TEXTURE IN THYROID ULTRASONOGRAPHY AND ITS RELATION TO BCL-2 PROTEINS IN PATIENTS WITH BREAST CANCER AND AUTOIMMUNE THYROID DISEASES

D. Smutek, J. Jiskra

3rd Department of Medicine, 1st Medical Faculty, Charles University, Prague, Czech Republic

smutek@cesnet.cz

Abstract: Properties of ultrasound images of thyroid gland in 11 subjects with breast cancer and autoimmune thyroid diseases were compared with antiapoptotic protein Bcl-2 serum levels. Statistical texture features were used for ultrasound description. A high correlation with coefficient of determination 0.89 between four texture features and Bcl-2 levels was found. The significant correlation was detected in both groups of women. These findings suggest the possibility of using of quantitative ultrasound indicators as an attribute for evaluating the degree of thyroid apoptosis in patients with thyroid autoimmune diseases, similarly as the Bcl-2 serum levels may be used as a laboratory marker. The significant correlation in women with breast cancer could indicate a possible suppression of apoptosis of thyrocytes and thyroid lymphocytes, which may play an important role in thyroid autoimmunity initiation. Finally, because the higher expression of Bcl-2 is probably associated with longer overall survival in women with breast cancer and the strong positive correlation between Bcl-2 and texture features was found, there is a possibility that the changes in ultrasound image of the thyroid gland (found in patients with autoimmune thyroid diseases) may have a relation to the prognosis of women with breast cancer.

Introduction

Autoimmune thyroid diseases are chronic inflammations of the thyroid gland [1]. They usually lead to hypothyroidism, which causes serious health problems to the patient. The autoimmune process in the gland changes the structure of the thyroid tissue. The changes can be either focal or, most often, diffuse. The tissue changes can be mostly detected by an established method in the diagnosis of thyroid disorders - by ultrasonography [2-4]. The disease can also be diagnosed from clinical examination, hormonal and immunological analyses of blood, and from cytological examination using fine needle aspiration biopsy.

Bcl-2 family proteins are important regulators of apoptosis. Apoptosis play an important role in pathogenesis of both autoimmune and oncological diseases. Dysregulation of apoptosis may be involved in the development of subacute thyroid inflammation [5] and is associated with the pathogenesis of organ-specific autoimmune diseases, through altered target organ susceptibility [6]. The differential expression of Bcl-2 family proteins in both thyrocytes and lymphoid follicles may be involved in the pathology of Graves disease [7]. Thyrocytes in Graves diseases express higher levels of Bcl-2 compared with thyrocytes in lymphocytic thyroiditis. The opposite pattern was observed in thyroid lymphoid follicles: low Bcl-2 in Graves diseases and high Bcl-2 in lymphocytic thyroiditis [6]. Consistently, thyrocyte apoptosis was marked in lymphocytic thyroiditis and poor in Graves disease thyroids, and thyroid-infiltrating lymphocytes apoptosis was marked in Graves diseases and poor in lymphocytic thyroiditis [6]. In patients with breast cancer, higher expression of Bcl-2 is probably associated with longer overall survival and relapse-free survival in women with breast cancer [8].

The aim of the study was to evaluate a relation between quantitative descriptors of thyroid ultrasound image and serum levels of Bcl-2 as a possible marker of better survival in women with breast cancer and as a marker of apoptosis in Graves diseases and lymphocytic thyroiditis.

We have previously shown that the information related to diagnosis of autoimmune thyroid disease can be adequately extracted from sonographic images of thyroid gland and that it can be further used as quantitative (objective) indicator of sonographic examination [9] or even for the automatic classification of thyroid inflammations [10-11]. For the quantitative characteristics of ultrasound images description we use statistical texture features.

Subjects and Methods

Subjects: We investigated 11 randomly chosen women with breast cancer (5 patients) and with autoimmune thyroid disease such as lymphocytic thyroiditis or Graves disease (6 patients). The patients were diagnosed by clinical examination and laboratory tests.

Family and personal history with respect to autoimmune, endocrine and oncological diseases were obtained from the subjects, and they all underwent clinical examination and thyroid ultrasonography. An informed verbal consent was obtained from all

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participants and the procedures followed were in accordance with the ethical standards of our institution's committee on human experimentation.

