Feature Analysis and Automatic Identification of Leukemic Lineage Blast Cells and Reactive Lymphoid Cells from Peripheral Blood Cell Images

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Background: Automated peripheral blood (PB) image analyzers usually underestimate the total number of blast cells, mixing them up with reactive or normal lymphocytes. Therefore, they are not able to discriminate between myeloid or lymphoid blast cell lineages. The objective of the proposed work is to achieve automatic discrimination of reactive lymphoid cells (RLC), lymphoid and myeloid blast cells and to obtain their morphologic patterns through feature analysis. **Methods:** In the training stage, a set of 696 blood cell images was selected in 32 patients (myeloid acute leukemia, lymphoid precursor neoplasms and viral or other infections). For classification, we used support vector machines, testing different combinations of feature categories and feature selection techniques. Further, a validation was implemented using the selected features

over 220 images from 15 new patients (five corresponding to each category). **Results:** Best discrimination accuracy in the training was obtained with feature selection from the whole feature set (90.1%). We selected 60 features, showing significant differences ($P < 0.001$) in the mean values of the different cell groups. Nucleus-cytoplasm ratio was the most important feature for the cell classification, and color-texture features from the cytoplasm were also important. In the validation stage, the overall classification accuracy and the true-positive rates for RLC, myeloid and lymphoid blast cells were 80%, 85%, 82% and 74%, respectively. **Conclusion:** The methodology appears to be able to recognize reactive lymphocytes well, especially between reactive lymphocytes and lymphoblasts. J. Clin. Lab. Anal. 31:e22024, 2017. 2016 Wiley Periodicals, Inc.

Key words: cytology; hematology; image processing; leukemia; pathology

INTRODUCTION

Although immunological, cytogenetic, and molecular tests are being increasingly used, the morphologic analysis of peripheral blood (PB) is still an important initial step for rapid morphologic diagnosis, selection of additional techniques and follow-up of the patients with malignant blood diseases, including acute leukemia (1, 2). Automated PB image analyzers have been integrated into the daily routine in some clinical laboratories. This represents a valuable technological advance, since they are able to pre-classify most of the normal blood cells (3–6). Nevertheless, these automatic analyzers usually underestimate the total number of blast cells in PB, mixing them up with reactive or normal lymphocytes and they are not able to distinguish between myeloid or lymphoid blast cell lineages. Briggs et al. (3) reported that the preclassifying

agreement for blast cell class in Cellavision DM96 was at 76.6%. It also depends on operator's morphology experience.

The challenge to solve the open problem of blast cell automatic recognition is twofold: (a) the difficult differentiation between reactive lymphocytes and blast cells, since they share some morphologic similarities, such as the diffuse pattern chromatin, the presence of nucleoli or the basophilic cytoplasm; and (b) the discrimination between myeloid or lymphoid origin, since

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these subtypes of blast cells exhibit very similar patterns.

Recent works have proposed a methodology for blood cell automatic recognition based on the pattern recognition paradigm, which obtained very accurate results in the classification of different lymphoid cell subtypes (7–9). In the present article, we adapt this methodology to achieve the automatic discrimination between blast cells from lymphoid and myeloid origin and their separation with respect to the reactive lymphoid cells. This work includes a detailed analysis of the features that characterize the target cells. These features involve both nucleus and cytoplasm. The cytoplasm segmentation has not been always addressed in the literature (10, 11), while it may be important not only to extract geometrical insight provided by the nucleus/cytoplasm ratio, but also for the color-texture characterization. The information in both the nucleus and the cytoplasm has been essential for the automatic recognition of the cell types included in this study. This is emphasized through different experiments, which are performed to investigate the best quantitative descriptors for the discrimination between reactive lymphocytes and blast cells from both myeloid and lymphoid lineage.

The automatic classification of the cell image groups proposed in this article is a new contribution to the state of the art since, up to the author’s knowledge, it has not been jointly considered before. Their differentiation is relevant since it allows: (a) to discern among malignant and non-malignant diseases; and (b) the recognition of the myeloid or lymphoid morphologic pattern in malignancy.

MATERIALS AND METHODS

Our classification method was performed following the subsequent steps: (a) blood sample preparation,

patient selection, and image acquisition; (b) segmentation; (c) feature extraction; (d) feature analysis; and (e) classification. A block scheme of these steps is given in Figure 1.

