The detection of cervical intraepithelial neoplasia by electrical impedance spectroscopy: The effects of acetic acid and tissue homogeneity

Balasubramani L, Brown BH, Healey J, Tidy JA

GYNECOLOGIC ONCOLOGY Volume: 115 Issue: 2 Pages: 267-271
Published: NOV 2009 Publisher: ELSEVIER

Abstract

Objective: To evaluate the efficacy of an electrical impedance probe in the diagnosis of high grade cervical intraepithelial neoplasia (CIN) in women referred with cervical smear abnormalities and to assess the effect of acetic acid (AA) on the measurements.

Methods: This was a prospective observational study done in the colposcopy clinic, Royal Hallamshire Hospital, Sheffield, UK. 165 women, either with a clinical indication or abnormal cervical cytology, were recruited into the study. A pencil probe, incorporating four gold electrodes, was used to record impedance spectra from 12 points on the cervix both before and after the application of 5% AA. Spectra were also recorded from tissue boundaries. Colposcopic examinations, including probe positioning, were video recorded to allow for correlations between histopathological diagnosis of colposcopically directed biopsies, colposcopic impression and the diagnosis based on impedance measurements.

Results: Receiver operating characteristic (ROC) curves were derived. The areas under the curves to discriminate original squamous from high grade CIN were 0.80 (pre AA) and 0.79(post AA). Comparison of these curves showed no significant difference, indicating that application of AA does not produce a large change in spectra. The probe could distinguish tissue boundaries from homogeneous tissue points.

Conclusions: The electrical impedance probe has the potential to be used as an adjunct to colposcopy in the diagnosis of high grade CIN. It has the advantage of real time results, decreasing the need for diagnostic cervical biopsies, and facilitates a wider use of the ‘see and treat’ policy without the risk of over treatment.

Keywords: cervical intraepithelial neoplasia, electrical impedance, colposcopy.

Introduction

Cancer of the cervix remains the second most common cancer among women worldwide. Cervical cancer is potentially preventable by screening and treatment of high grade cervical intraepithelial neoplasia (CIN) [1]. Conventional cervical cytology when used as a single test has a reported specificity of 95-98% but a sensitivity of only 50% [2]. The effectiveness of the cervical programme therefore relies on multiple opportunities at identifying and treating CIN. The use of liquid based cytology (LBC) has shown a reduction in the number of inadequate smears, but a recent systematic review and meta-analysis by Arbyn et al found that LBC did not improve the detection of high grade CIN and that LBC was neither more sensitive nor specific than conventional cytology [3].

Cervical screening programmes using either conventional or LBC are highly expensive and resource poor countries find it financially and logistically impossible to organise such nationwide screening programmes, leading to a high incidence and mortality from cervical cancer. Cytological screening also has a ‘test to result delay’, leading to anxiety in women. There is therefore, the need to develop other tests that not only improve the detection of high grade CIN but also provide real time results, so that women with abnormal smears can be appropriately managed.

Colposcopic examination including directed biopsies has long been proposed as the gold standard in the evaluation of abnormal cervical cytology. However there is increasing evidence demonstrating its poor performance with only 54.8% of women with CIN3 being diagnosed in the colposcopy arm of the ALTS study[4]. Various new technologies, usually employing optical spectroscopy, have been investigated to improve the detection of CIN and have reported sensitivity to detect high grade CIN in the range of 70-95% and specificity of 50-83% [5].

The current study was aimed at the detection of high grade CIN with a probe that measures tissue electrical impedance. Biological tissue has electrical impedance, which is a measure of the total opposition a circuit presents to electric current. Electrical impedance depends on the frequency of current applied. At frequencies of a few kHz to 1MHz, known as the β dispersion region, cell structure and arrangement are the main determinant of tissue impedance. At higher frequencies, electric current penetrates cell membranes and impedance will be determined by intracellular structures and possibly the size of the nucleus. At lower frequencies, electric current is unable to penetrate cell membranes and current therefore, flows mainly through the extra cellular space. Impedance at lower frequencies thus depends on cell spacing and arrangement [6].

