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Prognosis of chronic lymphocytic leukemia from infrared spectra of lymphocytes

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Abstract

Peripheral mononuclear cells obtained from blood of normal individuals and from patients with chronic lymphocytic leukemia (CLL) were investigated by infrared spectroscopy and multivariate statistical analysis. Not only are the spectra of CLL cells different from those of normal cells, but hierarchical clustering also separated the CLL cells into a number of subclusters, based on their different DNA content, a fact which may provide a useful diagnostic tool for staging (progression of the disease) and multiple clone detection. Moreover, there is evidence for a correlation between the increased amount of DNA in the CLL cells and the in-vivo doubling time of the lymphocytes in a given patient. © 1997 Elsevier Science B.V.

Keywords: Chronic lymphocytic leukemia; Infrared spectroscopy; Linear discriminant analysis; DNA spectra

1. Posing the question

1.1. Why chronic lymphocytic leukemia?

Chronic lymphocytic leukemia (CLL), the most prevalent form of leukemia in Western Europe and North America, is the accumulation of non-proliferating, mature-looking but functionally immature lymphocytes in the peripheral blood, bone marrow and lymph nodes [1]. The disease shows a male dominance, with a male-to-female ratio of 2:1; cytogenetically, CLL cells show aberrations of chromosomes and/or additional copies of chromosomes (trisomy 12). The cause of CLL is still unclear though viruses may play a role in the pathogenesis of the disease. The clinical manifestation of CLL can differ considerably

in that some patients may have a short survival rate of only a few months after diagnosis, while others may live with the disease for more than 10 years. It is therefore vital to find some sort of parameter that can be used for a prognosis of the disease.

1.2. How can infrared spectroscopy contribute to the prognosis of CLL?

At present the diagnosis of CLL is based on the following criteria (i) an absolute lymphocyte count greater than 10^9 L^{-1} and (ii) when either 30% of the bone marrow is replaced by CLL cells or the blood lymphocytes present clonality as determined by the phenotype. Morphologically, the CLL cells are almost identical to normal lymphocytes, however they have lost the ability to undergo the final step of maturation [2]. The CLL cells also persist for a longer time in the

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peripheral blood stream due to lack of normal activation of apoptosis (programmed cell death), possibly caused by DNA hypomethylation [2,3]. Presently, the prognosis for patients with CLL is based on two observations: (i) a short lymphocyte doubling time (less than 12 months) and/or (ii) the detection of basic chromosomal abnormalities such as trisomy 12. Both procedures are time- and labor-intensive. Since any change in these structurally modified cells should be reflected in their infrared spectra, a multivariate statistical analysis of these spectra should provide access to such information.

2. Methodology

Peripheral lymphocytes, free of monocytes and T-cells, were isolated from blood samples using a Ficoll–Hypaque density gradient [4]. The lymphocytes were then washed twice with saline to remove the medium and concentrated by centrifugation. A drop of 5 μL of the cell suspension, containing about 3×10^3 cells, was placed on an infrared-transparent BaF_2 window and dried down under mild vacuum. The homogeneity of this thin film was checked by infrared microscopy. In a separate set of experiments, a concentrated cell-suspension from the same sample was placed between two CaF_2 windows. All IR measurements were performed on an FTS-60 Biorad spectrometer with an MCT detector, at a nominal resolution of 4 cm^{-1} (with triangular apodization) using 256 co-added scans.

Cluster analysis was performed using Ward's minimum variance algorithm and Euclidean distances as distance measure [4]. Linear discriminant analysis was performed using the optimal region selection algorithm with cross-validation, and applying the stringent leave-one-out method [5].

3. Results

Representative spectra of normal and leukemic lymphocytes are shown in Fig. 1. Traces a and b represent, respectively, spectra of normal and CLL cells obtained from dried films while traces c and d are spectra obtained from cell suspensions. The

Fig. 1. Infrared spectra of normal (a,c) and leukemic (b,d) lymphocytes. Traces a and b represent spectra of dried films of CLL cells, traces c and d are spectra of CLL cells in suspension. Trace e is part of a pure bovine DNA spectrum.

Fig. 2. Relative DNA content found in CLL cells of two different patients during a year of disease. Bottom panel: marker band for DNA double strands. Top panel: bar diagram (each from three separate measurements) of the integrated intensity of the DNA marker band.

Fig. 3. Dendrogram representation of normal lymphocytes and CLL cells, created by cluster analysis of first derivative spectra in the 900–1300 cm⁻¹ region. The five panels A, B, C, D and E show the mean spectra of each sub-cluster.

spectral profile of isolated lymphocytes contains regions characteristic for lipids (I), proteins (II) and DNA (III). The features between 800 and 1300 cm⁻¹ are almost pure DNA bands, as is apparent from a comparison with trace e, the spectrum of bovine DNA. In addition, there is a DNA band at 1717 cm⁻¹ which is often used as a marker characteristic for base pairing in DNA double strands. As shown in Fig. 2, this band can be conveniently used for following and quantitating the amount of DNA in leukemic cells. When monitored over time, this band can indicate (for the same patient) DNA stability or significant changes in the leukemic cell population, which in turn can be related to a change in the cell-doubling time. The bar diagrams for the two patients in Fig. 2 demonstrate that the relative amount of DNA

can be constant or it can continuously increase over a period of time, which in turn correlates either with a stable or with a more aggressive form of the disease. The overall differences between the spectra of normal lymphocytes and CLL cells are clearly evident from the nature of their DNA bands, as is illustrated in Fig. 3. The cluster analysis performed on spectra of normal and CLL cells clearly separates these two classes. Interestingly, the CLL spectra fall into a number of stable subclusters, suggesting that infrared spectroscopy can detect small differences within the leukemic cell population. Linear discriminant analysis was performed on the same set of spectra to determine whether there is a correlation between the spectral information and the doubling-time of the CLL cells (see Table 1). In order to establish the

Table 1
LDA-based prediction of doubling time from IR spectra of CLL cells

Entire Data Set (92)	Slow	Progressive	Accuracy (%)
Slow	<u>58</u>	1	98.3
Progressive	5	<u>28</u>	84.8
Training Data Set (55)	Slow	Progressive	Accuracy (%)
Slow	<u>41</u>	0	100
Progressive	0	<u>14</u>	100
Validation Data Set (61)	Slow	Progressive	Accuracy (%)
Slow	<u>38</u>	6	86.4
Progressive	5	<u>12</u>	70.6

The range selected for LDA by the Optimal Region Selection algorithm comprised 30 data points in the region 900–1300 cm⁻¹.

percentage of positive classification, the spectra were subdivided into training and validation sets. Each set had two group classifiers, spectra recorded from cells with doubling times above or below 12 months. The results were 100% correct classification for the training set and 82% correct classification for the validation set, a clear indication of a strong correlation between the doubling time and the spectral character of the CLL cells.

4. Medical impact

The present course of establishing a prognosis for CLL patients is based on a short lymphocyte doubling-time, usually over the period of a year. Instead of having to wait months for the results of the doubling time of CLL cells, infrared spectroscopy, coupled with a multivariate statistical analysis, may be able to provide this information within a much shorter time. Furthermore, spectra of CLL cells

obtained from the same patient over a given period of time could be used to determine whether the DNA content increases or whether it remains stable (high or low) over time. This can be associated with an aggressive or a less aggressive form of the disease, and thus become a useful parameter in a clinical setting.

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