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Original Articles

Development and Validation of a Computerized Cytomorphometric Method to Assess the Maturation of Vaginal Epithelial Cells

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Background: After menopause, declining levels of estrogens may cause vaginal discomfort, or so-called ''vaginal atrophy.'' Evaluation of therapies for vaginal atrophy may be performed using the so-called ''maturation index.'' The maturation index is expressed as the percentage of (para)-basal, intermediate, and superficial epithelial cells in a vaginal smear. Manual assessment of the maturation index is subject to inter- and intraobserver variations. In this study, assessment of the maturation of cells in vaginal smears using automated image analysis was investigated.
Materials and Methods: Automated assessment, using a commercially available image analysis system, was performed on hematoxylin-eosin-stained cytospin specimens. A training set was constructed by an experienced cytotechnologist, based upon visual classification of stored grey value images. From this, two discriminant functions (DFs) were calculated capable of classifying cells in one of the three types. These cell classifiers were capable of classifying 97% of the cells correctly. Data from automated assessment were compared with those of classical manual counting. Specimens of 13 mature and 6 atrophic vaginal specimens were assessed in duplicate, both manually and by image analysis, using the DFs.
Results: No significant interobserver effect was found for image analysis, whereas a significant effect was found for manual counting. Both methods were able to distinguish between matured and atrophic specimens.
Conclusions: It was concluded that for assessment of vaginal maturation, the use of automated image analysis systems is recommended. Besides increased reproducibility, image analysis systems yield additional data describing the size and shape of the cytoplasm and nucleus of cells, which might increase discriminating power. Cytometry 35:196–202, 1999. © 1999 Wiley-Liss, Inc.

Key terms: postmenopausal atrophy; vaginal maturation; maturation index; automated image analysis; therapy evaluation

Menopause in women is associated with the occurrence of climacteric complaints (hot flashes, night sweats, and palpitations), bladder problems, accelerated bone loss (osteoporosis), and vaginal discomfort. These symptoms are related to an abrupt loss of ovarian function at the onset of menopause, resulting in a sharp decline in the endogenous production of ovarian hormones, especially of serum 17β-estradiol and circulating conjugates of this hormone. Low estradiol levels may lead to a deficient maturation of vaginal mucosa, or so-called ''vaginal atrophy'' (1), a condition that is clinically presented by dryness, irritation, and itch. To compensate for declining levels of endogenous estrogens, women can be treated by exogenous estrogens. Clinical studies have demonstrated that estrogens are effective in relieving (post)menopausal vaginal discomfort (2); they induce proliferation of vaginal epithelium, and thus reestablish a normal appearance of the vaginal mucosa. Also, the local application of a moisturizing bioadhesive gel has been shown to be effective against vaginal discomfort (3,4). The vaginal smear provides an accurate estimate of the proliferative state of the vaginal epithelium. The effect of treatment for postmenopausal vaginal atrophy may be accurately monitored by evaluating subsequent vaginal smears (5).

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Cytomorphologically, vaginal atrophy is defined as a condition with high numbers of (para)basal and intermediate cells and very low numbers of superficial cells (6). The maturation of epithelial cells can be analyzed in vaginal smears by manual assessment (MA) of the fraction of (para)basal, intermediate, and superficial epithelial cells (maturation index, or MI (7)). Therapy for vaginal atrophy encourages the maturation of the epithelium, resulting in a shift in the MI from high percentages of (para)basal and intermediate cells to higher percentages of superficial cells. Recently, interest in the treatment of postmenopausal complaints, such as vaginal atrophy, has increased. In order to optimize treatment, and prevent occurrence of unwanted side effects as much as possible, accurate monitoring of treatment-effects is required. Cytomorphological analysis has been shown to be subject to inter- and intraobserver variability. Therefore, an automated and standardized image analysis procedure is desired (8,9).