Cervical epithelium is a highly structured stratified tissue that exhibits changes as it progresses from normal epithelium to high grade CIN. Changes include loss of stratification, increase in the nuclear cytoplasmic ratio and an increase in the extra cellular space. This formed the basis of our finite element modelling (FEM), that is a computational tool that predicts the electrical properties of biological tissues.

In our previously reported work, FEM in 3-D was used to predict the impedance spectra from squamous, columnar, metaplastic, low and high grade CIN tissues [7] (Fig 1).

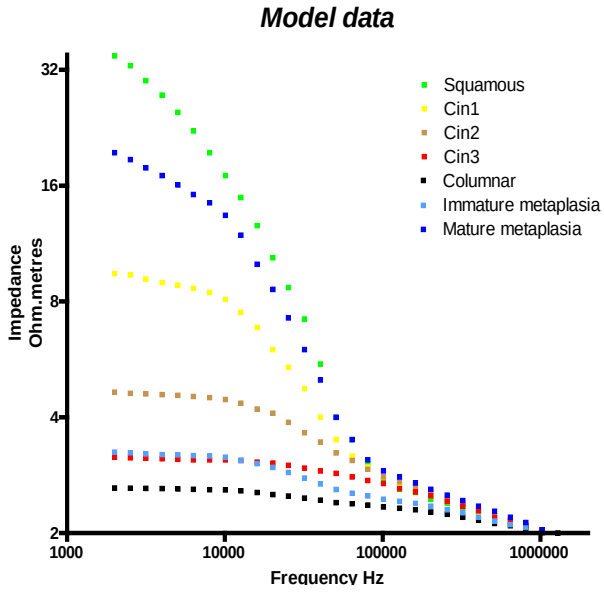


Figure 1: Impedance spectra derived from finite element modelling.

The initial version of the probe (frequency range 4.8- 614 kHz), used in a pilot study of 124 women and a subsequent study on 87 women, showed that sensitivity and specificity obtained in the detection of high grade CIN were as least as good as those obtained from conventional Pap testing in women attending colposcopy clinics [8,9] . There were, however, difficulties in separating columnar and metaplastic epithelium from CIN. The second version used higher frequencies (2- 1200kHz) to determine if better separation of tissue types could be obtained by exploiting the abnormal nuclear changes, which would be interrogated by the use of higher frequencies. While the use

of higher frequencies did help in the discrimination of columnar from CIN tissue, using higher frequencies did not appear to improve other tissue separations [10].

The aim of the current study was to evaluate the performance of the ‘epitheliometer’ in discriminating between tissue types and to assess its potential for use as an adjunct to colposcopy by taking impedance measurements over a range of frequencies (76.3Hz to 625 kHz) where tissue impedance depends mainly on cell spacing and cellular arrangement. Secondary outcomes were to determine if application of acetic acid to the cervix altered tissue impedance and to assess if the probe was able to identify tissue boundaries in order to remove ambiguous measurements

Methods

One hundred and sixty five women attending the colposcopy clinic, with any cervical smear abnormality or a clinical indication for colposcopy, were recruited into the study. Women pregnant at the time of colposcopy and post menopausal women were excluded. Post menopausal women were excluded as lack of oestrogen may lead to atrophy of the cervical epithelium and this was not factored into our model. Pregnant women were excluded as the cervix tends to become more vascular during pregnancy and this will change impedance spectra. Participants were given a study information leaflet, study details were discussed and written informed consent was taken. Ethics approval was obtained. Of the 165 women, 124 formed the main study group and 41 were used in the boundary detection investigation.

Impedance measurements were made using a 5.5mm diameter pencil probe with four 0.6 mm diameter gold electrodes mounted flush with the face of the probe. A current of <20 µA p-p was passed between an adjacent pair of electrodes and the resulting potential measured between the two remaining electrodes. Measurements were made at 16 frequencies by doubling the frequency in steps from 76.3Hz to 625 kHz.

