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Added value of electrical impedance spectroscopy in adjunction of colposcopy: a prospective cohort study

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 Added value of electrical impedance spectroscopy in adjunction of colposcopy: prospective cohort study Laura Bergqvist, ¹ Annu Heinonen, ¹ Xavier Carcopino, ² Charles Redman, ³ Karoliina Aro, ¹ Mari Kiviharju, ¹ Se Virtanen, ¹ Pirjo-Liisa Omar, ¹ Laura Kotaniemi-Talonen, ^{4,5} Karolina Louvanto, ^{4,5} Pekka Nieminen, ^{*1} Kalliala^{*1,6} ¹Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, 00 Helsinki, Finland ²Department of Obstetrics and Gynecology, Hôpital Nord, APHM, Aix-Marseille University (AMU), U 	
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33 34 18 *Equal contribution	
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1 2		
3 4 5	31	Abstract (247)
5 6 7	32	Objective : To assess whether electrical impedance spectroscopy (EIS) as an adjunctive technology enhances
8 9	33	the performance of colposcopy.
10 11	34	Design: Prospective cohort study.
12 13 14	35	Setting: University Hospital colposcopy clinic.
15 16	36	Participants: Colposcopy with EIS for 647 women and conventional colposcopy for 962 women.
17 18	37	Interventions: Comparison of the performance of colposcopy by referral cervical cytology in two cohorts,
19 20	38	with and without EIS as an adjunctive technology.
21 22 23	39	Outcome measures: Prevalence of cervical intraepithelial neoplasia grade 2 or worse (CIN2+), diagnostic
23 24 25	40	testing accuracy to detect CIN2+ with and without EIS and their relative differences between cohorts.
26 27	41	Results: The prevalence of CIN2+ varied between the cohorts according to referral cytology: 17.0% after
28 29	42	abnormal squamous cells of unknown significance (ASC-US) referral cytology in EIS cohort and 9.1% in the
30 31	43	reference cohort, 16.5% and 18.9% after low-grade squamous intraepithelial lesion (LSIL), 44.3% and 58.2%
32 33 34	44	after atypical squamous cells, cannot exclude HSIL (ASC-H), and 81.9% and 77.0% after high-grade
35 36	45	squamous intraepithelial lesion (HSIL) cytology, respectively. Sensitivity to detect CIN2+ was higher in the
37 38	46	EIS cohort, varying from 1.79 (95% CI 1.30-2.45) after LSIL referral cytology to 1.16 (95% CI 1.09-1.23) after
39 40	47	HSIL referral cytology, with correspondingly lower specificity after any referral cytology.
41 42 43	48	Conclusions: Colposcopy with EIS had overall higher sensitivity but lower specificity to detect CIN2+ than
44 45	49	conventional colposcopy. CIN2+ prevalence rates were, however, not consistently higher in the EIS cohort,
46 47	50	suggesting innate differences between the cohorts or truly lower detection rates of CIN2+ for EIS,
48 49 50	51	highlighting the need for randomized controlled trials on the effectiveness of EIS.
51 52 53	52	Keywords: Electrical impedance spectroscopy (EIS), colposcopy, cervical intraepithelial neoplasia (CIN),
54 55 56	53	cervical cytology, sensitivity, specificity
57 58 59 60	54	

1 2 3 4 5	55	Strengths and limitations of this study
6 7	56	1. The performance and prevalence results of electrical impedance spectroscopy in adjunction with
8 9	57	colposcopy were stratified by referral cytology.
10 11	58	2. The results were compared to a cohort with conventional colposcopy of the same colposcopy clinic
12 13 14	59	representing similar populations.
14 15 16	60	3. The prevalence of CIN2+ was based on the first visit histological data.
17 18	61	4. The results offer information only in women with transformation zone type 1-2.
19 20 21	62	
21 22 23	63	4. The results offer information only in women with transformation zone type 1-2.
24 25	64	
26 27 28	65	
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38 39	60	
40 41 42	69	
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1 2 3 4	77	Introduction
5 6	78	During the next decades, the incidence and prevalence of high-grade cervical disease will decrease in the
7 8 9	79	developed countries due to human papillomavirus (HPV) vaccination programs [1, 2] and transition to
10 11	80	primary high-risk HPV (hrHPV)-DNA test -based screening.[3] Consequently, colposcopy will become more
12 13	81	challenging due to resulting lower positive predictive value. Therefore, to detect those in need of
14 15 16	82	treatment, it will be essential to correctly identify the high-grade lesions and take biopsies at
17 18	83	representative locations. Also, reliable means to rule out high-grade lesions without excessive number of
19 20 21	84	biopsies or frequently repeated tests or colposcopies are needed.
21 22 23	85	The sensitivity and specificity of colposcopy in identifying uterine cervical high-grade precancerous lesions
24 25	86	have been previously reported to vary between 66% to 80% and 63% to 95%, respectively.[4-7]
26 27 28	87	Furthermore, the probability of detecting a high-grade disease at colposcopy is affected by the referral
20 29 30	88	cytology, being higher after high-grade squamous intraepithelial lesion (HSIL) cytology than after atypical
31 32	89	squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesion (LSIL)
33 34 35	90	cytology results.[8]
36 37	91	ZedScan (Zillico Ltd.)[9] is a hand-held device using electrical impedance spectroscopy (EIS) in identifying
38 39	92	cervical pathology.[10] It is designed to provide guidance to colposcopist in biopsy taking by indicating the
40 41 42	93	most abnormal cervical tissue area.[10] ZedScan measures the electrical properties of the cervical
43 44	94	epithelium to differentiate pre-cancerous and cancerous tissue from normal epithelium.[10-12] The area
45 46	95	with the most abnormal impedance is reported visually, aiding the colposcopist in targeting biopsies.
47 48 49	96	The sensitivity of colposcopy has been suggested to increase with the use of EIS [10, 13-16] even in women
50 51	97	with low probability of high-grade cervical disease and with minor colposcopic changes, as its use is
52 53	98	independent of visual findings in colposcopy.[12, 17] The developers of the technology have been involved
54 55 56	99	in most of the published studies. In women with persistent hrHPV positivity without cervical cytological
57 58	100	changes, EIS has detected additional cases of cervical intraepithelial neoplasia grade 2 or worse (CIN2+)
59 60	101	compared to women without EIS examination.[17] The benefit of EIS seems to vary depending on the

referral cervical cytology, being most useful in terms of finding extra cases of CIN2+ in women with lowgrade referral cervical cytology. [13, 14, 16] NICE guidelines recommend further research on EIS. [18] Our objective was to assess, stratified according to referral cytology, whether EIS combined with colposcopy increases the diagnostic testing accuracy of CIN2+ compared to conventional colposcopy in women referred to colposcopy for abnormal cervical cytology.

Methods

Participants

All women (n=1609) in this study were examined between 2013-21 at the outpatient colposcopy clinic of Helsinki University Hospital for a new referral for abnormal cytology. We included women if their cervical transformation zone (TZ) was type 1 or 2 (TZ1-2) and the information on both colposcopic impression and histopathological results were available. Exclusion criteria were transformation zone type 3 (TZ3) and pregnancy. Women referred for persistent hrHPV positivity without cytological changes were excluded due to the lack of sufficient control cohort as high-risk HPV testing as a part of primary screening was implemented in Helsinki region only in 2019.

The EIS cohort consisted of 647 women with colposcopy and ZedScan examination successfully performed between September 2018 and August 2021. The cohort was collected prospectively with non-consecutive patient recruitment. Under the study period ZedScan equipment was available at the colposcopy and used at the decision of the individual colposcopist. EIS examinations were done according to the manufacturer's protocol and all colposcopists had an adequate training prior using the device. If active bleeding during colposcopy occurred, the EIS procedure was omitted.

We could not directly compare the performance of colposcopy alone against colposcopy with ZedScan as an adjunctive tool using only the EIS cohort, as these two events were not truly independent of each other in the routine clinical setting applied here. Therefore, we used a previously collected prospective cohort of 962 patients examined with conventional colposcopy in the colposcopy clinic of Helsinki University

3 4	126	Hospital, Finland, between 2013 and July 2017 as the reference cohort (ISRCTN10933736),[19] with all
5 6	127	women fulfilling the inclusion criteria included. Only the primary colposcopy after referral and its
7 8 9	128	histological results were included in both cohorts.
10 11 12	129	Abnormal cervical cytology results were categorized according to the Bethesda system as ASC-US or worse.
	130	Histological results were reported according to WHO 2003, 2013 and 2020 classification. The evaluation of
	131	histopathological specimens, biopsies, and large loop excision of the transformation zone (LLETZ) cones,
18		was done by the gynaecological histopathologists of Helsinki University Hospital. The most severe
19 20	133	histological diagnosis of all biopsies or LLETZ was recorded.
21 22	174	
22 23 24		Clinical procedures
26	135	All participants had a colposcopic examination with the application of acetic acid to the cervix.
27 28 29		Subsequently, participants in the EIS cohort underwent a ZedScan examination. ZedScan readings were
	137	made from 10 to 12 points clockwise around the cervix. On the Zedscan reading, red colour points out the
	138	area with the highest probability of high-grade disease, amber colour indicates possible high-grade areas
35		and the absence of high-grade disease is indicated with green colour. In the EIS cohort, cervical biopsy sites
36 37	140	were determined by the colposcopist based on both ZedScan results and colposcopic impression. The most
38 39 40	141	severe histological diagnosis of all biopsies was recorded.
	142	Random biopsies were not routinely taken in either of the cohorts. Colposcopy examination in both cohorts
43 44 45	143	was based on Finnish Current Care Guidelines.[20] Five percent acetic acid and Lugol's iodine were
	144	available at the discretion of individual colposcopist to assess the abnormal cervical areas for biopsy. The
48 49		colposcopic impression was recorded as high-grade, low-grade, or normal. Immediate LLETZ at initial visit
50 51	146	('select and treat'-approach) was performed when evaluated necessary according to Finnish Current Care
52 53 54	147	Guidelines: HSIL referral cytology with a colposcopic impression of CIN2+ entitled to perform LLETZ at the
	148	initial colposcopy with consent from the patient. [20] After cervical cytology with glandular atypia favouring
	149	neoplasia (AGC-FN) the Finnish Current Care Guidelines recommends immediate LLETZ irrespective of the
59 60	150	age of the referred woman.[20]

151 Data analysis

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5 6 We compared the prevalence of histologically confirmed CIN2+ lesions between the EIS and reference 152 7 8 153 cohorts and calculated the sensitivity, specificity, positive and negative predictive values for colposcopy in 9 10 154 both cohorts for the detection of CIN2+ lesions, both overall and stratified according to the referral cervical 11 12 13 155 cytology. The positive test result for EIS cohort was defined as suspected presence of CIN2+ either by 14 15 156 ZedScan and/or via colposcopic inspection. The test result was negative if both the colposcopic impression 16 17 157 and ZedScan agreed on low-grade lesion or normal cervical finding, i.e. absence of CIN2+ lesion. In the 18 19 158 reference cohort, positive test result was defined as a colposcopic impression of CIN2+ while negative test 20 21 ₂₂ 159 result was defined as the absence of changes suggesting CIN2+ lesions. The most advanced 23 24 160 histopathological result of the biopsies or LLETZ specimen taken at the initial visit were used as a reference 25 ²⁶ 161 standard in both cohorts. Women without biopsies and with negative ZedScan result and normal 27 28 162 colposcopic impression as well as low-grade referral were considered true negatives. Even though 29 30 ₃₁ 163 colposcopy and EIS examination were not truly independent tests in the setting used, we still performed a 32 33 164 sensitivity analysis within the EIS cohort and assessed separately diagnostic testing accuracy of colposcopy 34 ³⁵ 165 and EIS in that cohort alone as well. 36 37 38 166 Risk ratio and risk difference were used to compare the sensitivity and specificity between the EIS and 39 ⁴⁰ 167 reference cohorts. The p-values <0.05 were considered statistically significant. All statistical analyses were 41 42 168 performed using STATA/SE 15 (StataCorp, College Station TX, USA) and all statistical tests used were two-43 44 45 169 sided. 46 47 48 170 Results 49 50 171 There were 1027 eligible women with adequate colposcopy and Zedscan examination performed In the EIS 51 52 cohort. Altogether 68 women with other referral reasons than abnormal cervical cytology, 215 women with 53 172 54 55 173 follow-up colposcopy visits and 97 women with missing data were excluded. In total, 647 women with new 56 ⁵⁷ 174 colposcopy referrals of abnormal cytology were included in the analysis (Table 1). Of all ZedScan 58

⁵⁹ ₆₀ 175 procedures 75% were conducted by three individual colposcopists. The reference cohort included 1383 Page 9 of 26

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Table 1. Characteristics of the electrical impedance spectroscopy (EIS) cohort and the reference cohort.

