivity, and multiple-angle light-scattering parameters. The light scattering parameters indicate morphological changes reflected by cellular complexity, granularity, cell size, and nuclear structure [8, 24]. On DxH 900, users can define customized CPD rules, including 70 CPD and CBC parameters, for abnormal cell detection without additional middleware [22, 23]. In this study, we constructed nine CPD rules on the basis of our own data, the International Consensus Group for Hematology (ICGH)-suggested criteria for MSR, and the manufacturer-provided resources for detecting abnormal cells and blasts [25]. For the rules, we used five CPD: four CPD of neutrophil volume and light scatter (MN-V-NE, MN-LMALS-NE, MN-MALS-NE, and MN-AL2-NE) and one CPD of lymphocyte volume (SD-V-LY). Platelet count and hemoglobin level were used as CBC parameters for neutrophils, and lymphocyte count and proportion for lymphocytes. Using these components, we constructed nine CPD rules (five neutrophil-related rules and four lymphocyte-related rules) (Table 2). With these rules, we focused on improving the detection capability of myeloblasts and lymphoblasts; considering the rarity of monoblasts, we did not use any monocyte parameters.

Statistical analysis

The distribution of data and the presence of outliers were checked using the Shapiro–Wilk test and the Reed method, respectively [26, 27]. All data exhibited non-parametric distributions, and no outlier was detected. Data were expressed as medians with interquartile ranges (IQR) for the continuous variables or as numbers with proportions for the categorical and binary variables. Our sample size was thought to have approximately 95 % power (1 – β) with 95 % confidence interval (CI) (n<0.05) [28].

MSR was considered as a gold standard, and samples were considered as containing blasts, if there were more than 1 % blasts. Abnormal cells were defined as left-shifted neutrophils, blasts, atypical or abnormal lymphocytes, and NRBCs. For detecting abnormal cells using flags and/or CPD rules on DxH 900, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), efficiency, and areas under the curve (AUC) in receiver operating characteristic (ROC) curves were calculated with their 95 % CIs, according to the CLSI guidelines (EP2-ED3 and EP24-A2) [28, 29]. AUC were interpreted as follows: 0.9≤AUC<1, excellent; 0.8≤AUC<0.9, good; 0.7≤AUC<0.8, fair; 0.6≤AUC<0.7, poor; 0.5≤AUC<0.6, fail [30]. The blast-containing samples were divided into quartiles according to the blast count, and a chi-squared test was used to compare the number of positive results of flagging, CPD rules, and the combination of flagging and CPD rules across quartiles. All statistical analyses were conducted using the MedCalc Statistical Software (version 20.210; MedCalc Software Ltd, Ostend, Belgium). Rounding rules were applied to summary statistics, and a two-tailed p-value<0.05 was considered statistically significant [31].

Results

Among the 239 samples, abnormal cells were detected in 51 (21.3 %) samples and blasts in 40 (16.7 %) samples by MSR; WBC-related flags and CPD rules were positive in 75 (31.4 %) samples and 64 (26.8 %) samples, respectively, by the DxH 900 (Table 3). Among the 51 samples containing abnormal cells, both flags and CPD rules were positive in 31 (60.8 %) samples. Two samples showed negative results of both flags and CPD rules, although one sample contained blasts (1.5 %), and the other sample contained NRBCs (1 NRBC/100 WBCs). Among the 40 samples containing blasts (25 samples containing myeloblasts and 15 samples containing lymphoblasts), both flags and CPD rules were positive in 27 (67.5 %) samples; however, one sample (blasts of 1.5 %) showed negative results of both flags and CPD rules.

Table 3: Distribution of flags and CPD rules in all samples.

<table>
<thead>
<tr>
<th>Total (n=239)</th>
<th>Abnormal cells (n=51)</th>
<th>Blasts (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPD rules (+)</td>
<td>CPD rules (−)</td>
</tr>
<tr>
<td>Flags (+)</td>
<td>44</td>
<td>31</td>
</tr>
<tr>
<td>(−)</td>
<td>20</td>
<td>144</td>
</tr>
</tbody>
</table>

Abnormal cells were defined as left-shifted neutrophils, blasts, atypical or abnormal lymphocytes, and nucleated red blood cells. For the definition of flags and CPD rules, see Table 2. CBC, complete blood count; CPD, cell population data; n, number.
Flags, CPD rules, and the combination of flags and CPD rules all showed high NPV (>90 %) in detecting abnormal cells and blasts (Table 4). Compared with flags, the combination of flags and CPD rules significantly reduced the number of false-negative samples in detecting abnormal cells and blasts (27.5 vs. 3.9 %, p-value=0.001; 27.5 vs. 2.5 %, p-value=0.002, respectively).