The probe was used to take 12 measurements from the cervix (Fig 2). 5% acetic acid was then sprayed on the cervix and the probe was used to take 12 further measurements. Impedance measurements were automatically transferred to a laptop. Colposcopy was performed and cervical biopsies taken if clinically indicated. Colposcopy was satisfactory and the entire transformation zone was visible in all the cases. The entire procedure from visualisation of the cervix and probe positioning, to haemostasis at the site of cervical biopsies, was video recorded for subsequent correlation between results obtained from colposcopic impression, histopathological diagnosis of colposcopically directed biopsies and impedance.

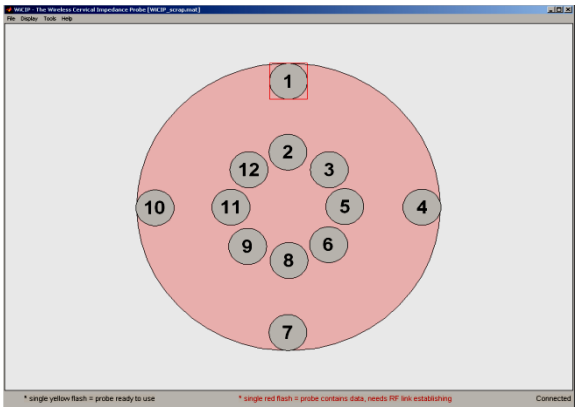


Figure 2: Probe positioning on the cervix

The probe assesses tissues lying beneath the area covered by the four electrodes. In the transformation zone, this area may have more than one histological type as a result of either physiological metaplasia or CIN. A separate probe with the capability of injecting current through both adjacent and diagonal drives was used to evaluate if the probe could differentiate tissue boundaries. This probe was identical to the first, but included the means to switch the current injection and voltage measurement circuitry between electrodes. Boundary data was collected from 41 women but was only performed post acetic acid as areas of CIN become distinct only after acid application. The probe was deliberately placed on 258 homogenous tissues and 209 tissue boundaries- squamo-columnar(n=112), squamo-CIN (n=78) and columnar-CIN (n=19) . Data analysis was used to determine if the probe could differentiate between homogeneous and tissue boundary sites.

Results

One hundred and twenty four women were recruited into the main study. A ‘learning curve’ was noticed for impedance measurements taken from the first 20 women of the study and these women were excluded from further analysis. Of the 104 women who remained in the study, 99 were referred with an abnormal ‘Pap’ smear and five were seen with post coital bleeding. The types of cervical smear abnormality are shown in Table 1.

Impedance spectra were recorded from a total of 1093 points prior to the application of acetic acid. Of these, 204 points were rejected. Twenty five points were rejected as the probe was unable to take an impedance measurement. This happened if there was poor contact between the cervix and the probe tip or if the cervix was covered with blood. A further 179 points were rejected as a definite colposcopic impression could not be assigned either due to poor quality of the video recording or if the probe was placed on a tissue boundary as identified during the video analysis. A total of 889 points with a clear colposcopy result and good impedance data were analysed, of which 636 points were squamous, 45 columnar, 85 high grade CIN and 113 immature metaplasia . In addition, 10 points were classified as ‘others’ consisting of points of HPV, inflammation, mature metaplasia or low grade CIN. Similarly, a total of 1068 post acetic acid impedance measurements were collected from the 104 women. After rejecting unsuitable points as with the pre acetic acid data, a total of 839 points were analysed with 597 squamous, 37 columnar, 76 high grade, 114 immature metaplasia and 15 points classified as ‘others’ consisting of mature metaplasia, HPV, inflammation or low grade CIN.

