ORIGINAL PAPER

Morphometric parameters and silver stain used in diagnosis of thyroid follicular diseases

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Abstract

Fine needle aspiration biopsy (FNAB) of the thyroid is limited in distinguishing hyperplastic nodular goiter from true follicular neoplasm and in separating follicular adenoma from follicular carcinoma. The present study was done to evaluate if the morphologic and morphometric investigations and silver staining of nucleolar organizer regions (NORs), either alone or in association would help to differentiate the thyroid follicular diseases. 40 FNAB smears of thyroid follicular diseases, histopathologically diagnosed as nodular goiter, lymphocytic thyroiditis, follicular adenoma and follicular carcinoma, were analyzed using the standard cytological exam, quantitative analysis and NORs assessment. The qualitative evaluation, correlated with the numerical results obtained from the quantitative analysis, revealed that the cellular pattern, mean nuclear diameter and NORs area are valuable criteria in the diagnosis of the benign and malign follicular lesions, respectively. The results attained through morphometry increase the sensibility and the specificity of FNAB in the diagnosis of thyroid follicular carcinomas.

***Keywords*:** fine needle aspiration biopsy, thyroid follicular neoplasm, morphometry, NORs.

Introduction

During the last decade, the importance of fine needle aspiration biopsy (FNAB) has notably grown, coming to be considered the most accurate method in the management of thyroid nodular diseases. The percentage of patients guided to thyroidectomy has come down by 25%, while the cases of carcinoma that were surgically excised have increased by 15–30% [1–4].

The most difficult problem concerning the cytological diagnosis is making the difference between the follicular injuries (adenomas and follicular carcinomas). This is why these lesions are usually described as follicular neoplasms or thyroid follicular diseases, term introduced by Löwhagen T in 1979 [5], which includes hyperplastic nodules, follicular adenomas and follicular carcinomas. Beginning with the 80', many researchers have tried to increase the efficiency of thyroid FNAB's, by introducing new methods of evaluation (imunocytochemistry, morphometry or cytochemistry) [6–10].

The current study focus on the evaluation of thyroid follicular diseases (hyperplasias, adenomas, chronic lymphocytic thyroiditis and follicular carcinomas), through a panel of morphological, morphometrical and special stains (silver stain) for identification of the nucleolar organizer regions (NORs), in the FNAB.

The originality of the study consists of using the same smears for the morphometrical and the cytochemical methods, the smears initially colored through the classical May-Grunwald Giemsa (MGG) method being discolored and argentically stained afterwards.

Material and methods

The study group was composed of 40 cases of thyroid follicular diseases diagnosed in the Department of Pathology of the „Sf. Spiridon" University Hospital Iassy during five years (1999–2003).

The selection of the cases was based on the following criteria: all the cases were histopathologically confirmed as thyroid follicular diseases and were expelled from the cytological study the cases with oncocytic (oxyphilic) metaplasia (Hürthle cells) which showed obvious nuclear anomalies and inequalities, while excluding the errors of statistical analysis.

We considered for each analyzed case: the morphological characters (the cellularity and the presence of the colloid), the dominant cellular arrangement ("honeycomb", syncytial, microfollicular, or isolated patterns), morphometric features (nuclear diameter and nuclear area [11]).

The cellularity of the smears was classified in four categories: rich, moderate, poor and acellular smears,

depending on minimum quantity accepted in literature – five or six cellular groups, with well preserved follicular cells, each group containing around 10 cells [1, 12, 13].

In the selected smears, we have enough cellular material for the morphological interpretation, for the morphometrical analysis and, further on, for the accomplishment of the cytochemical method.

The 40 smears were standard colored with MGG stain. To evaluate the usefulness of the NORs stain, the smears were decolorized with a mixture of ethylic alcohol and 1% chlorhydric acid, fixed in ethylic alcohol 95% and rehydrated in alcohol 80%, for 15 minutes afterwards. There followed the stage of the washing in bidistilled dedurised water (10 minutes). The technique we applied was similar to the method of the silver stain on smears [14]. Additionally, we realized the coloring of the nuclei with methyl green for their better visualization.

The microscopical images were acquired with a Nikon Eclipse 600 microscope, endowed with a Nikon DN 100 video camera, by using the Lucia Net software (running on the Department of Pathology, "Sf. Spiridon" University Hospital, Iassy).

