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The use of electrical impedance spectroscopy in the detection of cervical intraepithelial neoplasia

S. ABDUL*, B.H. BROWN†, P. MILNES† & J.A. TIDY*
**Sheffield Gynaecological Cancer Centre, Royal Hallamshire Hospital, Sheffield, United Kingdom; and*
†Department of Medical Physics, University of Sheffield, Royal Hallamshire Hospital, Sheffield, United Kingdom

Abstract. Abdul S, Brown BH, Milnes P, Tidy JA. The use of electrical impedance spectroscopy in the detection of cervical intraepithelial neoplasia. *Int J Gynecol Cancer* 2006;**16:**1823–1832.

The objective of this study was to assess the performance of cervical impedance spectroscopy in the detection of cervical intraepithelial neoplasia (CIN) using the new MKIII impedance probe. A prospective observational study recruited women referred to colposcopy with an abnormal Papanicolaou smear. A pencil probe incorporating four gold electrodes was used to measure electrical impedance spectra from cervical epithelium. Colposcopy examinations, including probe positioning, were video recorded to allow for correlation between results obtained from colposcopic impression, histopathologic examination of colposcopic punch biopsies, and impedance measurements. Cervical impedance–derived parameters *R*, *S*, *R*/*S*, *C*, and F_c were assessed to see if significant difference in values obtained in CIN and normal epithelium existed. The performance of the probe in identifying women with CIN was also assessed. One hundred seventy-six women were recruited and 1168 points analyzed. Parameters *R*, *S*, and F_c showed significant separation of CIN or squamous intraepithelial lesion (SIL) from squamous, mature metaplastic, and columnar epithelium. Sensitivities of 74% and specificity of 53% can be achieved in identifying CIN 2/3 (High-grade SIL) in screened women. We conclude that cervical impedance spectrometry provides a potentially promising real-time screening tool for CIN with similar sensitivity and specificity to currently used screening tests. Further research is ongoing to develop the probe for potential clinical use.

KEYWORDS: cervical impedance spectrometry, cervical intraepithelial neoplasia, CIN, screening, SIL.

Cervical cancer is the second most common female cancer worldwide, affecting 471,000 women per year and is the commonest cause of cancer-related mortality (233,000 deaths per year). The United States had an estimated 12,200 new cases and 4100 deaths annually[1,2].

The detection and treatment of the precursor lesion, cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesion, can prevent the development of cancer. Screening programs based on the use of exfoliative cytology (the Papanicolaou [Pap] smear) to detect CIN have been shown to reduce the incidence of and mortality from cervical cancer, resulting in thousands of lives being saved every year[3]. When used as a single test, however, the Pap smear has a reported specificity of 95–98% but a sensitivity of only 50%[4]. The effectiveness of screening programs therefore relies on multiple opportunities at identifying underlying CIN by regular screening through an organized computerized call and recall system. Such screening programs are highly costly, with an estimated cost of $240 million a year in the UK alone. The costs and logistical organization needed make such screening programs difficult to organize in the developing countries where 80% of cervical cancer cases occur. Cytologic screening also has a delay from the test being taken to the results being made available resulting in significant patient anxiety.

There is therefore a need to develop other tests for the detection of CIN that may improve both the efficiency and the reliability of screening but in addition may also provide instant results.

Screening methods that have been investigated include the detection of human papillomavirus (HPV

Address correspondence and reprint requests to: Summi Abdul, MB, ChB, BSc(Hon), DFFP, MRCOG, G18 Royal Hallamshire Hospital, Sheffield S10 2JF, UK. Email: SummiAbdul@hotmail.com

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DNA), optical techniques (visual inspection of the cervix following the application of acetic acid [VIA], colposcopy, cervicography, speculoscopy, fluorescence spectroscopy, reflective spectroscopy, Raman spectroscopy, confocal imaging, and optical coherence tomography), optoelectronic techniques (TruScreen, Polartechnics Ltd, Sydney, Australia), and electrical measurement techniques[5].