Data Analysis:

The impedance spectra were compared with a template for CIN3 determined from the Finite Element Model of cervical epithelium. The mean deviations of the measured spectra from the template were used to categorise the measurements as normal squamous epithelium, columnar, high grade CIN [CIN2+] or immature metaplasia. The deviations were used point by point and compared with the colposcopic impression

obtained from the videos and histological diagnoses obtained from directed punch biopsies. To evaluate if the probe could differentiate CIN from all other tissue types, receiver operating characteristic (ROC) curves were drawn using the Medcalc statistical package. A per woman analysis was also performed which classified women with a diagnosis of either high grade CIN or normal using the impedance probe results.

Pre acetic acid data:

ROC curves derived for normal squamous epithelium and high grade CIN showed an AUC of 0.80 indicating a good separation between the two tissue types. The sensitivity was 78.8% with a specificity of 73%. ROC curves were also derived for CIN *vs* all other tissue types. The AUC was 0.77 with a sensitivity of 85.9% and a specificity of 62.6%. For the per woman analysis, women were divided into either the normal group or the high grade CIN group depending on the colposcopic impression. The AUC was 0.75 with a sensitivity of 88.4% and a specificity of 63.9%. The sensitivity and specificity values quoted are those where the sum of the sensitivity and specificity values was at a maximum. The corresponding value of deviation from the high grade CIN template was used as the threshold when separating the women into the normal or CIN groups.

Post acetic acid data:

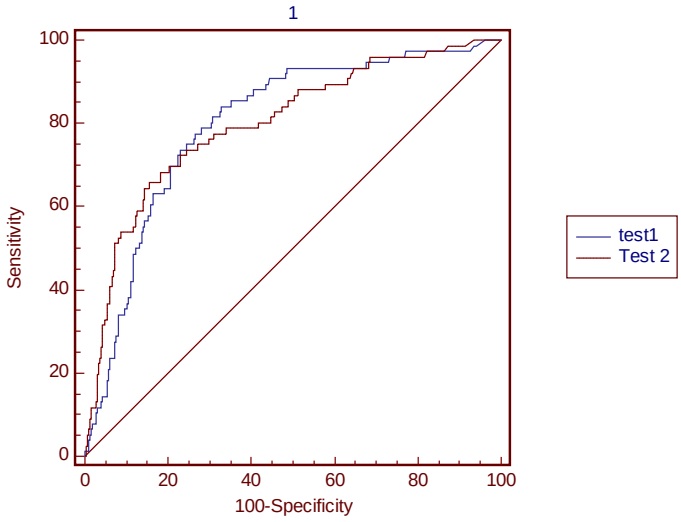
ROC curves derived to discriminate original squamous from high grade CIN showed an AUC of 0.79 with a sensitivity of 78.9% and a specificity of 66%. The AUC for high grade CIN *vs* all other tissue was 0.74 with a sensitivity of 73.6% and a specificity of 63%. The per woman analysis gave an AUC of 0.74 with a sensitivity of 89.7% and a

specificity of 50% showing that the probe may be used as an adjunct to colposcopy to clinically diagnose women with high grade CIN.

Comparison of pre and post acetic acid data:

Figure 3 shows the comparison of the pre and post acetic acid ROC curves derived to differentiate original squamous from high grade CIN. The difference between the two ROC curves was not found to be statistically significant (p=0.98). Similarly, there was no significant difference between the pre and post acetic acid ROC curves derived to differentiate between high grade CIN *vs* all other tissue (p=0.68), showing that acetic acid does not cause a large change in impedance spectra.

Figure 3: Comparison of pre & post AA ROC curves- squamous Vs HG



Test 1- pre AA data, Test 2-Post AA data.

Comparison of Epitheliometer diagnosis with histology:

Epitheliometer diagnosis of tissue type and histological diagnosis at the site of biopsy were compared directly by studying data from 22 points where the epitheliometer reading was almost coincident with the biopsy site. Both the epitheliometer and histological diagnosis concurred with 18 sites identified by both as high grade CIN and 4 points identified as non-CIN, suggesting a 100% sensitivity and specificity, albeit on a small sample set.