Image Acquisition: A sonographic imaging system Philips Envisor M254DA equipped with an 8-MHz linear probe was used for acquisition of B-mode ultrasound images. In order to determine reproducible echo levels, constant sonographical operating conditions were defined. The images were directly saved from ultrasound scanner in DICOM format. MATLAB software (The MathWorks, Inc., Nattick, MA, USA) was then used to analyze the digitized images.

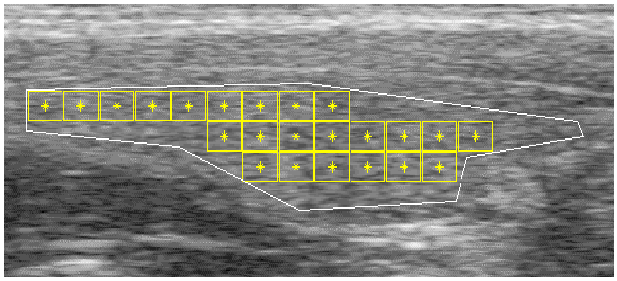

Figure 1: Sonographic image (longitudinal scan of right lobe) with drawn boundary of thyroid gland and texture samples (rectangular windows of 41x41 pixels).

Texture features: Co-occurrence matrices [12] were computed from a set of the non-overlapping fixed-size rectangular regions (referred as texture samples) obtained from a manually segmented thyroid gland (Figure 1).

For each *N*x*N* texture sample *W* taken from an image *I*, a set of gray level co-occurrence matrices $C_d(i,j)$ is calculated for a given separation vector $\vec{d}$ as follows:

$$C_{\vec{d}}(i,j) = \frac{1}{(N-a)(N-b)}$$

$$card\left\{\left(\vec{r},\vec{r}+\vec{d}\right): \vec{r},\vec{r}+\vec{d} \in W \text{ and } \begin{array}{l} I(\vec{r}) = i \text{ and } \\ I(\vec{r}+\vec{d}) = j \end{array}\right\}$$

where $\vec{d}$ =(*a*,*b*), $I(\vec{r})$ is the gray level of pixel $\vec{r}$, from the interval of 0,1,…, *G*-1. Optimal histogram resolution, *G* = 64, according to Scott's rule [13] was used for the corresponding feature vector dimension and the number of samples. *Card X* is the size of the set *X*. The elements of C_d represent the frequencies of occurrence of different gray level combinations at a distance $\vec{d}$.

The texture features (see the definition in Table 1) were computed from the co-occurrence matrices corresponding to twelve different separations $\vec{d}$. These features are of statistical nature and do not require any image pre-processing.

Table 1: Description of Haralick texture features [12]. Given separation vector $\vec{d}$, $C_{\vec{d}}(i,j)$ is the co-occurrence matrix, i.e. the 2-dimensional histogram of simultaneous occurrence of values *i* and *j* in all image pixel pairs separated by $\vec{d}$.

Feature name	**Definition**
Cluster tendency	$\sum_i \sum_j \left(i-\mu_i + j-\mu_j\right)^2 C_{\vec{d}}(i,j)$
Texture entropy	$-\sum_i \sum_j C_{\vec{d}}(i,j) \log C_{\vec{d}}(i,j)$
Texture contrast	$\sum_i \sum_j \lvert i-j \rvert C_{\vec{d}}(i,j)$
Texture correlation	$\frac{\sum_i \sum_j (i-\mu_i)(j-\mu_j) C_{\vec{d}}(i,j)}{\sqrt{\text{var(i)var(j)}}}$
Texture homogeneity	$\sum_i \sum_j \frac{C_{\vec{d}}(i,j)}{1+\lvert i-j \rvert}$
Inverse difference moment	$\sum_{i,i\neq j} \sum_{j,i\neq j} \frac{C_{\vec{d}}(i,j)}{\lvert i-j \rvert}$
Maximum probability	$\max_i \max_j C_{\vec{d}}(i,j)$
Probability of run length of 2	$\sum_i \frac{\left(C_i - C_{\vec{d}}(i,i)\right)^2 C_{\vec{d}}(i,i)}{\left(C_{\vec{d}}(i)\right)^2}$
Uniformity of energy	$\sum_i \sum_j \left(C_{\vec{d}}(i,j)\right)^2$

where $\mu_i = \sum_i \sum_j i C_{\vec{d}}(i,j)$;

$\mu_j = \sum_i \sum_j j C_{\vec{d}}(i,j)$; $\quad C_i = \sum_j C_{\vec{d}}(i,j)$

$\text{var}(i) = \sum_i \sum_j (i-\mu_i)^2 C_{\vec{d}}(i,j)$;

$\text{var}(j) = \sum_i \sum_j (j-\mu_j)^2 C_{\vec{d}}(i,j)$

$i, j = 0,1,\ldots,G-1.$

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Statistic: Forward stepwise multiple regression was carried out for evaluating the correlations between Bcl-2 serum level and texture features. The level of significance was taken as $p < 0.05$.