Biologic tissue has electrical impedance, which is a function of frequency of electrical current applied. The reason for this dependence is that tissues contain components that have both resistive and capacitive (charge storage) properties. Both size of the impedance, as well as the dependence of impedance on frequency, are related to tissue composition and structure.

Cervical epithelium is a highly structured, stratified tissue that exhibits changes associated with CIN, including an increase in the nuclear cytoplasmic ratio, loss of the layer of flattened cells close to the surface, and an increase in the volume of extracellular space. The geometric arrangement of cells is known to affect tissue impedance, and this formed the basis for predicting the associated tissue impedance spectra likely with CIN.

Our previous study[6] has shown that a pencil probe incorporating four gold electrodes can be designed and used to measure an electrical impedance spectrum from cervical epithelium. In the pilot study on 124 women and subsequent study on 87 women[7,8], changes in the spectra were observed in association with premalignant changes. The sensitivity and specificity obtained in the detection of premalignant changes were at least as good as those obtained from cervical smear testing. The observed changes in impedance spectra can be explained by modeling the known structural changes in the top 400 μm of cervical epithelium associated with CIN 1, 2, and 3[9,10]. There were, however, difficulties with the probe separating metaplastic and columnar epithelium from CIN.

Separation of tissue types not achieved by making measurements at eight frequencies between 4.8 and 614 kHz may be achieved by taking a greater number of measurements over a wider frequency range. This may allow separation to be achieved by exploiting the abnormal nuclear changes seen in CIN that differ from the normal nuclei of metaplastic tissue.

As a result, we have designed a new probe that takes impedance measurements at 30 frequencies over a range of 2–1200 kHz. The aims of the current study were to determine the performance of the new probe in separating normal tissue from CIN and to assess potential for its use as a screening tool.

Materials and methods

Impedance measurements were made using a 5.5-mm-diameter pencil probe (Fig. 1) with four 1-mm-diameter gold electrodes mounted flush with the face of the probe and spaced equally on a circle. A current of <25 μA peak-to-peak was passed between an adjacent pair of electrodes and the resulting potential measured between the two remaining electrode. Measurements were taken at 30 frequencies in the range 2–1200 kHz. Calibration was performed by placing the probe in saline of known electrical conductivity. A four-electrode measurement of the transfer impedance spectrum is essentially independent of the contact impedance between electrode and tissue (which is of the order of 1 kΩ compared to the transfer impedance of 100Ω).

Patients

Women with abnormal Pap smears were recruited after the study had been approved by an ethics committee and informed consent was obtained. Patient measurements were made in the colposcopy clinic.

Impedance measurements were made prior to the application of acetic acid that is needed for the purpose of colposcopy. The probe was placed in eight positions on the cervix. These were as for the cardinal points of the compass with four positions close to the border with the endocervical canal and the remaining four well into the normal squamous epithelial surface of the cervix. Impedance measurements were made serially at 80 frames per sec and recorded onto computer files. Colposcopy examinations, including probe positioning, were recorded by video to allow for correlation between the results obtained from colposcopic impression, histopathologic examination of colposcopically directed punch biopsies, and impedance.

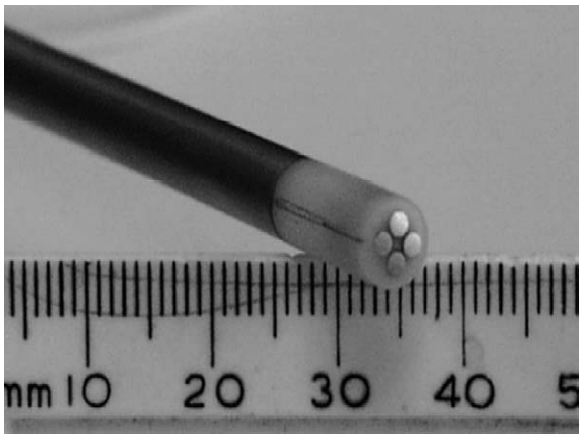

Figure 1. Cervical impedance spectroscopy four-electrode probe.