Blood Sample Preparation, Patient Selection, and Image Acquisition

Blood samples from a total of 47 patients were included in the present study, 32 patients for the training and 15 for the validation stage, which were obtained from the routine workload of the Core laboratory of the Hospital Clínic of Barcelona. Venous blood was collected into tubes containing K₃EDTA as anticoagulant. Samples were analyzed on the Advia 2120 (Siemens Healthcare Diagnosis, Deerfield, IL, USA) analyzer, and PB films were automatically stained with May Grünwald-Giemsa in the SP1000i (Sysmex, Kobe, Japan) within 4 hr of blood collection.

The training set included a number of 696 images obtained from 11 patients with acute myeloid leukemia (AML), six with precursor lymphoid neoplasms (ALL) and 15 with viral or other infections. The validation was carried out using 220 new images from five new patients for each of the studied groups. Acute leukemia diagnosis was established by clinical and morphologic findings, immunophenotype, cytogenetic and molecular analysis, and other complementary tests following the WHO classification publication (See Table 1; 12).

Pathologists (LB, AM) selected a total of 916 digital images of blast cells or reactive lymphoid cells from the remaining blood cells in the PB smears. A number of 359 were blast cell images from patients with AML, 336 were blast cell images from patients with ALL and 221 images were reactive lymphoid cells (RLC) from patients with viral or other infections. Table 1 shows the distribution of the final diagnosis, the number of

Fig. 1. The complete methodology for the training stage, steps, and categories in each one is described in the scheme. A training set (TS) of 696 images were used to obtain a database of features, and a detailed feature analysis was performed: PCA, feature experiments, feature selection, and the statistical analysis of the selected features. The SVM classifier is tuned using the TS for further validation classification.

TABLE 1. Diagnosis of the Patients in the Respective Training and Validation Sets: (a) Acute Leukemia According to the WHO 2008 Classification; and (b) Viral or Other Infections

Leukemia diagnosis (myeloid or lymphoid antigens expression)		Training		Validation	
		P	I	P	I
Acute myeloid leukemia, Not otherwise specified (NOS)	AML with myelodysplasia-related changes (panmyeloid markers)	4	100	1	20
	Therapy-related myeloid neoplasms (CD34+, CD13+, CD33+)	1	21	1	20
	AML without maturation (CD34+, HLA-DR+, CD13+)	2	50	1	20
	AML with maturation (CD13+, CD33+, CD65+, CD11b+, CD15+)	1	25	1	20
	AML with recurrent genetic abnormalities	2	38	1	20
AML with recurrent genetic abnormalities	AML with mutated NPM1 (panmyeloid markers, CD14+, CD11b+)	2	38	1	20
	AML with t(6;9)(p23;q34) (panmyeloid markers, CD38+, HLA_DR+, CD117+, CD34+, CD15+)	1	25	–	–
B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities	B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q 11.2) (CD10+, CD19+, TdT+, CD25+)	2	100		
	B lymphoblastic leukemia/lymphoma with t(v;11q23) (CD19+, CD10–, CD24–)			2	24
B lymphoblastic leukemia/lymphoma, NOS (CD19+, cytoplasmic CD79a+ and CD22+, CD10+, surface CD22+, and CD24+)		2	60	2	29
T lymphoblastic leukemia/lymphoma (TdT+, CD34+, CD1a+, cytoplasmic CD3+, CD4+, CD5+, CD7, and CD8)		2	103	1	20
Viral or other infections		15	174	5	47
TOTAL		32	696	15	220

P, patients; I, images; AML, acute myeloid leukemia; NOS, not otherwise specified; FAB, French–American–British; NPM1, nucleophosmin.

patients included in each group and the number of cell images selected. Individual blast cells and reactive lymphoid cell images from PB were obtained using the CellaVision DM96 system (CellaVision, Lund, Sweden). The image resolution was 363×360 pixels.

Segmentation

We performed a segmentation method based on spatial kernel Fuzzy-C means (13, 14), which was developed in (7–9). The final outcome was the automatic separation of three different regions of interest (ROI) of the cell: nucleus, cytoplasm and peripheral zone around the cell.

Feature Extraction

The main objective of this step was to obtain quantitative information from the ROI of the image. We used geometric and color-texture features. Geometric features are quantitative measures related to the morphology of the different regions of the cell, including the cytoplasmic profile feature described for the first time in (7) and adding the compactness of the nucleus and the cytoplasm (9).

Color-texture features are divided into statistical and granulometric. Statistical features were calculated for each color component on the image (15, 16). They

were also applied over the six sub-images resulted from a two level wavelet decomposition of each color component on the image (17–20). Granulometric features were obtained from the granulometric and the pseudo-granulometric curves (9, 20). All the above features were calculated for the nucleus and the cytoplasm as described in (9).