	EIS cohort						
	n=647	%	n=962	%			
Mean age	35.7		35.4				
Std.deviation, Range	9.3(20.3-76.4)		9.6(19.2-67.8)				
Referral cervical cytolog	BY						
ASC-US	94	14.5	99	10.3			
LSIL	236	36.5	381	39.6			
ASC-H	192	29.7	237	24.6			
HSIL	94	14.5	200	20.8			
AGC-NOS	28	4.3	28	2.9			
AGC-FN	3	0.5	17	1.8			
	647	100	962	100			
Age		100	502	100			
-30 γ	175	27.0	295	30.7			
<30 y 30-44 y	366	56.6	295 495	30.7 51.5			
>45 y	106	16.4	172	17.9			
	647	100	962	100			
TZ type							
TZ type 1	446	68.9	620	64.4			
TZ type 2	201	31.1	342	35.6			
	647	100	962	100			
Biopsies and LLETZ							
No biopsy	22	3.4	10	1.0			
1 biopsy	165	25.5	109	11.3			
2 biopsies	263	40.6	420	43.7			
3 biopsies	83	12.8	257	26.7			
4 biopsies	1	0.2	43	4.5			
5 biopsies	0	0	5	0.5			
LLETZ	113	17.5	118	12.3			
	647	100	962	100			
Histology							
No biopsy	22	3.4	10	1.0			
	222	34.3	248	25.8			
Normal histology CIN1 (LSIL)	181	28.0	313	32.5			
CIN2 (HSIL)	95						
····= (···=·)		14.7	210	21.8			
CIN3 (HSIL)	107	16.5	154	16.0			
Glandular atypia	1	0.2	5	0.7			
AIS	14	2.2	15	1.6			
Adenocarcinoma	3	0.5	3	0.3			
Sq. cell carcinoma	2	0.3	4	0.4			
177 Std. deviation: standard of	647	100	962	100			

Std. deviation: standard deviation; EIS: electrical impedance spectroscopy; CIN: cervical intraepithelial neoplasia; TZ: transformation zone; ASC-US: ⁵⁵ 178 atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial 56 179 lesion; ASC-H: atypical squamous cells that cannot exclude HSIL; AGC-NOS: atypical glandular cells not otherwise specified; AGC-FN: atypical 57 180 glandular cells that favor neoplasia; AIS: adenocarcinoma in situ; sq. cell carcinoma: squamous cell carcinoma; LLETZ: large loop excision of the

58 181 transformation zone

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2 3 182 eligible women. Of these, 86 women were excluded due to other referral reasons than abnormal cervical 4 5 183 cytology, 174 for having TZ3, 143 for missing relevant clinical data and 18 for pregnancy. As a result, a total 6 7 184 of 962 women fulfilled the inclusion criteria (Table 1). 8 9 10 185 At least one biopsy was taken or imminent LLETZ made in 625 (96.6%) women in the EIS cohort and 952 11 12 13 186 (99.0%) in the reference cohort. Only one biopsy was taken from one quarter of women 165 (25.5%) in the 14 15 187 EIS cohort and among 109 (11.3%) in the reference cohort, whereas twenty-two (3.4%) women in the EIS 16 17 188 cohort and 10 (1.0%) in the reference cohort had no biopsy. The average number of biopsies was 1.8 if at 18 19 189 least one biopsy was taken in the EIS cohort and 2.3 in the reference cohort. (Table 1) 20 21 22 190 Altogether 222 (34.3%) women in the EIS cohort had CIN2+, including 5 (0.8%) cervical carcinomas and 14 23 24 25 191 (2.2%) adenocarcinoma in situ cases. In the reference cohort 391 (40.6%) women had CIN2+, including 7 26 27 192 (0.7%) cervical carcinomas and 15 (1.6%) adenocarcinoma in situ cases. (Table 1). The prevalence of CIN2+ 28 29 193 was higher in the reference cohort among those referred for LSIL or ASC-H cytology, whereas the 30 31 194 prevalence of CIN2+ was higher in the EIS cohort after ASC-US and HSIL referral cytology (Table 2). 32 33 34 195 In the EIS cohort the overall sensitivity to detect CIN2+ was 94% (95% CI 90-97%) with corresponding 35 36 196 specificity of 34% (95% Cl 29-39%) (Table 2). The sensitivity varied according to referral cytology, being the 37 38 ₃₉ 197 lowest, 77%, for LSIL cytology (95% CI 61-89%) and the highest for HSIL cytology with 100% sensitivity (95% 40 41 198 CI 95-100%) (Table 2). The specificity was lowest for HSIL cytology, 6% (95% CI 0-29%), and highest for ASC-42 ⁴³ 199 US, 47% (95% CI 36-59%). EIS missed 3 low-grade referral cases of CIN2+ identified by the colposcopist (two 44 45 200 cases if CIN2 and one CIN3). Colposcopic impression was less than CIN2 in 43 CIN2+ cases that were 46 47 ₄₈ 201 detected by ZedScan. A total of 13 cases (5.9%) of CIN2+ were missed by both ZedScan and the colposcopist 49 (biopsies still taken due to suspicion of low-grade lesion), including two adenocarcinoma in situ cases and 50 202 51 52 203 eleven high-grade lesions (nine CIN2 and two CIN3 cases). 53 54 55 204 In the reference cohort, the overall sensitivity to detect CIN2+ was 68% (95% CI 63-73%) with 56 ⁵⁷ 205 corresponding specificity of 84% (95% CI 81-87%) (Table 2). The sensitivity to detect CIN2+ by colposcopic 58 59 206 impression of CIN2+ was the lowest after LSIL cytology, 43%, and the highest after HSIL cytology, 86% 60

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Table 2. Sensitivity, specificity, negative and positive predictive value of the electrical impedance spectroscopy (EIS) cohort and the reference cohort for the detection of CIN2+ lesions by

cervical cytology, TZ type and age group.

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5							EIS (n=6	547)							Ref	erence coh	ort (n=96	2)		
6					Colpo+ZS			Colpo +ZS						Colpo			Colpo			
7			CIN2+/n	CIN2%	CIN2+1	Sensitivity	<cin2 n<="" td=""><td><cin2<sup>3</cin2<sup></td><td>Specificity</td><td>PPV</td><td>NPV</td><td>CIN2+/n</td><td>CIN2%</td><td>CIN2+²</td><td>Sensitivity</td><td><cin2 n<="" td=""><td><cin2<sup>4</cin2<sup></td><td>Specificity</td><td>PPV</td><td>NPV</td></cin2></td></cin2>	<cin2<sup>3</cin2<sup>	Specificity	PPV	NPV	CIN2+/n	CIN2%	CIN2+ ²	Sensitivity	<cin2 n<="" td=""><td><cin2<sup>4</cin2<sup></td><td>Specificity</td><td>PPV</td><td>NPV</td></cin2>	<cin2<sup>4</cin2<sup>	Specificity	PPV	NPV
8		ASC-US	16/94	17.0	15	94(70-100)	78/94	37	47(36-59)	27(16-40)	97(86-100)	9/99	9.1	5	56(21-86)	90/99	87	97(91-99)	63(25-92)	96(89-99)
9		LSIL	39/236	16.5	30	77(61-89)	197/236	82	42(35-49)	21(14-28)	90(82-95)	72/381	18.9	31	43(31-55)	309/381	285	92(89-95)	56(42-70)	87(83-91)
10		ASC-H	85/192	44.3	84	99(94-100)	107/192	12	11(6-19)	47(39-55)	92(64-100)	138/237	58.2	87	63(54-71)	99/237	64	65(54-74)	71(62-79)	56(46-65)
11		HSIL	77/94	81.9	77	100(95-100)	17/94	1	6(0-29)	83(74-90)	100(3-100)	154/200	77.0	133	86(80-91)	46/200	21	46(31-61)	84(78-90)	50(34-66)
12		AGC-NOS	3/28	10.7	2	67(9-99)	25/28	11	44(24-65)	13(2-38)	92(62-100)	5/28	17.9	3	60(15-95)	23/28	22	96(78-100)	75(19-99)	92(73-99)
		AGC-FN	2/3	66.7	1	50(1-99)	1/3	1	100(3-100)	100(3-100)	50(1-99)	13/17	76.5	8	62(32-86)	4/17	1	25(1-81)	73(39-94)	17(0-64)
13 14		ALL	222/647	34.3	209	94(90-97)	425/647	144	34(29-39)	43(38-47)	92(86-96)	391/962	40.6	267	68(63-73)	571/962	480	84(81-87)	75(70-79)	80(76-83)
15		TZ1	156/446	35.0	146	94(89-97)	290/446	90	31(26-37)	42(37-48)	90(82-95)	279/620	45.0	187	67(61-73)	341/620	287	84(80-88)	78(72-83)	76(71-80)
16		TZ2	66/201	32.8	63	96(87-99)	135/201	54	40(32-49)	44(36-52)	95(85-99)	112/342	32.7	80	71(62-80)	230/342	193	84(79-88)	68(59-77)	86(81-90)
17																				
18		<30 y	60/175	34.3	56	93(84-98)	115/175	40	35(26-44)	43(34-52)	91(78-98)	134/295	45.4	96	72(63-79)	161/295	124	77(70-83)	72(64-80)	77(69-83)
		30-44 y	131/366	35.8	124	95(89-98)	235/366	78	33(27-40)	44(38-50)	92(84-97)	211/495	42.6	144	68(62-75)	284/495	244	86(81-90)	78(72-84)	79(74-83)
19 20		>45 y	31/106	29.2	29	94(79-99)	75/106	26	35(24-47)	37(27-49)	93(77-99)	46/172	26.7	27	59(43-73)	126/172	112	89(82-94)	66(49-80)	86(78-91)
			/																	
21		HG cytology	164/289	56.7	162	99(96-100)	125/289	14	11(6-18)	59(53-65)	88(62-98)	305/454	67.2	228	, ,	149/454	86	58(49-66)	78(73-83)	53(45-61)
22		LG cytology	58/358	16.2	47	81(69-90)	300/358	130	43(38-49)	22(16-28)	92(87-96)	86/508	16.9	39	45(35-57)	422/508	394	93(91-96)	58(46-70)	89(86-92)
23			102/200	FC C	101	00/07 100)	124/200	10	11(6 17)		02/00 100	202/427	66.0	220		145/427	05			
24		ASC-H, HSIL	162/286	56.6	161	99(97-100)	124/286	13	11(6-17)	59(53-65)	93(66-100)	292/437	66.8	220	75(70-80)	145/437	85	59(50-67)	79(73-83)	54(46-62)
25		ASC-US,LSIL	55/330 5/31	16.7	45 3	82(69-91)	275/330	119	43(37-49)	22(17-29)	92(86-96)	81/480	16.9	36	44(33-56)	399/480	372	93(90-96)	57(44-70)	89(86-92)
26		Glandular	5/31	16.1	3	60(15-95)	26/31	12	46(27-67)	18(4-43)	86(57-98)	18/45	40.0	11	61(36-83)	27/45	23	85(66-96)	73(45-92)	77(58-90)
27		1 biopsy	11/165	6.7	7	64(31-89)	154/165	78	51(43-59)	8(4-17)	95(88-99)	14/109	12.8	5	36(13-65)	95/109	94	99(94-100)	83(36-100)	91(84-96)
28		2 biopsies	78/263	29.7	70	90(81-96)	185/263	43	23(17-30)	33(27-40)	84(71-93)	112/420	26.7	66	59(49-68)	308/420	276	90(86-93)	67(57-77)	86(81-89)
29		≥3 biopsies	43/84	51.2	43	100	41/84	0	0	51	0	168/305	55.1	113	67(60-74)	137/305	92	67(59-75)	72(64-78)	63(54-70)
30		LLETZ	90/113	79.6	89	99(94-100)	23/113	1	4(0-22)	80(72-87)	50(1-99)	97/118	82.2	83	86(77-92)	21/118	8	38(18-62)	87(78-93)	36(17-59)
31	209	EIS: electrical	impedance	spectroso	copy; CIN: co	ervical intraepit	helial neopla	asia; TZ: trar	nsformation zo	ne; PPV: positi	ve predictive v	alue; NPV: n	egative p	redictive	value; ASC-L	JS: atypical	squamou	us cells of und	etermined	
31	210		•	•		ithelial lesion				•	•		• .				•			

