Quantitative evaluation of immuno- and histochemical reaction intensity by spatial visualization techniques

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Abstract

The aim of this study was an application of spatial visualization techniques for quantitative measurements of immuno- and histochemical reactions. For a quantitative histochemical study, specimens, collected from patients with chronic gastritis, were stained with paS/AB, while for immunohistochemical evaluation, specimens were used, collected from patients with chronic parathyroiditis and were analyzed with Ki-67 proliferation marker and apoptosis bcl-2 protein. The new technique permitted to obtain quantitative objective results. Statistical cluster analysis of those results indicated small groups of cases for reevaluation and supported the final diagnosis.

Key words: quantitative histochemistry, measure of marker expression, spatial image analysis.

Introduction

Quantitative measurement of the intensity of immuno- and histochemical staining is a significant problem in pathomorphological diagnosis. Routinely, visual evaluation is performed in light microscopy and semi-quantitative assessment of reaction strength is expressed in scores, usually from 0 to +++. In order to improve the objectivity of assessment, a computer technique was developed for raw colour images, extended to three-dimensional space by introducing image intensity as the third dimension [1]. In our study, this method was used for cases of intestinal metaplasia, illustrating the evaluation of histochemical reaction, and for cases with hyperparathyroidism, presenting the application for immunohistochemical investigation.

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Material

For quantitative histochemical study, twenty needle biopsy specimens, obtained from patients with chronic gastritis, were stained with paS/AB pH 2,5 (Fig. 1). For quantitative immunohistochemical evaluation, our study was conducted on resected goitres from patients with chronic parathyroiditis. Immunohistochemical staining was performed for apoptosis bcl-2 protein (Dako). Proliferating cells were detected by immunostaining for Ki-67 (Dako). Images of 640x480 pixels each (at 400x magn.), were acquired by the use of a digital light microscope, running under the Motic Images v. 1.2 software (Micro Optic Industrial Group Co) for Windows.

Methods

The acquired images were visualized in three-dimensional space by introducing image brightness as the third dimension. The colours of immuno- or histochemical reactions were then exposed by reducing the remaining elements to the background with the use of three filters. A filter of brightness extracted the pixels with brightness, exceeding the defined threshold value and corresponding to the colour of the reaction. The remaining elements were reduced. The second, colour filtering, gave pixels with colours compatible with the reaction, present in histological specimen. Third, a filter of saturation was used, removing reaction free elements. The filters were fixed for each series of images acquired from a specimen. The surface of extracted positive reaction was computed as the total number of pixels in colours of the reaction. In order to compute the reaction volume, its spatial representation was divided into small prisms and

Table 1. Quantitative assessment of paS/AB in cases of intestinal metaplasia

Intestinal metaplasia	Group I (n=13)	Group II (n=6)	p-level
Measurements	Mean ± SD	Mean ± SD	
Area of paS/AB [per 1 μm^2]	0.021 ± 0.014	0.079 ± 0.032	p<0.001
Expression of paS/AB	86 ± 10	93 ± 17	ns

Table 2. Area and expression of Ki-67 and bcl-2 in cases of parathyroiditis

Hyperparathyroidism	Hyperplasia		Adenoma		p-level
Measurements	Mean ± SD	Min-Max	Mean ± SD	Min-Max	
Area of bcl-2+ [per 1 μm^2]	0.077±0.041	0.010-0.122	0.087±0.006	0.039-0.125	ns
Expression of bcl-2+	125 ± 12	100 - 137	130 ± 3	125 - 134	ns
Area of Ki-67+ [per 1 μm^2]	0.004±0.002	0.001-0.007	0.006±0.002	0.002-0.009	p<0.05
Expression of Ki-67+	152 ± 22	113 - 180	144 ± 23	101 - 169	ns

Table 3. Clustering of hyperplasia and adenoma cases in parathyroiditis.