Feature Analysis

The purpose of this step was to analyze the quantitative features obtained from the images of the training set to identify the most relevant for the further classification. The analysis was carried out in three steps: (a) considering the whole group of features and applying *principal component analysis* (PCA; 21) to reduce the data dimension as a tool to visualize all the features corresponding to the three different groups of cells included in this study; (b) performing a series of experiments dividing the features into categories; and (c) applying feature selection using the mutual information maximization criterion (CMIM; 22, 23) to reduce the redundancy of the variables and the complexity of the classification. Finally, a statistical analysis of the most relevant features was performed. Chi-Square test was used to test the hypothesis that the samples were normally distributed, and non-parametric Kruskal–Wallis test was used to compare the

means in the three different groups (RLC, AML and ALL).

Classification

In the training stage, the classification step was the automatic recognition of the different blast cell lineages and RLC in PB images using the selected features. We used support vector machines (SVM) with a radial basis function as kernel for the classification (24, 25). Linear SVM were used only when the number of features was very high. The training classification performance was evaluated by the application of the 10-fold cross validation technique (8, 9) over the training set of 696 images. After this process, a confusion matrix with the values of the classification results was calculated. The outcome of this stage was a classifier appropriately tuned for further use.

The validation of the final classifier was carried out following the same steps previously used (see Fig. 1), except the feature analysis, since the best features were already obtained in the training stage. The new 220 cell images (validation set) were processed to obtain their best features, which were used by the classifier to automatically recognize the different abnormal cells.

RESULTS

As we show in Figure 2, three different regions were obtained after the segmentation of the individual cell images included in the present study: (a) nucleus; (b) cytoplasm; and (c) peripheral zone around the cell.

In the training stage, a set of 2,379 features was extracted from the segmented 696 images: 2,366 color-texture features from the “L*a*b*” and “CMYK”

color spaces and 13 geometrical features. For the feature analysis, we applied a PCA dimension reduction over the whole set to obtain the first and second principal components. Figure 3 shows a representation of both components. While RLC had a distinct pattern with respect to myeloid (MBC) and lymphoid (LBC) blast cells, MBC and LBC showed an overlapping region between them in this representation.

The second step in the feature analysis comprised a series of experiments summarized in Table 2. The upper part of the table shows the classification accuracy for the first group of experiments (1–3) in which three different categories of features were considered: (a) the whole set; (b) geometrical features only; and (c) color-texture features only. The classification results obtained in the experiments 1 and 3 were very similar, showing a global accuracy of 81.9% and 81.6%, respectively. In addition, when only geometrical features were used (experiment 2), the global accuracy was slightly lower (78.4%). On the other hand, the lowest part of Table 2 shows the accuracy classification results obtained in the experiments 4 and 5, in which 60 most relevant features were obtained applying the feature selection procedure over the whole set and the color-texture set, respectively. Both experiments exhibited greater global accuracy (90.1% and 88.9%) in comparison to the previous ones without feature selection.

From all the experiments in Table 2, the best global accuracy in the classification was obtained in the experiment 4. Table 3 shows the 10 out of 60 most relevant features selected in the experiment 4. Two of these features were geometric (nucleus/cytoplasm ratio and nucleus area) and the remaining eight corresponded to the color-texture category, seven related to

Fig. 2. Examples of segmented cells: myeloid blast cells (MBC), lymphoid blast cells (LBC), and reactive lymphoid cells (RLC), respectively. The images show three different regions: nucleus (yellow line), cytoplasm (blue line), and peripheral zone around the cell (red line). (X 1,000, May Grünwald-Giemsa stain).

Fig. 3. First and second principal components of all set of features obtained by principal component analysis (PCA), showing a different position regarding these principal components in the groups of cells analyzed. Reactive lymphoid cells (RLC) showed a different pattern with respect to blast cells. Myeloid blast cells (MBC) and lymphoid blast cells (LBC) shared a small area in this PCA representation.

TABLE 2. Global Classification Accuracy Results Considering Different Feature Categories and Using Feature Selection

Experiment	Feature category	Number of features	SVM type	Global classification accuracy (%)
1	Whole set	2379	Linear	81.9
2	Geometric	13	RBF Kernel	78.4
3	Color- texture	2366	Linear	81.6
4	Whole set	60	RBF Kernel	90.1
5	Color- texture	60	RBF Kernel	88.9

SVM, support vector machine; RBF, radial basis function.

the nucleus and one to the cytoplasm. Within the color-texture category, five were statistical and three were granulometric features. The nucleus/cytoplasm ratio was the most significant feature. When mean values were compared for the most relevant features obtained in RLC, MBC, and LBC groups, we found significant differences ($P < 0.001$) in 59 out of 60 features. This fact is illustrated in Figure 4, which shows box plots for six of the most relevant features.

