The Correlation Dimension Identifies B-Cell Immunoglobulin Light Chain Restriction in Peripheral Blood Flow Cytometry Data

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Abstract

The correlation dimension (D2) is a mathematical tool that quantifies dimensional properties of fractals. We set out to determine whether immunoglobulin light chain restriction (LCR) in B-cell lymphoproliferative disorders (BCLPDs) could be identified using the D2.

The D2 was calculated from flow cytometry data files (FCS 2.0) from a training set of peripheral blood specimens from 22 patients with and 23 patients without BCLPD. A cutoff value derived from the training set was applied to a validation set of 69 patients with and 64 patients without BCLPD.

In the training set, all BCLPDs had a D2 of less than 0.72 (CD19-gated \( \kappa/\lambda \) data). When this cutoff was applied to the validation set, the D2 had sensitivity and specificity values of 88% and 98%, respectively.

The D2 can identify LCR in peripheral blood flow cytometry data. It offers operator-independent data analysis and may be useful in objectively quantifying differences in data distribution.

In B-cell lymphoproliferative disorders (BCLPDs), immunophenotypic analysis of the peripheral blood using flow cytometry shows changes in cell population distribution and complexity. An altered \( \kappa/\lambda \) immunoglobulin light chain ratio (KLR) is a commonly used indicator of monoclonality.\(^1\) This measure requires operator-set boundaries defining positive and negative cell populations, which may be subjective, especially if antigen expression is a continuous spectrum of negative to positive.\(^2\) We sought to determine whether the correlation dimension (D2), a mathematical tool developed to quantify the complexity of fractals, could be used as an operator-independent means to identify B-lymphocyte immunoglobulin light chain restriction in peripheral blood flow cytometric data.

Many natural phenomena exhibit the fractal characteristics of self-similarity and scalability.\(^3\) Fractals are mathematical objects that look roughly the same at any magnification (scale). The branching pattern of a fractal tree, for example, would look similar whether one observed the tree as a whole or microscopically examined the xylem and phloem in the leaves. While real-world objects lack the same degree of self-similarity over a wide scale that mathematical fractal constructs do, real-world objects may exhibit fractal properties using a limited perspective.\(^4\)

The fractal dimension (FD) is a characteristic of a fractal that corresponds to its complexity. More complex fractals have a higher FD than do less complex fractals.\(^1\) The FD represents the relationship of a fractal’s geometry, such as perimeter or area, to the scale in which the geometry is measured. Many mathematical formulas have been developed to measure the FD, one of which is the D2.

Grassberger and Procaccia\(^4,5\) designed the D2 to measure the dimension of a type of fractal known as a strange...
attractor. Strange attractors are seen in data consisting of large sets of points, and this technique is easily applied to flow cytometric data that also may be represented by many points. The D2 is derived from all points within a set of data and outputs a single number representing the data’s overall fractal complexity.

Some physiologic phenomena exhibit fractal properties; therefore, analysis of the D2 may prove useful in medical science.6,7 The FD has been used to quantitate lung abnormalities on chest radiographs,8 identify abnormalities in heart rate variability associated with increased mortality,9,10 and analyze electroencephalograms.11 To our knowledge, no studies have attempted to analyze flow cytometric data patterns using the D2. Peripheral blood samples from patients with BCLPDs may be less complex immunophenotypically than those from healthy control subjects. In BCLPDs, a single clone of lymphocytes replaces the various cellular components of the peripheral blood. We undertook this study to determine whether the D2 could be reasonably applied to flow cytometric data patterns using CD19 κ/λ immunoglobulin light chain expression in BCLPD as the model system.

Materials and Methods

Case Selection

A training set composed of peripheral blood specimens from 22 patients with B-cell chronic lymphocytic leukemia (CLL) and 23 patients with no immunophenotypic evidence of a lymphoproliferative disorder (control group) was selected from archived material at the Cleveland Clinic Foundation, Cleveland, OH. The patients were being evaluated for evidence of a lymphoproliferative disorder between November 2003 and February 2004. All diagnoses were based on the World Health Organization criteria.12 The control group included 11 cases that were essentially normal, 7 cases with anemia, 2 cases with neutrophilic leukocytosis, 1 case with absolute lymphocytosis, 1 case with absolute lymphopenia, and 1 case with thrombocytopenia.