The primary aim of this study was to describe the development of a computerized cytomorphometric method for fully automatic assessment of the maturation of cells as present in vaginal smears (automated cytomorphometric analysis, or CMA). The applicability of the method was studied by comparing data from smears containing many atrophic cells of postmenopausal women with data from matured specimens of premenopausal women. Development of this method also included its validation against the classical manual method.

MATERIALS AND METHODS

Patients and Cell Preparation

Scrapes of vaginal mucosa were used from 19 patients who had visited a gynecologist with diverse complaints, not related to vaginal atrophy. Samples of the vaginal mucosa were obtained by rotating a special brush (Vibabrush, Rovers B.V., Oss, The Netherlands) in the middle portion of the vaginal wall. Smears were put on a glass slide, fixed with a spray fixative (Pro-Fixx, Lerner Laboratories, Pittsburgh, PA), and stained by the Papanicolaou procedure. After preparation of the smear, the brush with adhering cells was put into ethanol-carbowax fixation fluid, and subsequently cytospins were prepared. The cytospin device deposits the cell material into a well-defined square area of the slide, facilitating fully automated measurement. The specimens were stained using the hematoxylin-eosin (H&E) procedure. The spectral characteristics of the H&E staining allow separate identification of nuclei and cytoplasm by CMA very well, as illustrated in Figure 1.

Of 19 patients, 12 were postmenopausal and 7 were premenopausal.

Cytomorphometric Analysis

System setup. CMA was performed with a Discovery 2.6 Fluorbance system (10) (Becton Dickinson Cellular Imaging Systems, Leiden, The Netherlands). The cytospin specimens were scanned fully automatically with a 25× objective (NA = 0.7) using a motorized stage and automatic focusing. Microscopic fields measuring 550 × 550 µm were digitized by a Xillix MicroImager 1400 monochromatic CCD camera (Xillix Technologies Corp, Vancouver, British Columbia, Canada; pixel size 6.8 × 6.8 µm, 8-bit sampling), resulting in an effective resolving power of 0.54 × 0.54 µm.

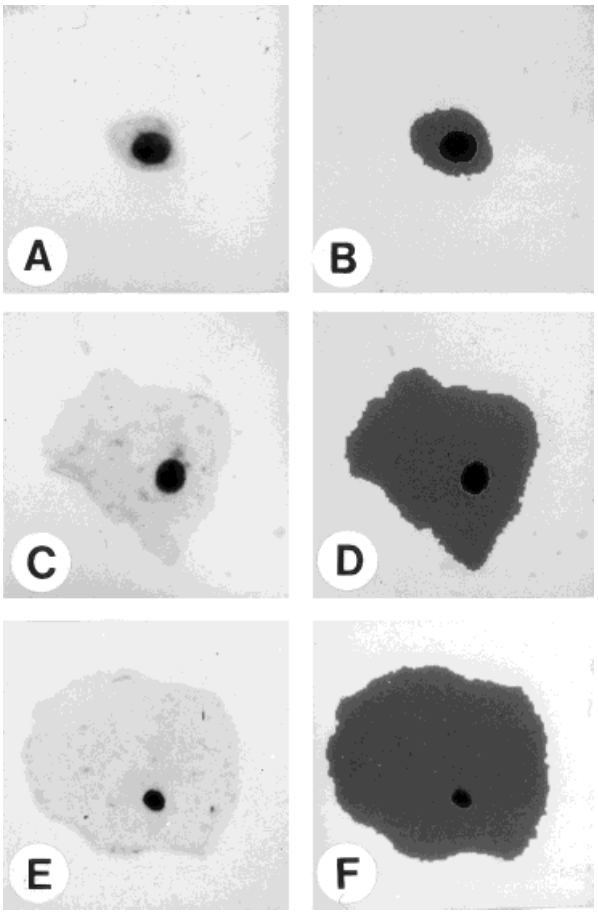

FIG. 1. Examples of microscopic images of hematoxylin-eosin-stained (para)basal (**A**), intermediate (**C**), and superficial (**E**) cells, and the same cells after segmentation (**B**, **D**, and **F**).