Boundary Data:

Apart from the 124 women recruited to the main study, data were also collected from a further 41 women, giving spectra from 209 tissue boundaries and 258 sites of homogeneous tissue. The data sets were analysed by using a quantity *(n)* where *n* is the ratio between null and the normal measurements.

$$n = \left| \frac{null}{norm} \right|$$

Where:

null is the measurement taken from diagonally opposing electrodes at a frequency of 78.1kHz.

norm is the measurement taken from adjacent electrodes.

n was calculated for all the data and the boundary and homogeneous sets were then compared. The best separation of the two sets was found using a value of *n*=0.18. All of

the homogeneous data set was analysed initially by deriving ROC curves to discriminate between squamous and high grade CIN (AUC=0.86). ROC curves were then derived using the same dataset after excluding impedance measurements where *n* was greater than 0.18. Exclusion of inhomogeneous data appeared to improve the sensitivity of the probe in the detection of high grade CIN but this improvement of AUC was not statistically significant. Using a more severe limit by decreasing the threshold for *n* increased the AUC (0.98) significantly, but this involved rejecting 85% of the measurements and might prolong the measurement time excessively.

Discussion

Histologically, the development of CIN is associated with the loss of stratification and differentiation, increased nuclear cytoplasmic ratio and an increase in the extracellular space. Previous studies have suggested a six-fold increase in the extra-cellular space when compared to normal squamous tissue [9]. It is this increase in extra-cellular space that decreases impedance at low frequencies in CIN. Thus, original squamous epithelium has a high impedance while high grade CIN, that has more extra-cellular space, has a lower impedance. Finite element modeling provided the templates for impedance spectra for the various cervical tissue types and the templates were similar to impedance spectra recorded from the cervix. The best fit with the measured data was obtained for models with an epithelium of 300-400µm in depth with a surface layer of mucus of 10µm [10]. Previous studies showed that impedance measurements taken at various times during the menstrual cycle did not vary significantly [8]. The probe was

therefore used on all women who consented to participate in the study irrespective of the phase of their menstrual cycle.

A small pressure has to be applied to the probe in order to get a good contact between the electrodes and the cervix. Studies by Gonzalez-Correa et al showed that reliable readings could be obtained at pressures above 3kPa [11]. In their study, the authors observed that when a small depression was seen on the tissue underneath the probe tip, the pressure was usually above 11kPa [11,12]. A depression caused by the probe tip was seen on the cervix in the clinical situation and this was a factor that was used when taking impedance measurements. A 'learning curve' was noticed for the impedance measurements taken from the first 15-20 women and this was borne out by the fact that more variability was seen in the impedance spectra in the first twenty women of the study. These women were excluded and do not form part of the 104 women analysed.

Analysis was initially performed on a per point basis and the AUC for the pre AA ROC was 0.80 and for the post AA ROC 0.79, showing a good discrimination between the two tissue types. In a clinical setting it is important to diagnose if a woman referred with smear abnormality has high grade CIN as this will inform further management. To assess if the probe would be able to identify women with high grade CIN, a per woman analysis was performed. The AUC was 0.75 with a sensitivity of 88.4% and a specificity of 63.9% showing that the probe may be used as an adjunct to colposcopy to clinically diagnose women with CIN.

We compared results obtained from pre AA and post AA data to evaluate if the application of AA would alter impedance measurements. Clinically, the application of acetic acid removes mucus and debris from the surface of the cervix, causes a coagulation of cell proteins and leads to an aceto white change in areas with CIN. It might be expected that post AA values would be better than pre AA values as we should be able to visualise the aceto-white area denoting CIN. Surprisingly, results showed that it was the pre AA ROC curves that gave a slightly better discrimination. It is possible that because of the thinner layer of mucus after the application of AA, the pathways of electrical current in the cervical epithelium are altered and this needs to be studied further with separate templates for post acetic acid analysis. Balas et al studied the structural and functional alterations in cervical epithelium following the application of acetic acid. Their results suggest that application of acetic acid will result in some closing of gap junctions and an increase in proton concentration in both intracellular and extracellular spaces[13]. Gap junctions have been shown by tissue modeling to have only a small effect [14]. The increased proton concentration will cause a decrease in tissue impedance but hydrogen ions have relatively low mobility and so the change in impedance will be small. We can conclude that a small decrease in tissue impedance, leading to small changes in spectral shape, might be expected following the application of acetic acid but these changes are likely to be much less than the changes associated with the large structural differences between normal and CIN epithelium. While the pre AA AUC showed slightly better discrimination of tissue types than the post AA AUC, comparison of the ROC curves found no statistical significance, demonstrating that the application of acetic acid does not markedly change impedance measurements.