EIS: electrical impedance spectroscopy; CIN: cervical intraepithelial neoplasia; TZ: transformation zone; PPV: positive predictive value; NPV: negative predictive value; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells that cannot exclude HSIL; AGC-NOS: atypical glandular cells not

otherwise specified; AGC-FN: atypical glandular cells that favor neoplasia; LLETZ: large loop excision of the transformation zone; HG: high grade; LG: low grade

¹Colposcopic impression and/or ZedScan result of CIN2+ of histologically confirmed CIN2+ cases.

²Colposcopic impression of CIN2+ of histologically confirmed CIN2+ cases.

³Colposcopic impression and ZedScan result less than CIN2 of histologically confirmed cases <CIN2.

⁴Colposcopic impression less than CIN2 of histologically confirmed cases <CIN2

(Figure 1, Table 2). Overall, the colposcopic impression was less than CIN2+ in 31.7% (124/391) of CIN2+
cases and biopsies were taken due to suspicion of a low-grade lesion. Results stratified according to TZ
type, age, and referral cytology are presented in Table S1.

Compared to the referral cohort, the sensitivity to detect CIN2+ was higher in the EIS cohort overall, with
risk ratio (RR) of 1.38 (95% CI 1.28-1.49), and after LSIL, ASC-H and HSIL referral cervical cytologies (Table
3). TZ 1 and taking two or more biopsies were associated with higher observed sensitivity (Table 3).
Specificity was correspondingly lower in the EIS cohort overall as well as when stratified according to
referral cytology (Table 3).

In the EIS cohort, colposcopic impression of high-grade disease (CIN2+) was present with EIS indicating the ₂₅ 225 presence of CIN2+ in 73.4% of all histologically confirmed CIN2+ cases. In the sensitivity analysis within the EIS cohort, colposcopy alone was indicative for the presence of CIN2+ in 166 of 222 CIN2+ cases (74.8%) 27 226 29 227 and ZedScan in 206 of 222 (92.8%) of CIN2+ cases, suggesting an additional 40 cases (24.1%) detected by ZedScan only. The additional cases increased the detection of CIN2+ from 30 to 44 in women with low-grade cytology and from 136 to 162 in women with high-grade cytology (Figure 1). The sensitivity to detect 36 230 CIN2+ by colposcopy alone according to referral cytology was otherwise similar between the cohorts, 38 231 except for women with ASC-H cervical cytology the colposcopy alone in the EIS cohort seemed to detect ⁴⁰ 232 more CIN2+ cases (p=0.02) (Figure 1).

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3	234	risk ratios of s	ensitivity and sp	ecificity.								
1			EIS	Reference	Sens	sitivity		EIS	Reference	Spec	ificity	
5			Sensitivity	Sensitivity	Risk difference (95%) ¹	RR (95%) ¹	p.	Specificity	Specificity	Risk difference (95%) ¹	RR (95%) ¹	р.
7		ASC-US	94(70-100)	56(21-86)	0.38(0.04-0.73)	1.69(0.93-3.07)	0.0219	47(36-59)	97(91-99)	-0.49(-0.610.38)	0.49(0.39-0.62)	<0.0001
3		LSIL	77(61-89)	43(31-55)	0.34(0.16-0.51)	1.79(1.30-2.45)	0.0006	42(35-49)	92(89-95)	-0.51(-0.580.43)	0.45(0.38-0.53)	<0.0001
)		ASC-H	99(94-100)	63(54-71)	0.36(0.27-0.44)	1.57(1.38-1.78)	<0.0001	11(6-19)	65(54-74)	-0.53(-0.650.42)	0.17(0.10-0.30)	<0.0001
0 1		HSIL	100(95-100)	86(80-91)	0.14(0.08-0.19)	1.16(1.09-1.23)	0.0007	6(0-29)	46(31-61)	-0.40(-0.580.22)	0.13(0.02-0.89)	0.0033
1 2		AGC-NOS	67(9-99)	60(15-95)	0.07(-0.62-0.75)	1.11(0.38-3.25)	0.8504	44(24-65)	96(78-100)	-0.52(-0.730.30)	0.46(0.29-0.72)	0.0001
3		AGC-FN	50(1-99)	62(32-86)	-0.12(-0.86-0.63)	0.81(0.19-3.47)	0.7565	100(3-100)	25(1-81)	0.75(0.33-1.17)	4.0(0.73-21.84)	0.1709
4		ALL	94(90-97)	68(63-73)	0.26(0.20-0.31)	1.38(1.28-1.49)	<0.0001	34(29-39)	84(81-87)	-0.50(-0.560.45)	0.40(0.35-0.46)	<0.0001
5												
6		TZ1	94(89-97)	67(61-73)	0.27(0.20-0.33)	1.40(1.27-1.53)	<0.0001	31(26-37)	84(80-88)	-0.53(-0.600.47)	0.37(0.31-0.44)	<0.0001
7 8		TZ2	96(87-99)	71(62-80)	0.24(0.14-0.34)	1.34(1.18-1.57)	0.0001	40(32-49)	84(79-88)	-0.44(-0.530.34)	0.48(0.38-0.59)	<0.0001
9			(,	()					- ()		(
0							0.0007					
1		<30 y	93(84-98)	72(63-79)	0.22(0.12-0.32)	1.30(1.15-1.48)		35(26-44)	77(70-83)	-0.42(-0.530.31)	0.45(0.35-0.59)	<0.0001
2 3		30-44 y	95(89-98)	68(62-75)	0.26(0.19-0.34)	1.39(1.25-1.53)	<0.0001	33(27-40)	86(81-90)	-0.53(-0.600.45)	0.39(0.32-0.47)	<0.0001
4		≥45 y	94(79-99)	59(43-73)	0.35(0.18-0.52)	1.59(1.23-2.07)	0.0008	35(24-47)	89(82-94)	-0.54(-0.660.42)	0.39(0.28-0.54)	<0.0001
5		,	, , , , , , , , , , , , , , , , , , ,	()	, , , , , , , , , , , , , , , , , , ,	, , , , , , , , , , , , , , , , , , ,		, ,			, , , , , , , , , , , , , , , , , , ,	
6		HG cytology	99(96-100)	75(70-80)	0.24(0.19-0.29)	1.32(1.24-1.41)	<0.0001	11(6-18)	58(49-66)	-0.47(-0.560.37)	0.19(0.12-0.32)	<0.0001
7		LG cytology	81(69-90)	45(35-57)	0.36(0.21-0.50)	1.79(1.37-2.33)	< 0.0001	43(38-49)	93(91-96)	-0.50(-0.560.44)	0.46(0.41-0.53)	
8		La cytology	01(05 50)	45(55 57)	0.30(0.21 0.30)	1.75(1.37 2.33)	0.0001	45(50 45)	55(51 50)	0.50(0.50 0.44)	0.40(0.41 0.55)	0.0001
9 0		1 biopsy	64(31-89)	36(13-65)	0.28(-0.10-0.66)	1.78(0.77-4.10)	0.1654	51(43-59)	99(94-100)	-0.48(-0.560.40)	0.51(0.44-0.60)	<0.0001
1		2 biopsies	90(81-96)	59(49-68)	0.31(0.19-0.42)	1.52(1.28-1.81)	< 0.0001	23(17-30)	90(86-93)	-0.66(-0.730.59)	0.26(0.20-0.34)	<0.0001
2		≥3 biopsies	100	67(60-74)	0.33(0.26-0.40)	1.49(1.34-1.65)	< 0.0001	0	90(80-93) 67(59-75)	-0.67(-0.750.59)	0.20(0.20-0.34)	<0.0001
3	235	•		, , ,	, , , , , , , , , , , , , , , , , , ,	, ,			. ,	, , , , , , , , , , , , , , , , , , ,		<0.0001

Table 3. Sensitivity and specificity of the electrical impedance spectroscopy cohort (EIS) and the reference cohort by cytology, TZ type and age group in identifying CIN2+, with corresponding risk differences and the rick ratios of consitivity and considering

EIS: electrical impedance spectroscopy; CIN: cervical intraepithelial neoplasia; TZ: transformation zone; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells that cannot exclude HSIL; AGC-NOS: atypical glandular cells not otherwise specified; AGC-FN: atypical glandular cells that favor

neoplasia; HG: high grade; LG: low grade

¹The values of risk difference >0 or the values of risk ratio >1 imply better/improved effect with ZedScan.

239 Discussion

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We compared the performance of colposcopy in detecting CIN2+ according to referral cervical cytology 240 241 with and without EIS as an adjunctive technology. Colposcopy combined with EIS seemed to have a higher 242 sensitivity, but a lower specificity compared to conventional colposcopy, regardless of the referral cervical 13 243 cytology. The prevalence of CIN2+ lesions was higher in the EIS cohort after ASC-US and HSIL referral, but 15 244 lower after LSIL and ASC-H cervical cytology. The average number of biopsies was lower in the EIS cohort.