Images were digitized at $\lambda = 580$ nm for detection of nuclear staining and $\lambda = 540$ nm for detection of cytoplasmic staining. At these wavelengths the dyes showed maximum absorbance. Detection of nuclei was performed in three steps:

1) By automated adjustment of the lamp voltage and by use of a shading correction algorithm, the intensity of image points (pixels) belonging to the background (i.e., no staining was present) was kept at grey value 200. Next, all pixels in the image acquired at $\lambda = 580$ nm with a grey value below a threshold value were considered to be hematoxylin-positive. This grey value threshold was set manually for each specimen by examining the histogram of a number of digitized microscopic fields. Threshold values for hematoxylin ranged between 178–188, due to staining variations among specimens. Therefore, fixed threshold values could not be used.

2) Neighboring (8 connected) image pixels that were positive for hematoxylin were grouped together to form objects that possibly corresponded to nuclei.

3) For each object, the area and the contour ratio were determined. Contour ratio was defined as

$$1{,}000 * \text{perimeter}/(2 * \sqrt{(\pi * \text{area})}),$$

describing the roughness of the contour of an object. Artifacts were removed by applying thresholds to these parameters. Parameter threshold values were determined for use in the entire study from manually selected, correctly recognized objects from a number of specimens. Objects with an area of more than 50 pixels and contour ratio smaller than 1,300 were used.

Objects found in this way were considered to represent correctly recognized, single-lying (i.e., nonoverlapping) nuclei. The same procedure was applied to the image acquired at 540 nm for detection of the cytoplasm of cells. For this purpose, grey value thresholds were found ranging from 115–125. For artifact removal, a minimum area of 850 pixels was used. When only single-lying epithelial cells were required, a maximum contour ratio of 1,500 was used as well.

For all cells and cell clumps detected by CMA, the area, perimeter, contour ratio, and bending energy of the nucleus and of the cytoplasm were calculated. Also, the ratio between the area of the nucleus and the area of the cytoplasm and the ratio between the perimeter of the nucleus and the perimeter of the cytoplasm were calculated.

Cell classification by cytomorphometric or automated analysis (training set). In order to obtain a statistical cell classifier, a number of nonoverlapping, single cells of the training set were classified manually by an experienced cytotechnologist on the basis of stored grey level images. These 623 cells of 5 patients, both from atrophic and mature specimens, were selected, measured by CMA, and subsequently classified as either (para)basal, intermediate, or superficial. All measured nuclear and cytoplasmic features of these cells were used in a forward stepwise logistic regression analysis in order to select the best subset of features to discriminate between (para)basal cells and the non(para)basal cells (both intermediate cells and superficial cells). The selected features were used in logistic regression analysis to obtain a discriminant function DF(1) to classify the measured cells as (para)basal cells and non(para)basal cells. The same procedure was used to obtain a second DF(2) for the discrimination of intermediate cells and superficial cells.

The aforementioned DFs are not suitable for classification of cells in clumps, because no data regarding the cytoplasm as calculated for the single cells are available for these clumped cells. Therefore, two new DFs were determined by logistic regression, based on nuclear parameters only.

The same-image cytometric data were used to construct statistical cell classifiers, as well as to evaluate the performance of the cell classifiers. This procedure may give classification results that are optimistically biased. The ''leave-one-out'' cross-validation procedure was used in the logistic regression analyses to reduce this bias.

CMA-assessment of maturation index and maturation value (test set). Two measurement runs were performed for each specimen: 1) assessment of single-lying (i.e., nonoverlapping) epithelial cells (single-cell CMA), and 2) assessment of both clusters of epithelial cells (two or more cells with touching or overlapping cytoplasms) and single cells (clump CMA). For single-cell CMA, the contour ratio limitation was applied, and only cytoplasmic objects encompassing exactly one single nucleus were allowed. For clump CMA, all objects with an area greater than the threshold value were assessed.