Biopsy was used where colposcopy alone did not offer a clear categorization of the tissue. Tissues at each impedance measurement point were classified as a result of video analysis into following epithelial groups: normal original squamous epithelium, high-grade CIN (CIN 2/3), low-grade CIN (CIN 1), mature metaplastic, immature metaplastic, columnar, inflammatory, HPV infected, Nabothian follicle, high-grade cervical glandular intraepithelial neoplasia, and invasive cancer. To qualify as "normal," squamous epithelium has to lie outside the transformation zone, show no evidence of change with acetic acid, and have a positive staining with Lugol's iodine. The whole of the transformation zone was visible in all cases. In nearly all cases, two separate sets of data (each of 80 measurements recorded over 1 sec) were recorded in succession in order to check reproducibility of the measurements. Only the best fitted of the two measurements was used for the results presented in this study.

Analysis

The 80 measurements forming the first data set recorded at each measurement position were averaged to give mean values of impedance at each of the 30 frequencies. These data, forming an impedance spectrum, were then fitted by a least square deviation method to a Cole equation to give estimates of R_0, R_∞, and F_c. R_0 and R_∞ are the impedances (real part) at very low and very high frequencies, respectively; F_c is the frequency at which the impedance spectra has its maximum gradient. In this case, an equivalent electrical circuit consisting of a resistor R placed in parallel with a resistor S and capacitor C in series will have an impedance Z, given by the Cole equation[11]:

$$Z = R_\infty + \frac{(R_0 - R_\infty)}{\left(1 + (jF/F_c)^{(1-\alpha)}\right.}$$

where, α is a constant that increases with the inhomogeneity of tissue, but we assumed a value of zero.

$$R_0 = R, \quad R_\infty = \frac{RS}{R+S}, \; F_c = \frac{1}{2\pi C(R+S)}$$

Parameters R, S, C, and F_c can thus be determined from the fitted Cole equation. Because the probe was calibrated in saline of known conductivity, R and S are inversely proportional to conductivity and have the units of Ωm. They can be related to the extracellular and intracellular spaces, respectively, as described earlier. C is related to the cell membrane capacitance and is given in units of Fm^{-1}. F_c is the frequency, in Hz, at which current is able to penetrate the cell membrane and pass through both the intracellular and the extracellular space.

Results

A clear colposcopy result and good impedance data were available for 1168 measurements made on 176 women. The maximum possible number of measurements was 1408 (ie, 8×176); thus, 240 measurements could not be assessed because either the point where the probe had been placed was not clearly identified by biopsy or colposcopy to be in one histologic group (105 measurements) or data were rejected on technical grounds (135 measurements). Technical rejection of data occurred if there was poor contact of electrodes at the time of the measurement being taken or the impedance spectra produced could not be fitted to the Cole's equation with the software used.

After comparing colposcopic and histology results, there were found to be 680 measurements from normal squamous epithelium, 178 from CIN 2/3 (high grade), and 39 from CIN 1 (low grade). In addition, 135 points were classified as mature metaplastic, 79 as immature metaplastic, and 28 as columnar tissue.

The derived Cole equation parameters for the six tissue groups are shown in Table 1, Table 2, and Figure 2. From normal squamous epithelium through CIN 1 to CIN 2/3, mean values for R decrease by a factor of 3.2; S increases by a factor of about 2.0, F_c increases by a factor of about 4.5; R/S decreases by a factor of about 4.0, and C does not change greatly. Nonparametric Mann–Whitney U test (two-tailed tests) was performed and showed that there are several significant separations of the groups ($P < 0.0001$).