The images were made with 400x magnification, and the measurements were carried out on at least 15–20 cells per each microscopic field, with a total of 50 cells for each case. Concerning the silver stain, 50 cells were randomly chosen for each case and NORs were visualized with 100× magnification (with immersion). We counted the number of NORs per nucleus and computed the total NORs and nucleus areas, respectively. The marking of the nuclear contours was hand-made, and the measurement of the diameters and of the nuclear areas was made automatically, using the MICROIMAGE software (IMAGE ANALYSIS SOFWARE).

The results obtained for each category of lesions were statistically processed with EXCEL and EPIINFO functions (t–Student test, χ^2 test).

Results

Synoptic presentation of the morphological and morphometrical characteristics of the studied groups

The diagnosis of the 40 cases, based on the pathological exam, was nodular goiter – 11 cases, adenoma – 10 cases, lymphocytic thyroiditis – nine cases and follicular carcinoma 10 cases.

The analyzed cases of **nodular goiter** presented, from a qualitative point of view, rich or moderate cellularity of the smears (81.8%); the colloid was loose, abundant, palely basophilic, and represented the background of the smears in four cases (36.4%). The dominant cellular arrangement was the honeycomb pattern – seven cases (63.63%), microfollicular – three cases (27.27%) and had a syncytial pattern in one case (9.1%). We did not identify any isolated cellular arrangement.

Table 1 presents, in addition, the qualitative aspects and the numerical results of the quantitative analysis.

Table 1 – ***The studied cases of nodular goiter***

Case	Sex	Age	Cellularity	Colloid	Pattern	Measurement values for nuclei (pixels)		
						diameter	*area*	*ratio*
1	f	74	1	2	honeycombs	107.9 ± 11.5	8515.9 ± 1598.5	78.8
2	f	41	2	3	honeycombs	90.1 ± 14.9	5370.6 ± 1701.8	59.7
3	f	44	2	1	microfollicular	61.8 ± 23.3	2751.8 ± 2011.3	44.4
4	m	36	1	1	microfollicular	88.5 ± 14.0	5087.2 ± 1488.3	57.2
5	f	53	2	2	microfollicular	88.7 ± 10.8	5415.4 ± 1251.2	60.8
6	f	56	3	3	honeycombs	100.0 ± 13.5	6788.1 ± 1834.5	67.9
7	f	45	3	3	honeycombs	118.2 ± 18.0	9400.9 ± 2971.8	79.7
8	m	41	2	3	honeycombs	96.6 ± 16.7	6040.1 ± 2307.1	62.3
9	f	64	2	2	honeycombs	104.0 ± 17.6	6898.1 ± 2032.2	66.3
10	f	58	3	2	syncytial	108.7 ± 22.5	8062.3 ± 4015.0	73.9
11	f	64	3	2	honeycombs	100.7 ± 16.9	6488.6 ± 2100.5	64.2
Total						95.4 ± 20.8	6253.2 ± 2710.6	65.8

The microscopic images, illustrating the morphometrical analysis, are presented in the Figures 1 and 2.

By using the statistical methods of calculation in the cases of nodular goiter, the correlation coefficient had the value r = 0.92, which demonstrated the strong connection between the nuclear diameter and the nuclear area. We have also observed strong statistical correlation in all cases between the nuclear area and the NORs area, as well as between the number of NORs and their medium size ($p < 0.001$). In the analyzed cases of **adenoma,** the cellularity of the smears was rich or moderated in 90%, while the colloid was abundant or moderately present in seven cases.

The most frequent cellular pattern was the microfollicular one (50% of the cases), followed by the one in "honeycomb" present in four cases (40%), and isolated – in one case (10%). No case was identified in which the dominant arrangement of the follicular epithelial cells would be syncytial.

Table 2 presents, in addition, the qualitative aspects and the numerical results of the quantitative analysis.

The microscopic images, illustrating the morphometrical analysis, are presented in the Figures 3 and 4.

In these cases, the value of the correlation coefficient (r = 0.94) demonstrated the strong positive correlation between the diameter and the area of the

nuclei. We obtained statistically significant correlation between the nuclear area and the NORs area, as well as between the number of NORs and their mean area ($p < 0.001$).