If possible, for each specimen 300 cells (single-cell CMA) or cell clumps (clump CMA) were measured; otherwise, the entire measurement area of the slide was scanned and analyzed. MI consists of the fractions of (para)basal, intermediate, and superficial epithelial cells in a smear (7). From this, the MV is determined by $0.5 \times$ fraction of intermediate cells + fraction of superficial cells (11). Cells from each measurement were classified using the DFs, and MI and MV were determined for single-cell CMA and for clump CMA.

Classical Manual Assessment

The maturation of vaginal cells in terms of the maturation index was assessed manually by an experienced cytotechnologist. Maturation was expressed by the maturation index, applying the procedure described elsewhere (7) to 200 vaginal cells. In short, cells were visually classified as (para)basal, intermediate, or superficial, and the percentage of each of the three types was calculated. From this, the maturation value was determined as described above. Assessment was performed in duplicate with a 2-month interval, by the same cytotechnologist.

Statistical Methods

All statistics were performed using SPSS for Windows (SPSS, Inc., Chicago, IL). The Shapiro-Wilks test for normality showed a significant deviation from normality for a number of parameters; therefore, nonparametric tests were used throughout the study. Correspondence between measurement sessions for MA and CMA and between MA and CMA were studied using Spearman rank correlation coefficients and Wilcoxon matched-pairs signed-rank tests. Differences between patient classes were studied using the Mann-Whitney U-test.

RESULTS

Automated Cytomorphometric Analysis

CMA cell classification (training set). In order to discriminate between selected (para)basal, intermediate, and superficial cells of the training set of 623 cells, a stepwise logistic regression analysis was performed. This resulted in the following parameters, with decreasing significance: perimeter of the cytoplasm, ratio between the perimeter of the nucleus and the perimeter of the

Table 1
*Results of Classification of Epithelial Cells in the Training Set, Using Discriminant Functions Based on Morphometric Parameters**

				Predicted class:			
	(Para)basal			Intermediate		Superficial	
Actual class	Total Number	N	%	N	%	N	%
Single-cell CMA							
(Para)basal	177	170	96	7	4	0	0
Intermediate	208	4	2	192	92	12	6
Superficial	238	0	0	10	4	228	96
	95% overall correct classification						
Clump CMA							
(Para)basal	177	96	54	77	44	4	2
Intermediate	208	45	22	151	73	12	6
Superficial	238	0	0	10	4	228	96
	73% overall correct classification						

*Results from both single-cell CMA and clump CMA of cytospins are shown. Listed are the number of cells (N) and the percentage.

cytoplasm, and ratio between the area of the nucleus and the area of the cytoplasm. Differences in significance levels between these parameters were small. In general, area is a more stable parameter than perimeter; therefore, we used the ratio between the area of the nucleus and the area of the cytoplasm to classify cells as (para)basal:

$$DF(1) = -103 * \text{area of nucleus/area of cytoplasm} + 11.0.$$

Cells with $DF(1) < 0$ were classified as (para)basal, and cells with $DF(1) \geq 0$ as non(para)basal cells. The DF to classify non(para)basal cells in intermediate cells and superficial cells is given by:

$$DF(2) = -0.085 * \text{area of nucleus} + 19.5.$$

Cells with $DF(2) < 0$ were classified as intermediate, and with $DF(2) \geq 0$ were classified as superficial cells. Using these two DFs to classify the 623 cells in the training set resulted in 95% overall correct classification (Table 1). None of the (para)basal cells were classified as superficial cells, and vice versa. With regard to the intermediate cells, only 2% of the cells were classified as (para)basal cells and 6% as superficial cells. The ''leave-one-out'' cross-validation procedure gave the same classification results, so it was concluded that classification by DF(1) and DF(2) is unbiased.