An independent validation data set of peripheral blood specimens from 69 patients with a BCLPD and 64 patients with no immunophenotypic evidence of a lymphoproliferative disorder was selected from archived material from our institution. T-lymphocyte disorders and specimens suggestive but not diagnostic of a BCLPD were excluded. The BCLPD group included 42 cases of CLL, 3 cases of hairy cell leukemia, 8 cases of leukemic mantle cell lymphoma, 2 cases of splenic marginal zone lymphoma, 1 case of lymphoplasmacytic lymphoma, 1 case of B-cell prolymphocytic leukemia, and 12 cases of BCLPD that were not further classifiable. The 64-case control group included 11 peripheral blood specimens with no identifiable abnormality, 9 cases of leukopenia, 8 cases of anemia, 6 cases of thrombocytopenia, 4 cases of pancytopenia, 8 cases of neutrophilic leukocytosis, 4 cases of absolute lymphocytosis, and 14 cases with various combinations of leukocytosis, leukopenia, anemia, and thrombocytopenia.
Flow Cytometric Procedure

Four-color flow cytometric immunophenotypic analysis was performed using fluorescein (FITC)-, phycoerythrin (PE)-, peridinin chlorophyll protein (PerCP)-, and allophycocyanin (APC)-conjugated antibodies to CD79b (Beckman Coulter, Fullerton, CA), CD3, CD4, CD5, CD8, CD10, CD16/56, CD19, CD20, CD45, FMC7, and κ and λ immunoglobulin light chains (Becton Dickinson [BD] Biosciences, San Jose, CA). Samples of EDTA-anticoagulated whole blood were obtained, and the WBC concentration was adjusted to less than 20 million cells per milliliter. Then 2 mL of the specimen was washed twice in phosphate-buffered saline–1% bovine serum albumin wash buffer (Sigma-Aldrich, St Louis, MO) and reconstituted to the original volume in the wash buffer. We added 150 to 200 µL of the cell suspension to 12 × 75 mm BD Falcon polystyrene staining tubes (BD Biosciences) containing 5, 10, or 20 µL of FITC-, PE-, PerCP-, and APC-conjugated antibodies, according to the manufacturer’s instructions.

The samples were incubated for 15 minutes in the dark at room temperature. Then, 2 mL of FACSlyse (BD Biosciences) was added, and the specimens were mixed and incubated for 10 minutes in the dark at room temperature. Specimens were centrifuged for 2 minutes at 1,000g, and the supernatant was discarded. We added 2 mL of wash buffer, the specimen was centrifuged at 1,000g for 2 minutes, and the supernatant was discarded. We then added 100 µL of wash buffer to each tube, each tube was mixed, and 100 µL of 1% paraformaldehyde–phosphate-buffered saline was added to each tube. Up to 20,000 events per tube were acquired using a Becton Dickinson FACSCalibur flow cytometer and saved into an FCS 2.0 data file.

Computation of the D2

An Intel-compatible personal computer running a program written in the Java programming language by one of us (D.G.R.) calculated the D2 of CD45 side-scatter and CD19-gated κ/λ light chain data. The program was written using an object-oriented structure. The user chose 2 parameters to analyze, eg, CD45 and side-scatter values, in a data file. The D2 was calculated as described by Grassberger and Procaccia. Each event recorded by the flow cytometer in the FCS file was treated as a point in a 2-dimensional coordinate system. The x and y values of each point equaled the value of the 2 parameters of interest associated with each flow cytometry event. These values ranged between 0 and 1,023. The program then calculated the Euclidean distance between all possible point pairs. The number of point pairs less than 1 unit of distance (r) from one another was recorded, and this value was divided by the total number of point pairs to yield the probability (P) that any pair of points in the data file would be within 1 unit of distance from each other. This procedure was repeated, counting all point pairs within 1.5 units of one another, then determining the probability a pair of points would be within 1.5 units of each other. The test distance was increased incrementally, and probabilities were determined for distances (r) between 1 and 1,000 in intervals of 0.5. The program saved the calculation results in a text file, which then was imported into a Microsoft Excel spreadsheet (Microsoft, Redmond, WA). The D2 equaled the slope of the line, computed using linear regression, representing the relationship between log(r) and log(P).