Table 1. Statistical data for parameters R (Ωm), S (Ωm), C (μFm^{-1}), F_c (Hz), and R/S in different tissue groups

	Original squamous (680)					Low grade (39)					High grade (178)				
Parameter	R	S	C	F_c	R/S	R	S	C	F_c	R/S	R	S	C	F_c	R/S
Mean	23.87	3.29	0.89	13,247	9.57	11.12	4.29	1.02	21,096	3.46	7.50	6.04	0.71	58,787	2.33
Median	20.05	2.99	0.67	8904	6.98	8.49	3.67	0.80	12,790	2.24	3.99	5.88	0.49	28,691	0.63
SE	0.57	0.07	0.03	539	0.34	1.36	0.29	0.11	4560	0.54	0.70	0.20	0.06	6273	0.38

Table 2. Statistical data for parameters R (Ωm), S (Ωm), C (Fm^{-1}), F_c (Hz), and R/S in different tissue groups

	Columnar (28)					Mature metaplastic (135)					Immature metaplastic (79)				
Parameter	R	S	C	F_c	R/S	R	S	C	F_c	R/S	R	S	C	F_c	R/S
Mean	5.18	7.22	0.39	88,823	1.09	18.94	3.86	1.10	17,243	7.49	7.65	5.90	0.57	71,571	2.14
Median	2.61	7.73	0.24	69,648	0.37	13.76	3.14	0.84	9778	4.63	3.48	5.81	0.40	40,409	0.52
SE	1.46	0.44	0.08	13,943	0.41	1.27	0.37	0.11	2852	0.70	1.14	0.26	0.06	9414	0.44

CIN 2/3 can be separated from normal cervical tissue by a number of parameters: R, S, C, F_c, and R/S separate CIN 2/3 tissue from normal squamous epithelium ($P < 0.0001$) as well as from mature metaplastic tissue ($P < 0.0001$). CIN 2/3 also shows significant separation from columnar epithelium using R ($P = 0.0023$), S ($P = 0.0074$), C ($P = 0.0047$), F_c ($P = 0.0051$), and R/S ($P < 0.0001$). CIN 2/3 could not be separated by any of the parameters from immature metaplastic tissue.

CIN 1 can also be separated from normal cervical tissue by a number of parameters. R, S, R/S ($P < 0.0001$), *and* F_c ($P = 0.0078$) separate CIN 1 tissues from normal squamous epithelium. All five parameters separate ($P < 0.0001$) CIN 1 from columnar epithelium and immature metaplastic tissues. R, S, C, F_c, and R/S separate CIN 1 from CIN 2/3 tissues ($P < 0.0001$).

To assess the usefulness of the technique as a screening test, we derived receiver operating characteristic (ROC) curves[12] for the normal cervical epithelium and CIN tissue groups. ROC curves show the sensitivities (1—the fraction of false negatives) and the specificities (1—the fraction of false positives), with these variables used as discriminants between the normal squamous epithelium and the CIN 2/3 tissue groups. If there is discrimination between the two groups, the curve is displaced upward and to the left. The area under the curve (AUC) indicates the degree of separation, an area of 0.5 corresponds to no discrimination and an area of 1.0 to perfect separation. The AUC for separation of CIN from normal tissue types are shown in Table 3. R/S appears to be the best single parameter for separation of CIN from original squamous, mature metaplastic, and columnar tissue with AUC values of 0.865, 0.784, and 0.712, respectively.

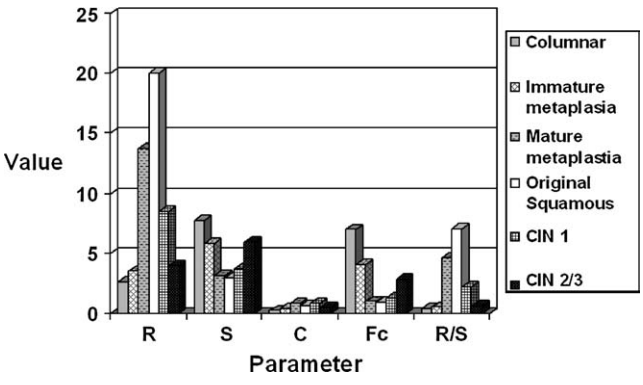

Figure 2. Median values for parameters R (Ωm), S (Ωm), C (μFm^{-1}), F_c (Hz), and R/S in different tissue groups.

Analysis per woman

The data were also grouped for each woman so that we could make comparisons between the electrical impedance measurements and the results of both the referral smear test and the outcome of the colposcopy examination.