In a small sample of 22 measurements where histological diagnosis was compared with the epitheliometer diagnosis, there was 100% concordance between histology and probe diagnosis. The overall results are encouraging and larger numbers of patients are needed to explore this further.

Good discrimination of immature metaplasia from high grade CIN using the probe remains a problem. This is a difficult area in colposcopy as well, as both these tissue types take up acetowhite and look similar on visual inspection. Immature metaplasia is a highly active tissue undergoing transformation and can prove quite challenging for histopathologists as well. In our study, ROC curves to differentiate immature metaplasia from high grade CIN, using any of the study parameters gave an AUC ranging from 0.50-0.65. While this is not ideal, in previous studies the AUC for these two tissue types has only been 0.51-0.55 [8] showing a definite improvement in the discrimination between these two tissues.

The ability of the probe to diagnose a tissue boundary was evaluated in our study. In a perfectly homogeneous tissue there is a balance between the two receiving electrodes. When the probe is placed on a boundary between tissues of different resistance, the system is unbalanced and this is detected by the probe. It is essential that impedance measurements are not taken from tissue boundaries to reduce the possibility of a false positive diagnosis of CIN. Improved AUCs were obtained when impedance measurements taken from inhomogeneous tissues were excluded. This capability will be incorporated into the next version of the impedance probe, with a view to reducing the number of false positive results.

Previously published work [8-10,15] used older versions of the electrical impedance probe. Our current data are slightly worse when compared with our previous published data but this may be accounted for by the inclusion of women with borderline and mild dyskaryosis smears in our study group [8,9]. Our study sample is more representative of a population attending the colposcopy clinics but results in an overall lower prevalence of high grade CIN. Results published by Abdul et al showed a good discrimination of original squamous and high grade CIN with an AUC of 0.88 [10]. However, the per woman analysis was slightly worse at AUC=0.65 in the previous study when compared to the per woman AUC in the current study (AUC=0.75). It is therefore possible that the use of a wider range of frequencies in the current probe has improved tissue differentiation.

We have demonstrated that the epitheliometer has the potential to act as an adjunctive test in the colposcopy clinic to diagnose CIN without the need for biopsies, take better targeted biopsies if required and guide management at first visit. In poor countries, where organized call and recall screening programmes are not in place, visual inspection of the cervix with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI) have been used as screening tools. A recent study established a sensitivity of VIA to be 79% and a specificity of 85% with VILI on average 10% more sensitive and equally specific[16]. The impedance probe is a self contained, battery powered, portable device which does not require cervical biopsies for diagnosis of CIN. It can thus be used as an inexpensive tool for the diagnosis of CIN. As the probe gives real time results,

women diagnosed with high grade CIN can be treated at the same visit without the risk of overtreatment. The development of such a device is especially important as the major burden of cervical cancer is borne by poor countries.

Conclusion

The main advantages of the epitheliometer are that it is objective and is capable of real time diagnosis without the need for colposcopically directed biopsies. It is portable, user friendly, has a short learning curve and can be used as an adjunct to colposcopy. It could be used with VIA in resource poor countries and women diagnosed with high grade CIN can be offered treatment at the same visit, without the risk of over treatment.

Conflict of Interests:

Acknowledgements:

DO30, NHS, New and Emerging Applications of Technology, NEAT Detection of pre-malignant changes in epithelium using Impedance Spectroscopy, 1st Jan 2004 to 31st July 2006,

B H Brown, £230,900.