Table 2 – *The studied cases of follicular adenoma*

Case	Sex	Age	Cellularity	Colloid	Pattern	Measurement values for nuclei (pixels)		
						diameter	*area*	*ratio*
1	f	36	2	3	honeycombs	88.7 ± 10.5	5130.9 ± 1220.7	57.7
2	f	34	2	2	microfollicular	118.0 ± 19.6	8716.3 ± 2218.9	73.9
3	f	52	2	3	honeycombs	97.6 ± 11.8	5966.5 ± 1609.9	60.9
4	f	40	1	2	microfollicular	90.0 ± 9.2	5455.2 ± 810.5	60.6
5	m	64	3	2	microfollicular	100.5 ± 19.9	6704.3 ± 2174.9	66.4
6	f	45	3	2	honeycombs	106.2 ± 14.5	7758.8 ± 2224.4	73.2
7	f	63	3	1	isolated	83.4 ± 7.4	4929.5 ± 770.8	59.4
8	f	34	3	1	microfollicular	111.9 ± 9.4	8776.4 ± 1392.3	78.4
9	f	50	2	2	honeycombs	74.3 ± 8.1	3838.0 ± 840.0	51.9
10	f	35	2	1	microfollicular	106.2 ± 10.1	8001.8 ± 1996.6	75.5
Total						97.9 ± 18.6	6625.3 ± 2374.4	67.6

In the analyzed cases of **lymphocytic thyroiditis**, the smears situated themselves between rich (five cases – 55.6%) or moderate (four cases – 44.4%), while the colloid was abundant in 55.6% of the cases. The most frequent cellular arrangement was that of "honeycomb" (five cases – 55.5%), followed by the isolated pattern (two cases – 22.2%) and by the microfollicular and syncytial cellular arrangement, one case each (11.1%).

Table 3 presents, in addition, the qualitative aspects and the numerical results of the quantitative analysis.

Table 3 – *The studied cases of lymphocytic thyroiditis*

Case	Sex	Age	Cellularity	Colloid	Pattern	Measurement values for nuclei (pixels)		
						diameter	*area*	*ratio*
1	f	37	2	1	honeycombs	99.7 ± 19.2	6669.8 ± 2664.8	66.7
2	f	36	2	2	microfollicular	67.7 ± 9.5	3020.5 ± 976.3	44.4
3	f	47	2	1	honeycombs	109.1 ± 20.5	8353.4 ± 3383.2	76.6
4	f	59	3	3	honeycombs	102.1 ± 13.9	7724.4 ± 1839.6	75.7
5	f	48	3	2	isolated	102.6 ± 14.8	7780.2 ± 1985.8	75.7
6	f	45	3	1	honeycombs	102.0 ± 10.0	7118.0 ± 1252.5	69.8
7	f	56	3	2	honeycombs	90.1 ± 8.3	5663.7 ± 1016.2	62.9
8	f	59	2	1	isolated	115.0 ± 12.7	9210.7 ± 1582.6	80.1
9	f	50	3	1	syncytial	100.2 ± 20.4	7211.3 ± 2969.1	72.1
Total						97.8 ± 17.9	6766.4 ± 2470.8	69.0

The microscopic images, illustrating the morphometrical analysis, are presented in the Figures 5 and 6. The value of the correlation coefficient, $r = 0.94$, demonstrated the strong correlation between the diameter and the area of the nuclei. We obtained statistically significant correlation between the nuclear area and the NORs area, as well as between the number of NORs and their mean area ($p < 0.001$).

The 10 cases chosen to illustrate the **malign thyroid pathology** presented either rich smears (six cases – 60%) or moderate ones (four cases – 40%). The colloid was missing in 30% of the cases, and the dominant cellular architecture was the syncytial one (five cases – 50%), followed by the microfollicular pattern (three cases – 30%). We have not identified the „honeycomb" arrangement in any case.

Table 4 presents, in addition, the qualitative aspects and the numerical results of the quantitative analysis.

