Distinguished expert series

How to improve cytologic screening for endocervical adenocarcinoma?

M. E. Boon[1], E. Ouwerkerk-Noordam[1], A. W. F. M. van Leeuwen[2], L. P. Kok[3], C. van Haaften-Day[1]

[1]Leiden Cytology and Pathology Laboratory, Leiden; [2]Pathological Laboratory Dordrecht, Dordrecht; [3]Department of Biomedical Engineering, University of Groningen, Groningen (The Netherlands)

Summary

Aim: A retrospective study was undertaken to investigate how to improve the diagnosis of endocervical adenocarcinoma in screening programs.

Material and Methods: The study group consisted of 29 slides of women diagnosed with cancer but who had negative smears. The slides were subdivided in 12 smears taken less than one year before diagnosis by histology and 17 smears taken between one and 10 years prior to diagnosis. A hundred smears of healthy women were used for comparison. All smears were studied macroscopically after which both groups of smears were scanned by the Neural Network Scanner (NNS). Differences between groups were studied for statistical significance using Pearson's Chi-squared test.

Findings: The macroscopic parameter of these smears found to be present most frequently was a heavy admixture of blood. The presence of blood (lysed or not) in the smears was equally consistently highlighted by the NNS. Statistical significance of the association of this parameter, with the presence of cancer, was demonstrated.

Conclusion: The awareness of blood as a background feature of adenocarcinoma of the cervix will help to select cases needing special attention. These difficult bloody smears, studied by light microscopy and by NNS images can also be selected for additional MiB-1 staining. With this approach, blood in smears, otherwise frequently leading to a compromise of classification, can become a blessing in disguise. The diagnosis of endocervical adenocarcinoma in screening smears will therefore be improved.

Key words: Adenocarcinoma of the endocervix; Bloody; NNS.

Introduction

The diagnosis of adenocarcinoma of the endocervix in cervical smears can be difficult [3]. The awareness and retrospective analysis of so-called false-negative smears hidden in cytological archives has shed new light on the problems in the diagnostic processes [7, 10 ,12]. For instance, in highly differentiated adenocarcinomas cancer cells are not recognized by the screener as being malignant [4, 8]. The precursor lesions, adenocarcinoma in situ (AIS), similarly are often not detected in the smears [5, 9]. The purpose of screening programs is to diagnose developing malignancy at the stage of AIS. We feel however that in the Netherlands screening programs for AIS may still be limited with respect to sensitivity. For instance, between 1991 and 2001, 634,325 smears were screened in the Leiden Cytology and Pathology Laboratory (LCPL), and only 50 cases of AIS and 45 cases with adenocarcinoma of the endocervix were found. These data suggest that many cases of adenocarcinoma remain undetected. In the same period, as many as 641 cases with (squamous cell) carcinoma in situ (CIS) were detected and 98 squamous cell carcinomas (SCCs). Thus only 44 of the 142 (31%) of the cases with invasive carcinoma from the archives of the LCPL were adenocarcinomas of the endocervix. Therefore it is important to establish the right sample parameters for screening for adenocarcinoma of the cervix. One way to achieve insight in the diagnostic problems encountered in routine screening is to re-analyze the smears preceding a diagnosis of malignancy.

We present an analysis of 29 smears from a Laboratory in The Netherlands, all diagnosed as cancer-negative, of patients who by histology were proven to have adenocarcinoma of the endocervix. All 29 slides were evaluated macroscopically and scanned with the NNS system for the analysis of the background features.

Material and Methods

Smears of 29 patients with adenocarcinoma of the cervix were collected. The histology of these cases was performed between 1989 and 1999 at the Pathology Laboratory in Dordrecht, the Netherlands. In a quality control project, in total 38 smears were identified of women with adenocarcinoma of the endocervix, of which only nine were immediately diagnosed as cancer-positive (Papanicolaou classification IIIB, IV, and V). The remaining 29 smears were so-called cancer-negative. Of these cases, accordingly there was no histological investigation following the cytology. In 15 of these 29 cases, no follow-up smear was asked for. In four cases, the smear was of poor quality and on advise of the laboratory had to be repeated. In three cases the diagnosis was "atypical cells of unknown significance" (ASCUS and AGUS). In the remaining seven a follow-up smear was taken because the cytological diagnosis was of "low-grade intraepithelial lesion" (L-SIL and L-AIL). The 29 patients were divided into a group of 12 cases in which the smear preceded the diagnosis of adenocarcinoma by one year or less, and a group of 17 in which the diagnostic histology was performed one-10 years after the date of smear.