20 Overall, EIS performed well with a high sensitivity (94%) but had a low specificity (34%) consistent with the 21 246 22 23 247 previous studies.[13, 14, 16] Here, the sensitivity might have been overestimated in both cohorts as the 24 25 248 true positive result was based on histology data at first visit only and lesions missed at first visit and 26 27 28 249 detected during the follow-up were not included in either cohort. Still, this would not affect the estimates 29 30 250 of relative performance. The sensitivity (68%) and specificity (84%) of colposcopy in the reference cohort 31 32 251 was as well in line with existing data.[5, 7, 21]

The increased detection of CIN2+ cases by EIS has been reported as most pronounced in women with low-35 252 36 37 253 grade cytology [13, 14, 16] or with high-risk HPV positivity without cytological changes. [16, 17] In our study, 38 39 254 additional cases of CIN2+ detected by EIS were also most frequent among low-grade referrals. 40 41 42 255 Furthermore, the sensitivity to detect CIN2+ with EIS was higher in most cervical cytology groups (ASC-US, 43 44 256 LSIL, ASC-H, HSIL) compared to colposcopy alone. Only within HSIL cytology EIS combined with colposcopy 45 46 257 detected all CIN2+ cases. In women with other referral cytology (ASC-US, LSIL, ASC-H) there were cases of 47 48 258 CIN2+ that EIS did not detect, but where biopsy of CIN2+ was warranted based on colposcopic diagnosis. 49 50 259 Nevertheless, missed cases of CIN2+ were even more frequent in the reference cohort, where more CIN2+ 51 52 53 260 lesions were detected in biopsies with colposcopic impression of CIN1 or lower. Contrary to expectations, 54 55 261 the prevalence of CIN2+ was higher in EIS cohort only after ASC-US and HSIL referral cytology. One 56 ⁵⁷ 262 explanation for lower prevalence of CIN2+ lesions in the EIS cohort after LSIL and ASC-H cytology could be 58 59 263 that routine practice in Finland is to take biopsies also from low-grade lesions, rather than to abstain from

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taking biopsies when CIN2+ lesions are not colposcopically suspected. Biopsies even from mild acetowhite
lesions are important in excluding a high-grade disease as the sensitivity of colposcopy to detect CIN2+ is
far from 100%. Such biopsies could well have been more frequent without than with EIS as an additional
confirmation on suspected absence of CIN2+. This is supported by the observation that two or more
biopsies were taken from 54% of women in the EIS cohort, whereas up to 75% of women in the reference
cohort had at least two biopsies. Multiple biopsies are known to increase the sensitivity of colposcopy as at
least small lesions can easily be missed.[22, 23] In women with low-grade referral cervical cytology, a single
biopsy has shown to be insufficient to rule out a high-grade disease.[24] A British survey has also reported
experienced colposcopists to take mostly two biopsies in diagnosing high-grade disease.[25] The average
number of biopsies in the EIS cohort was higher (1.84) compared to previous reports (1.07 and 1.51),[23,
14] but still lower than in the reference cohort (2.3).

Our observation of overall fewer biopsies along with fewer CIN2+ lesions detected in the EIS cohort can either indicate a true difference in CIN2+ prevalence between the cohorts, selection bias towards using EIS preferably on patients in whom CIN2+ lesion is not clearly present, or that CIN2+ lesions could have been missed in the EIS cohort, especially after LSIL and ASC-H referral cytology. If lesions were missed, it could possibly be due to a higher biopsy threshold in the EIS cohort, as indicated by lower number of biopsies. Without longitudinal data we still cannot be certain whether prevalent CIN2+ cases were indeed more frequently missed at the first visit in the EIS cohort. The prevalence of CIN2+ in EIS cohort in women with high-grade cytology (ASC-H and HSIL) is below previous observations (56.6% vs. 79.1-84.0%).[13, 16] 283 However, when restricted to only women with HSIL referral cervical cytology or low-grade (ASC-US and 284 LSIL) cytology, the prevalence for CIN2+ here did not differ from previous reports.[13, 16] Cytological diagnoses may well vary between cytopathologists as well as between countries and this possible difference in classification might also explain the observed difference in CIN2+ prevalence, especially after ASC-H cytology. [26] The longitudinal data on EIS results are scarce. In women referred with low-grade cytology, the future risk of CIN2+ was increased in up to 36 months follow-up if both colposcopic impression and EIS results were indicative for CIN2+ compared with women with other combinations of

290 these two parameters, suggesting that EIS might provide new information on the future risk of high-grade 291 disease.[27]

292 **Strengths and limitations**

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293 Most previous studies have compared the performance of EIS as an adjunctive technology for colposcopy 294 against conventional colposcopy within the cohort where EIS was used, even though in clinical setting EIS is 295 not a truly independent measurement from colposcopy. To our knowledge this is the first report on the 18 296 performance of EIS as an adjunctive technology for colposcopy stratified according to referral cytology and 20 297 compared to an external reference cohort. Even though our cohorts were collected at different time 298 periods, they both represent women in the same catchment area referred to colposcopy due to abnormal ₂₅ 299 cervical cytology. All colposcopies were performed in the same clinic by experienced colposcopists. Furthermore, none of the authors of this work have financial conflicts of interest with the technology 27 300 29 301 studied. Our study also has some limitations. When the cervical transformation zone is not fully visible, TZ 302 type 3, ZedScan technology cannot be reliably applied and the results are not applicable to this population. 303 CIN2+ lesions could well have been missed in both cohorts since the results are based on data collected on 36 304 the initial visit. However, complete certainty of the histology would have required LLETZ for all participants 38 305 which would not have been ethically just.

41 306 Conclusions

43 44 307 Colposcopy with EIS has a higher sensitivity and a lower specificity in identifying CIN2+ compared to 45 46 308 conventional colposcopy, irrespective of cervical cytology. EIS can, therefore, be assumed to be of clinical 47 48 309 benefit in colposcopy, particularly in women with low-grade cervical cytology where the prevalence of 49 50 310 CIN2+ is low. We also observed an overall lower prevalence of CIN2+ lesions in the EIS cohort compared to 51 52 53 311 a reference cohort with conventional colposcopy. The performance of EIS as an adjunctive technology for 54 55 312 colposcopy has not been previously compared by cytology to an external reference cohort. While the 56 ⁵⁷ 313 observation of lower CIN2+ rate could be explained by different CIN2+ prevalence between the cohorts or 58 59 314 selection bias, the finding is important and warrants further research, especially along with the observed 60

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² ³ 315	lower number of biopsies in the EIS cohort. Adjunctive technologies are likely to become increasingly
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5 6 7	appealing in colposcopy, as the prevalence of high-grade cervical lesions is declining. Randomised
7 8 317 9	controlled trials comparing EIS with a conventional colposcopy, including women referred due to persistent
9 10 318 11	HPV infection without cytological changes are warranted. Before such further evidence, firm
12 319 13 14	recommendations on applicability of EIS as an adjunctive technology for colposcopy cannot be made.
15 320 16	Figure 1. Numbers and rates of CIN2+ lesions detected in the electrical impedance spectroscopy cohort
¹⁷ 321 18	(EIS) and in the reference cohort according to referral cytology. (A) Numbers and rates of CIN2+ detected
¹⁹ 20 322	by ZedScan alone and reference cohort stratified according to referral cytology (B) Numbers and rates of
21 22 323 23	CIN2+ detected by colposcopy alone in EIS and reference cohorts stratified according to referral cytology.
23 24 324 25	Numbers of patients are given in the columns.
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27 325	Contributors
28 29	
30 326 31	PN and IK were responsible for the conceptualisation and design of the study as well as methodology. XC,
32 327 33	KL, LKT contributed to conceptualisation. LB performed the statistical analysis with the aid of IK. LB, PN,
³⁴ 328 35	MK, PLO, SV and AH were responsible for data collection. LB drafted the original manuscript and IK, PN, CR,
36 37 329 38	XC, KL, LKT, AH and KA participated in writing, reviewing and editing. All authors listed qualify for
39 330 40	authorship and approved the final version of the paper.
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42	
44	
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49	
50	Competing interests None declared.
51 334 52	competing interests None declared.
52 53	
54 335 55	Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or
⁵⁶ 336 57	reporting, or dissemination plans of this research.
58 59	
⁵⁹ 337 60	Patient consent for publication Not required.

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2 3 4	338	Ethics approval This study and data collection on patients where EIS was used was considered as a service
5 6	339	evaluation and therefore a separate ethical approval was not required as per consultation with Helsinki-
7 8 9	340	Uusimaa Hospital District Ethical Committee. For the historical reference cohort an ethical approval was
	341	received from Helsinki-Uusimaa Hospital District Ethical Committee (ref. no. 130/13/03/03/2013).
12 13 14	342	Data availability statement Data are available upon reasonable request.
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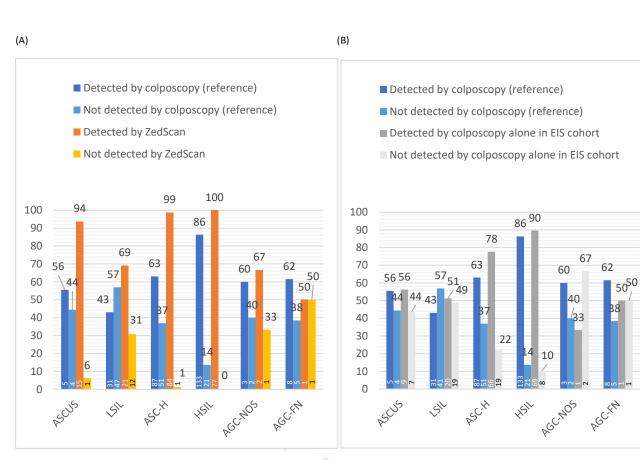


 Table S1. Sensitivity, specificity, positive and negative predictive value of the electrical impedance spectroscopy (EIS) cohort and the reference cohort for the detection of CIN2+ lesions within

different cervical cytology by TZ type and age group.

		EIS cohort (n=647)								Reference cohort (n=962)						
		Colpo+ZS ¹			Colpo+ZS ³					Colpo ²			Colpo ⁴			
	CIN2+/n	CIN2+	Sensitivity	<cin n<="" th=""><th><cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th><th>CIN2+/n</th><th>CIN2+</th><th>Sensitivity</th><th><cin2 n<="" th=""><th><cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th></cin2<></th></cin2></th></cin2<></th></cin>	<cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th><th>CIN2+/n</th><th>CIN2+</th><th>Sensitivity</th><th><cin2 n<="" th=""><th><cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th></cin2<></th></cin2></th></cin2<>	Specificity	PPV	NPV	CIN2+/n	CIN2+	Sensitivity	<cin2 n<="" th=""><th><cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th></cin2<></th></cin2>	<cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th></cin2<>	Specificity	PPV	NPV
ASC-US	16/94	15	94(70-100)	78/94	37	47(36-59)	27(16-40)	97(86-100)	9/99	5	56(21-86)	90/99	87	97(91-99)	63(25-92)	96(89-99
TZ1	11/66	10	91(59-100)	55/66	24	44(30-58)	24(12-40)	96(80-100)	7/57	3	43(10-82)	50/57	47	94(84-99)	50(12-88)	92(81-98
TZ2	5/28	5	100(48-100)	23/28	13	57(35-77)	33(12-62)	100(75-100)	2/42	2	100(16-100)	40/42	40	100(91-100)	100(16-100)	100(91-10
<30 y	6/28	5	83(36-100)	22/28	12	55(32-76)	33(12-62)	92(64-100)	1/43	0	0(0-98)	42/43	39	93(81-99)	0(0-71)	98(87-100
30-44 y	10/52	10	100(69-100)	42/52	21	50(34-66)	32(17-51)	100(84-100)	7/28	4	57(18-90)	21/28	21	100(84-100)	100(40-100)	88(68-97
>45 y	0/14	0	0	14/14	4	29	0	100	1/28	1	100(3-100)	27/28	27	100(87-100)	100(3-100)	100(87-10
LSIL	39/236	30	77(61-89)	197/236	82	42(35-49)	21(14-28)	90(82-95)	72/381	31	43(31-55)	309/381	285	92(89-95)	56(42-70)	87(83-91
TZ1	26/157	20	77(56-91)	131/157	53	41(32-49)	20(13-30)	90(79-96)	53/235	23	43(30-58)	182/235	169	93(88-96)	64(46-79)	85(79-90
TZ2	13/79	10	77(46-95)	66/79	29	44(32-57)	21(11-36)	91(75-98)	19/146	8	42(20-67)	127/146	116	91(85-96)	42(20-67)	91(85-96
<30 y	4/39	1	25(1-81)	35/39	21	60(42-76)	7(0-32)	88(68-97)	17/79	9	53(28-77)	62/79	54	87(76-94)	53(28-77)	87(76-94
30-44 y	28/153	24	86(67-96)	125/153	46	37(28-46)	23(16-33)	92(81-98)	46/224	19	41(27-57)	178/224	166	93(89-97)	61(42-78)	86(80-91
>45 y	7/44	5	71(29-96)	37/44	15	41(25-58)	19(6-38)	88(64-99)	9/78	3	33(8-70)	69/78	65	94(86-98)	43(10-82)	92(83-97
ASC-H	85/192	84	99(94-100)	107/192	12	11(6-19)	47(39-55)	92(64-100)	138/237	87	63(54-71)	99/237	64	65(54-74)	71(62-79)	56(46-65
TZ1	57/134	56	98(91-100)	77/134	6	8(3-16)	44(35-53)	86(42-100)	100/167	64	64(54-73)	67/167	42	63(50-74)	72(61-81)	54(42-65
TZ2	28/58	28	100(88-100)	30/58	6	20(8-39)	54(40-68)	100(54-100)	38/70	23	61(43-76)	32/70	22	69(50-84)	70(51-84)	60(42-75
<30 y	24/72	24	100(86-100)	48/72	5	10(4-23)	36(25-49)	100(48-100)	57/90	34	60(46-72)	33/90	18	55(36-72)	69(55-82)	44(29-60
30-44 y	46/90	45	98(89-100)	44/90	4	9(3-22)	53(42-64)	80(28-100)	67/120	45	67(55-78)	53/120	37	70(56-82)	74(61-84)	63(49-75
>45 y	15/30	15	100(78-100)	15/30	3	20(4-48)	56(35-75)	100(29-100)	14/27	8	57(29-82)	13/27	9	69(39-91)	67(35-90)	60(32-84
HSIL	77/94	77	100(95-100)	17/94	1	6(0-29)	83(74-90)	100(3-100)	154/200	133	86(80-91)	46/200	21	46(31-61)	84(78-90)	50(34-66
TZ1	58/67	58	100	9/67	0	0	87	0	104/131	88	85(76-91)	27/131	15	56(35-75)	88(80-94)	48(30-67
TZ2	19/27	19	100(82-100)	8/27	1	13(0-53)	73(52-88)	100(3-100)	50/69	45	90(78-97)	19/69	6	32(13-57)	78(65-88)	55(23-83
<30 y	25/31	25	100	6/31	0	0	81	0	54/75	48	89(77-96)	21/75	10	48(26-70)	81(69-90)	63(35-85
30-44 y	45/54	45	100(92-100)	9/54	1	11(0-48)	85(72-93)	100(3-100)	84/102	72	86(76-92)	18/102	9	50(26-74)	89(80-95)	43(22-66
>45 y	7/9	7	100	2/9	0	0	78	0	16/23	13	81(54-96)	7/23	2	29(4-71)	72(47-90)	40(5-85

