Towards an automated procedure for the quantitative cytological screening of cervical neoplasms

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SUMMARY Integrating microdensitometry has been used to quantify the glycoprotein, epithelial membrane antigen (EMA), which is frequently expressed on the cytoplasmic membrane of neoplastic cells of the cervix uteri. The technique was able to identify and quantify the antigen over a wide concentration range and could also be semi-automated using a microcomputer interfaced to the microdensitometer and programmed with the simple high level language BASIC.

Widespread reference has been made elsewhere to the application of microcomputer technology to quantitative cytological assessment.[1-3] More specifically, the quantitative screening of neoplasms has also been referred to[4] and in particular, the concept of automated analysis of exfoliated cells of the cervix uteri.[5] We have investigated the possibility of quantifying epithelial membrane antigen (EMA) glycoprotein bound to the plasma membrane of neoplastic cells of the cervix by means of an integrating microdensitometer. In order to test whether our quantification technique could be automated we have also used a computer programme written in BASIC.

The EMA glycoprotein is confined to but widely distributed in epithelial tissues and the tumours derived from them.[6] It has been identified in the metastatic deposits of primary breast carcinoma,[6,7] some carcinomas of the skin[8] and in cervical intraepithelial neoplasia.[8,9]

Material and methods

Cervical smears were taken in the conventional manner using a wooden Ayres spatula which was immediately placed in 5 ml of Cellfix[10] solution where it remained for at least 24 h. Smears were then prepared as described previously.[9] Those from women with known cervical intraepithelial neoplasia (CIN) were taken at the Colposcopy Clinic just prior to assessment and treatment. The normal smears were taken at routine gynaecology clinics as second samples, the first being sent for Papanicolaou staining classification.[11] In this preliminary study we

analysed 11 smears, three with each grade of CIN and two which were cytologically normal.

The indirect alkaline phosphatase method for immunocytochemistry was used for the detection of EMA.[12] Briefly, smears were incubated for 60 min with a suitable dilution of rabbit antiserum, then washed and incubated for the same period with the enzyme-conjugated second antibody. The stain was developed in a solution of naphthol AS-BI phosphate and Brentamine Fast Red. All smears were processed together to eliminate variation between experiments. At least 20 EMA-negative, and where possible 20 EMA-positive cells, were measured from each smear using a Vickers M86 microdensitometer with a ×40 objective, a scanning spot size of 0·5 μm and a closing mask of 13 μm diameter (type A3). Previous calibration with an EMA-positive cell (Fig. 1) had indicated an optimal wavelength of 500 or 570 nm. Four measurements were made on the membrane of each cell and the results expressed in terms of the optical density recorded by the microdensitometer. Readings were also taken with the microdensitometer interfaced with a Research Machines 380Z microcomputer (Fig. 2). Under the control of a BASIC programme the microcomputer could process results taken directly from the instrument.

Results

The calibration curve for the alkaline phosphatase reaction product (Fig. 1) was plotted using the mean of readings taken in triplicate. The curve showed peaks of maximum absorption at wavelengths of 500 and 570 nm, all measurements being subsequently carried out at 500 nm. The results from the smears
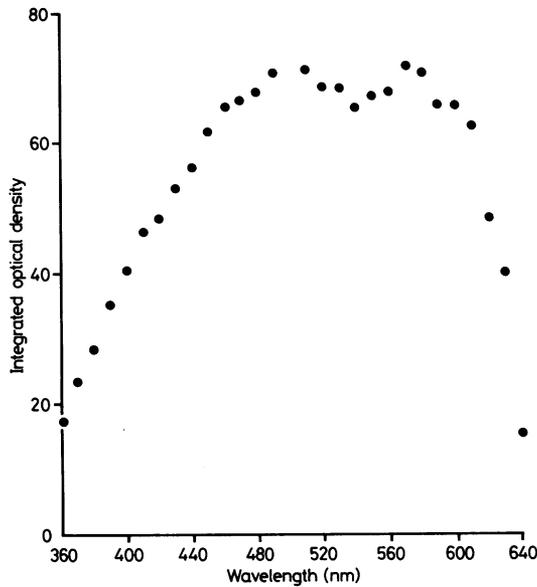
Fig. 1 *Integrated optical density in arbitrary units for an EMA-positive cell sample over a range of wavelength settings.*
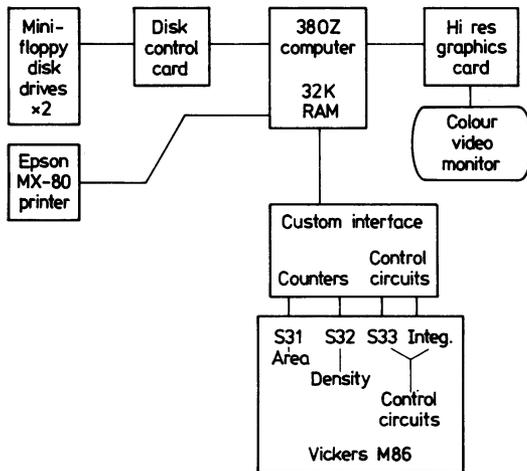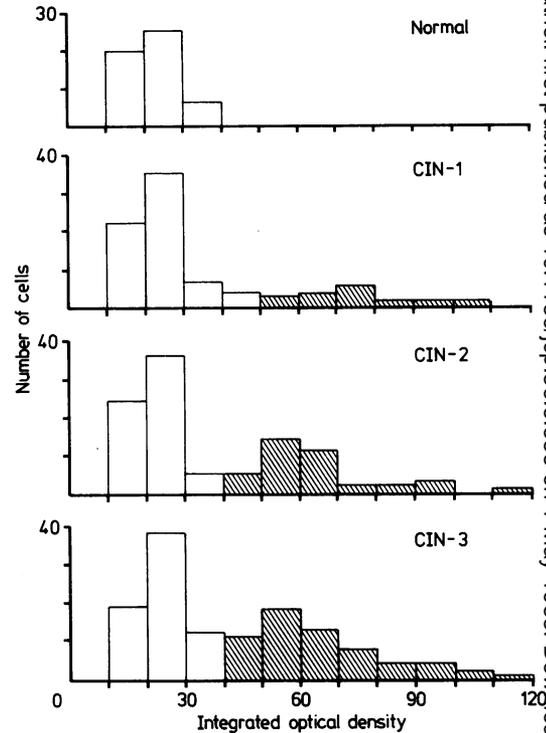
Fig. 3 *Histograms showing the integrated optical densities for cytologically normal and abnormal cells from both normal and CIN grade 1–3 patients. The bars representing cytologically abnormal cells are shaded while those representing normal cells are unshaded.*