Derivation of Sensitivity and Specificity

Each case was classified as BCLPD or control based on the “gold standard,” a combined morphologic and immunophenotypic diagnosis based on World Health Organization criteria. The D2 of each case in the training set was calculated, and a receiver operating characteristic (ROC) curve identified the optimum cutoff value separating the CLL group from the control group. This cutoff value was applied to D2 values derived from an independent validation set, and the sensitivity and specificity were determined.

Statistics

The D2s of the BCLPD and control groups were compared with one another using the Student 2-tailed t test. Significance was set at a P value of less than .05.

In Vitro Lower Limit of Detection

The lower limit of detection was determined by diluting neoplastic lymphocytes from a lymph node involved by B-cell lymphoma with lymphocytes from a control peripheral blood sample. The D2 was calculated for suspensions in which κ-restricted B lymphocytes accounted for 0%, 9%, 40%, 52%, 76%, 82%, and 100% of all B lymphocytes. Linear regression was used to determine the relationship between the percentage of neoplastic lymphocytes and the D2. The lower limit of detection was the dilution at which the D2 equaled the cutoff value identified in the training set.

Results

For the training set of 22 CLL and 23 control samples, the CLL group demonstrated less complexity, as measured by D2, than the control group. The overall WBC pattern was studied using CD45 side-scatter data, and the B lymphocyte distribution was assessed using CD19-gated κ/λ immunoglobulin light chain data. We recorded 20,000 CD45 side-scatter events for all cases, and a mean of 10,750 (range, 36-20,000) CD19-gated κ/λ light chain events per case were analyzed. The CLL group had a statistically significantly lower D2 than the control group when the D2 was calculated using CD45 side-scatter data or CD19-gated κ/λ immunoglobulin light chain.
data ($P < .001$ for both comparisons). These results are consistent with an overall simplification of the sample to predominance of a single cell type. For CD45 side-scatter data, the CLL group had a mean D2 of 1.02 with an SD of 0.08 and the control group had a mean D2 of 1.12 with an SD of 0.10. For CD19-gated $\kappa/\lambda$ light chain data, the CLL group had a mean D2 of 0.58 with an SD of 0.04 and the control group had a mean D2 of 0.88 with an SD of 0.08. **Figure 2** illustrates the distribution of the D2 in the CLL and control groups. A low D2 corresponded to a well-defined light chain–restricted population seen on a CD19-gated $\kappa/\lambda$ scatter plot **Figure 3**.

We generated an ROC curve from the training set results and determined a cutoff value based on this curve **Figure 4**. There was no overlap between the 2 groups using CD19-gated $\kappa/\lambda$ data, and choosing a cutoff value of 0.72 yielded sensitivity and specificity values of 100% for each. A D2, calculated from CD19-gated $\kappa/\lambda$ immunoglobulin light chain data, greater than the cutoff value indicated a polyclonal data pattern. A D2 less than or equal to the cutoff value indicated a monoclonal data pattern.

Using the D2 of CD45 side-scatter data from the training set to construct an ROC curve showed maximum sensitivity and specificity values of 100% and 30%, respectively. These

**Figure 2** A, By using CD45 side-scatter data to assess the overall distribution of WBCs, we found less complexity in the chronic lymphocytic leukemia (CLL) group ($n = 22$; mean, 1.02; SD, 0.08), as measured by the correlation dimension (D2), than in the control group ($n = 23$; mean, 1.12; SD, 0.10; $P < .001$). B, CD19-gated $\kappa/\lambda$ immunoglobulin light chain data were used to examine the B lymphocyte population. The D2 of the CLL group ($n = 22$; mean, 0.58; SD, 0.04) was less than that of the control group ($n = 23$; mean, 0.88; SD, 0.08), showing that CLL cells have a relatively more simple light chain expression pattern than do control B lymphocytes ($P < .001$).

