Determination of Acute Leukemia Lineage with New Morphologic Parameters Available in the Complete Blood Cell Count

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Abstract. Goals: Cell population data (CPD) are new morphologic parameters including volume, conductivity, and five light scattering characteristics used for leukocyte classification by an automated hematology analyzer, the UniCel DxH 800. We developed a discriminating CPD model to predict the leukemia lineage during routine complete blood cell count (CBC). Procedures: We analyzed the CPD of 405 blood samples containing more than 10% blasts that were randomly divided into test and validation sets. With the test set, we produced a model for categorizing acute lymphoblastic leukemia (ALL) or acute promyelocytic leukemia (APL), using ranges of the CPD and regarding the remainder as non-APL acute myeloid leukemia. We verified these models against the validation set. Results: In the test set, we formulated a 21-parameter model which identified 43 of 47 ALL cases (91.5% sensitivity) and ruled out 151 of 156 other leukemia cases (96.8% specificity), and a 13-parameter model which distinguished all 10 APL cases (100% sensitivity) and excluded 193 other leukemia cases (100% specificity). In the validation set, the ALL model showed 85.1% sensitivity and 94.2% specificity, and the APL model 100% sensitivity and 100% specificity. Conclusions: This study demonstrated a new solution for predicting blast lineage using the CPD on a CBC and leukocyte differential.

Key words: Acute leukemia, ALL, AML, and Leukocytes morphology.

Introduction

Acute leukemia can occur in any age group, with a predominance of lymphoblastic leukemia in children [1], while myeloid malignancies are more common in adults [2,3]. Over the past decades, the prognosis for patients suffering from all types of acute leukemia has improved significantly as standardized treatment protocols have been developed which concurrently allow for higher remission rates, minimize acute toxicities, and carry lower risks of late-occurring complications. This success is due in great part to a better understanding of the pathophysiology of acute leukemia, and to newer diagnostic techniques allowing for more precise and reproducible sub-classification of individual disease [4-7].

The sub-classification of acute leukemia is very complex and takes into account the information obtained from various laboratory techniques such as morphologic examination of the leukemic blasts, review of bone marrow biopsy specimens, immunophenotyping by flow cytometry [8-10], and identification of specific cytogenetic and molecular abnormalities [11,12]. Employing diagnostic arsenals, hematopathologists and clinicians are able to predict disease prognosis for each patient, assess the likelihood of relapses, and offer more targeted therapy.
Despite the current complexity of the complete classification scheme, clinicians need to know the lineage the leukemia belongs to in order to administer appropriate treatment. Acute leukemia is categorized as either acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML). In the case of AML, further identification of acute promyelocytic leukemia (APL), one of AML’s subtypes, is very important, since the therapeutic regimen is different from other types of AML and its associated disseminated intravascular coagulation necessitates therapy not to be delayed [13-16]. Though the diagnosis of AML can be ascertained when Auer rods are found, it can be difficult to morphologically confirm APL because in some APL cases, the morphology of leukemic cells mimic leukemic monocytes, and even agranular or microgranular variants are present. Because a subset of blasts in B-ALL can have morphologic similarities to myeloid blasts, the differentiation of ALL from AML is not easy either. Therefore, initial assessment must often rely on morphology and flow cytometry results of the peripheral blood or on bone marrow aspirate material before the outcomes of cytogenetic and molecular tests are available.

Flow cytometry, however, is not feasible in all hospitals and laboratories as it requires modern instrumentation and specialized technologists and pathologists. Even in large academic institutions, the flow cytometry service typically operates during regular work hours, which can pose a problem on weekends. The limitation of flow cytometry is more pronounced in developing nations. The choice of therapy will be mainly based on the morphologic diagnosis by a hematopathologist. For these reasons, it is possible that induction therapy for a patient in a critical situation may be delayed.

It has already been documented that each sub-type of acute leukemia shows morphologic features of blasts [11]. The presence of Auer rods is diagnostic for non-lymphoid leukemia, while the presence of abundant cytoplasm makes classification difficult. Whereas morphologic features were the standard of care for many decades before the advent of flow cytometry, we now know that this approach is not as accurate and reproducible as once thought. Some institutions do not have staff hematopathologists, especially on weekends, and as a result, the morphologic diagnosis often falls to inexperienced personnel.