Table 4 – *The studied cases of follicular carcinoma*

Case	Sex	Age	Cellularity	Colloid	Pattern	Measurement values for nuclei (pixels)		
						diameter	*area*	*ratio*
1	f	62	3	2	syncytial	112.9 ± 12.7	7785.8 ± 1425.4	68.9
2	f	21	2	1	microfollicular	103.3 ± 15.5	7553.0 ± 2105.2	73.3
3	f	70	2	0	syncytial	112.6 ± 10.4	8282.5 ± 1470.2	73.3
4	f	41	2	0	syncytial	110.7 ± 6.97	7900.1 ± 1212.1	71.2
5	f	22	3	2	syncytial	96.4 ± 10.3	6281.6 ± 1342.0	65.4
6	f	26	3	1	microfollicular	102.3 ± 8.17	7375.3 ± 1084.9	72.3
7	f	32	2	2	syncytial	104.2 ± 7.15	7883.6 ± 869.1	75.8
8	m	52	2	1	isolated	106.8 ± 10.5	8005.7 ± 1659.8	74.8
9	f	61	2	0	isolated	102.8 ± 11.8	7460.9 ± 1651.7	72.4
10	m	54	3	1	microfollicular	117.4 ± 12.0	9459.4 ± 1709.2	80.8
Total						108.9 ± 16.5	8087.0 ± 2507.8	74.2

The microscopic images, illustrating the morphometrical analysis, are presented in the Figures 7 and 8.

In follicular carcinoma as well the correlation coefficient (r = 0.92) demonstrated the strong correlation between the diameter and the area of the nuclei. A positive correlation was observed (r = 0.34) between the **area of the nuclei** and the **area of NORs,** and an inverse correlation (r = -0.46) between the mean value of the NORs and their number.

The calculation of the NORs area shows the converse proportionality between their number and the determined area.

Discussions

Value of the quantitative analysis in the differential diagnosis of the thyroid follicular diseases

The numerical results obtained through morphometry provided interdependency between the carcinoma and the rest of the thyroid nodular lesions. By using the t-Student test, we revealed statistically significant differences between the tumoral pathology (follicular carcinoma) and other categories of thyroid nodular diseases.

The statistical analysis showed the fact that the nuclear area and the nuclear diameter, in the cases of follicular carcinoma, are significantly larger than in the case of any other type of thyroid nodular diseases.

The area of NORs and the nuclear area have showed the same statistical significance, while the number of NORs has showed a statistical significance only in the case of thyroid inflammatory diseases (Table 5).

Evaluation of the personal results, in comparison with similar studies reported in literature

Even though the data of the specialized literature confirm a high level of accuracy for the FNAB in the thyroid diseases, there are still discussions concerning the incapacity of giving a **final malignity diagnosis** for the thyroid follicular lesions, only on the basis of the morphological characteristics [15].

Table 5 – ***Statistical differences between carcinoma and other thyroid nodular diseases***

Measurement values	Thyroid diseases		t-Student; p
	Carcinoma (603)	***Nodular goiter (511)***	
Nuclei area (pixels)	35423 ± 11926	30515 ± 11482	6.18; $p < 0.001$
NORs area (pixels)	1533.1 ± 898.7	1065.0 ± 745.0	9.50; $p < 0.001$
NORs number	2.1 ± 1.0	2.2 ± 1.2	1.49; N S
Mean NORs area (pixels)	864.8 ± 691.1	632.8 ± 534.6	6.31; $p < 0.001$
	Carcinoma (603)	Adenoma (676)	
Nuclei area (pixels)	35423 ± 11926	30760 ± 13523	6.55; $p < 0.001$
NORs area (pixels)	1533.1 ± 898.7	1048.6 ± 700.4	10.66 ; $p < 0.001$
NORs number	2.1 ± 1.0	2.0 ± 1.0	1.79; NS
Mean NORs area (pixels)	864.8 ± 691.1	656.4 ± 602.9	5.71; $p < 0.001$
	Carcinoma (603)	Thyroiditis (545)	
Nuclei area (pixels)	35423 ± 11926	24746 ± 16446	12.48; $p < 0.001$
NORs area (pixels)	1533.1 ± 898.7	1116.7 ± 1374.3	6.01; $p < 0.001$
NORs number	2.1 ± 1.0	1.2 ± 0.5	19.56 ; $p < 0.001$
Mean NORs area (pixels)	864.8 ± 691.1	964.1 ± 849.3	2.16; $p < 0.05$

The multiple secondary changes founded in nodular goiter are well known. So, there are nodules composed only of large, cystic dilated follicles, with a flattened epithelium, while other nodules are densely and present, sometimes, solid areas as well. If the aspiration involved such areas, the differential diagnosis with a follicular adenoma or with a follicular carcinoma is difficult.