As a control series, 100 smears were used from healthy women. All smears were first evaluated macroscopically. The samples were coded as a) insufficient (very little cellular material), b) sufficient, and c) very bloody. Secondly, the smears were scanned with the NNS bought from the firm NSI (New York, USA) to allow analysis of the background features. This computerized PAPNET® system scans with the aid of neural network technology a selection of 128 cellular fields (PAPNET images). This may include diagnostic cells, epithelial fragments or background information such as old blood, fibrin or necrosis. These 128 images (tiles) are then collected in two sets of 64 images on the computer screen of the diagnostic unit of the NNS system and the 16 most significant images are selected for the "summary screen" [6]. Based on the NNS background images of the 29 smears, the cases were coded as clean, inflammatory, and bloody [11] The latter were further subdivided into: fresh blood, old blood (lysed erythrocytes and fibrin), both fresh and old blood, and blood and necrotic material.

Pearson's Chi-squared test: Samples were measured by the two times four table with three degrees of freedom.

Results

In Table 1 the macroscopic and microscopic smear parameters of the 29 patients and the 100 healthy women are presented. The percentage of bloody smears of the cancer patients was ten times that of the healthy women. The differences between these two groups by macroscopy was significant, as shown by the application of Pearson's Chi-squared test: $p < 0.0001$. Also the differences of the microscopic background NNS images between the patients and the healthy women was significant: $p < 0.0001$.

Table 1. — *Macroscopic and microscopic sample parameters.*

	Patients with adenocarcinoma		Healthy women	
	n	%	n	%
Macroscopy	*29*		*100*	
• Insufficient	6	21	1	1
• Sufficient	13	45	96	96
• Bloody	10	34	3	3
Microscopy	*29*		*100*	
• Clean	15	52	85	85
• Inflammatory	3	10	11	11
• Bloody	11	38	4	4

Table 2. — *Macroscopic sample parameters: one year (≤ 1 year) versus many years (1-10 years) preceeding the diagnosis of adenocarcinoma.*

Smear	≤ 1 year	1-10 years
Insufficient	2	4
Sufficient	4	9
Bloody	6	4
Total	12	17

Table 3. — *Macroscopic sample parameters: one year (≤ 1 year) versus many years (1-10 years) preceeding the diagnosis of adenocarcinoma.*

Smear	≤ 1 year	1-10 years
Clean	5	10
Inflammatory	1	2
Bloody	6	5
Total	12	17

Table 4. — *Subtypes of bloody smears.*

	Patients with adenocarcinoma	Healthy women
Fresh blood	5	3
Old blood	2	1
Fresh and old blood	2	0
Blood and necrosis	2	0
Total	11	4

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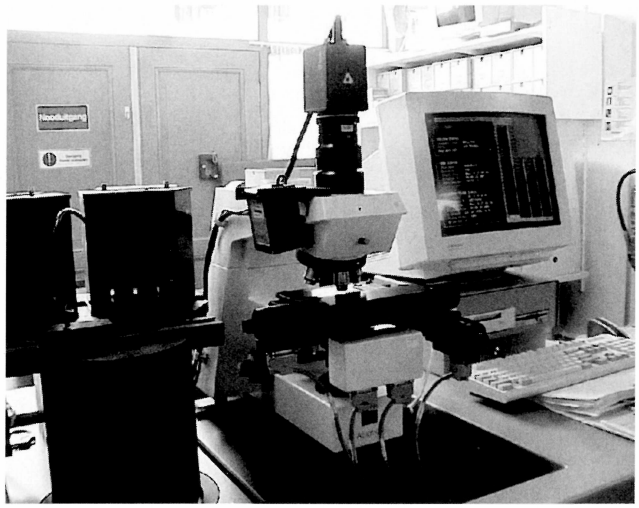

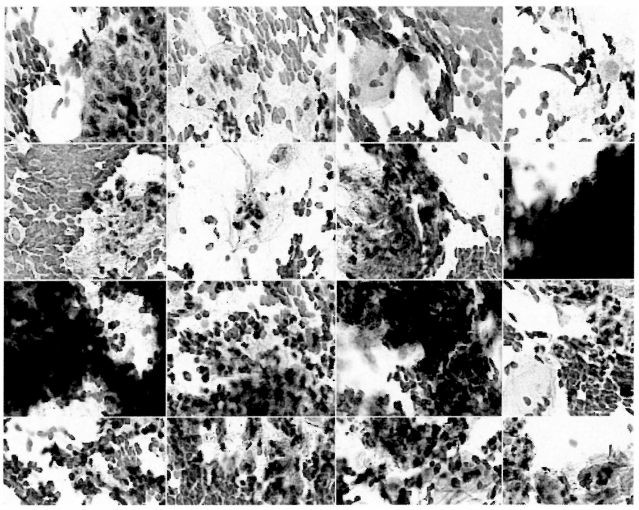

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Figure 1. — The Neural Network Scanner at the Leiden Cytology and Pathology Laboratory, consisting of a) two cassettes, b) a robot finger, c) a microscope with motorized stage, d) a computer screen showing the graphics-user interface.
Figure 2. — The "summary screen" of 16 images all containing blood. Three of the images contain cancer fragments which cannot be recognized because they are covered by a thick layer of blood.