To provide a single indicator for each woman, R/S was first calculated for each of the eight measurement sites. This was an attempt to take into account the fact that R decreases and S increases as we progress from normal squamous epithelium through CIN 1 to CIN 2/3. The lowest value of R/S (R/S *minimum*) was then taken as the outcome for each woman on the basis that this should identify the greatest abnormality. However, we found that this method of identification included a number of tissue sites that were identified by colposcopy as columnar or immature metaplastic tissues. To limit this confusion, we excluded sites where R was less than or equal to 2.95 Ωm (the 25% percentile for the CIN 2/3 group) when taking the minimum value of R/S in each woman. In this study, we also analyzed the data by defining CIN by percentile limits on R, S, and R/S. If any of the measurements on a particular woman were within these limits then a positive impedance result was returned. In addition, we assessed the AUC curves for R/S to define the cutoff value needed to differentiate between the normal and the CIN tissue to give a per point sensitivity of 50% and specificity of 98.4%. We obtained a value of 0.64, so we classified tissue on the basis of R/S values into original squamous and nonsquamous epithelium based on an R/S cutoff value of 0.64. Again, if any of the measurements on a particular woman were below a R/S value of 0.64, then a positive impedance result was returned.

Table 3. AUC values of ROC separating high- and low-grade CIN from different tissue types

Epithelium	High-grade CIN					Low-grade CIN				
Parameter	*R*	*S*	*C*	F_c	*R/S*	*R*	*S*	*C*	F_c	*R/S*
Original squamous	0.88	0.83	0.63	0.79	0.89	0.78	0.70	0.58	0.63	0.77
Columnar	0.68	0.65	0.67	0.67	0.69	0.83	0.83	0.84	0.86	0.83
Mature metaplastic	0.80	0.80	0.69	0.77	0.81	0.65	0.64	0.51	0.61	0.66
Immature metaplastic	0.54	0.51	0.55	0.55	0.55	0.71	0.71	0.74	0.74	0.72

The colposcopy and biopsy results were used to place women into either a CIN group or a normal group. If any tissue of CIN 1 or CIN 2/3 was identified then the woman was placed in the CIN group. There were 104 women in the CIN group and 55 in the normal group. Seventeen were excluded from the total of 176 women. Women were excluded if we obtained fewer than six out of the possible eight measurements or the outcome of the colposcopy investigation was ambiguous.

The *R/S* minimum results are compared with the CIN and normal classification using the ROC curve is shown in Figure 3. The AUC is 0.652. A sensitivity of 60% can be obtained with a specificity of 60% if a cutoff of *R/S* minimum of 0.54 is used. Alternatively if a cutoff of 0.43 is used then a sensitivity of 50% and specificity of 75% can be obtained.

If we define CIN using the 25 and 75 percentiles on *R*, *S*, and *R/S*, ie, $2.95 < R < 7.34$, $3.79 < S < 5.88$, and $0.41 < R/S < 2.02$, then we obtain sensitivity 89% (96/108), specificity 15% (8/55), positive predictive value 67% (96/143), and negative predictive value 50% (8/16).

If the women were then categorized on the basis of an impedance results for *R/S* of 0.64 as cutoff between classifying points as normal or CIN, then the following performance measures are obtained for detection of CIN: sensitivity 66.3% (69/104), specificity 49% (27/55), positive predictive value 67.7% (69/96), and negative predictive value 55% (35/63).

For clinical practice, it is the identification of CIN 2/3 that is important in determining cases that are treated. If we perform the same analysis using *R/S* as the borderline but for identification of CIN 2/3, we obtain sensitivity of 74%(58/78), specificity of 53%(42/80), positive predictor values of 60%, and negative predictor values of 67%(42/62). In this study, cervical cytology had a positive predictive value of 67% (103/154).