Figure 1 shows the distribution of blast counts, flags, and CPD rules in blast-containing samples (n=40), and the median blast count was 4.5 % (range, 1–35.9 %). The blast count in each quartile was: Q1<2 % (n=10); 2 %<Q2<4 % (n=10); 4 %<Q3<8 % (n=10); and Q4>8 % (n=10). As the blast counts increased, the number of positive results of CPD rules and the combination of flags and CPD rules showed a statistical significance; but the flags did not (data not shown).

In these blast-containing samples, the most frequently observed flag was ‘atypical lymphocytes ≥5 %’ that was observed in 19 samples (47.5 %), followed by ‘NRBCs (≥1 %)’ in 17 samples (42.5 %) and ‘Blasts ≥1 % in 12 samples (30 %). Regarding CPD rules, the most frequent one was CPD rule #3 (if MN-LMALS-NE<122 and PLT#<80) that was observed in 22 samples (55 %), followed by CPD rule #9 (if SD-V-LY>14.2 and LY%>65 %) in 19 samples (47.5 %) and CPD rule #4 (if MN-MALS-NE<127 and PLT#<80) in 17 samples (42.5 %). In the samples with lower blast counts (blasts ≤2 %, n=13), all five WBC-related flags were equally noted in twice, and the most frequently observed CPD rule was #9 (if SD-V-LY>14.2 and LY%>65 %) that was observed in six samples (46.1 %). Collectively, the flags detected six out of 13 samples, and the CPD rules detected 10 out of 13 samples (46.2 vs. 76.9 %, p-value=0.05).

Figure 2 compares the flow diagram between flags and the combination of flags and CPD rules. In samples with abnormal CBC results (regardless of WBC differential, n=203), 134 samples showed no flags; however, 14 out of these 134 samples showed abnormal cells (blasts, n=11; atypical lymphocytes, n=1; NRBCs, n=2). When the combination of flags and CPD rules was applied to the samples with abnormal CBC results, 115 samples did not show any flags and CPD rules. However, two out of these 115 samples contained abnormal cells; one contained blasts (1.5 %), and the other contained NRBCs (1 NRBC/100 WBCs).

**Discussion**

This is the first study that evaluated the combined performance of both flags and customized CPD rules on DxH 900 for detecting blasts. Using the samples from the patients with hematologic diseases, we also explored how we can optimize

<table>
<thead>
<tr>
<th>Presence of abnormal cells</th>
<th>Abnormal cells</th>
<th>Total</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Efficiency</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>37</td>
<td>38</td>
<td>75</td>
<td>72.5</td>
<td>79.8</td>
<td>49.3</td>
<td>91.5</td>
<td>78.2</td>
</tr>
<tr>
<td>(-)</td>
<td>14</td>
<td>150</td>
<td>164</td>
<td>(58.3–84.1)</td>
<td>(73.3–85.3)</td>
<td>(41.2–57.5)</td>
<td>(87.2–94.4)</td>
<td>(72.5–83.3)</td>
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<tr>
<td>CPD rules</td>
<td>(+)</td>
<td>43</td>
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<td>64</td>
<td>84.3</td>
<td>88.8</td>
<td>67.2</td>
<td>95.4</td>
</tr>
<tr>
<td>(-)</td>
<td>8</td>
<td>167</td>
<td>175</td>
<td>(71.4–93.0)</td>
<td>(83.4–93.0)</td>
<td>(57.4–75.7)</td>
<td>(91.7–97.5)</td>
<td>(83.0–91.7)</td>
</tr>
<tr>
<td>Flags+</td>
<td>(+)</td>
<td>49</td>
<td>46</td>
<td>95</td>
<td>96.1</td>
<td>75.5</td>
<td>51.6</td>
<td>98.6</td>
</tr>
<tr>
<td>CPD rules (-)</td>
<td>2</td>
<td>142</td>
<td>144</td>
<td>(86.5–99.5)</td>
<td>(68.7–81.5)</td>
<td>(45.2–57.9)</td>
<td>(94.8–99.6)</td>
<td>(74.3–84.8)</td>
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<table>
<thead>
<tr>
<th>Presence of blasts</th>
<th>Blasts</th>
<th>Total</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Efficiency</th>
<th>AUC</th>
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<tbody>
<tr>
<td>(+)</td>
<td>29</td>
<td>46</td>
<td>75</td>
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<td>93.3</td>
<td>76.2</td>
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<tr>
<td>(-)</td>
<td>11</td>
<td>153</td>
<td>164</td>
<td>(56.1–85.4)</td>
<td>(70.4–82.6)</td>
<td>(31.5–46.4)</td>
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<td>92.5</td>
<td>86.4</td>
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<tr>
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<td>175</td>
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<td>(80.9–90.9)</td>
<td>(48.8–66.3)</td>
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<td>143</td>
<td>144</td>
<td>(86.8–99.9)</td>
<td>(65.1–78.0)</td>
<td>(35.7–46.6)</td>
<td>(95.4–99.9)</td>
<td>(70.2–81.4)</td>
</tr>
</tbody>
</table>