EIS: electrical impedance spectroscopy; CIN: cervical intraepithelial neoplasia; TZ: transformation zone; PPV: positive predictive value; NPV: negative predictive value; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells that cannot exclude HSIL; AGC-NOS: atypical glandular cells not otherwise specified; AGC-FN: atypical glandular cells that favor neoplasia; LLETZ: large loop excision of the transformation zone; HG: high grade; LG: low grade

¹Colposcopic impression and/or ZedScan result of CIN2+ of histologically confirmed CIN2+ cases.

²Colposcopic impression of CIN2+ of histologically confirmed CIN2+ cases.

³Colposcopic impression and ZedScan result less than CIN2 of histologically confirmed cases <CIN2.

⁴Colposcopic impression less than CIN2 of histologically confirmed cases <CIN2

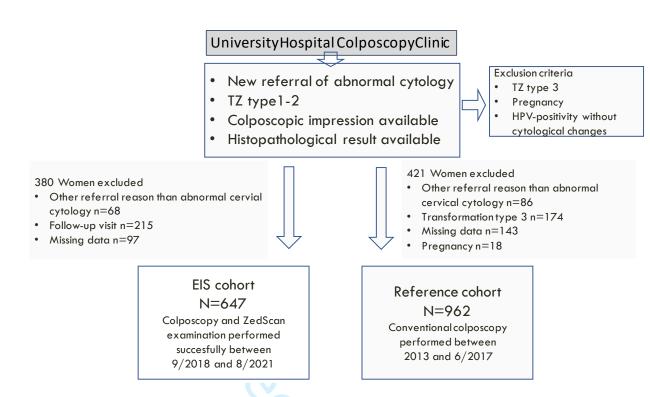


Figure S1. Flow-chart of the study comparing the performance of colposcopy by referral cervical cytology in two cohorts with and without electrical impedance spectroscopy as an adjunctive technology.

R. R. ONL

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Section & Topic	No	Item	Reported on pa #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy	2
		(such as sensitivity, specificity, predictive values, or AUC)	
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions	2
		(for specific guidance, see STARD for Abstracts)	
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4
	4	Study objectives and hypotheses	4
METHODS			
Study design	5	Whether data collection was planned before the index test and reference standard	5
		were performed (prospective study) or after (retrospective study)	
Participants	6	Eligibility criteria	5-6
	7	On what basis potentially eligible participants were identified	5
		(such as symptoms, results from previous tests, inclusion in registry)	
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5-6
	9	Whether participants formed a consecutive, random or convenience series	5-6
Test methods	10a	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	5-6
	12a	Definition of and rationale for test positivity cut-offs or result categories	7
		of the index test, distinguishing pre-specified from exploratory	
	12b	Definition of and rationale for test positivity cut-offs or result categories	7
		of the reference standard, distinguishing pre-specified from exploratory	
	13a	Whether clinical information and reference standard results were available	6
		to the performers/readers of the index test	
	13b	Whether clinical information and index test results were available	5-6
		to the assessors of the reference standard	
Analysis	14	Methods for estimating or comparing measures of diagnostic accuracy	7
	15	How indeterminate index test or reference standard results were handled	5-6
	16	How missing data on the index test and reference standard were handled	5-6
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	7
	18	Intended sample size and how it was determined	5-6
RESULTS			
Participants	19	Flow of participants, using a diagram	Figure S1,
			submitted as a
	20	Baseline demographic and clinical characteristics of participants	separate file 8, Table 1
	21a	Distribution of severity of disease in those with the target condition	8, Table 1
	21b	Distribution of alternative diagnoses in those without the target condition	8, Table 1
	210	Time interval and any clinical interventions between index test and reference standard	5-6
Test results	23	Cross tabulation of the index test results (or their distribution)	8,10,12 Table1-3
i con i courto	23	by the results of the reference standard	0,10,12 TOUCT-
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	10,12 Table 2-3
	24	Any adverse events from performing the index test or the reference standard	NA
DISCUSSION			
DISCUSSION	26	Study limitations, including sources of potential bias, statistical uncertainty, and	13-15
	20	generalisability	1, 1, 1,
	27	Implications for practice, including the intended use and clinical role of the index test	15-16
OTHER	-1	החקווסטוסוז זיו אישרוכי, ווכוסטוואַ גור ווגרוועכע עשב מוע כוווועם דטוב טו גור וועבא נבשנ	10 10
INFORMATION			
	28	Registration number and name of registry	6 ISRCTN for the
	20	For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	reference cohor

29	Where the full study protocol can be accessed	Submitted as a separate file
 30	Sources of funding and other support; role of funders	16

STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on <u>http://www.equator-network.org/reporting-guidelines/stard.</u>



BMJ Open

Added value of electrical impedance spectroscopy in adjunction of colposcopy: a prospective cohort study

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Primary Subject Heading :	Obstetrics and gynaecology
Secondary Subject Heading:	Obstetrics and gynaecology, Oncology
Keywords:	Colposcopy < GYNAECOLOGY, Gynaecological oncology < GYNAECOLOGY, Community gynaecology < GYNAECOLOGY

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review only

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3 4	1	Added value of electrical impedance spectroscopy in adjunction of colposcopy: a
5 6	2	prospective cohort study
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11 12	4	Laura Bergqvist, ¹ Annu Heinonen, ¹ Xavier Carcopino, ² Charles Redman, ³ Karoliina Aro, ¹ Mari Kiviharju, ¹ Seppo
13	5 6	Virtanen, ¹ Pirjo-Liisa Omar, ¹ Laura Kotaniemi-Talonen, ^{4,5} Karolina Louvanto, ^{4,5} Pekka Nieminen, ^{*1} Ilkka Kalliala ^{*1,6}
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2 3 4 5	31	Abstract
6 7	32	Objective: To assess whether electrical impedance spectroscopy (EIS) as an adjunctive technology enhances
8 9	33	the performance of colposcopy.
10 11	34	Design: Prospective cohort study.
12 13 14	35	Setting: University Hospital colposcopy clinic.
14 15 16	36	Participants: Colposcopy with EIS for 647 women and conventional colposcopy for 962 women.
17 18	37	Interventions: Comparison of the performance of colposcopy by referral cervical cytology in two cohorts,
19 20	38	with and without EIS as an adjunctive technology.
21 22 22	39	Outcome measures: Prevalence of cervical intraepithelial neoplasia grade 2 or worse (CIN2+), diagnostic
23 24 25	40	testing accuracy to detect CIN2+ with and without EIS and their relative differences between cohorts.
26 27	41	Results: The prevalence of CIN2+ varied between the cohorts according to referral cytology: 17.0% after
28 29	42	abnormal squamous cells of unknown significance (ASC-US) referral cytology in EIS cohort and 9.1% in the
30 31 22	43	reference cohort, 16.5% and 18.9% after low-grade squamous intraepithelial lesion (LSIL), 44.3% and 58.2%
32 33 34	44	after atypical squamous cells, cannot exclude HSIL (ASC-H), and 81.9% and 77.0% after high-grade
35 36	45	squamous intraepithelial lesion (HSIL) cytology, respectively. Sensitivity to detect CIN2+ was higher in the
37 38	46	EIS cohort, varying from 1.79 (95% CI 1.30-2.45) after LSIL referral cytology to 1.16 (95% CI 1.09-1.23) after
39 40	47	HSIL referral cytology, with correspondingly lower specificity after any referral cytology.
41 42 43	48	Conclusions: Colposcopy with EIS had overall higher sensitivity but lower specificity to detect CIN2+ than
44 45	49	conventional colposcopy. CIN2+ prevalence rates were, however, not consistently higher in the EIS cohort,
46 47	50	suggesting innate differences between the cohorts or truly lower detection rates of CIN2+ for EIS,
48 49 50	51	highlighting the need for randomized controlled trials on the effectiveness of EIS.
51 52 53	52	Keywords: Electrical impedance spectroscopy (EIS), colposcopy, cervical intraepithelial neoplasia (CIN),
54 55 56	53	cervical cytology, sensitivity, specificity
57 58 59 60	54	

1 2 3 4 5	55	trengths and limitations of this study	Strengt
5 6 7	56	1. The intervention and reference cohorts were both collected within the daily patient flow at the	1.
8 9	57	same colposcopy clinic.	
10 11	58	2. The reference cohort was collected between 2013-2017 (n=962) and the EIS cohort between 2018-	2.
12 13 14	59	2021 (n=647).	
15 16	60	3. The prevalence of CIN2+ in both cohorts was based on the histopathological data obtained at the	3.
17 18	61	first visit.	
19 20 21	62	4. Diagnostic testing accuracy was calculated for the detection of CIN2+ in both cohorts.	4.
21 22 23	63	5. We estimated the added value of electrical impedance spectroscopy compared to conventional	5.
24 25	64	colposcopy within and between cohorts stratified according to the referral cytology.	
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83 Introduction

During the next decades, the incidence and prevalence of high-grade cervical disease will decrease in the developed countries due to human papillomavirus (HPV) vaccination programs [1, 2] and transition to primary high-risk HPV (hrHPV)-DNA test -based screening.[3] Consequently, colposcopy will become more challenging due to resulting lower positive predictive value. Therefore, to detect those in need of treatment, it will be essential to correctly identify the high-grade lesions and take biopsies at representative locations. Also, reliable means to rule out high-grade lesions without excessive number of biopsies or frequently repeated tests or colposcopies are needed.

have been previously reported to vary between 66% to 80% and 63% to 95%, respectively.[4-7]
Furthermore, the probability of detecting a high-grade disease at colposcopy is affected by the referral
cytology, being higher after high-grade squamous intraepithelial lesion (HSIL) cytology than after atypical
squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesion (LSIL)
cytology results.[8]

2 2edScan (Zillico Ltd.)[9] is a hand-held device using electrical impedance spectroscopy (EIS) in identifying 3 98 cervical pathology.[10] It is designed to provide guidance to colposcopist in biopsy taking by indicating the 3 99 most abnormal cervical tissue area.[10] ZedScan measures the electrical properties of the cervical 3 100 epithelium to differentiate pre-cancerous and cancerous tissue from normal epithelium.[10-12] The area 3 with the most abnormal impedance is reported visually, aiding the colposcopist in targeting biopsies.