The follicular adenomas, in their turn, are classified in four categories: macrofollicular, mediofollicular, microfollicular and trabecular, and the cytological material obtained through FNAB fluctuate according to their cytological type.

The different histopathological aspects in the follicular carcinomas represent a nightmare for the cytologist. The carcinomas can be well differentiated, if formed out of "normal" follicles, or poorly differentiated, with frequent solid and trabecular areas. As in the follicular carcinomas the polarity is lost in the follicles, there appears a syncytial or microfollicular aspect.

The great majority of studies on cellular distribution show the presence of the „honeycomb" pattern in the nodular goiter, in the thyroiditis and in the follicular adenoma, while it is missing in the smears that belong to the follicular carcinomas [16, 17].

In our study, the „honeycomb" pattern was present with a percentage of 63.63% in the nodular goiter, of 40% in the adenoma, of 55.6% in the thyroiditis and was absent in the cases of carcinoma, in which the syncytial (50%) and microfollicular (30%) patterns dominated.

The data we obtained are similar to those reported in the literature [18].

The syncytial pattern represents densely cellular areas, made out of disorganized follicles. For this pattern, the information is contradictable: Kini SR *et al.* [16], concerning a studied series, do not report any case of adenoma with a syncytial pattern, while Ravinsky E and Safneck JR [17] find this pattern in all studied categories.