Results

Forward stepwise multiple regression (see Table 2) was performed for the serum level of Bcl-2 protein in ng/ml (dependent variable) and all image features (independent variables). Level of Bcl-2 protein correlated with Probability of run length of 2 with separation vector $\vec{d}$ (2,2) (beta coefficient -1,6), Texture homogenity with separation vector $\vec{d}$ (1,1) (beta coefficient 5), Texture correlation with separation vector $\vec{d}$ (4,0) (beta coefficient -0.3). Multiple R-Square (coefficient of determination) for this correlation was 0.89 (F-test 5.9; $p < 0.028$).

Table 2: Forward stepwise multiple regression for the serum level of Bcl-2 protein in ng/ml (*dependent variable*) and all image features (*independent variables*). R-Square (coefficient of determination) for this correlation was 0.89

Texture feature (independent variable)	**Separation vector $\vec{d}$**	**Beta coefficient**
Probability of run length of 2	(2,2)	-1,6
Texture homogenity	(1,1)	5
Texture correlation	(4,0)	-0.3

Discussion and Conclusion

It was recently proposed that Bcl-2 could inhibit cancer progression. Higher expression of Bcl-2 is probably associated with longer overall survival and relapse-free survival in women with breast cancer [8]. Similarly, the measurement of Bcl-2 serum levels in women with metastatic breast cancer showed a trend toward improvement of survival in patients with higher levels of Bcl-2 [14].

An extensive retrospective study of 9520 women with breast cancer in Massachusetts General Hospital has suggested that the survival rate of patients with contemporary autoimmune thyroid disorders is higher in comparison with women with healthy thyroid glands [15]. Similar results have been obtained in Smyth's and Shering's studies [16-17]. In a recent study, the presence of thyroid disease in Turkish women with breast cancer was associated with reduction of the number of metastatic lymph nodes, vascular invasion and tumor size [18]. However, there is not a sufficient prospective mortality study, and thus, the influence of the presence of autoimmune thyroiditis on the outcome of women with breast cancer is controversial.

In our previous study the significant negative correlation between tumor marker CA 15-3 and gray pixel value in B-mode ultrasound image and positive correlation between CA 15-3 and one of spatial texture feature (F7) were found. According to our opinion, increased gray pixel value and decreased F7 values (usually found in autoimmune thyroiditis) could be linked with the advanced stages of breast cancer [19].

The aim of the study was to evaluate a possible relations between quantitative descriptors of thyroid ultrasound image and serum levels of Bcl-2 as a possible marker of better survival in women with breast cancer and as a marker of apoptosis in Graves diseases and lymphocytic thyroiditis.

The significant correlation of the Bcl-2 serum levels and quantitative descriptors of thyroid ultrasound image was found in both groups of women – with autoimmune thyroid diseases and breast cancer. These findings suggest the possibility of using quantitative indicators of thyroid ultrasound image as a clinical attribute for evaluating degree of thyroid apoptosis in patients with thyroid autoimmune diseases, similarly as the Bcl-2 serum levels maybe used as a laboratory marker. The significant correlation of Bcl-2 serum levels and quantitative indicators of thyroid ultrasound image in women with breast cancer could indicate a possible suppression of apoptosis of thyrocytes and thyroid lymphocytes, which may play an important role in an initiation of thyroid autoimmunity.

Finally, because the higher expression of Bcl-2 is probably associated with longer overall survival in women with breast cancer [8] and the strong positive correlation of Bcl-2 and the statistical texture features was found, there is a possibility that the changes in thyroid ultrasound image (described by texture features) found in patients with autoimmune thyroid diseases may have a relation to the prognosis of women with breast cancer.

This finding leads us to future research work where we would like to verify a usability of this finding in a clinical practice.

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