In Figure 1 is the Neural Network Scanner shown with microscope and motorized stage, while Figure 2 gives the 16 images of the so-called "summary screen", all containing blood. Three of the images could not be recognized because they were covered by a thick layer of blood.

The 29 patients were further divided in 12 and 17 cases (Table 2). By macroscopy six of the smears of the 12 cases of the first group were bloody, while only four smears of the 17 cases were found bloody. Figures 3 and 4 show such a smear, of a patient with an adenocarcinoma fragment, completely covered by blood. These findings were confirmed in the NNS background images (Table 3). There were 11 smears with blood in the patient group and four in the group of healthy women. Table 4 gives the results of the subtypes of these 15 smears. In the group of patients, smears with old blood (lysed erythrocytes) were frequently encountered while in the healthy women such was seen in only one smear.

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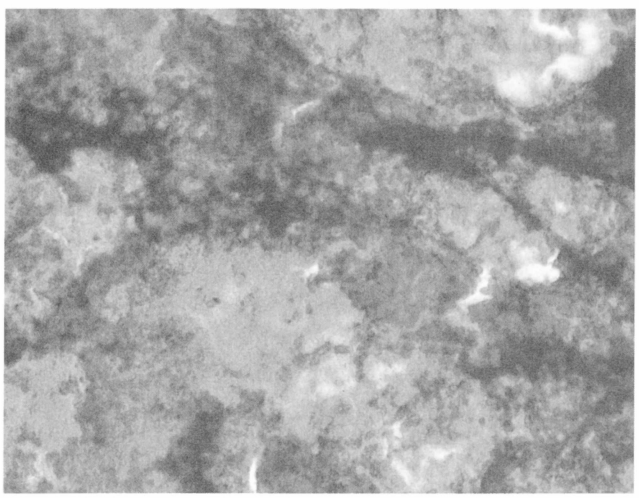

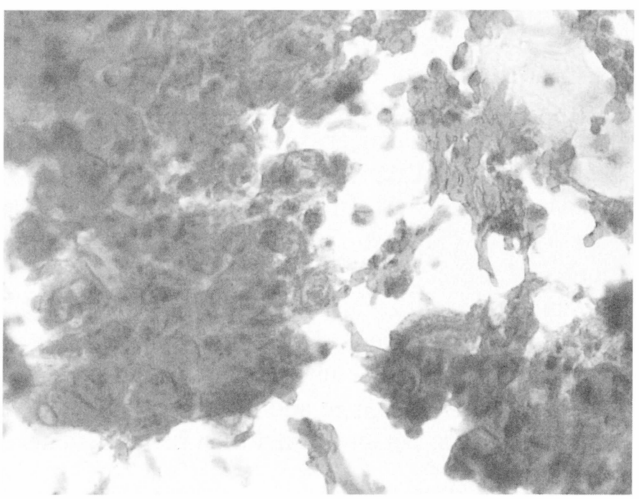

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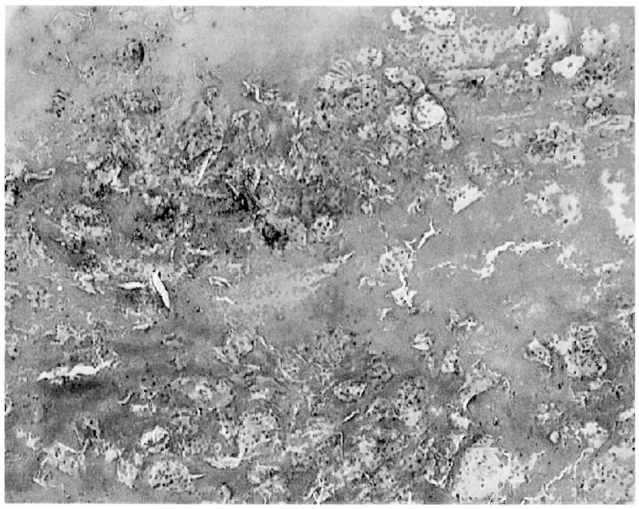
Figure 3. — A smear of a patient with an adenocarcinoma fragment completely covered by blood.
Figure 4. — A magnification of a part of slide 3. The nuclei of the carcinoma cells are completely blurred.