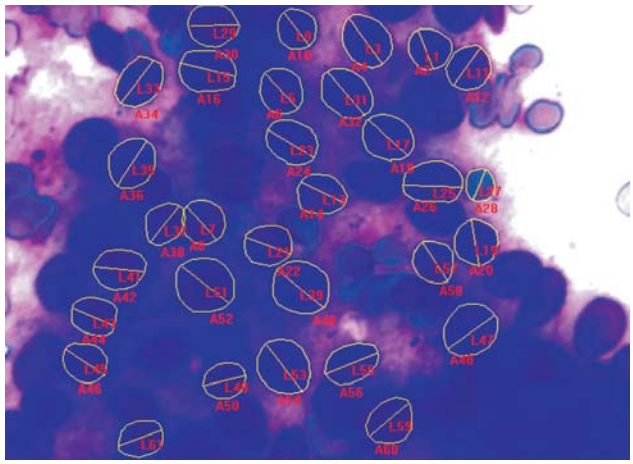

Figure 1 – ***Nodular goiter (MGG, ×400)***

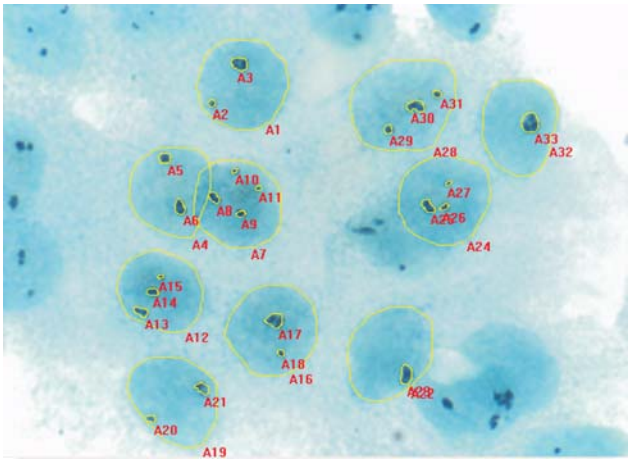

Figure 2 – ***Nodular goiter (silver stain, ×1000)***

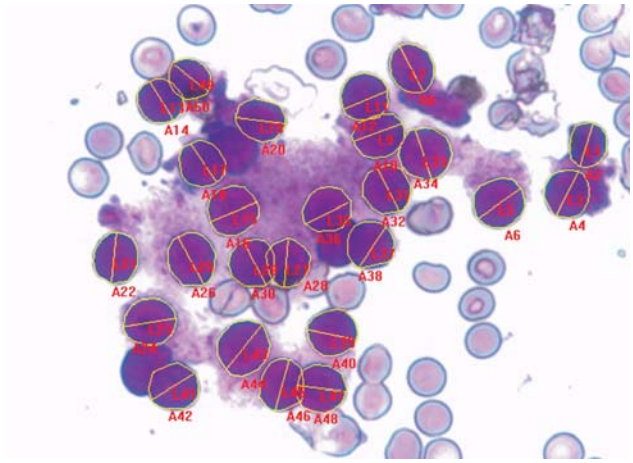

Figure 3 – ***Follicular adenoma (MGG, ×400)***

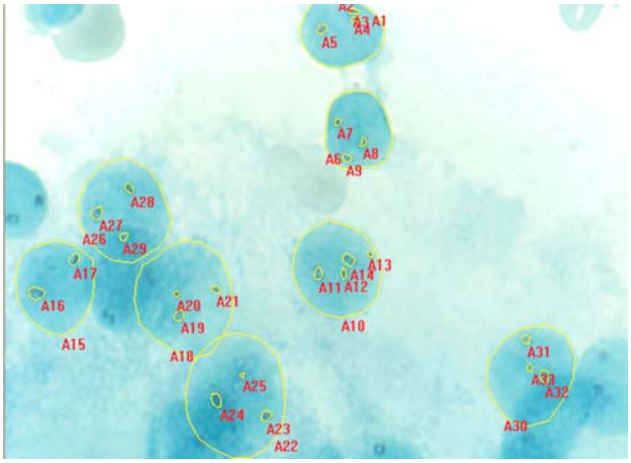

Figure 4 – ***Follicular adenoma (silver stain, ×1000)***

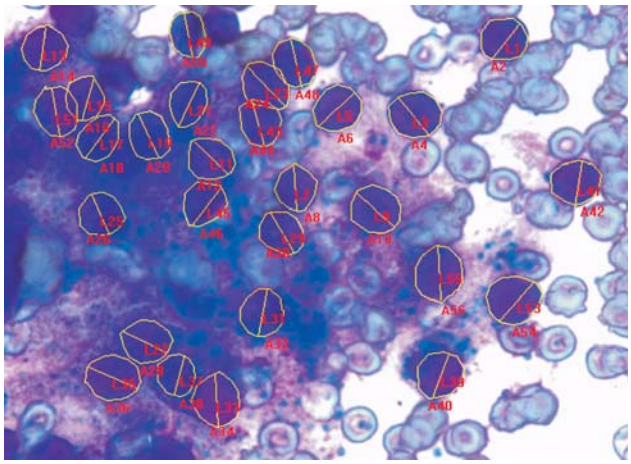

Figure 5 – ***Lymphocytic thyroiditis (MGG, ×400)***

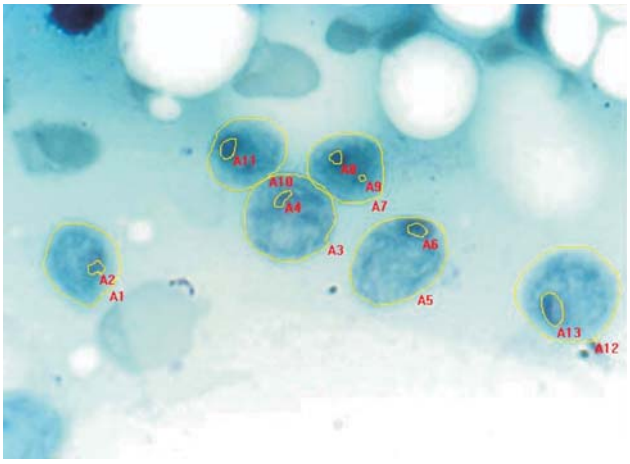

Figure 6 – ***Lymphocytic thyroiditis (silver stain, ×1000)***

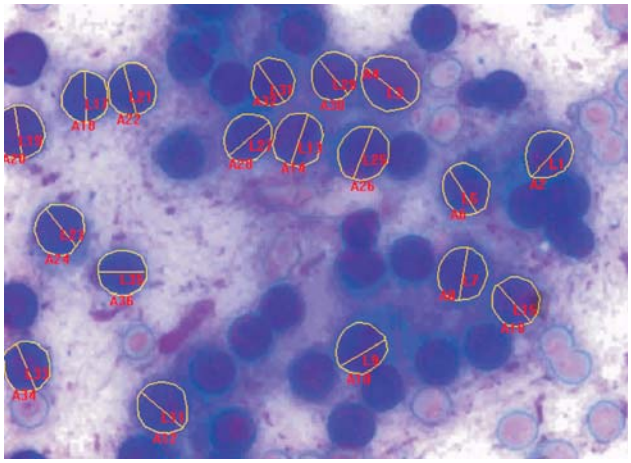

Figure 7 – ***Follicular carcinoma (MGG, ×400)***

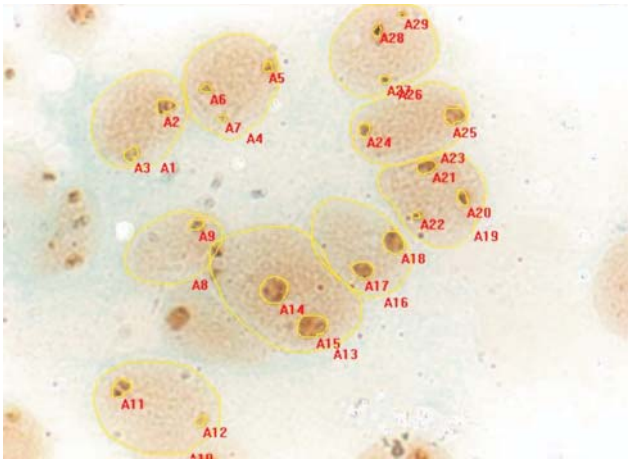

Figure 8 – ***Follicular carcinoma (silver stain, ×1000)***

The results obtained in our study tend to be in accordance with Kini SR *et al.* [16].

In our opinion, the syncytial pattern can sometimes be an artifact – due to an aggressive cellular manipulation, that can determine a cellular disorganization or characteristic superposition.

The study of the diameter and of the nuclear area made by Boon ME *et al.* [19] and Kini SR *et al.* [16] has shown statistically significant differences between the malign tumoral diseases and the benign ones, our study having reached the same statistically significant results.

Preceding studies, dedicated to NORs morphometry [20–22] have allowed the differentiation of thyroid benign and malign lesions, by the correlation of the nuclear area with the NORs area.

Our data are partially in accordance with these references. In our study, the ratio between the NOR area and their mean number per nucleus has had an inverse correlation, meaning that the number of NORs has decreased while they were growing in size.

For the NORs number, we have obtained statistically significant values ($p < 0.001$) only between the follicular carcinoma (2.1 ± 1.0) and the thyroiditis (1.2 ± 0.5).