In the midst of complex tests, part of the routine work-up for a newly-diagnosed patient is the complete blood count with differential (CBC-diff) by automated hematology analyzers with different technological solutions for recognizing the various white blood cell (WBC) types. This study utilized the DxH 800 Hematology Analyzer (Beckman Coulter Inc, Brea, CA), which is able to directly recognize the morphologic features indicative of the sub-types of WBCs and collect cell population data (CPD), new morphologic parameters. The DxH 800 incorporates advanced hardware technology, innovative computer algorithms, impedance technology, flow cell volume, conductivity, and five light scatter measurements to perform CBC. The light scatter measurements are median angle light scatter (MALS), lower median angle light scatter (LMALS), and upper median angle light scatter (UMALS), all which are informative for granularity and membrane surface, as well as the axial light loss (AL2) measurement, which analyzes cellular transparency, and the low angle light scatter (LALS), a
These seven cell population data (CPD) are displayed as mean (MN) values within 254 channel detectors and standard deviation (SD) values, which mean degree of in vivo variation of cells on that parameter [17]. Therefore, CPD from each cellular event by the DxH 800 compiled 14 parameters consisting of the MN and SD value of 7 characteristics: volume, conductivity, and 5 scatters. Previous studies have shown how the CPD can be used to analyze cellular morphology in a quantitative, objective, and automated manner, free from the subjectivity of interpretation, which is time-consuming, expensive and not consistently reproducible [17-26].

Blasts may be counted in one or several of the WBC sub-populations, affecting the CPD of that population. If only a few blasts are present in a leukocyte population, it would not make sufficient change in the CPD of that cell population. The objective of this study is to develop a multi-parametric discriminating model based on CPD that can reliably predict the lineage in new cases of acute leukemia that have an adequate number of blasts. Therefore, we included only cases showing more than 10% blasts in the blood.

### Materials and Methods

**Study population and group assignment.** In this study, we included newly diagnosed cases of acute leukemia that presented to the Seoul St. Mary’s Hospital, Seoul, Korea, between July 2009 and August 2012. A total of 405 cases in this study received a complete routine diagnostic work-up. For inclusion, a minimum requirement in acute leukemia was a blast percentage of at least 10% in the peripheral blood, since smaller percentages of blasts would not be sufficient to impact the CPD. The leukemia sub-type was identified based on laboratory tests including CBC-diff, microscopic review of the peripheral blood and bone marrow aspirate, bone marrow biopsy, flow cytometry, and cytogenetic and molecular results. This study was approved by the Institutional Review Board.

Based on the final hematopathology report, all cases were assigned to one of the three major treatment groups that require different induction regimens (ALL, AML and APL). The AML group included four cases diagnosed as mixed phenotype acute leukemia (MPAL); these cases received an identical induction regimen.

Once cases were assigned to their respective diagnostic groups, the data were randomly split into 2 similarly sized datasets by alternative grouping using serial
The final breakup of patients was as such:
- In the test set: 47 ALLs, 146 AMLs, 10 APLs
- In the validation set: 47 ALLs, 145 AMLs, 10 APLs.

Data collection and analysis for the development of multiparametric discriminating models. We calculated the ratio of CPD to the mean of normal samples analyzed in the same day to compare data between analysts. To avoid artificial variation due to a very small number of cells analyzed, we deleted the SD parameters derived from leukocyte subpopulations composed of less than 100 cells. Since the DxH 800 examines 8,192 leukocytes per sample, we erased the SD parameters of cell subpopulations reported as less than 1.3% in the differential count. We also removed both MN and SD parameters in cases of 0% cells in a subpopulation.

We labelled the CPD of neutrophils (NE), lymphocytes (LY), monocytes (MO), and eosinophils (EO) using uppercase letters. Each parameter changed in a different way in specific disease. Therefore, we measured the ratio of MN value of a parameter to that of another parameter. That ratio was defined as calculated parameter. We labelled the calculated parameters of neutrophils (ne), lymphocytes (ly), monocytes (mo), eosinophils (eo) and non-nucleated red blood cell (nnr) using lowercase letters. We calculated the MN value of light scattering per cell volume estimated by absorbed light (AL2) and electric high voltage (V), e.g. mals/al2, lals/al2, umals/v, lmals/v and lals/v. The amount of light scattering at a specific angle to that detected at median angle was calculated as umals/mals, lmals/mals and lals/mals. We counted the conductivity per cell volume (c/al2 and c/v).