**Figure 3** This CD19-gated plot shows that a low correlation dimension (0.53) was associated with a well-defined cluster of light chain–restricted B lymphocytes (k/\l ratio, 0.01). FITC, fluorescein isothiocyanate; PE, phycoerythrin.

**Figure 4** A receiver operating characteristic curve was generated using CD19-gated $\kappa/\lambda$ data from the training set. Evaluation of different cutoff points showed that a correlation dimension of 0.72 was optimal, yielding a sensitivity and specificity of 100%.
statistics were attained using a cutoff value of 1.15 (nonneoplastic pattern if $D_2 > 1.15$). The poor discrimination of CD45 side-scatter $D_2$ data was attributed to limitations in the diagnostic threshold. Because of the superior performance of the immunoglobulin light chain data, we focused on this parameter in a new series of cases.

The 0.72 $D_2$ cutoff value for CD19-gated $\kappa/\lambda$ light chain data was applied to the independent validation set of 69 cases with a BCLPD and 64 with no immunophenotypic evidence of a lymphoproliferative disorder (control group). By using this cutoff value to indicate a BCLPD, the $D_2$ had a sensitivity of 88% and a specificity of 98%.

The 8 false-negative $D_2$ samples consisted of 2 hairy cell leukemias, 2 CLLs, 2 BCLPDs (not further classifiable), 1 splenic marginal zone lymphoma, and 1 mantle cell lymphoma. These patients had low-level involvement by BCLPD (1 case) Figure 5A, showed highly variable light chain expression intensity (5 cases) Figure 5B, or had a lymphocyte population with a distinct subclone (2 cases) Figure 5C. The preceding patterns increased the heterogeneity of the data and, therefore, the $D_2$.

<table>
<thead>
<tr>
<th></th>
<th>Control Cases</th>
<th>BCLPD Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_2+$</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td>$D_2-$</td>
<td>63</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>69</td>
</tr>
</tbody>
</table>

BCLPD, B-cell lymphoproliferative disorder; $D_2$, correlation dimension.

* Sensitivity, 88%; specificity, 98%.

**Table 1**

**Sensitivity and Specificity of $D_2$ in the Validation Set**

![Figure 5A](image1.png) **A**, A false-negative correlation dimension ($D_2$) result occurred in this case of low-level hairy cell leukemia (HCL). The CD19+CD25+CD103+ HCL cells are shown in red. **B**, This case of chronic lymphocytic leukemia (CLL) had an abnormal $\kappa/\lambda$ ratio and a falsely negative $D_2$. It showed variable light chain expression intensity, causing an increase in the $D_2$. **C**, This case of CLL with a distinct subclone showed 2 CD19+ populations: 1 with (red) and 1 without (purple) surface $\kappa$ light chain expression. This pattern elevated the $D_2$, and caused a false negative result. APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin.
We compared the D2 with a commonly used tool in the assessment of B-cell malignancy, the κ/λ immunoglobulin light chain ratio. Using the KLR as the sole test for a BCLPD generated results slightly better than the D2 (sensitivity, 93%; specificity, 100%) Table 2. A KLR indicative of a BCLPD was defined as 4 or more or less than 0.5. Alternative cutoffs and their associated sensitivities and specificities are listed in Table 2. There were 5 false-negative KLR cases, and all were positive when assessed using the D2. All 5 were involved by BCLPDs with no surface light chain expression, resulting in a falsely negative KLR owing to analysis of residual nonneoplastic B cells. The simplification of the data distribution was detected by the D2. Although lack of surface light chain expression is atypical, it is not always indicative of a BCLPD. Therefore, we treated these situations as falsely negative for the purpose of comparison. The discrepancies between the D2 and the KLR are detailed in Table 3. These cases demonstrate that although the D2 and KLR are useful ways of looking at data, flow cytometric interpretation requires the complex integration of expression patterns of many markers combined with morphologic findings. Used together, the D2 and KLR identified all BCLPDs in the validation set.