The 60 features selected in experiment 4 were used in the classification step. Table 4 shows the confusion

matrix that summarized the results for the three cell subsets analyzed. The rows represent the confirmed diagnosis established by clinical, morphologic characteristics, immunophenotype, cytogenetic and molecular studies (“gold standard”) and the columns show the predicted diagnosis given by the classification algorithm. The data were normalized to show the percentages of true diagnosis. The true-positive rate for each cell type was 97% for RLC, 87% for LBC, and 88% for MBC. Figure 5 shows some examples of different cell subset images, which were correctly identified by the classification system. Each row corresponds to RLC, LBC, and MBC, respectively.

The classifier obtained in the previous steps was finally validated using a set of 220 cell images from new patients, which were not used in the training stages. Table 5 gives the classification results, in which the overall accuracy was 80%. The true-positive rate for each cell type was 85% for RLC, 74% for LBC, and 82% for MBC, respectively. These results indicate that the proposed method could recognize the difference between reactive lymphocytes and blasts in general, observing some misclassification of reactive lymphocytes as myeloblasts. Some overlapping features

TABLE 3. First 10 Most Relevant Features Obtained in the Experiment 4. Feature Type, Color Component, and Color Space (“L*a*b*” or “CMYK”) in Which This Feature Was Calculated is Shown. Information Provided by Each Feature Related to the Nucleus or Cytoplasm is Reported. Mean Values for Each Feature in Reactive Lymphoid Cell (RLC), Lymphoid Blast Cell (ALL) and Myeloid Blast Cell (AML) Images are Shown

Feature	Type	Color component (Color space)	Nucleus or cytoplasm	RLC	ALL	AML
				Mean	Mean	Mean
Nucleus/cytoplasm ratio	Geometric	NA	NA	1.28	4.21	2.98
Homogeneity ^a	Statistical. 2nd order	K (CMYK)	Nucleus	0.79	0.73	0.76
Correlation ^a	Statistical. 2nd order	a (Lab)	Nucleus	0.69	0.61	0.62
Nucleus Area ^b	Geometric	NA	NA	10227	8883	10671
Correlation ^a	Statistical. 2nd order	C (CMYK)	Nucleus	0.79	0.76	0.75
Skewness ^a	Pseudogranulometric	a (Lab)	Nucleus	−0.56	−0.86	−0.66
Entropy ^a	Statistical. 1st order	K (CMYK)	Nucleus	4.98	5.42	5.20
Sum Average ^a	Statistical. 2nd order	C (CMYK)	Cytoplasm	6.43	10.33	9.25
Mean ^a	Pseudogranulometric	M (CMYK)	Nucleus	0.03	0.03	0.03
Skewness ^a	Pseudogranulometric	Y(CMYK)	Nucleus	0.45	0.38	0.27

RLC, reactive lymphoid cell; ALL, precursor lymphoid neoplasms; AML, acute myeloid leukemia; NA, not apply; N, nucleus; C, cytoplasm.

^aFeatures that correspond to probability distributions have not units.

^bArea is measured in pixels.

Fig. 4. Feature comparison using box plots of features containing the central 50% of the images in the group. The line in the box represents the median.

between these two cell groups, as observed in Figure 3, could explain the relative lower recognition of lymphoblasts and myeloblasts.

DISCUSSION

Recently we have published a complete method that reaches high precision in the recognition of different types of abnormal lymphoid cells (7–9). In the current article, for the first time, we used this method

combining segmentation, feature extraction and classification algorithms for the automatic discrimination of reactive lymphocytes from blast cells in general and for the recognition between myeloblasts and lymphoblasts. Besides, within the myeloid and lymphoid blast cells, we considered different subtypes following the 2008 WHO publication (12) to increase the heterogeneity of the features.

The segmentation method was very effective in separating three regions for each blast cell and RLC images:

nucleus, cytoplasm and external region of the cell, whereas other authors only segmented one (nucleus; 10, 11) or two regions (nucleus and cytoplasm; 24–30).