Figure 1 displays clustering of ALL and APL cases using ascending data of all parameters. Using clustering plots of the test set, we selected several CPD parameter ranges for predicting ALL by diagnosis of differentiation. We chose cut-off ranges to exclude other types of acute leukemia and include the maximum number of ALL cases using the KYL program, an Excel macro program. We repeated the process to generate another model for identifying APL. Once these models were developed, we calculated the sensitivity and specificity for identifying the corresponding leukemia sub-type. Finally, cases that would neither fit the category of ALL nor APL were classified as either AML (vast majority of cases) or MPAL. In order to verify the developed models, we used the validation set and calculated the sensitivity and specificity, therefore reflecting its expected performance in a clinical situation.

Results

Using the test set, we found that ALL cases showed clustering based on CPD (Figure 1) and were able to identify 20 morphologic parameter ratios plus monocyte count that could be incorporated into a multi-parametric discriminating model for detecting cases of ALL among acute leukemia. We applied these parameters along with the cut-off points in the characterization of ALL (Table 1). In this test set, the 21-parameter model identified 43 out of 47 ALL cases (91.5% sensitivity) and ruled out 151 of 156 cases of other acute leukemia (96.8% specificity).

Also using the test set, we were able to identify 13 parameter ratios that could be incorporated into a multi-parametric discriminating model for detecting cases of APL among acute leukemia. Table 2 shows the list of parameter ratios and cut-off points utilized. In the test set, this 13-parameter model
distinguished all 10 cases of APL, including a case of hypogranular variant APL, (Figure 2, Table 3) and excluded all 192 cases of other acute leukemia (100% sensitivity and specificity).

The performance of these models in the validation set was as follows: the ALL model identified 40 out of 47 ALL cases (85.1% sensitivity) and ruled out 146 out of 155 cases of other acute leukemia (94.2% specificity); the APL model distinguished all 10 cases of APL (100% sensitivity) and excluded all 192 cases of other acute leukemia (100% specificity).

Discussion

For many years, microscopic review of all blood samples was routine practice, and thus allowed the medical community to gather a vast body of knowledge on how cells change in various disease states. Laboratories have faced increasing workloads and economic pressures over the past decades. Along with the advent of automated cell counters capable of reporting a CBC-diff, the diagnostic use of morphologic information has steadily decreased. Today only a minority of blood samples actually come under the microscope [27,28].

The same is true when it comes to the differential diagnosis of the various sub-types of acute leukemia. Historically, the sub-classification of blasts into lymphoid or myeloid lineage was based predominantly on morphology, not to mention the identification of promyelocytic leukemia. The serious limitations cannot be overstated for this standard of care. Morphologic analysis is subjective and heavily dependent on the personal experience of the reviewer, and the identification of features in either lymphoid or myeloid lineage is poorly reproducible. In addition, the number of blasts analyzed is limited to a few hundred cells. From a practical perspective, this is a very time-consuming and expensive approach. As the standard method for the sub-classification of acute leukemia, morphology was largely replaced initially by cytochemistry and later by flow cytometry immunophenotyping. As previously discussed, these new technologies may not be readily available, causing the initiation of therapy to be delayed for several days.

The findings of our study show promise for these situations, since we were able to predict the lineage of unknown cases of acute leukemia accurately (85.1 - 100% sensitivity), using data of a routine CBC-diff by the hematology analyzer only. Of

Figure 2. A case of hypogranular acute promyelocytic leukemia showing a typical agranular cytoplasm with deeply indented nuclei (left) and PML-RARA fusion (right) using Vysis LSI PML/RARA dual color translocation probe (orange, 15q22-24 PML; green, 17q21.1 RARA).
particular importance was the performance of the APL discriminating model, which was able to classify all cases of APL in both the test and validation sets.

Most APL cells have been reported to be deposited in the neutrophil population [29]. Therefore, the neutrophil population of APL samples would be a mixture of APL cells and neutrophils, causing higher MN-V-NE and SD-V-NE. APL is a hematological emergency, and any delays in treatment can have devastating consequences due to the severe coagulopathy associated with the accumulation of abnormal promyelocytes. The coagulopathy is curable with the use of all-trans retinoic acid in the vast majority of cases [13-15]. Sometimes, treatments of APL are started solely based on morphologic analysis, and in many situations, induction is delayed until genetic results are available, thus placing the patient at serious risk. It might be reassuring to the medical practitioner that the use of CPD models allows a blast morphologic analysis to identify APL cases with 100% sensitivity and specificity, especially hypogranular variant of APL.