To understand the relationship between the D2 and the KLR, we determined the correlation coefficient (r) between the D2 and the KLR (for non-λ-restricted cases). In general, there was an inverse relationship between the KLR and the D2. Although the 2 values shared some variability, the relationship was imperfect (r = −0.73). The D2 gave information in addition to that provided by the KLR. The KLR described the relative “magnitudes” of κ and λ light chain expression, whereas the D2 provided information on the “shape” of the data distribution.

Dilution of neoplastic B lymphocytes from a nodal marginal zone lymphoma into a normal B-lymphocyte population from peripheral blood revealed that the 0.72 D2 cutoff was reached when neoplastic B lymphocytes accounted for 74% of all B lymphocytes Figure 6. This corresponded to a KLR of 6.3.

**Discussion**

We demonstrated that the D2, a mathematical tool developed to measure the complexity of fractals can, in principle, be applied to flow cytometric data pattern analysis. Specifically, the D2 can identify B-cell light chain restriction in peripheral blood using CD19-gated κ/λ immunoglobulin data. We realized that examining only CD19 κ/λ information in the diagnosis of B-cell neoplasia is artificial and fails to use all the analytic power offered by multiparameter flow cytometry. The

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**Table 2**

Comparison of KLR Cutoffs in the Validation Set*

<table>
<thead>
<tr>
<th></th>
<th>≥2 or &lt;0.5</th>
<th>≥4 or &lt;0.5</th>
<th>≥6 or &lt;0.5</th>
<th>≥8 or &lt;0.5</th>
<th>≥10 or &lt;0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>94</td>
<td>93</td>
<td>88</td>
<td>88</td>
<td>84</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>88</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

KLR, κ/λ ratio.  
*A KLR cutoff of ≥4 or <0.5 was used as positive in this analysis (bold).

**Table 3**

Discrepancies Between D2 and KLR (≥4 or <0.5)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>D2</th>
<th>KLR</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>–</td>
<td>+</td>
<td>CLL (CD19+CD20+CD5+CD23+, FMC7–, slg–) with distinct subclone (κ light chain–restricted)</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>+</td>
<td>HCL with variable light chain expression intensity</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>+</td>
<td>BCLPD with variable light chain expression intensity</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>+</td>
<td>CLL with variable light chain expression intensity</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>+</td>
<td>SMZL with variable light chain expression intensity</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>+</td>
<td>BCLPD with variable light chain expression intensity</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>+</td>
<td>BCLPD (CD45[dim], CD19+CD20+CD10+, CD34–, TdT–, slg–) with a subclone (CD45[bright], κ light chain–restricted)</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>+</td>
<td>Low-level involvement by HCL with a characteristic phenotype (CD19+CD20+CD11c+CD22+CD25+CD103+)</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>–</td>
<td>CLL lacking surface light chain expression</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>–</td>
<td>CLL lacking surface light chain expression</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>–</td>
<td>CLL lacking surface light chain expression</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>–</td>
<td>BCLPD lacking surface light chain expression</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>–</td>
<td>Mantle zone lymphoma lacking surface light chain expression</td>
</tr>
</tbody>
</table>

BCLPD, B-cell lymphoproliferative disorder; CLL, chronic lymphocytic leukemia; D2, correlation dimension; HCL, hairy cell leukemia; KLR, κ/λ ratio; SMZL, splenic marginal zone lymphoma; slg, surface immunoglobulin; TdT, terminal deoxynucleotidyl transferase; +, positive; –, negative.
purpose of this study was not to attempt to replace current flow cytometric data analysis with D2 analysis or advocate its immediate use for light chain expression evaluation. Rather, it was to show proof of the principle that D2 can be applied to flow cytometric data. This seems to be the case using CD45 side-scatter and CD19 κ/λ data.