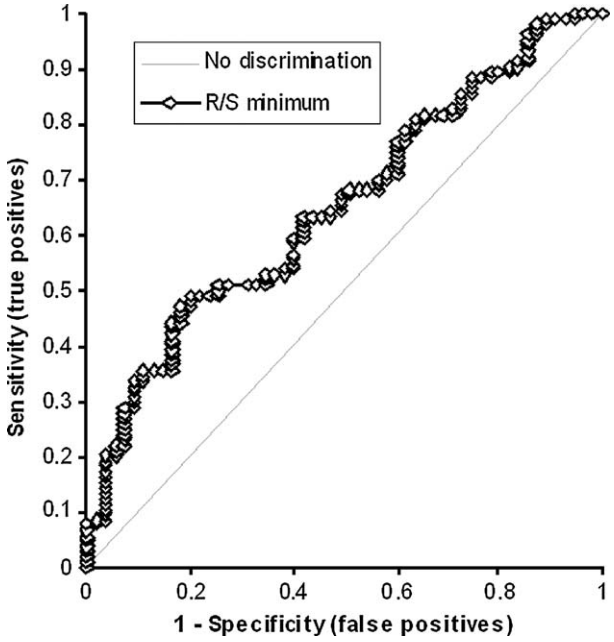

Figure 3. ROC for detection of CIN 2/3 (high-grade squamous intraepithelial lesion) in women when using *R/S* minimum as a discriminant. (AUC = 0.652).

Discussion

The cervix has a number of normal tissue types from columnar epithelium, immature, and mature metaplastic epithelium at the transformation zone, to original squamous epithelium. The development of CIN is associated with the loss of superficial cell layering, an increase in nuclear cytoplasmic ratio of cells, and increase in the volume of extracellular space. The parameters *R*, *S*, *C*, and F_c have been derived by the fitting of the Cole's equation to the measured impedance spectra. *R* is determined by conduction through the extracellular space and therefore is sensitive to the layering of cells. Normal squamous epithelium would be expected to have a high value for *R* as current has to track around cells and take a long resistive pathway. Columnar epithelium is a monolayer with underlying stroma and would be expected to have the lowest *R* value. Metaplastic changes occur with reverse cell hyperplasia, cellular layering, and subsequent differentiation leading to immature and mature metaplastic tissue. We would expect a gradual increase in *R* with the transformation of tissue from columnar to immature metaplastic and then to mature metaplastic tissue.

In CIN, the superficial layering of cells is lost and there is an increase in extracellular space, thus the value of *R* is greatly reduced with the progression of CIN compared to normal squamous epithelium. The observed changes in *R* fit well with the cellular modeling.

S is determined by conduction through the intracellular space. There is a well documented increase in mean nuclear size in CIN[13,14], and this can be expected to cause a decrease in intracellular conductance resulting in an increase in the value of *S* compared to original squamous epithelium. Modeling work shows that the value of *S* is expected to fall with increasing metaplastic maturation from columnar to mature metaplastic tissue. The observed values in *S* are in keeping with this. *C* is determined by the structure of the cell membrane, but no significant changes were seen in *C*.

F_c is the frequency at which current is able to penetrate the cell membrane. Modeling work has shown that F_c is expected to increase from normal original squamous epithelium to CIN and is expected to decrease from columnar epithelium to immature and mature metaplastic tissue. The observed results are in keeping with changes expected with modeling work.

The values and trends seen in *R* and *S* are similar in this study compared with our previous study, although the absolute values are slightly higher, but this may be explained by slight changes in the probe construction and the wider range of measured frequency. The current study also reports F_c values obtained and gives parameter values obtained in normal columnar and metaplastic tissue.

Good separation can be achieved of CIN from original squamous epithelium, mature metaplastic, and columnar epithelium using the various parameters. ROC curves show sensitivities, and specificities of 70–87% can be obtained in detecting changes associated with CIN in individual biopsy-proven sites. The analysis per woman gives sensitivity and specificity of 60% if using *R*/*S* minimum values.