Fig. 2 *Schematic diagram of the Research Machines 380Z computer system and its interface coupling to a Vickers M86 integrating microdensitometer.*

(Fig. 3) showed that while no positive cells were detected in the cytologically normal smears it was possible using microdensitometry to distinguish quantitatively between EMA-positive and -negative cells in the CIN grades. Where possible 20 positive cells were measured for each patient, although this became impractical at the lower CIN grades where the number of cells expressing the antigen became severely reduced. In some cases the values of positive results differed only slightly from the negative readings. However, the smears derived from the CIN grade patients always included positive cells whose optical density per unit area of membrane was at least double that of the highest reading negative cells in the same smear. Positive readings showed a relatively wide distribution which contrasted with the narrower spread of negative results and appeared unrelated to CIN grade. The maximum standard deviations for the positive cells did not indicate wide differences between the four membrane measurements made for each cell.

Our results confirmed earlier reports[13] that the concentration of EMA was highest for cells occurring singly without contact with other malignant cells. In some instances we observed weak antigen expression which was associated with obviously damaged or injured tissues, results which were excluded from this present study.
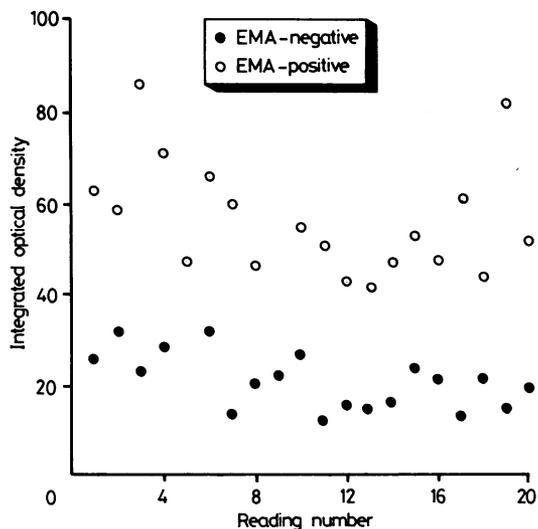
Fig. 4 *A computer graphics display illustrating the ability of the microcomputer to distinguish between EMA-negative (●) and -positive (○) cells on the basis of integrated optical density.*

A Research Machines 380Z computer interfaced with the microdensitometer and using a simple programme written in BASIC could reliably distinguish between EMA-positive and -negative cells on the basis of integrated optical density measurements (Fig. 4). The Figure shows two superimposed high resolution graphic displays of positive and negative results for one of the CIN 3 grade patients.

Discussion

The rationale behind the current investigation was to test the possibility of quantifying the EMA glycoprotein test by using integrating microdensitometry. Traditionally this has usually been applied to the measurement of nuclear rather than membrane chromophores; however, quantification of membrane areas may be sucessfully carried out with this technique. In the current investigation we were assisted by the homogeneity of the alkaline phosphatase reaction product which gave a standard deviation in the quadruple sets of readings carried out on each cell that was small in relation to the magnitude of the readings obtained. Similarly, the maximal expression of the antigen appeared with isolated cells rather than dense aggregates. This assisted microdensitometry but may also have explained the relatively wide distribution of positive readings, lower positive values of antigen being expressed by some attached cells nevertheless suitable for analysis. Even at the lower values the reac-

tion product could still be distinguished and quantified by microdensitometry. We noted the weak expression of antigen in some obviously damaged cells which may be linked to the protective role previously cited for EMA.[8]

A preliminary test of the method as a candidate for automated cytological approaches showed that it was possible by using a simple programme written in BASIC for a microcomputer to distinguish between EMA-positive and -negative readings taken directly from the microdensitometer. Recent developments in automated cytology procedures have adopted the Quantimet 720D Image Analysis system combined with a Plumbicon light pen selection technique.[14] The results obtained using a rudimentary automated approach indicate that there appears to be no reason why a sophisticated microcomputer system of this type could not be applied to the EMA glycoprotein test.

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