References

1. Quinn M, Babb P, Jones J, Allen E. Effect of screening on incidence of and mortality from cancer of cervix in England: evaluation based on routinely collected statistics. Br Med J (Clinical research ed). 1999 Apr 3;318(7188):904-8.

2. Nanda K, McCrory DC, Myers ER, Bastian LA, Hasselblad V, Hickey JD, et al. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Annals Int Med. 2000 May 16;132(10):810-9.

3. Arbyn M, Bergeron C, Klinkhamer P, Martin-Hirsch P, Siebers AG, Bulten J. Liquid Compared With Conventional Cervical Cytology: A Systematic Review and Meta-analysis. Obstet Gynecol. 2008 Jan;111(1):167-77.

4. Louwers JA, Kocken M, ter Harmsel WA Verheijen RHM. Digital colposcopy:ready for use? An overview of literature. Br J Obstet Gynaecol 2009; 116:220-29

5. Wang SS, Walker JL, Schiffman M, Solomon D. Evaluating the risk of cervical precancer with a combination of cytologic, virologic, and visual methods. Cancer Epidemiol Biomarkers Prev. 2005 Nov;14(11 Pt 1):2665-8.

6. Walker DC, Brown BH, Blackett AD, Tidy J, Smallwood RH. A study of the morphological parameters of cervical squamous epithelium. Physiological measurement. 2003 Feb;24(1):121-35.

7. Walker DC, Brown BH, Smallwood RH, Hose DR, Jones DM. Modelled current distribution in cervical squamous tissue. Physiol Meas. 2002 Feb;23(1):159-68.

8. Brown BH, Tidy JA, Boston K, Blackett AD, Smallwood RH, Sharp F. Relation between tissue structure and imposed electrical current flow in cervical neoplasia. Lancet. 2000 Mar 11;355(9207):892-5.

9. Brown BH, Milnes P, Abdul S, Tidy JA. Detection of cervical intraepithelial neoplasia using impedance spectroscopy: a prospective study Br J Obstet Gynaecol. 2005 Jun;112(6):802-6.

10. 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 Sep-Oct;16(5):1823-32.

11. Gonzalez-Correa CA, Brown BH, Smallwood RH, Kalia N, Stoddard CJ, Stephenson TJ, et al. Assessing the conditions for in vivo electrical virtual biopsies in Barrett's oesophagus. Medical & Biological Engineering & Computing. 2000 Jul;38(4):373-6.

12. Gonzalez-Correa CA, Brown BH, Smallwood RH, Walker DC, Bardhan KD. Electrical bioimpedance readings increase with higher pressure applied to the measuring probe. Physiological measurement. 2005 Apr;26(2):S39-47.

13. Balas C PG, Potirakis A. in Vivo molecular imaging of cervical neoplasia using acetic acid as biomarker. IEEE Journal of selected topics in quantum electronics. 2008;14:29-42.

14. White FH, Gohari K. Alterations in the volume of the intercellular space between epithelial cells of the hamster cheek-pouch: quantitative studies of normal and carcinogen-treated tissues. J Oral Pathol. 1984 Jun;13(3):244-54.

15. Abdul S, Brown BH, Milnes P, Tidy JA. A clinical study of the use of impedance spectroscopy in the detection of cervical intraepithelial neoplasia (CIN). Gynecol Oncol. 2005 Dec;99(3 Suppl 1):S64-6.

16. Arbyn M, Sankaranarayanan R, Muwonge R, Keita N, Dolo A, Mbalawa CG, et al. Pooled analysis of the accuracy of five cervical cancer screening tests assessed in eleven studies in Africa and India. International journal of cancer. 2008 Apr 10;123(1):153-60.

Table 1: Results of LBC samples for women recruited into the study.

Mild dyskaryosis	34
Moderate dyskaryosis	16
Severe dyskaryosis	27
Borderline	20
Post coital bleeding	5
Inflammatory	1
Glandular neoplasia	1