The sensitivity of colposcopy has been suggested to increase with the use of EIS [10, 13-17] even in women with low probability of high-grade cervical disease and with minor colposcopic changes, as its use is independent of visual findings in colposcopy.[12, 18, 19] The developers of the technology have been involved in most of the published studies. In women with persistent hrHPV positivity without cervical cytological changes, EIS has detected additional cases of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to women without EIS examination.[18] The benefit of EIS seems to vary depending on

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2 3 4	108	the referral cervical cytology, being most useful in terms of finding extra cases of CIN2+ in women with low-
5 6 7	109	grade referral cervical cytology.[13, 14, 16, 17] NICE guidelines recommend further research on EIS.[20]
8 9	110	Our objective was to assess, stratified according to referral cytology, whether EIS combined with
10 11	111	colposcopy increases the diagnostic testing accuracy of CIN2+ compared to conventional colposcopy in
12 13 14	112	women referred to colposcopy for abnormal cervical cytology.
15 16	113	Methods
17		
18 19 20	114	Participants
21 22	115	All women (n=1609) in this study were examined between 2013-21 at the outpatient colposcopy clinic of
23 24 25	116	Helsinki University Hospital for a new referral for abnormal cytology. We included women if their cervical
	117	transformation zone (TZ) was type 1 or 2 (TZ1-2) and the information on both colposcopic impression and
29	118	histopathological results were available. Exclusion criteria were transformation zone type 3 (TZ3), previous
30 31 32	119	history of cervical cancer or large loop excision of the transformation zone (LLETZ) and pregnancy. Women
33 34	120	referred for persistent hrHPV positivity without cytological changes were excluded due to the lack of
36	121	sufficient control cohort as high-risk HPV testing as a part of primary screening was implemented in Helsinki
37 38 39	122	region only in 2019.
40 41	123	The EIS cohort consisted of 647 women with colposcopy and ZedScan examination successfully performed
43	124	between September 2018 and August 2021. The cohort was collected prospectively with non-consecutive
44 45	125	patient recruitment. Under the study period ZedScan equipment was available at the colposcopy and used
46 47 48	126	at the decision of the individual colposcopist. EIS examinations were done according to the manufacturer's
	127	protocol and all colposcopists had an adequate training prior using the device. If active bleeding during
51 52 53	120	colposcopy occurred, the EIS procedure was omitted.
54 55	129	We could not directly compare the performance of colposcopy alone against colposcopy with ZedScan as
56 57	130	an adjunctive tool using only the EIS cohort, as these two events were not truly independent of each other
58 59 60	131	in the routine clinical setting applied here. Therefore, we used a previously collected prospective cohort of

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~	132	962 patients examined with conventional colposco
5 6	133	Hospital, Finland, between 2013 and July 2017 as t
0	134	women fulfilling the inclusion criteria included. On
9 10 11	135	histological results were included in both cohorts.
12 13 14	136	Abnormal cervical cytology results were categorize
	137	Histological results were reported according to WH
17 18	138	histopathological specimens, biopsies, and LLETZ c
19 20 21	139	of Helsinki University Hospital. The most severe hi
22	140	Clinical procedures
25 26	141	All participants had a colposcopic examination with
20	142	Subsequently, participants in the EIS cohort under
29 30 31	143	made from 10 to 12 points clockwise around the co
	144	area with the highest probability of high-grade dise
34 35	145	and the absence of high-grade disease is indicated
37	146	cover well the junction area of the cervix. However
38 39 40	147	case of very large cervix. After routine measureme
	148	presence of CIN2+ by ZedScan, a particular single p
	149	most abnormal area to be biopsied. In the EIS coho
45 46	150	colposcopist based on both ZedScan results and co
47 48 49	151	diagnosis of all biopsies was recorded.
51	152	Random biopsies were not routinely taken in eithe
	153	was based on Finnish Current Care Guidelines.[22]
54 55 56	154	available at the discretion of individual colposcopis
F7	155	colposcopic impression was recorded as high-grade
59	156	('select and treat'-approach) was performed when

1 2

> ents examined with conventional colposcopy in the colposcopy clinic of Helsinki University , Finland, between 2013 and July 2017 as the reference cohort (ISRCTN10933736),[21] with all fulfilling the inclusion criteria included. Only the primary colposcopy after referral and its

al cervical cytology results were categorized according to the Bethesda system as ASC-US or worse. ical results were reported according to WHO 2003, 2013 and 2020 classification. The evaluation of hological specimens, biopsies, and LLETZ cones, was done by the gynaecological histopathologists nki University Hospital. The most severe histological diagnosis of all biopsies or LLETZ was recorded.

procedures

cipants had a colposcopic examination with the application of acetic acid to the cervix. Jently, participants in the EIS cohort underwent a ZedScan examination. ZedScan readings were om 10 to 12 points clockwise around the cervix. On the Zedscan reading, red colour points out the h the highest probability of high-grade disease, amber colour indicates possible high-grade areas absence of high-grade disease is indicated with green colour. In most women, 12 measurements ell the junction area of the cervix. However, it might be possible that minor areas are omitted in very large cervix. After routine measurements (10-12 around the cervix) in case of suspected e of CIN2+ by ZedScan, a particular single point mode can be used to localise more carefully the normal area to be biopsied. In the EIS cohort, cervical biopsy sites were determined by the opist based on both ZedScan results and colposcopic impression. The most severe histological is of all biopsies was recorded.

biopsies were not routinely taken in either of the cohorts. Colposcopy examination in both cohorts ed on Finnish Current Care Guidelines. [22] Five percent acetic acid and Lugol's iodine were e at the discretion of individual colposcopist to assess the abnormal cervical areas for biopsy. The opic impression was recorded as high-grade, low-grade, or normal. Immediate LLETZ at initial visit and treat'-approach) was performed when evaluated necessary according to Finnish Current Care

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1 2		
2 3 4	157	Guidelines: HSIL referral cytology with a colposcopic impression of CIN2+ entitled to perform LLETZ at the
5 6	158	initial colposcopy with consent from the patient.[22] After cervical cytology with glandular atypia favouring
7 8	159	neoplasia (AGC-FN) the Finnish Current Care Guidelines recommends immediate LLETZ irrespective of the
11	160	age of the referred woman.[22]
12 13 14	161	Data analysis
15 16 17	162	We compared the prevalence of histologically confirmed CIN2+ lesions between the EIS and reference
	163	cohorts and calculated the sensitivity, specificity, positive and negative predictive values for colposcopy in
	164	both cohorts for the detection of CIN2+ lesions, both overall and stratified according to the referral cervical
22 23	165	cytology. The positive test result for EIS cohort was defined as suspected presence of CIN2+ either by
	166	ZedScan and/or via colposcopic inspection. The test result was negative if both the colposcopic impression
	167	and ZedScan agreed on low-grade lesion or normal cervical finding, i.e. absence of CIN2+ lesion. In the
28 29 30	168	reference cohort, positive test result was defined as a colposcopic impression of CIN2+ while negative test
31 32	169	result was defined as the absence of changes suggesting CIN2+ lesions. The most advanced
	170	histopathological result of the biopsies or LLETZ specimen taken at the initial visit were used as a reference
	171	standard in both cohorts. Women without biopsies and with negative ZedScan result and normal
37 38 39	172	colposcopic impression as well as low-grade referral were considered true negatives. Even though
	173	colposcopy and EIS examination were not truly independent tests in the setting used, we still performed a
42 43	174	sensitivity analysis within the EIS cohort and assessed separately diagnostic testing accuracy of colposcopy
44 45 46	175	and EIS in that cohort alone as well.
	176	Risk ratio and risk difference were used to compare the sensitivity and specificity between the EIS and
49 50 51	177	reference cohorts. The <i>p</i> -values < 0.05 were considered statistically significant. All statistical analyses were
	178	performed using STATA/SE 15 (StataCorp, College Station TX, USA) and all statistical tests used were two-
54 55	179	sided.
56 57 58 59 60	180	Patient and public involvement

1 2

3 181 Patients and/or the public were not involved in the design, conduct, reporting, or dissemination of this 4 5 182 research. 6 7 8 183 Results 9 10 11 184 There were 1027 eligible women with adequate colposcopy and Zedscan examination performed In the EIS 12 13 185 cohort. Altogether 68 women with other referral reasons than abnormal cervical cytology, 215 women with 14 15 186 follow-up colposcopy visits and 97 women with missing data were excluded. In total, 647 women with new 16 17 18 187 colposcopy referrals of abnormal cytology were included in the analysis (Figure S1, Table 1). Of all ZedScan 19 20 188 procedures 75% were conducted by three individual colposcopists. The reference cohort included 1383 21 22 189 eligible women. Of these, 86 women were excluded due to other referral reasons than abnormal cervical 23 24 ₂₅ 190 cytology, 174 for having TZ3, 143 for missing relevant clinical data and 18 for pregnancy. As a result, a total 26 27 191 of 962 women fulfilled the inclusion criteria (Figure S1, Table 1). 28 29 30 192 At least one biopsy was taken or imminent LLETZ made in 625 (96.6%) women in the EIS cohort and 952 31 32 193 (99.0%) in the reference cohort. Only one biopsy was taken from one quarter of women 165 (25.5%) in the 33 34 194 EIS cohort and among 109 (11.3%) in the reference cohort, whereas twenty-two (3.4%) women in the EIS 35 36 195 cohort and 10 (1.0%) in the reference cohort had no biopsy. The average number of biopsies was 1.8 if at 37 38 ₃₉ 196 least one biopsy was taken in the EIS cohort and 2.3 in the reference cohort (Table 1). 40 41 42 197 Altogether 222 (34.3%) women in the EIS cohort had CIN2+, including 5 (0.8%) cervical carcinomas and 14 43 44 198 (2.2%) adenocarcinoma in situ cases. In the reference cohort 391 (40.6%) women had CIN2+, including 7 45 46 199 (0.7%) cervical carcinomas and 15 (1.6%) adenocarcinoma in situ cases. (Table 1). The prevalence of CIN2+ 47 48 200 was higher in the reference cohort among those referred for LSIL or ASC-H cytology, whereas the 49 50 201 prevalence of CIN2+ was higher in the EIS cohort after ASC-US and HSIL referral cytology (Table 2, Table S1). 51 52 53 202 In the EIS cohort the overall sensitivity to detect CIN2+ was 94% (95% CI 90-97%) with corresponding 54 55 ₅₆ 203 specificity of 34% (95% Cl 29-39%) (Table 2, Table S1). The sensitivity varied according to referral cytology, 57 58 204 being the lowest, 77%, for LSIL cytology (95% CI 61-89%) and the highest for HSIL cytology with 100% 59 ⁶⁰ 205 sensitivity (95% CI 95-100%) (Table 2, Table S1). The specificity was lowest for HSIL cytology, 6% (95% CI 0

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Table 1. Characteristics of the electrical impedance spectroscopy (EIS) cohort and the reference cohort.