	Area of Ki-67+ [per 1 μm^2]	Expression of Ki-67+	Area of bcl-2+ [per 1 μm^2]	Expression of bcl-2+
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Hyperplasia (cluster I)	0.003 ± 0.002	117 ± 14	0.143 ± 0.052	130 ± 17
Hyperplasia (cluster II)	0.004 ± 0.003	165 ± 13	0.038 ± 0.029	123 ± 13
Adenoma (cluster I)	0.006 ± 0.005	161 ± 13	0.157 ± 0.038	134 ± 6
Adenoma (cluster II)	0.005 ± 0.004	113 ± 20	0.069 ± 0.029	128 ± 5

pyramids. The volume of each solid shape was computed. The reaction volume was derived as the sum of all prism and pyramid volumes. The expression of the reaction was assessed as the ratio of its volume divided by its surface. Image processing was performed by the use of a computer program, designed and programmed in C++ language by Strzelczyk [1]. The obtained results were compared between groups by the Mann-Whitney test. A consistency between semi-qualitative evaluation of specimens (Sydney scale) and the computed spatial technique was assessed by Spearman's correlation coefficient. Cluster analysis (the K-means method) was also performed to assess homogeneity of the results, based on the following two features: the area and colour intensity of investigated reactions. The statistical analyses were carried out with the Statistica PL v.6 (StatSoft Inc.) computer program.

Results

The area of paS/AB in intestinal metaplasia was, on the average, 0.039 ± 0.034 per 1mm^2 and ranged from 0.002 to 0.138 per 1mm^2. The intensity of reaction colour measured in 256 colours' scale was on average 88 ± 12 and ranged from 75 to 112. K-means clustering showed a non homogeneity of results and revealed two groups among them. (Table 1). The area of paS/AB per 1 μm^2 was significantly different in those groups, while the intensity of reaction was similar.The area of paS/AB reaction correlated well with the Sydney scale assessment (r=0.759, p<0.05) in Group I, while it was not correlated in Group II (r=0.086, p<0.05). In immunohistochemical studies of hyperparathyroidism cases, a significant difference was found between parathyroid hyperplasia and adenoma for Ki-67+ (Table 2). The analysis of area and of the expression of Ki-67 and bcl-2 by the use of K-means clustering showed heterogeneity of cases with parathyroiditis. Adenoma cases were divided into two clusters. Similarly, the cases with hyperplasia were also divided into two subgroups (Table 3).

Final remarks

The technique of spatial visualization allowed us to obtain quantitative and more objective results. Cluster analysis indicated a small group of cases with intestinal metaplasia, showing advanced changes, associated with additional disease symptoms. Quantitative immunohistochemical analysis, performed in our study in chronic hyperthyroiditis, permitted to find groups of cases with hyperplasia and adenoma, showing a minor intensity and area of the markers. That was helpful in the final diagnosis. The evaluation by the the use of spatial techniques, as presented in this paper, is more objective than semi quantitative evaluation [2].