These are slightly worse figures compared with our pilot study, but this may be accounted for by an inclusion of borderline and mildly dyskaryotic smear referrals, with a lower overall prevalence of CIN and a greater number of immature metaplastic points. The difficulty arises with separation of immature metaplastic tissue and CIN resulting in an increased false-positive rate. The use of a wider frequency range and increased measurement in our current study was hoped to have exploited the nuclear changes that occur in CIN to differentiate it from immature metaplastic tissue. This appears not to have improved separation, and as a result, the per woman analysis shows slightly worse figures compared with our previous studies. This may be due to the inability of the Cole's equation fitting software to fit the impedance curve in cases where two or more dispersions were seen. Use of a wider frequency range produces two dispersion curves in some cases, and so use of improved software to allow fitting to two dispersion curves may improve separation of immature metaplastic tissue.

The probe assesses tissue lying beneath the area covered by the four electrodes at the tip. In the transformation zone, this area may well have more than one histologic tissue type as a result of either physiologic or pathologic changes. As a consequence, false-positive results may be produced.

The detection of CIN has been approached primarily by the use of cytologic screening in the form of the Pap smear and subsequent colposcopy for abnormal smears. Newer methods have concentrated on improvement of results of cytologic screening by liquid-based cytology, HPV DNA detection and optical techniques (VIA, colposcopy, and cervicography). The advantages and disadvantages of the various screening methods available for the detection of CIN are summarized in Table 4.

Although there are many advantages to the current screening methods in use, the disadvantages are that they require extensive training of staff, do not provide instant results, and use in developing countries is impractical due to cost and logistical problems. As a result, there has been an increased interest in the development of real-time screening technologies, providing instant results. The potential advantages of real-time screening tests include a reduction in patient anxiety, improved patient compliance, ability to repeat inadequate tests immediately, use in one-stop screening/diagnostic/counseling/treatment clinic thus avoiding repeat visits, and potential use as secondary screening tool as an adjunct or triage tool. These new technologies also have potential disadvantages that often include the need for expensive specialized equipment, the inability of the techniques to assess endocervical lesions, and the lack of large population–based studies assessing performance of the techniques.

Techniques assessed include optical techniques (speculoscopy, fluorescence spectroscopy, reflective spectroscopy, Raman spectroscopy, confocal imaging, and optical coherence tomography), Optoelectronic techniques (TruScreen), and electrical measurement techniques[5].

Table 4. A comparison of the screening methods for the detection of CIN: Advantages, disadvantages, sensitivities, and specificities

Screening method	Advantages	Disadvantages	Sensitivity (%)	Specificity (%)	Reference
Cervical cytology	Successfully used in mass screening programs and shown to reduce cervical cancer rates, and detects endocervical abnormalities	Costly screening programs, extensive training of staff needed, subjective interpretation of cytology, and lacks sensitivity, reliability, and repeatability	44–99	91–98	Nanda *et al.*[4]
HPV testing	Objective diagnosis, self-testing possible	Costly, low positive predictive value, high prevalence in women with no abnormalities, and testing is lab dependent	97.1	93.3	Cuzick *et al.*[22]
Colposcopy	Current gold standard for diagnosis of CIN, size and location of CIN lesions identified	Expensive, nonmobile equipment needed, extensive training needed, subjective diagnoses, high cost, not a screening tool but used to assess women with abnormal cytology	79–96	25–71	Hopman *et al.*[23] and Mitchell *et al.*[15],[24]
Cervicography	Permanent documentation of examination and nontrained staff may take images	Trained staff needed for analysis of images, two-dimensional images affect interpretation, and subjective diagnoses	45	95	Schneider *et al.*[25]
Visual inspection with acetic acid	Low cost, minimal training needed, real time	Limited ability to detect endocervical lesions, subjective	70–90	64–94	University of Zimbabwe/ JHPIEGO Cervical Cancer Project[26]
Fluorescent spectroscopy	Real-time, objective diagnosis, minimal training needed	Expensive specialized equipment, diagnosis/training of machine based on subjectivity of histology and colposcopy, endocervical lesions not assessed	91	75	Coppleson *et al.*[16]