In our opinion, the absence of correlation in the NORs/nucleus ratio comes from the fact that, in the thyroid carcinomas, NORs have frequently appeared in-groups and bigger. Consecutively, the choice of the best microscopical image to measure the nuclear area has imposed a general preview, losing some of its accuracy.

This is why we find it necessary to realize the quantitative analysis of NORs and nucleus, outside the count of NORs, so as to minimize the risks of falsely positive results.

Conclusions

The association of morphometry and cytochemistry in order to establish a significant preoperatory cytological diagnosis system for the follicular lesions suspected of malignity need the effort of the pathologist, but the present study demonstrates the effectiveness of this association.

The results attained through morphometry increase the sensibility and the specificity of FNAB in the diagnosis of thyroid follicular carcinomas.

The technique we propose allow the use of the same smears classically colored by MGG for the silver stain as well.

The technique is elaborate, it is particularly recommended for clinically controversial cases, for patients with an unbalanced metabolism or whether the surgical intervention presents the risk for developing postoperatory complications.

Although our conclusions cannot be generalized for the moment, as the evaluation of a larger number of cases is compulsive, the data are stimulating.

References

[1] GOELLNER J. R., GHARIB H., GRANT C. S., JOHNSON D. A., *Fine needle aspiration cytology of the thyroid, 1980 to 1986*, Acta Cytol, 1987, 31:587–590.

[2] CARUSO D., MAZZAFERRI E. L., *Fine needle aspiration in management of thyroid nodules*, Endocrinologist, 1991, 1:194–199.

[3] ROSAI J., CARCANGIU M. S., DELELLIS R. A., *Tumors of thyroid gland. Atlas of tumor pathology*, Armed Forces Institute Pathology, Washington, 1992, 31–275.