Figure 1. Reaction paS/AB in intestinal metaplasia

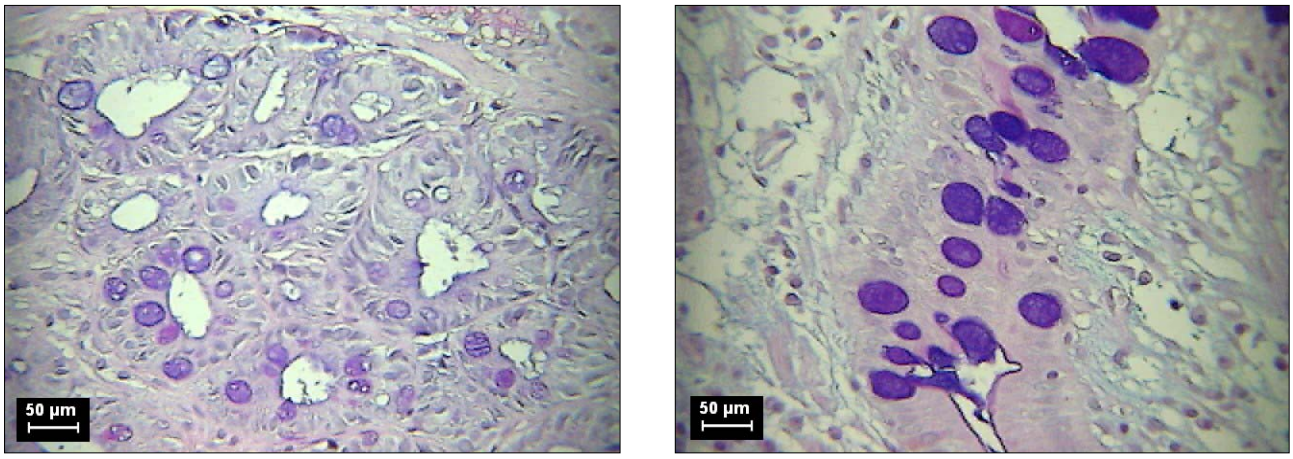


Figure 2 Spatial representation of paS/AB in Figure 1.

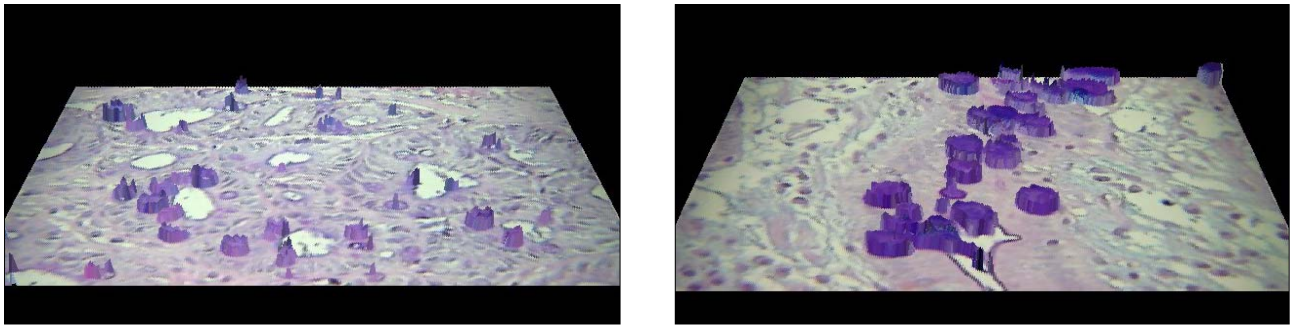


Figure 3. Orthogonal projection of paS/AB (Fig. 2) onto the plane

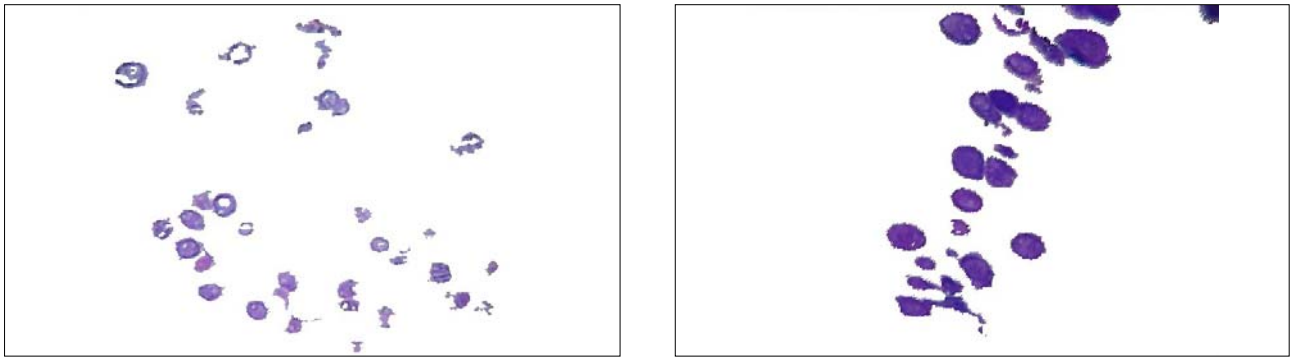


Figure 4. Expression of bcl2 in adenoma case (left) and the segmented reaction (right).

