Morphometric analysis of thyroid cell aspirates

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SUMMARY One hundred and nineteen patients with thyroid nodules underwent fine needle aspiration cytology. Fifty eight were subsequently shown to have multinodular goitre: 36 had a follicular adenoma; 12 follicular carcinoma; and 13 papillary carcinoma on paraffin section. Morphometry performed on the aspirated cells stained by Papanicolaou and Giemsa methods showed significant differences in mean nuclear area and nuclear perimeter between groups of patients with benign thyroid nodules and those with malignant nodules. The wide variation in the mean nuclear areas and perimeters, however, severely limits the diagnostic use of morphometry in individual aspirates.

Fine needle aspiration cytology has been widely used in the assessment of thyroid nodules. Many investigators have shown that the technique is valuable,¹⁻⁵ but others have emphasised limitations and urged caution.⁶ Although false positive results are rare, the usefulness of the technique has been limited to some extent by difficulties in distinguishing follicular adenomas and multinodular goitres from well differentiated follicular carcinoma and papillary carcinoma of the thyroid. Even in centres where large numbers of aspirates have been processed over many years,^{7,8} histologically incorrect diagnosis of malignancy occurs in up to 25% of cases.

Computed morphometric methods have been introduced to try to improve the diagnostic yield from fine needle aspirates of the thyroid. Some authors have found this to be useful,⁹⁻¹¹ but others have not.^{12,13} These published series used Giemsa stained preparations. As most cytopathologists are familiar with Papanicolaou stained preparations, we present the results of morphometric analysis of both Giemsa and Papanicolaou stained preparations of aspirated cells from thyroid nodules of patients subsequently shown by histology to have multinodular goitre, follicular adenoma, follicular carcinoma, or papillary carcinoma.

Material and methods

STUDY POPULATION

One hundred and nineteen patients with thyroid nodules were studied. These included 29 men (mean age 44.9 years) and 90 women (mean age 43.7 years).

Aspirates were performed using a 23 gauge needle. The smears were immediately fixed in 95% ethanol and stained using a standard Papanicolaou method.¹⁴ In addition, smears from 49 patients were air dried and stained using a commercial variation of Wright's Giemsa stain (Diff-Quik Solution 1, Lab Aids, Narrabeen, New South Wales, Australia).

MORPHOMETRIC ASSESSMENT

The smears were evaluated and reported in the usual manner using diagnostic criteria outlined by Miller *et al.*¹⁵ In addition, morphometry was carried out using the MOP-Videoplan image analysis system (Zeiss, Oberkochen, West Germany). At least 25 randomly selected thyroid cells were selected for diagnosis cell groupings on each slide. All had intact nuclei. The figure of 25 was chosen on the basis of preliminary studies and the results published by Luck *et al.*¹² The nuclei were displayed on a graphics tablet and outlined manually using a cursor at a magnification factor of 400. Nuclear area and perimeter were then computed and data stored. Attempts were made to estimate whole cell area and perimeter so that cytoplasmic area and nucleus:cytoplasm ratio could be calculated. It was difficult, however, to identify precisely the thyroid cell boundaries (fig 1).

Calibration was checked regularly by using a slide micrometer. Reliability was confirmed by repeated measurements of a control group of aspirates randomly interspersed among the study samples. Measurements were carried out by a single observer (HC), who had no knowledge of the results of cytological assessment of the aspirates or the histological diagnosis.

Fig 1 Fine needle aspiration from thyroid nodule of patient subsequently shown to have multinodular goitre. Cytoplasmic boundaries cannot be identified. (Giemsa stain). $\times 960$.

HISTOLOGICAL ASSESSMENT

Histological classification of the subsequent surgical resection specimens was carried out using the WHO classification¹⁶ by a single observer (RGW), who was not aware of the results of the morphometric analysis of the thyroid aspirates.