[4] ***, *AACE/AAES Medical Surgical Guidelines for Clinical Practice: Management of Thyroid Carcinoma*, Endocr Pract, 2001, 7:203–217.

[5] LÖWHAGEN T., GRANBERG P., LUNDELL G. *et al.*, *Aspiration biopsy cytology (ABC) in nodules of the thyroid gland suspected to be malignant*, Surg Clin North Am, 1979, 59:3–7.

[6] BACKDAHL M., WALLIN G., LÖWHAGEN T., WILLEMS J., *Planimetric studies of fine needle aspirates from follicular adenoma and carcinoma of the thyroid*, Acta Cytol, 1980, 24:145–148.

[7] FERRER–ROCA O., BALLESTER–GUARDIA E., MARTIN–RODRIGUEZ J., *Morphometric, densitometric and flowcytometric criteria for the automated classification of the thyroid lesions*, Analyt Quant Cytol Histol, 1990, 12:48–55.

[8] MONTIRONI R., ALBERTI R., SISTI S. *et al.*, *Discrimination between follicular adenoma and follicular carcinoma of the thyroid. Preoperative validity of cytometry on aspiration smears*, Appl Pathol, 1992, 7:367–374.

[9] DEMICCO C., ZORO P., GARCIA S. *et al.*, *Thyroid peroxidase immunodetection as a tool to assist diagnosis of thyroid nodules of fine-needle aspiration biopsy*, Eur J Endocrinol, 1994, 131:474–479.

[10] POLYMOSI T., TOTH V., SAPI Z. *et al.*, *Diagnostic value of AgNOR method in thyroid cytopathology: correlation with morphometric measurements*, Diagn Citopathol, 1996, 14:140–144.

[11] CĂRUNTU I. D., *Morfometrie computerizată microscopică în histologie şi histopatologie*, Ed. "Gr. T. Popa" U.M.F. Iaşi, 2003, 99–134.

[12] HAMBERGER J. I., HUSAIN M., *Semiquantitative criteria for fine needle biopsy diagnosis: reduced false negative diagnosis*, Diagn Cytopathol, 1988, 4:14–17.

[13] NGUYEN G. K., GINSBERG J., CROCKFORD P. M., *Fine needle aspiration biopsy cytology of the thyroid: its value and limitations in the diagnosis and management of solitary thyroid nodules*, Pathol Ann, 1991, 26:63–91.

[14] RUSCHOFF J., PRASSER C., PLOTON D. *et al.*, *Diagnostic value of AgNOR staining in follicular cell neoplasm of the thyroid*, Am J Surg Pathol, 1993, 17:1281–1288.

[15] ROSEN J. B., AZADIAN A., WALFISH P. G. *et al.*, *Ultrasound fine needle aspiration biopsy in the management of thyroid disease*, Am J Surg, 1993, 166:346–349.

[16] KINI S. R., MILLER J. M., HAMBERGER J. I., *Cytopathology of follicular lesions of the thyroid*, Diagn Cytopathol, 1985, 1:123–132.

[17] RAVINSKY E., SAFNECK J. R., *Fine needle aspirates of follicular lesions in the thyroid gland: The intermediate cell type smear*, Acta Cytol, 1990, 34:813–820.

[18] SUEN K. C., *How does one separate cellular follicular lesions of the thyroid by fine needle aspiration biopsy?*, Diagn Cytopathol, 1988, 4:78–81.

[19] BOON M. E., LÖWHAGEN T., WILLEMS J. S., *Planimetric studies on fine needle aspirates from follicular adenoma and follicular carcinoma of the thyroid*, Acta Cytol, 1980, 27:145–148.

[20] SOLYMOSI T., TOTH V., SAPI Z. *et al.*, *Diagnostic value of AgNOR method in thyroid cytopathology: correlation with morphometric measurements*, Diagn Cytopathol, 1996, 14:140–144.

[21] SCHECHTMAN L., KOREN R., HOROWITZ A. *et al*., *Diagnostic value of AgNOR staining in thyroid cytology,* Anal Quant Cytol Histol, 1998, 20:187–197.

[22] ARACHO–PERULA E., ROLDAN–VILLALOBOS R., BLANCO–GARCIA F., BLANO–RODRIGUEZ A., *Objective differential classification of thyroid lesions by nuclear quantitative assessment*, Histol Histopathol, 1997, 12:425–431.

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