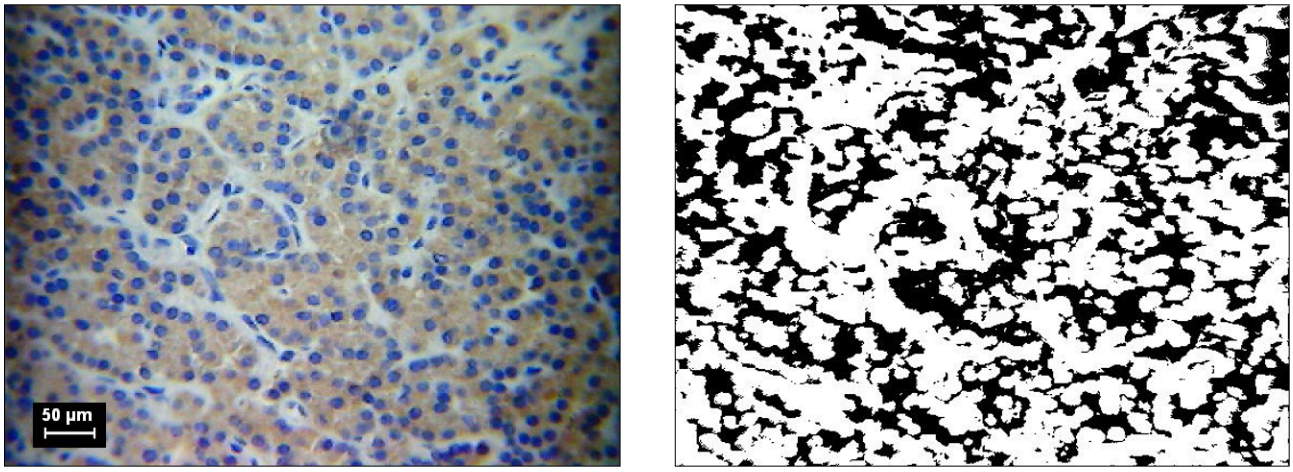
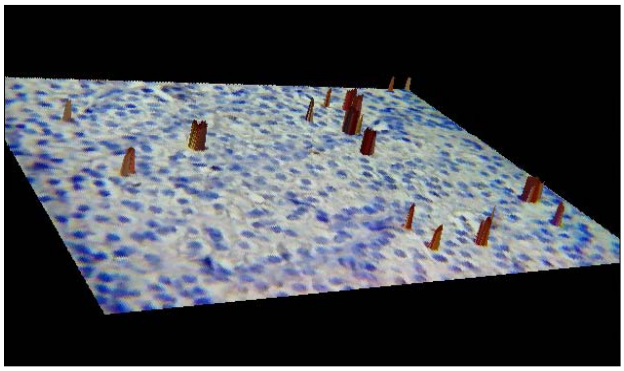
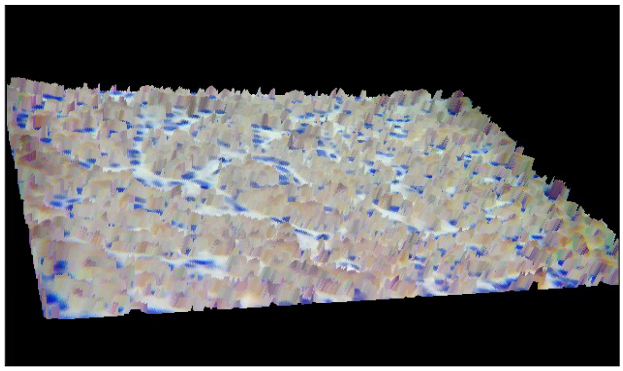


Figure 5. Spatial visualization of Ki-67 (left) and bcl-2 (right) in chronic parathyroiditis.





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