STATISTICAL ANALYSIS

A single factor analysis of variance test was performed using the Newman-Keuls multiple range

Fig 2 Scattergram showing mean nuclear area of Papanicolaou stained aspirated thyroid cells from 58 patients with multinodular goitre, 36 patients with follicular adenoma, 12 patients with follicular carcinoma, and 13 with papillary carcinoma. Diagnoses were established by histological examination of specimens at subsequent thyroid resection.

test¹⁷ for significance in difference in means between all groups.

Results

The table shows the distribution of histological diagnoses in the 119 subjects studied. Giemsa stained cytological preparations were available from 20 patients with multinodular goitre, 20 with follicular adenoma, six with follicular carcinoma, and three with papillary carcinoma. Mean nuclear areas and perimeters of aspirated thyroid cells using both Papanicolaou and Giemsa stains are also shown. The mean nuclear areas and perimeters of cells from follicular and papillary carcinomas were significantly larger

Table Nuclear measurements of Papanicolaou and Giemsa stained thyroid aspirates grouped by subsequent histological diagnosis

Histological diagnosis	Stain					
	Papanicolaou			Giemsa		
	No	Nuclear area (μm^2) Mean ($\pm$ SD)	Perimeter (μm) Mean ($\pm$ SD)	No	Nuclear area (μm^2) Mean ($\pm$ SD)	Perimeter (μm) Mean ($\pm$ SD)
Multinodular goitre	58	43.6 (7.6)	24.2 (2.1)	20	56.1 (10.7)	27.7 (2.4)
Follicular adenoma	36	50.1 (9.3)	26.1 (2.4)	20	66.4 (14.6)	30.2 (3.1)
Follicular carcinoma	12	64.2 (8.0)	29.2 (1.9)	6	84.0 (16.9)	33.8 (3.5)
Papillary carcinoma	13	57.1 (12.2)	27.8 (3.1)	3	97.4 (28.2)	36.4 (5.5)

The groups are significantly different (Papanicolaou $F = 20.14$, $p < 0.001$; Giemsa $F = 10.93$, $p < 0.001$).
Papanicolaou: 4 v 1, $p < 0.001$; 4 v 2, $p < 0.02$; 3 v 1, $p < 0.001$; 3 v 2, $p < 0.001$; 2 v 1, $p < 0.001$.
Giemsa: 4 v 1, $p < 0.001$; 4 v 2, $p < 0.005$; 3 v 1, $p < 0.001$; 3 v 2, $p < 0.025$; 2 v 1, $p < 0.05$.

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than those from follicular adenomas and multinodular goitre, and the mean areas and perimeters of follicular adenoma nuclei were significantly larger than nuclei from multinodular goitres. Despite these clear cut and significant differences between means there was a wide scatter of results within each diagnostic category (fig 2). Thus in any one patient measurement of nuclear area and perimeter was not helpful in diagnosis.

Discussion

The results showed significant differences in mean nuclear areas and nuclear perimeters between multinodular goitres and follicular and papillary neoplasms and significant differences between follicular adenomas and follicular and papillary carcinomas. Similar results were found both with air dried Giemsa stained preparations and Papanicolaou stained smears fixed in alcohol.

Clearly delineated cytoplasmic outlines were not obtained with either of our staining procedures, and consequently whole cell areas and derived nucleus: cytoplasmic ratios were not available. Similar difficulties in delineation of cell margins were noted by Luck *et al.*¹² Other workers¹⁰ have been able to measure cell areas. Examination of their published figures suggests, however, that considerable subjective interpretation was required in delineating cytoplasmic margins.

In the present study the mean nuclear areas and perimeters of cells aspirated from malignancies and stained by the Giemsa technique fell between those reported previously.^{10 12}

We have found, as have others,^{10 12} a wide variation in nuclear measurements from individual thyroid aspirates, and interpretation of nuclear measurements is difficult because of the wide overlap of individual mean values. Therefore, while groups of patients can be adequately separated statistically, we believe that morphometric assessment alone is inadequate to predict malignancy in thyroid aspirates.

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