Sensitivity, specificity, PPV, NPV, efficiency, and AUC are presented as percentages (95 % CI) or area under the curves (95 % CI). Abnormal cells were defined as left-shifted neutrophils, blasts, atypical or abnormal lymphocytes, and nucleated red blood cells. For the definition of flags and CPD rules, see Table 2. AUC, area under the curve; CI, confidence interval; CPD, cell population data; NPV, negative predictive value; PPV, positive predictive value.
Figure 1: Distribution of blast counts, flags, and CPD rules in blast-containing samples (n=40). The box indicates the relationship between blast quartiles and the number of positive results of flags, CPD rules, and the combination of flags and CPD rules using the chi-squared test. The lower quartile is equivalent to 2% of blasts. Blue boxes indicate samples with myeloblasts (n=25), and pink boxes indicate samples with lymphoblasts (n=15). CPD, cell population data.
Figure 2: Comparison of flow diagram between (A) flags and (B) flags and CPD rules. Abnormal cells were defined as left-shifted neutrophils including blasts, atypical lymphocytes, and nucleated red blood cells. CBC, complete blood count; CPD, cell population data; n, number.
the MSR-related laboratory workflow with combined flags and customized CPD rules. Our data showed that the MSR rate could be reduced without losing pathologic samples containing blasts or other abnormal cells, and such a finding could serve as a momentum to modify the current policy to conduct MSR for all samples from the department of hemato-oncology.

In the present study, the flags on the DxH 900 had a low PPV for detecting abnormal cells and blasts. On the contrary, flags had a high NPV, and no abnormal cells were found in 91.5 % of the samples without any flags (Table 4). Our data showed that the customized CPD rules and the combination of flags and CPD rules outperformed flags for detecting abnormal cells and blasts, in terms of sensitivity, specificity, efficiency, and AUC with a low false-negative rate. Flags alone could not alarm the presence of blasts in 11 samples, while the combination of flags and CPD rules could not alarm it only in one sample. These results suggest that the necessity of another complementary safeguard that can be added to the flagging on DxH 900. The CPD rules could compensate for the lack of flagging performance for detecting abnormal cells and blasts, especially when these cells are present in a small proportion.

In addition to the role of qualitative alarms for triggering MSR, we also explored the quantitative aspects of flags and CPD rules. In our data, the number of positive results of CPD rules and the combination of flags and CPD rules correlated with the blast counts (Figure 1). Of note, flags were not positive in the two samples with 7.9 and 11.5 % of blasts (both samples were from the patients with B-lymphoblastic leukemia); on the contrary, the customized CPD rules could detect all samples with 3 % or more of blasts. Some AHAs are equipped with their own tools that can detect abnormal findings quantitatively, such as the Q value or the I-MESSAGE [9, 32]. However, the DxH 900 does not provide such quantitative tools that may indicate the probable existence of abnormal findings; therefore, it can be assumed that the number of positive results of CPD rules could be alternatively used to compensate for its lack of quantitative information for abnormal findings. Of note, our data also showed that the nine customized CPD rules were all useful but not equally effective; the two CPD rules (#3 and #9) were thought to be more effective than the others for detecting blasts. Moreover, the rules composed of neutrophil-related CPD detected both myeloblasts and lymphoblasts, and vice versa. Considering that we used just five CPD among the 70 WBC-related CPD for customizing nice CPD rules, future researches are awaited to explore optimal combinations and algorithms using CPD rules.