	EIS co	hort	Reference cohort		
	n=647	%	n=962	%	
Mean age	35.7		35.4		
Std.dev., Range	9.3 (20.3-76	.4)	9.6 (19.2-67	7.8)	
Age					
<30 y	175	27.1	295	30.7	
30-44 y	366	56.6	495	51.5	
≥45 y	106	16.4	172	17.9	
	647	100.0	962	100.0	
Referral cervical cyt	ology stratified	by age			
ASC-US	20		12	4 5	
<30 y	28	4.3	43	4.5	
30-44 y	52	8.0	28	2.9	
≥45 y LSIL	14	2.2	28	2.9	
LSIL <30 γ	20	6.0	79	0.7	
30-44 y	39 153	6.0 23.6	224	8.2 23.3	
≥45 v	44	6.8	78	23.5 8.1	
ASC-H	44	0.8	78	0.1	
<30 y	72	11.1	90	9.4	
30-44 y	90	13.9	120	12.5	
≥45 y	30	4.6	27	2.8	
HSIL	50			2.0	
<30 y	31	4.8	75	7.8	
30-44 y	54	8.3	102	10.6	
≥45 y	9	1.4	23	2.4	
AGC-NOS					
<30 y	5	0.8	5	0.5	
30-44 y	15	2.3	12	1.2	
≥45 y	8	1.2	11	1.1	
AGC-FN					
<30 y	0	0.0	3	0.3	
30-44 y	2	0.3	9	0.9	
≥45 y	1	0.2	5	0.5	
	647	100.0	962	100.0	
TZ type					
TZ type 1	446	68.9	620	64.4	
TZ type 2	201	31.1	342	35.6	
	647	100.0	962	100.0	
Biopsies and LLETZ					
No biopsy	22	3.4	10	1.0	
1 biopsy	165	25.5	109	11.3	
2 biopsies	263	40.6	420	43.7	
3 biopsies	83	12.8	257	26.7	
4 biopsies	1	0.2	43	4.5	
5 biopsies	0	0.0	5	0.5	
LLETZ	113	17.5	118	12.3	
	647	100.0	962	100.0	
Histology					
No biopsy	22	3.4	10	1.0	
Normal histology	222	34.3	247	25.7	
CIN1 (LSIL)	181	28.0	312	32.4	
CIN2 (HSIL)	95	14.7	210	21.8	
CIN3 (HSIL)	107	16.5	154	16.0	
Glandular atypia	1	0.2	7	0.7	
AIS	14	2.2	15	1.6	
Adenocarcinoma	3 2	0.5	3	0.3 0.4	
Sq. cell carcinoma	2 647	0.3 100.0	4 962	0.4 100.0	
Std. dev.: standard de			962 ance spectrosco		

Std. dev.: standard deviation; EIS: electrical impedance spectroscopy; CIN: cervical intraepithelial neoplasia; TZ: transformation zone; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells that cannot exclude HSIL; AGC-NOS: atypical glandular cells not otherwise specified; AGC-FN: atypical glandular cells that favor neoplasia; AIS: adenocarcinoma in situ; sq. cell carcinoma: squamous cell carcinoma; LLETZ: large loop excision of the transformation zone

3 212 Table 2. Sensitivity, specificity of the electrical impedance spectroscopy (EIS) cohort and the reference cohort for the detection of CIN2+ lesions by cervical cytology, TZ type and age group.

5				•					
5			EIS cohor	t (n=647)			R	efence cohort	(n=962)
			Colpo+ZS				Colpo		
		CIN2+/n	CIN2+1	Sensitivity	Specificity	CIN2+/n	CIN2+ ²	Sensitivity	Specificity
	ASC-US	16/94	15	94(70-100)	47(36-59)	9/99	5	56(21-86)	97(91-99)
	LSIL	39/236	30	77(61-89)	42(35-49)	72/381	31	43(31-55)	92(89-95)
0	ASC-H	85/192	84	99(94-100)	11(6-19)	138/237	87	63(54-71)	65(54-74)
1	HSIL	77/94	77	100(95-100)	6(0-29)	154/200	133	86(80-91)	46(31-61)
2	AGC-NOS	3/28	2	67(9-99)	44(24-65)	5/28	3	60(15-95)	96(78-100)
3	AGC-FN	2/3	1	50(1-99)	100(3-100)	13/17	8	62(32-86)	25(1-81)
4	ALL	222/647	209	94(90-97)	34(29-39)	391/962	267	68(63-73)	84(81-87)
5	TZ1	156/446	146	94(89-97)	31(26-37)	279/620	187	67(61-73)	84(80-88)
6	TZ2	66/201	63	96(87-99)	40(32-49)	112/342	80	71(62-80)	84(79-88)
7					- ()			()	- ()
8	<30 y	60/175	56	93(84-98)	35(26-44)	134/295	96	72(63-79)	77(70-83)
-)	30-44 y	131/366	124	95(89-98)	33(27-40)	211/495	144	68(62-75)	86(81-90)
)	>45 y	31/106	29	94(79-99)	35(24-47)	46/172	27	59(43-73)	89(82-94)
1	HG cytology	164/289	162	99(96-100)	11(6-18)	305/454	228	75(70-80)	58(49-66)
2	LG cytology	58/358	47	81(69-90)	43(38-49)	86/508	39	45(35-57)	93(91-96)
3									
4	ASC-H, HSIL	162/286	161	99(97-100)	11(6-17)	292/437	220	75(70-80)	59(50-67)
5	ASC-US,LSIL	55/330	45	82(69-91)	43(37-49)	81/480	36	44(33-56)	93(90-96)
5	Glandular	5/31	3	60(15-95)	46(27-67)	18/45	11	61(36-83)	85(66-96)
7	1 biopsy	11/165	7	64(31-89)	51(43-59)	14/109	5	36(13-65)	99(94-100)
8	2 biopsies	78/263	70	90(81-96)	23(17-30)	112/420	66	59(49-68)	90(86-93)
9	≥3 biopsies	43/84	43	100	0	168/305	113	67(60-74)	67(59-75)
0	LLETZ	90/113	89	99(94-100)	4(0-22)	97/118	83	86(77-92)	38(18-62)
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LLETZ 90/113 89 99(94-100) 4(0-22) 97/118 83 86(77-92) 38(18-62)
 LLETZ 90/113 89 99(94-100) 4(0-22) 97/118 83 86(77-92) 38(18-62)
 EIS: electrical impedance spectroscopy; CIN: cervical intraepithelial neoplasia; TZ: transformation zone; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells that cannot exclude HSIL; AGC-NOS: atypical glandular cells not otherwise specified; AGC-FN: atypical glandular cells that favor

neoplasia; LLETZ: large loop excision of the transformation zone; HG high grade; LG: low grade

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 ¹Colposcopic impression and/or ZedScan result of CIN2+ of histologically confirmed CIN2+ cases.
 ²Colposcopic impression of CIN2+ of histologically confirmed CIN2+ cases.

26
29%), and highest for ASC-US, 47% (95% CI 36-59%). EIS missed 3 low-grade referral cases of CIN2+

³⁹ 221 identified by the colposcopist (two cases if CIN2 and one CIN3). Colposcopic impression was less than CIN2

⁴¹ 222 in 43 CIN2+ cases that were detected by ZedScan. A total of 13 cases (5.9%) of CIN2+ were missed by both

⁴³ ₄₄ 223 ZedScan and the colposcopist (biopsies were still taken due to suspicion of low-grade lesion), including two

46 224 adenocarcinoma in situ cases and eleven high-grade lesions (nine CIN2 and two CIN3 cases).

49 225 In the reference cohort, the overall sensitivity to detect CIN2+ was 68% (95% CI 63-73%) with

51 226 corresponding specificity of 84% (95% CI 81-87%) (Table 2, Table S1). The sensitivity to detect CIN2+ by

⁵³ 227 colposcopic impression of CIN2+ was the lowest after LSIL cytology, 43%, and the highest after HSIL 54

⁵⁵₅₆ 228 cytology, 86% (Figure 1, Table 2, Table S1). Overall, the colposcopic impression was less than CIN2+ in

57
 58 229 31.7% (124/391) of CIN2+ cases and biopsies were taken due to suspicion of a low-grade lesion. Results

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230 stratified according to TZ type, age, and referral cytology are presented in Table S2. There was no obvious

231 impact of age on specificity or sensitivity within different cytologies (Table S2).

Compared to the referral cohort, the sensitivity to detect CIN2+ was higher in the EIS cohort overall, with
 risk ratio (RR) of 1.38 (95% CI 1.28-1.49), and after LSIL, ASC-H and HSIL referral cervical cytologies (Table 3,
 Table S3). TZ 1 and taking two or more biopsies were associated with higher observed sensitivity (Table 3,
 Table S3). Specificity was correspondingly lower in the EIS cohort overall as well as when stratified
 according to referral cytology (Table 3, Table S3).

Table 3. Sensitivity and specificity of the electrical impedance spectroscopy cohort (EIS) and the reference cohort by cytology, TZ type and age
 group in identifying CIN2+, with corresponding risk ratios (RR) of sensitivity and specificity.

	EIS	Reference	Sensitivity		EIS	Reference	Specificity	
	Sensitivity	Sensitivity	RR (95%) ¹	p.	Specificity	Specificity	RR (95%) ¹	p.
ASC-US	94(70-100)	56(21-86)	1.69(0.93-3.07)	0.0219	47(36-59)	97(91-99)	0.49(0.39-0.62)	<0.000
LSIL	77(61-89)	43(31-55)	1.79(1.30-2.45)	0.0006	42(35-49)	92(89-95)	0.45(0.38-0.53)	<0.000
ASC-H	99(94-100)	63(54-71)	1.57(1.38-1.78)	<0.0001	11(6-19)	65(54-74)	0.17(0.10-0.30)	<0.000
HSIL	100(95-100)	86(80-91)	1.16(1.09-1.23)	0.0007	6(0-29)	46(31-61)	0.13(0.02-0.89)	0.003
AGC-NOS	67(9-99)	60(15-95)	1.11(0.38-3.25)	0.8504	44(24-65)	96(78-100)	0.46(0.29-0.72)	0.000
AGC-FN	50(1-99)	62(32-86)	0.81(0.19-3.47)	0.7565	100(3-100)	25(1-81)	4.0(0.73-21.84)	0.170
ALL	94(90-97)	68(63-73)	1.38(1.28-1.49)	<0.0001	34(29-39)	84(81-87)	0.40(0.35-0.46)	<0.00
TZ1	94(89-97)	67(61-73)	1.40(1.27-1.53)	<0.0001	31(26-37)	84(80-88)	0.37(0.31-0.44)	<0.00
TZ2	96(87-99)	71(62-80)	1.34(1.18-1.57)	0.0001	40(32-49)	84(79-88)	0.48(0.38-0.59)	<0.00
<30 y	93(84-98)	72(63-79)	1.30(1.15-1.48)	0.0007	35(26-44)	77(70-83)	0.45(0.35-0.59)	<0.00
30-44 y	95(89-98)	68(62-75)	1.39(1.25-1.53)	<0.0001	33(27-40)	86(81-90)	0.39(0.32-0.47)	<0.00
≥45 y	94(79-99)	59(43-73)	1.59(1.23-2.07)	0.0008	35(24-47)	89(82-94)	0.39(0.28-0.54)	<0.00
HG cytology	99(96-100)	75(70-80)	1.32(1.24-1.41)	<0.0001	11(6-18)	58(49-66)	0.19(0.12-0.32)	<0.00
LG cytology	81(69-90)	45(35-57)	1.79(1.37-2.33)	<0.0001	43(38-49)	93(91-96)	0.46(0.41-0.53)	<0.00
1 biopsy	64(31-89)	36(13-65)	1.78(0.77-4.10)	0.1654	51(43-59)	99(94-100)	0.51(0.44-0.60)	<0.00
2 biopsies	90(81-96)	59(49-68)	1.52(1.28-1.81)	<0.0001	23(17-30)	90(86-93)	0.26(0.20-0.34)	<0.00
≥3 biopsies	100	67(60-74)	1.49(1.34-1.65)	< 0.0001	0	67(59-75)	0	<0.00