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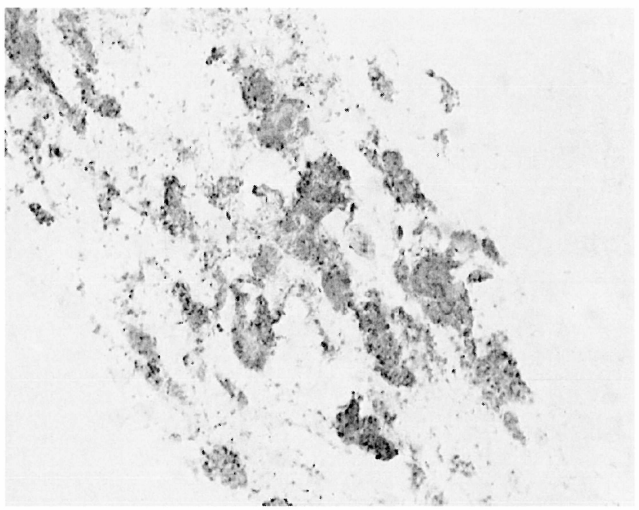

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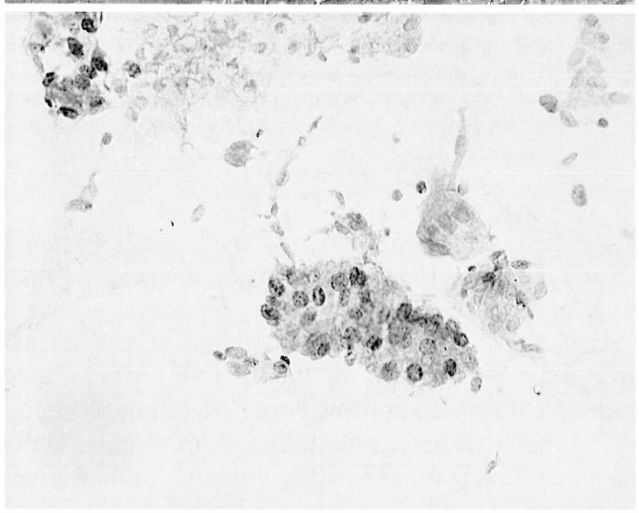

7

Figures 5, 6 and 7. — An exemplary case of the Leiden Laboratory.
Figure 5. — A microphotograph of a Papanicolaou-stained bloody smear of a patient.
Figure 6. — The same microphotograph, but with MiB-1 staining, with nuclei highlighted by the brown staining. Blood is no longer visible.
Figure 7. — A magnification of a cancer fragment with MiB-1-highlighted malignant nuclei.

Discussion

The purpose of cytological screening is to discover precancerous lesions of the uterine cervix. By removing the abnormal epithelium at this stage, the development of invasive cancer can be prevented. Nonetheless, in a proportion of women the first lesion diagnosed has been proven invasive cancer. This was also true for the 29 women with adenocarcinoma of the endocervix diagnosed in Dordrecht, the Netherlands, between 1989 and 1999.

Of these smears, sample parameters as “clean”, ”inflammatory”, and ”bloody” were studied. We did not attempt to identify cancer cells in these false-negative smears, but instead we focused completely on background features of the cell sample, as has been described by Kok *et al.*, 1999 [6].

Eleven of the 29 (38%) smears had many NNS tiles (images) with blood. In these cases, of the 64 tiles of page 2 of the diagnostic unit of the NNS system [6] between 55 and 64 contained erythrocytes of various degrees of preservation. In six of the 11 smears the blood was partly lysed. This gave the computer images an orange-like look, which was immediately evident, even at a distance. In nine of these 11 cases, the computer images contained a lot of dark blue components (Figure 2). In short, the combination of orange and dark blue was very striking. These six smears were conspicuous, even when observed macroscopically, due to their mixture of orange and dark red. Such smears should be approached more carefully and at least be evaluated by two persons rather than be immediately discarded as ”unsuitable for analysis” and ”non-informative”.

Four of the 11 bloody smears were confirmed at review to have been correctly classified at initial assessment as of very poor quality. Consequently a repeated smear had at that time been asked for. For some

reason, this recommendation to take a repeated smear did not result in a positive histology. It is our experience, that often the repeated smear is again too bloody for evaluation.

By using the MiB-1 staining method [1, 2] on these difficult smears, the fragments of the adenocarcinoma can be highlighted. The MiB-1 staining (Figures 5-7) shows the nuclear features of proliferating cancer cells by staining these with a dark brown color and thus the nuclear features of the cancer cells are no longer obscured by the presence of blood. With such an approach, equivocal cases can be solved in a relatively simple way.

In conclusion: The NNS is able to highlight background features which are statistically associated with and are indicative of difficult samples of cancer patients. With additional MiB-1 staining such cases can then be more precisely diagnosed. With this knowledge the quality and efficiency of the diagnostic process will improve.

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Address reprint requests to:
M. E. BOON, M.D.
Leiden Cytology and Pathology Laboratory
P.O. Box 16084
2301 Leiden - The Netherlands