Classification of cells in clumps (clump CMA) was based on nuclear parameters only, and resulted for the distinction between (para)basal cells and non(para)basal cells (intermediate cells and superficial cells) in the following discriminant function:

$$DF(3) = -0.017 * \text{area of nucleus} + 5.2.$$

For discrimination between intermediate cells and superficial cells in clump CMA, DF(2) could be used as this DF does not require cytoplasmic information. Classification results for this pair of DFs are shown in Table 1. Lacking cytoplasmic information, the distinction between (para) basal and intermediate cells was seriously hampered: 44% of (para)basal cells were falsely classified as intermediate, and 22% of intermediate cells were falsely classified as (para)basal.

CMA-assessment of MI and MV (test set). For single-cell CMA, the number of cells used for calculation of the MI and MV was over 200 for most of the cases of the test set, but was as low as 40 in one case. For clump CMA, all nuclei present in 300 clumps were taken into account, resulting in much higher numbers of cells. Results of CMA for individual patients are shown in Table 2. For mature specimens, hardly any (para)basal cells were detected by single-cell CMA. Atrophic specimens showed high percentages of (para)basal cells (>60% for all cases but one). Only one atrophic specimen (case 3) showed mainly intermediate cells. No apparent differences existed between the two sessions of single-cell CMA.

Results of Manual Assessment

Results of manual assessment are shown in Table 3. In most cases a mixture of superficial and intermediate cells or of intermediate and (para)basal cells was found. The smallest of the three values of the MI never represented more than 5% of the cells. In mature specimens no (para)basal cells were counted and in atrophic specimens hardly any superficial cells were seen. For atrophic specimens, the percentage of (para)basal cells was always over 60. Again, case 3 seems to be an exception in that it showed the lowest percentage of (para)basal cells and the highest MV values.

Reproducibility

Interobserver reproducibility. For the MI and MV values of single-cell CMA, no significant differences were seen between two sessions (all comparisons are listed in Table 4; see also Fig. 2). Correlations between the two automated sessions were high and significant for almost all parameters. ($r > 0.86$; $P < 0.05$). The only exception was the fraction of (para)basal cells in specimens from premenopausal patients. As this percentage was very close to 0, the correlation coefficient was entirely determined by noise, making it inaccurate. Results of clump CMA were not analyzed in this way because of their lower accuracy.

For the MI and MV values of manual counting, significant differences were seen for all parameters for at least one of the three classes of patients (premenopausal, postmenopausal atrophic, or postmenopausal mature). Correlations were less pronounced as for CMA and not always significant (Fig. 3 shows results for MV for all cases).

Comparison between manual and automated assessment. Comparison of the first sessions from both single-cell CMA and MA showed significant differences for most parameters for the three patient classes (Table IV; Fig. 4 shows comparison of MV values). Also, for all

Table 2
*Results of Automated Cytomorphometric Analysis**

		Single-cell CMA, session 1				Single-cell CMA, session 2				Clump CMA			
Number	Age	fr.P.	fr.I.	fr.S.	MV	fr.P.	fr.I.	fr.S.	MV	fr.P.	fr.I.	fr.S.	MV
Postmenopausal, atrophic specimens													
1	58	.65	.27	.08	.22	.70	.26	.05	.18	.56	.22	.22	.33
2	55	.63	.25	.12	.25	.63	.27	.10	.23	.27	.52	.21	.47
3	74	.21	.63	.16	.47	.19	.53	.29	.55	.12	.46	.43	.65
4	54	.78	.20	.03	.13	.72	.25	.04	.16	.26	.31	.44	.59
5	73	.99	.00	.01	.01	.99	.00	.01	.01	.18	.54	.28	.55
6	59	.86	.10	.04	.09	.88	.08	.05	.08	.36	.38	.26	.45
Postmenopausal, mature specimens													
7	68	.03	.62	.35	.66	.03	.55	.41	.69	.08	.46	.46	.69
8	61	.00	.27	.73	.86	.01	.30	.69	.84	.06	.34	.61	.78
9	66	.04	.84	.12	.54	.03	.83	.14	.55	.39	.39	.22	.42
10	76	.01	.31	.68	.83	.01	.28	.71	.85	.06	.26	.68	.81
11	52	.02	.23	.76	.87	.00	.01	.99	.99	.04	.20	.77	.86
12	57	.03	.62	.35	.66	.02	.62	.36	.67	.07	.44	.49	.71
Premenopausal													
13	27	.04	.48	.47	.71	.03	.51	.46	.71	.16	.50	.34	.59
14	35	.01	.54	.45	.72	.01	.69	.30	.65	.07	.43	.51	.72
15	30	.00	.06	.93	.96	.01	.05	.94	.97	.01	.06	.93	.96
16	39	.01	.25	.74	.87	.00	.34	.65	.83	.05	.29	.66	.80
17	50	.03	.59	.39	.68	.03	.45	.53	.75	.09	.46	.46	.69
18	43	.03	.69	.28	.62	.03	.64	.33	.65	.17	.49	.34	.58
19	38	.01	.10	.89	.94	.00	.18	.81	.91	.01	.15	.84	.91