Until now, the primary flow cytometric means used to evaluate a B-cell population for monoclonality has been the KLR.1 This sometimes is a subjective determination. First, it depends in large part on subjective operator-determined thresholds dividing light chain–positive cells from light chain–negative cells. Second, a threshold KLR must be defined. This ratio may vary among and within laboratories based on the individual operator’s experience or preference. The literature illustrates this large variation. Chizuka et al15 proposed using a KLR of greater than 2:1 to define a monotypic B-cell population. Robins et al16 used a KLR or a λ/κ ratio of greater than 3:1 in the assessment of the efficacy of flow cytometry in fine-needle aspiration for lymphoma diagnosis. In the diagnosis of lymphoid disorders of the spleen, Zeppa et al17 defined abnormal KLR and λ/κ ratios as greater than 6:1 or 4:1, respectively. Thus, one can see that there is little consensus on what the optimal ratio might be.

Because the D2 does not depend on determining thresholds for positivity (either in separating light chain–positive cells from light chain–negative cells or in the interpretation of the KLR) and data analysis is computerized, it offers a more operator-independent data analysis using preexisting hardware and is useful for objectively quantifying differences in data distribution. The D2 supplements the KLR in the assessment of B-lymphocyte clonality by describing the shape of the data distribution rather than the relative magnitudes of immunoglobulin light chain expression as defined by the KLR. Limitations of the D2 include a high detection threshold and the inability to differentiate symmetrical patterns. The relatively high detection threshold is inherent in the D2 determination. Because the algorithm gives each data point equal consideration, a population of abnormal cells can be lost in a background of normal cells. This problem can be minimized by taking advantage, as we did in this study, of the more specific gating that multiparameter flow cytometry allows.

The D2 describes the spatial relationship of one point to all other points within a set of data. Because symmetrical data sets have the same interrelationship of points, they cannot be distinguished using the D2. A set of lymphocytes showing strong κ expression could have the same D2 as a group of lymphocytes showing absent immunoglobulin light chain expression if the shape of the clusters were identical. Also, BCLPDs with 2 immunophenotypically distinct populations may have a D2 within the normal range because the additional subclone increases the complexity of the interrelationship of the points.

Fundamentally, the D2 condenses the information describing cell populations into a single number. Although individual detail is lost, collective knowledge of the global pattern is gained. Thus, in cases in which an overall view is desirable, the D2 is a useful tool. We demonstrate in this study that the D2 can be used to assist in identifying monotypic B-cell populations. The training set allowed us to empirically determine a reasonable threshold that, on testing in an independent data set, performed almost as well as the KLR determination. To be sure, the D2 is not perfect, but it can at least match standard methods. The limitations, as outlined, were apparent in the validation set. For example, the D2 failed to identify low-level involvement by hairy cell leukemia. Nevertheless, we contend that this new method removes a source of variation in flow cytometric data analysis and can be totally automated. It identified 5 BCLPDs not identified by the KLR. These samples showed absent immunoglobulin light chain expression, which is suggestive but not always diagnostic of a BCLPD.14 As shown in our data, the D2 seems to be an adjunct in the evaluation of samples for lymphoproliferative disorders. Together, the D2 and KLR identified all cases of BCLPD.

By using CD19 κ/λ expression in BCLPD as a model, we showed that the D2 can be applied successfully to analysis of peripheral blood flow cytometric data. In this context, it provides reasonable sensitivity and specificity in the identification of B-lymphocyte immunoglobulin light chain restriction by detecting global changes in the B-cell light chain expression. The D2 quantifies changes in distribution of complex

### Figure 6
The relative number of neoplastic lymphocytes necessary to result in a positive test for neoplasia using the correlation dimension (D2) was assayed in vitro by diluting a population of κ-restricted B cells into a polytypic lymphocyte population. The D2 cutoff value of 0.72 was reached when neoplastic B cells accounted for 74% of all B lymphocytes.
data patterns and offers useful information that potentially might be incorporated into a more sophisticated analysis combining the complementary power of multiparameter subpopulation analysis and data distribution analysis of D2s. A potential application is objectively measuring complex immunophenotypic abnormalities in maturing cell populations in myelodysplastic syndromes.

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References