Continued

Table 4. Continued

Screening method	Advantages	Disadvantages	Sensitivity (%)	Specificity (%)	Reference
TruScreen	Real-time, objective diagnoses, minimal training needed	Endocervical lesions not assessed, diagnosis/training of machine based on subjectivity of histology and colposcopy, direct contact with cervix needed so sterilization issues need to addressed or disposable tips/membranes used, area of cervix assessed is determined by number of measurements taken	70	81	Quek *et al.*[17] and Singer *et al.*[18]
Impedance spectroscopy	Real-time, objective diagnosis, low cost, minimal training needed	Endocervical lesions not assessed, diagnosis/training of machine based on subjectivity of histology and colposcopy, direct contact with cervix needed so sterilization issues need to be addressed or disposable tips/membranes used, area of cervix assessed is determined by number of measurements taken	74	53	Brown *et al.*[6,7,8]

Mitchell *et al.*[15] reviewed several methods and derived ROC curves. They gave areas under the ROC curves of 0.76 for Pap smear testing, 0.84 for diagnostic colposcopy, 0.75 for HPV testing, and 0.71–0.75 for a fluorescence spectroscopy technique they have developed.

Coppleson *et al.*[16] have shown that electrical and optical measurements can be used to detect precancerous changes in cervical tissue. Their probe, known as TruScreen[17], illuminates tissue at four different wavelengths, measuring reflective and backscatter light, and also makes bipolar electrical measurements of the response to a 0.8 volt pulse applied for 100 μs, subsequently observing the charge decay at the tissue/electrode interface. This response will be indirectly related to the low-frequency impedance of the electrodes and the underlying tissue. They report sensitivity of 70% and specificity of 81% for TruScreen in a study on 651 subjects[18].

Fluorescent spectroscopy has also been assessed in screening for CIN, with average sensitivity of 91% and specificity of 75% being reported. This technique has the advantage of being objective in reaching a diagnosis and needing little staff training; however, it also suffers with similar difficulties to those seen with our probe of separation of columnar and metaplastic tissue from CIN[19,20].

The prevalence of a condition within the test population will affect the performance of a screening tool, and this is highlighted by Belinson *et al.*[21] who found that although fluorescent spectroscopy had performed

well in colposcopy clinics, when tested in the Shanxi province cervical cancer screening study, a sensitivity of 94% was achieved but specificity was only 7%. As a result, it is clear that all new screening methods must be tested in realistic clinical settings where prevalence of CIN may be much lower than in many clinically reported studies.

Our probe showed a sensitivity of 74% and a specificity of 53% in the detection of CIN 2/3. Further work in improving software fitting of impedance to Cole's equation should allow improved separation of metaplastic tissue from CIN and improve probe performance.

The results of the current study using the new probe are similar to those we reported previously and again show sensitivities and specificities that are comparable with existing screening methods. The advantage of this method as a potential screening test is that it can provide an immediate result and may be used by those with minimal training in the setting of primary care or in the developing world. This would enable positively screened women to be investigated further by colposcopy or be treated in cases of high-grade CIN. This has an advantage over other potential real-time screening methods as the equipment needed would be relatively inexpensive, require little training, and thus could be easily used in primary care or in the developing countries where the organizational structure and economical factors limit national screening programs. Potential uses of the impedance probe include use as a primary screening in developing countries where no screening programs exist, use as an adjunct to cervical cytology in developed countries with established screening programs to improve sensitivity and specificity, and use in colposcopy or VIA clinics to allow immediate diagnosis and treatment of suspected lesions.

Research is ongoing into the development of our probe and software for use in clinical trials to assess real-time performance in prediction of CIN. In addition, the affects of acetic acid on cervical impedance are also being assessed. These results will be reported in due course.

Conclusions

Cervical impedance spectroscopy at 30 frequencies over a 2–1200 kHz frequency range produces similar sensitivity and specificity to the currently used screening methods with the advantage of providing real-time results and screening. Use of a wider frequency range has not improved separation of immature metaplastic tissue from CIN as had been hoped, but further work is ongoing to improve the software design to allow real-time screening and to improve fitting of impedance data to allow assessment in clinical studies. It holds potential as a relatively low-cost, real-time screening tool for detection of CIN that could be used both in primary care and in developing countries.

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