*Shown are the fractions of (para)basal (fr.P.), intermediate (fr.I.), and superficial (fr.S.) cells and the maturation value (MV) for two sessions of single-cell CMA and for clump CMA.

Table 3
*Results of Manual Assessment of Vaginal Maturation**

		Session 1				Session 2			
Number	Age	fr.P.	fr.I.	fr.S.	MV	fr.P.	fr.I.	fr.S.	MV
Postmenopausal, atrophic specimens									
1	58	.78	.22	.00	.11	.92	.08	.00	.04
2	55	.69	.27	.05	.18	.91	.10	.00	.05
3	74	.61	.39	.01	.20	.73	.28	.00	.14
4	54	.85	.15	.00	.08	.94	.07	.00	.03
5	73	.83	.17	.00	.09	.93	.07	.00	.04
6	59	.77	.19	.05	.14	.93	.08	.00	.04
Postmenopausal, mature specimens									
7	68	.00	.73	.28	.64	.00	.95	.06	.53
8	61	.00	.83	.18	.59	.00	.99	.02	.51
9	66	.00	.90	.11	.55	.00	.95	.05	.53
10	76	.00	.39	.62	.81	.00	.79	.22	.61
11	52	.00	.18	.82	.91	.00	.21	.79	.90
12	57	.00	.90	.10	.55	.00	.97	.03	.52
Premenopausal									
13	27	.00	.83	.17	.59	.00	.97	.03	.52
14	35	.00	.64	.36	.68	.00	.88	.12	.56
15	30	.00	.07	.93	.97	.00	.14	.87	.93
16	39	.00	.61	.39	.70	.00	.44	.56	.78
17	50	.00	.88	.13	.56	.00	.93	.07	.54
18	43	.00	.86	.14	.57	.00	.94	.06	.53
19	38	.00	.20	.80	.90	.00	.15	.85	.93

*Shown are the fractions of (para)basal (fr.P.), intermediate (fr.I.), and superficial (fr.S.) cells and the maturation value (MV) for two independent counting sessions.

parameters, correlations were not significant for specimens from postmenopausal patients. Especially in mature specimens, the intraobserver reproducibility of MA was remarkably poorer than for CMA, because higher fractions of intermediate cells were counted in the second measurement.

Analysis of atrophy. To evaluate the discriminating power of the MI and MV from MA and CMA, a comparison was made between specimens of premenopausal patients vs. atrophic specimens of postmenopausal patients. For manual assessment, highly significant differences between these two groups of specimens ($P < 0.003$) were seen for the fraction of (para)basal cells, the fraction of superficial cells, and the MV. For automated assessment of single cells, significant differences between the two groups of specimens ($P < 0.003$) were seen for the fraction of (para)basal cells, the fraction of superficial cells, and the maturation value. Less pronounced differences ($P < 0.015$) were seen for clump CMA for the fraction of (para)basal cells, the fraction of superficial cells, and the MV.