While the discrimination between ALL and AML is less time-sensitive when compared to the discernment of APL, our findings can still bring value to the diagnostic process for these cases. According to our results, the assortment of ALL and AML using CPD models can be performed with better sensitivity and specificity than human review [22,26].

Because small quantities of abnormal cells will not sufficiently impact the MN and SD measurements of the normal WBC subpopulations, a limitation for this CPD diagnostic approach is that it is only effective for a minimal percentage of circulating blasts. With more than 10% blasts in the blood, we executed this study for lineage determination, not for its detection sensitivity. The initial blood samples of acute leukemia cases, especially APL, could have less than 10% blasts, in which we do not recommend the usage of leukocyte CPD changes for subtype discrimination.

Our study showed how WBC morphology could still play a crucial role in the diagnosis of hematological disease. While it is not feasible to review every CBC-diff microscopically, we are now learning to use modern technologies available in hematology analyzers to evaluate these morphologic features in an automated, quantitative, fast, and inexpensive manner. Instead of analyzing 100 or 200 cells on a microscopic review, the DxH 800 examines 8,192 leukocytes in a matter of seconds, evaluating the morphologic features of cellular volume, cytoplasmic granularity, nuclear complexity, and internal density quantitatively via a point system, CPD. The advantages of a quantitative analysis are not only that the interpretation of the results is straightforward and can be automated via a set of numerical decision rules, but also that screening strategies can be implemented, whereby samples are flagged if they fall outside a set of CPD criteria chosen by the

### Table 3. The value of parameters in the multiparametric discriminating model for acute promyelocytic leukemia (APL) in a case of microgranular variant of APL.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN-MALS-NE</td>
<td>0.8</td>
<td>mo-lmals/v</td>
<td>0.4202</td>
</tr>
<tr>
<td>SD-AL2-NE</td>
<td>1.9295</td>
<td>eo-umals/mals</td>
<td>1.0241</td>
</tr>
<tr>
<td>MN-V-LY</td>
<td>1.1639</td>
<td>eo-lmals/mals</td>
<td>0.9578</td>
</tr>
<tr>
<td>ne-mals/al2</td>
<td>0.9492</td>
<td>nnr-umals/v</td>
<td>1.4783</td>
</tr>
<tr>
<td>ne-lals/v</td>
<td>0.6859</td>
<td>nnr-lmals/v</td>
<td>1.5797</td>
</tr>
<tr>
<td>ly-lals/al2</td>
<td>0.6140</td>
<td>nnr-lals/v</td>
<td>0.7971</td>
</tr>
<tr>
<td>ly-c/v</td>
<td>1.2551</td>
<td></td>
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</tbody>
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Abbreviations are as in Table 2.
pathologist. With the approach for early sub-classification of acute leukemia cases, our study displayed a potential clinical application using these morphologic parameters. The flow cytometry exhibited the value of assessing cellular morphology using the scattering histograms, especially in myelodysplasia [30,31]. Previous studies have demonstrated how decreased light scatter can be identified by the CPD at the time of a CBC-diff on a myelodysplastic sample [21,22].

Another feature of our study is the use of multiparametric discriminating models for disease classification. To the best of our knowledge, this is the first study that used data generated by a routine CBC-diff for the development of such models. Now that new technologies in hematological analyzers are capable of providing vast amounts of morphological data during a simple CBC-diff, analyzed data using software may shed new light on their value and power. Our study evidenced this potential, as we were able to detect patterns of morphological change by combining information from up to 21 different parameters. Using ratios of parameters, instead of their raw values, we automatically introduced internal controls into our data set similarly to flow cytometry, which was compared to population histograms. Recognizing these “morphological signatures” without an additional charge every time a routine CBC-diff is performed, this study potentially impacts further research from both economic and clinical perspectives.

In summary, we have described a new solution for evaluating blast morphology and predicting their lineage using information obtained at a regular CBC-diff. This finding may be of critical importance in allowing for faster and more accurate diagnosis and treatment of newly-diagnosed acute leukemia patients, especially in situations where modern tests such as flow cytometry are not available. We recommend that of further multi-centric studies be performed to evaluate the reproducibility of these models across institutions with different DxH800 sets and patient populations, to test clinical applicability.

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HK designed the research and analyzed data. YJH wrote the paper. KY, LJ, KM and OEJ performed laboratory work. LHK, PVJ, MWS and CB provided patient samples. LK provided analytical tools.

References