In the feature analysis, the two principal components of the whole feature set showed that the RLC had a different pattern in comparison to the blast cell groups (see Fig. 3). This is consistent with the existing

morphologic differences in the RLC cells with respect to the blast cells. Myeloid and lymphoid blast cells had an overlapping region in accordance with the morphologic similarities exhibited by some myeloblasts and lymphoblasts. The proposed series of experiments in this article show that the classification accuracy when color-texture features were used was greater than

TABLE 4. Confusion Matrix of the Support Vector Machine (SVM) Classification and 10-fold Cross Validation for the 696 Images Set (Experiment 4)

		Predicted diagnosis		
		RLC	LBC	MBC
Global accuracy: 90.1%				
Confirmed diagnosis	RLC	96.55	0.57	2.87
	LBC	1.14	87.83	11.02
	MBC	3.09	8.88	88.03

RLC, reactive lymphoid cell; LBC, lymphoid blast cell; MBC, myeloid blast cell.

The rows represent the confirmed diagnosis and the columns the predicted diagnosis given by the classification algorithm. The values are in percentage. The values in bold represent the global accuracy and the true positive rates in the classification for each cell type.

TABLE 5. Confusion Matrix of the Support Vector Machine (SVM) Classification and 10-fold Cross Validation for the 220 Images in the Validation Set

		Predicted diagnosis		
		RLC	LBC	MBC
Global accuracy: 80%				
Confirmed diagnosis	RLC	85.11	0.00	14.89
	LBC	4.11	73.97	21.92
	MBC	1.00	17.00	82.00

RLC, reactive lymphoid cell; LBC, lymphoid blast cell; MBC, myeloid blast cell.

The rows represent the confirmed diagnosis and the columns the predicted diagnosis given by the classification algorithm. The values are in percentage. The values in bold represent the global accuracy and the true positive rates in the classification for each cell type.

Fig. 5. Examples of individual cell images corresponding to the cells that were correctly classified. The first row corresponds to reactive lymphoid cells (RLC), the second to lymphoid blast cells (LBC) and the third row to myeloid blast cells (MBC). (X 1,000, May Grünwald-Giemsa stain).

the obtained with the geometric features, which is in accordance to the observations reported by other publications (10, 11, 28). The results demonstrate that the best classification is obtained when a reduced selected set of 60 features is used instead of the total number of the initially extracted features. This confirms that feature selection is appropriate not only to reduce the computational burden, but also to achieve satisfactory classification results as the most relevant information is maximized and redundancy is minimized. Nucleus-cytoplasm ratio was found to be the most important feature in the classification algorithm of blast and RLC cells, which was also the first feature in relevance in the automatic classification of abnormal lymphoid cells (9). It is also worth to remark the importance that obtaining information of the cytoplasm has in this work. The cytoplasm information not only was important for the geometrical information provided by the feature nucleus/cytoplasm ratio (the most relevant feature in the classification), but also for its color-texture information (see Table 3, feature 8). The “*Sum Average*” feature, related to the texture of the cytoplasm, provides essential information for the discrimination between myeloid and lymphoid lineages. The differences observed in this color-texture feature in myeloid or lymphoid blast cells may be related to the presence of an outline of immature granulation, which is more typical in myeloid lineage blast cells.

Previous studies (29, 30) have published satisfactory data related to the automatic recognition of lymphoid blast cells and normal lymphocytes using PB images. Nevertheless, morphologic differences between mature and immature lymphoid cells are more significant with respect to the groups selected in this article for the automatic classification. Markiewicz et al. (31) reported automatic image classification of myeloid blast cells and other myeloid cells at different maturation stages using bone marrow cell images. In other study (28), myeloid and lymphoid blast cell lineage images were considered as a single group and their automatic recognition with respect to other atypical lymphoid cell images was shown. Abdul Nasir et al. (10) considered lymphoid and myeloid blast cells as a single group in the classification step. The same authors in another study (11) reported good classification results of myeloid and lymphoid blast cells but considering normal blood cells (neutrophils) as a group, which are consistently different since the nucleus in these cells is lobulated. Finally, Reta et al. (26, 27) performed a binary classification of myeloid and lymphoid lineage blast cell images.

In conclusion, automated PB image analyzers are used in clinical laboratories as a screening tool to relieve the

burden of manual differential counting. High negative predictive value could prevent missing circulating blasts. Our methodology would be helpful to improve the current automated PB image analyzers as a screening tool to alert the possibility of circulating blasts. As shown in the validation step, the methodology described was able to recognize reactive lymphocytes well and discriminate them with respect to the lymphoblasts, with some misclassification of reactive lymphocytes as myeloblasts (as seen in Table 5). The relative lower recognition of lymphoblasts and myeloblasts could be explained by some overlapping features between these two cell populations as demonstrated at Figure 3.

Further work is in progress to extend the method to allow the automatic classification of other leukemic cells, such as atypical promyelocytes in acute promyelocytic leukemia or blast cells from monocytic origin.

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