DISCUSSION

In this study we have described an automated cytomorphometric method to fully assess the maturation of epithelial cells as present in vaginal smears. The results of the cytomorphometric method showed that geometric features of single cells present in hematoxylin-eosin-stained cytospin specimens can be used for automated assessment. The geometric features, describing both the nucleus and cytoplasm of cells, were successfully used to construct cell classifiers for the automatic identification of (para)basal (P), intermediate (I), and superficial (S) epithelial cells. Using only nonoverlapping single cells, the cells were classified in the S, I, and P classes with an overall correct classification of 95%.

Table 4
Results of Comparison Between Different Sessions for Cytomorphometric or Automated Analysis (CMA) and for Manual Assessment (MA) and Between Data From CMA and MA[†]

	CMA interobserver				MA interobserver				CMA vs. MA			
	fr.P.	fr.I.	fr.S.	MV	fr.P.	fr.I.	fr.S.	MV	fr.P.	fr.I.	fr.S.	MV
Postmenopausal, atrophic specimens												
Wilc.p.	ns	ns	ns	ns	*	*	ns	*	*	ns	*	ns
Spearm.r	1.0	.94	1.0	1.0	.94	1.0	–	.94	.71	.77	.58	.71
Spearm.p.	***	**	***	***	**	***	–	**	ns	ns	ns	ns
Postmenopausal, mature specimens												
Wilc.p	ns	ns	ns	ns	ns	*	*	*	*	*	*	*
Spearm.r.	.89	.94	.89	.89	–	.54	.54	.54	–	.66	.43	.43
Spearm.p	*	**	*	*	–	ns	ns	ns	–	ns	ns	ns
Premenopausal												
Wilc.p	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*	ns
Spearm.r	.54	.86	.86	.79	–	.93	.93	.93	–	.93	.93	.96
Spearm.p	ns	*	*	*	–	**	**	**	–	**	**	***
All specimens												
Wilc.p	ns	ns	ns	ns	*	ns	**	**	ns	**	***	**
Spearm.r	.94	.93	.96	.96	1.0	.89	.93	.94	.81	.86	.93	.95
Spearm.p	***	***	***	***	***	***	***	***	***	***	***	***

[†]For each specimen class and for all specimens, the *P*-values of Wilcoxon matched-pairs signed-rank test, Spearman correlation coefficient, and significance of the correlation are shown for the fractions of (para)basal (fr.P.), intermediate (fr.I.), and superficial (fr.S.) cells and the maturation value (MV). ns, $P > 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; –, this value could not be calculated.

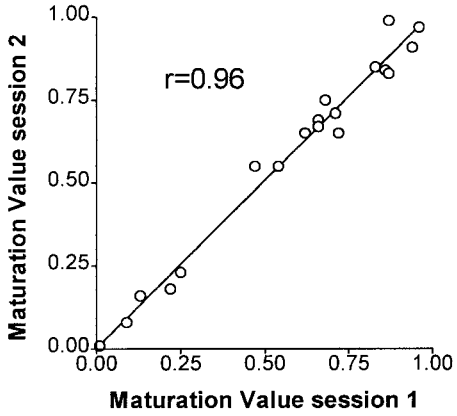

FIG. 2. Comparison of results of duplicate automated assessment of the maturation value of 19 vaginal specimens, both mature and atrophic.

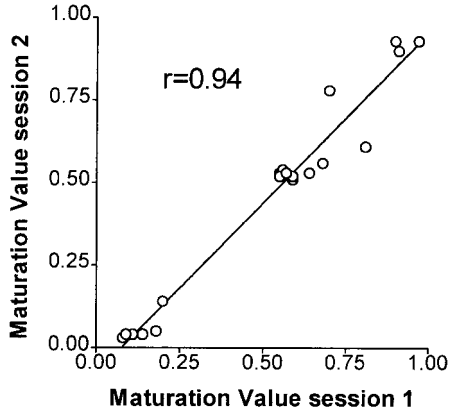

FIG. 3. Comparison of results of duplicate manual assessment of the maturation value of 19 vaginal specimens, both mature and atrophic.