Clinical hematology laboratories are faced to analyze a huge volume of samples quickly while maintaining high accuracy and minimizing false-negative results; accordingly, workflow optimization with increased efficiency is one of the key indicators in each laboratory [3, 6]. The ICGH suggested the criteria for MSR following automated CBC and WBC differential [25]; however, the proportion of samples requiring MSR depends on various factors in each laboratory or hospital, such as organization, technicians’ skills, and the patient population. Therefore, each laboratory should establish its own criteria for MSR and systematically verify the efficiency of the current criteria [1, 6–8, 33–35]. According to our own laboratory policy, MSR should have been conducted for all 239 samples from the hemato-oncology department included in this study, even if they met the ICGH rules. However, all samples with normal CBC results (n=36) showed no abnormal findings by MSR (Figure 2). In samples with abnormal CBC results (n=203), the number of false-negative samples decreased from 14 to two in terms of detecting abnormal cells when we applied the combination of flags and CPD rules. The present findings imply that the CPD rules can be customized in each laboratory reflecting its unique situation and need and can be used to modify the MSR criteria. To broaden such a beneficial application of CPD rules in addition to the flagging system, not just hematological or oncological samples but a larger number of samples including all kinds of patients should be explored in future researches, and department- or sample-specific application algorithms would be also necessary.

This study has several limitations. First, this was a single-center study and the results cannot be extrapolated directly to other laboratories. Considering the specific situation and need in each laboratory, some adjustment and modification would be necessary. Second, this study was performed using adults’ PB samples with a cut-off of 1 % for blasts. Because the flagging performance may be different depending on the sample type or cut-off for blasts, further evaluations would be required with various sample types and cut-offs [2, 9]. Third, this study did not explore the cost-effectiveness according to the different MSR strategies, which could be another essential consideration for laboratories with limited resources. Hematology laboratories need to establish their own criteria for MSR and consider implementing the proposed guidelines [1, 25, 33–35]. Additionally, laboratories should review their flagging criteria periodically to optimize the balance between detecting abnormal cells and minimizing the need for MSR. Last, we did not evaluate the effect of preanalytical storage condition on flags and CPD values and the precision of flags and CPD values [36]. Despite the fact that this missing information is relevant in all the studies with clinical samples, it can be crucial in those cases with a large burden of samples and a limited number of AHAs. A recent review noted that CPD values are...
affected according to the preanalytical storage condition [13]; the absence of harmonization or standardization of CPD values in the same AHAs as well as different AHAs should also be considered. Two recent studies evaluated the usefulness of CPD for screening myelodysplastic syndrome and showed different results in spite of using the same model of AHA [37, 38]. Therefore, further researches would be needed on changes in CPD values according to the preanalytical storage condition as well as on harmonization and/or standardization across different AHAs.

In conclusion, this is the first study that evaluated the performance of flags and customized CPD rules on DxH 900 for detecting abnormal cells and blasts. The customized CPD rules and the combination of flags and CPD rules outperformed flags alone for detecting abnormal cells and blast in the samples from the patients with hematologic diseases. The customized CPD rules can play a complementary role to WBC-related flags, and the combination of flags and CPD rules would be an improved safeguard for detecting pathologic samples. Each laboratory may optimize its own MSR criteria using customized CPD rules on DxH 900.

Research ethics: This study was conducted according to the Declaration of Helsinki, and the study protocol was approved by the Institution Review Board of KUMC (KUMC 2019-11-021). Informed consent: Written informed consent was waived for the use of blood samples that were routinely requested during clinical practice. Author contributions: Kim H collected the samples, analyzed the data, and wrote the draft; Hur M designed the study, analyzed the data, and finalized the draft; Yi JH, Lee GH, and Lee S participated in data collection and analysis; Moon HW and Yun YM participated in data analysis and reviewed the draft. All authors have accepted responsibility for the entire content of this manuscript and approved its submission. Competing interests: The authors state no conflict of interest. Research funding: This study was supported by the grant from Beckman Coulter, Inc. in 2019. The Beckman Coulter’s support did not affect the results and conclusions of this study. Data availability: The raw data can be obtained on request from the corresponding author.

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