Automated assessment of cells in clusters was found to be less reliable than measurement of single-lying cells (overall 73% correct classification), because of lack of cytoplasmic information. Clump CMA showed higher percentages of (para)basal cells in mature specimens and much lower percentages of (para)basal cells in atrophic specimens, underlining the lower accuracy of classification based on nuclear features only. Restriction to assessment of single-lying cells may theoretically result in an underestimation of the fraction of (para)basal cells in atrophic specimens, since these cells are more often disposed in cell clusters than single cells. Assessment of cells in clumps might therefore yield additional information to the measurement of single cells. This needs to be further investigated.

As cell maturation in the squamous epithelium of the vaginal mucosa is a continuous biological process, a perfect distinction in three disjunctive classes is not possible, neither by image analysis nor by a trained cytotechnologist. The major advantage of image analysis is that the cell class membership can be reproducibly determined by statistical cell classifiers, as shown in this study. Manual classification of individual cells, on the other hand, is subject to intra- and interobserver variations. In this study it was shown that the same experienced cytotechnologist applied cell classification criteria differently in the first and second measurements. In all atrophic specimens the fraction of (para)basal cells was greater in the second than in the first measurement session. In most cases of mature specimens, the fraction of intermediate cells was greater in the second measurement than in the first. These findings are suggestive of a learning effect. Therefore, the visual assessment of Papanicolaou-stained vaginal smears is not recommended for a reliable and reproducible measurement of the maturation indices for vaginal mucosa. For

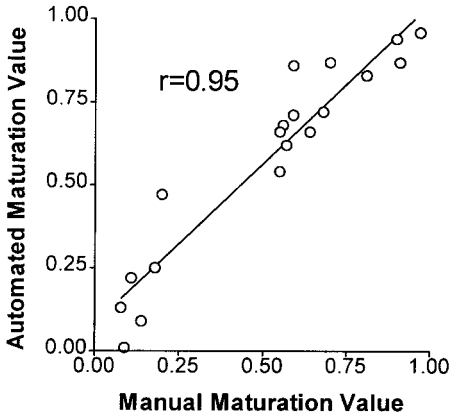

FIG. 4. Comparison of results of automated vs. manual assessment of the maturation value of 19 vaginal specimens, both mature and atrophic.

such purposes the use of image analysis systems seems to be much more appropriate.

These data support the idea that more accurate monitoring of therapy effects will be possible using cytomorphometrical parameters, such as size and shape, as opposed to classification of cells using DFs. Small changes in nuclear and cytoplasmic size and shape, not discernible by visual inspection, will be revealed by the automated procedure. The issues of discriminating power and the potential value of additional parameters yielded by the computerized cytomorphometric method in subsequent smears of women treated for vaginal atrophy will be the object of future studies.

An experienced cytotechnologist needed approximately 3–5 min to assess the maturation index manually. As it is, the automated measurement took in the order of 30–60 min for each measurement. Clearly, at this time the automated assessment of the maturation of vaginal smears costs more operator time than manual assessment. However, no special training is needed to operate the automated measurement device, whereas manual assessment requires a trained cytotechnologist. An even more important advantage of the automated method is the higher reproducibility and the possibility of studying more subtle morphological changes in addition to the rather coarse information present in the maturation index. Cost effectiveness of the automated method is likely to be improved in the near future, because of new developments in computer hard- and software.

In conclusion, this study showed that by using both the MA and CMA approaches, highly significant differences could be found between premenopausal smears and postmenopausal smears showing vaginal atrophy. Both approaches might thus be used to estimate the state of the vaginal epithelium, the cytomorphometric procedure being the more reproducible procedure. The developed computerized cytomorphometric method is a versatile and reliable tool in assessing the maturation of vaginal epithelial cells and allows a reproducible, automated throughput of vaginal smears which is of relevance in the conduct of clinical studies and screening programs.

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