EIS: electrical impedance spectroscopy; CIN: cervical intraepithelial neoplasia; TZ: transformation zone; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells that cannot exclude HSIL; AGC-NOS: atypical glandular cells not otherwise specified; AGC-FN: atypical glandular cells that favor neoplasia; HG: high grade; LG: low grade

54 243 ¹The values of risk ratio >1 imply better/improved effect with ZedScan

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244In the EIS cohort, colposcopic impression of high-grade disease (CIN2+) was present with EIS indicating the245presence of CIN2+ in 73.4% of all histologically confirmed CIN2+ cases. In the sensitivity analysis within the246EIS cohort, colposcopy alone was indicative for the presence of CIN2+ in 166 of 222 CIN2+ cases (74.8%)247and ZedScan in 206 of 222 (92.8%) of CIN2+ cases, suggesting an additional 40 cases (24.1%) detected by248ZedScan only. The additional cases increased the detection of CIN2+ from 30 to 44 in women with low-249grade cytology and from 136 to 162 in women with high-grade cytology (Figure 1). The sensitivity to detect250CIN2+ by colposcopy alone according to referral cytology was otherwise similar between the cohorts,251except for women with ASC-H cervical cytology the colposcopy alone in the EIS cohort seemed to detect252more CIN2+ cases (p=0.02) (Figure 1). Among colposcopists who performed colposcopies in both cohorts,253the average number of biopsies by cytology were higher in all cytology groups in the reference cohort254compared to the EIS cohort. The average number of biopsies varied between 1.7-2.3 in the EIS cohort and255between 2.2-2.8 in the reference cohort (Table S4).256Discussion257We compared the performance of colposcopy in detecting CIN2+ according to referral cervical cytology

with and without EIS as an adjunctive technology. Colposcopy combined with EIS seemed to have a higher sensitivity, but a lower specificity compared to conventional colposcopy, regardless of the referral cervical cytology. The prevalence of CIN2+ lesions was higher in the EIS cohort after ASC-US and HSIL referral, but lower after LSIL and ASC-H cervical cytology. The average number of biopsies was lower in the EIS cohort.

Overall, EIS performed well with a high sensitivity (94%) but had a low specificity (34%) consistent with the previous studies.[13, 14, 16] Here, the sensitivity might have been overestimated in both cohorts as the true positive result was based on histology data at first visit only and lesions missed at first visit and detected during the follow-up were not included in either cohort. Still, this would not affect the estimates of relative performance. The sensitivity (68%) and specificity (84%) of colposcopy in the reference cohort was as well in line with existing data.[5, 7, 23]

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The increased detection of CIN2+ cases by EIS has been reported as most pronounced in women with lowgrade cytology [13, 14, 16, 17] or with high-risk HPV positivity without cytological changes. [16, 18] In our study, additional cases of CIN2+ detected by EIS were also most frequent among low-grade referrals. Furthermore, the sensitivity to detect CIN2+ with EIS was higher in most cervical cytology groups (ASC-US, LSIL, ASC-H, HSIL) compared to colposcopy alone. Only within HSIL cytology EIS combined with colposcopy detected all CIN2+ cases. In women with other referral cytology (ASC-US, LSIL, ASC-H) there were cases of CIN2+ that EIS did not detect, but where biopsy of CIN2+ was warranted based on colposcopic diagnosis. Nevertheless, missed cases of CIN2+ were even more frequent in the reference cohort, where more CIN2+ lesions were detected in biopsies with colposcopic impression of CIN1 or lower. Contrary to expectations, the prevalence of CIN2+ was higher in the EIS cohort only after ASC-US and HSIL referral cytology. One explanation for lower prevalence of CIN2+ lesions in the EIS cohort after LSIL and ASC-H cytology could be that routine practice in Finland is to take biopsies also from low-grade lesions, rather than to abstain from taking biopsies when CIN2+ lesions are not colposcopically suspected. Biopsies even from mild acetowhite lesions are important in excluding a high-grade disease as the sensitivity of colposcopy to detect CIN2+ is far from 100%. Such biopsies could well have been more frequent without than with EIS as an additional confirmation on suspected absence of CIN2+. This is supported by the observation that two or more biopsies were taken from 54% of women in the EIS cohort, whereas up to 75% of women in the reference cohort had at least two biopsies. In addition, the average number of biopsies by cytology among colposcopists who performed colposcopies in both cohorts were constantly higher in the reference cohort compared to the EIS cohort reflecting a change in manner/threshold to take biopsies when ZedScan was used as an adjunct technology. Multiple biopsies are known to increase the sensitivity of colposcopy as at least small lesions can easily be missed. [24, 25] In women with low-grade referral cervical cytology, a single biopsy has shown to be insufficient to rule out a high-grade disease.[26] A British survey has also reported experienced colposcopists to take mostly two biopsies in diagnosing high-grade disease.[27] A Danish study found taking four biopsies to increases the detection rate of cervical dysplasia to 95.2%.[28] The average

number of biopsies in the EIS cohort was higher (1.84) compared to previous reports (1.07 and 1.51),[13,
14] but still lower than in the reference cohort (2.3).

296 Our observation of overall fewer biopsies along with fewer CIN2+ lesions detected in the EIS cohort can 297 either indicate a true difference in CIN2+ prevalence between the cohorts, selection bias towards using EIS preferably on patients in whom CIN2+ lesion is not clearly present, or that CIN2+ lesions could have been missed in the EIS cohort, especially after LSIL and ASC-H referral cytology. If lesions were missed, it could 300 possibly be due to a higher biopsy threshold in the EIS cohort, as indicated by lower number of biopsies. 301 Without longitudinal data we still cannot be certain whether prevalent CIN2+ cases were indeed more frequently missed at the first visit in the EIS cohort. The prevalence of CIN2+ in EIS cohort in women with high-grade cytology (ASC-H and HSIL) is below previous observations (56.7% vs. 79.1-84.0%).[13, 16] However, when restricted to only women with HSIL referral cervical cytology or low-grade (ASC-US and 305 LSIL) cytology, the prevalence for CIN2+ here did not differ from previous reports.[13, 16] Cytological diagnoses may well vary between cytopathologists as well as between countries and this possible difference in classification might also explain the observed difference in CIN2+ prevalence, especially after ASC-H cytology.[29] The longitudinal data on EIS results are scarce. In women referred with low-grade 309 cytology, the future risk of CIN2+ was increased in up to 36 months follow-up if both colposcopic impression and EIS results were indicative for CIN2+ compared with women with other combinations of these two parameters, suggesting that EIS might provide new information on the future risk of high-grade disease.[30]

7 313 Strengths and limitations

Most previous studies have compared the performance of EIS as an adjunctive technology for colposcopy against conventional colposcopy within the cohort where EIS was used, even though in clinical setting EIS is not a truly independent measurement from colposcopy. To our knowledge this is the first report on the performance of EIS as an adjunctive technology for colposcopy stratified according to referral cytology and compared to an external reference cohort. Even though our cohorts were collected at different time compared to an external reference cohort. Even though our cohorts were collected at different time

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periods, they both represent women in the same catchment area referred to colposcopy due to abnormal
cervical cytology. All colposcopies were performed in the same clinic by experienced colposcopists.
Furthermore, none of the authors of this work have financial conflicts of interest with the technology
studied. Our study also has some limitations. It is not possible to rule out that there would not have been
any variation in sensitivity or specificity between the cohorts in different time periods. EIS device is not
truly independent of colposcpic skills and the colposcopic performance can vary depending on the
colposcopist. Also, the referral cytology and the colposcopic impression are incorporated in the EIS analysis
by ZedScan. In order to take into account the variation of colposcopic performance and reliance on EIS
device we collected a large cohort representing routine work. Including colposcopic examinations by
several different colposcopists represents a real-life situation which could be considered as a strength
compared to studies where all colposcopies have been performed by a single colposcopist.
When the cervical transformation zone is not fully visible, TZ type 3, ZedScan technology cannot be reliably
applied and the results are not applicable to this population. CIN2+ lesions could well have been missed in
both cohorts since the results are based on data collected on the initial visit. EIS might miss some lesions

that either could have been detected with lower biopsy threshold or where biopsy would not have been
indicated even in conventional colposcopy. However, complete certainty of the histology would have

required LLETZ for all participants which would not have been ethically just.

36 Conclusions

⁵G 337 Colposcopy with EIS has a higher sensitivity and a lower specificity in identifying CIN2+ compared to
 ⁷G 338 conventional colposcopy, irrespective of cervical cytology. EIS can, therefore, be assumed to be of clinical
 ⁹benefit in colposcopy, particularly in women with low-grade cervical cytology where the prevalence of
 ¹CIN2+ is low. We also observed an overall lower prevalence of CIN2+ lesions in the EIS cohort compared to
 ⁴a 341 a reference cohort with conventional colposcopy. The performance of EIS as an adjunctive technology for
 ⁶colposcopy has not been previously compared by cytology to an external reference cohort. While the
 ⁸g 343 observation of lower CIN2+ rate could be explained by different CIN2+ prevalence between the cohorts or

2 3 344 4	selection bias, the finding is important and warrants further research, especially along with the observed
5 345 6	lower number of biopsies in the EIS cohort. Adjunctive technologies are likely to become increasingly
7 8 346 9	appealing in colposcopy, as the prevalence of high-grade cervical lesions is declining. Randomised
10 347 11	controlled trials comparing EIS with a conventional colposcopy, including women referred due to persistent
12 348 13	HPV infection without cytological changes are warranted. Before such further evidence, firm
14 349 15 16	recommendations on applicability of EIS as an adjunctive technology for colposcopy cannot be made.
¹⁷ 350 18	Figure 1. Numbers and rates of CIN2+ lesions detected in the electrical impedance spectroscopy cohort
19 20 351 21	(EIS) and in the reference cohort according to referral cytology. (A) Numbers and rates of CIN2+ detected
21 22 352 23	by ZedScan alone and reference cohort stratified according to referral cytology (B) Numbers and rates of
24 353 25	CIN2+ detected by colposcopy alone in EIS and reference cohorts stratified according to referral cytology.
26 354 27 28	Numbers of patients are given in the columns.
29 355 30	Contributors
31 32 356 33	PN and IK were responsible for the conceptualisation and design of the study as well as methodology. XC,
³⁴ 357 35	KL, LKT contributed to conceptualisation. LB performed the statistical analysis with the aid of IK. LB, PN,
36 37 358	MK, PLO, SV and AH were responsible for data collection. LB drafted the original manuscript and IK, PN, CR,
38 39 359 40	XC, KL, LKT, AH and KA participated in writing, reviewing and editing. All authors listed qualify for
41 360 42	authorship and approved the final version of the paper.
43 44 361 45 46	authorship and approved the final version of the paper. Funding
47 48 48	This work was supported by the Academy of Finland (IK), the Finnish Medical Foundation (IK), and Finnish
49 50 ³⁶³ 51	State Research Funding (IK).
52 53 364 54 55	Competing interests None declared.
56 365 57 58 59 60	Patient consent for publication Not required.

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2 3 4	366	Ethics approval This study and data collection on patients where EIS was used was considered as a service
5 6	367	evaluation and therefore a separate ethical approval was not required as per consultation with Helsinki-
7 8 9	368	Uusimaa Hospital District Ethical Committee. For the historical reference cohort an ethical approval was
	369	received from Helsinki-Uusimaa Hospital District Ethical Committee (ref. no. 130/13/03/03/2013).
12 13	370	Data availability statement Data are available upon reasonable request.
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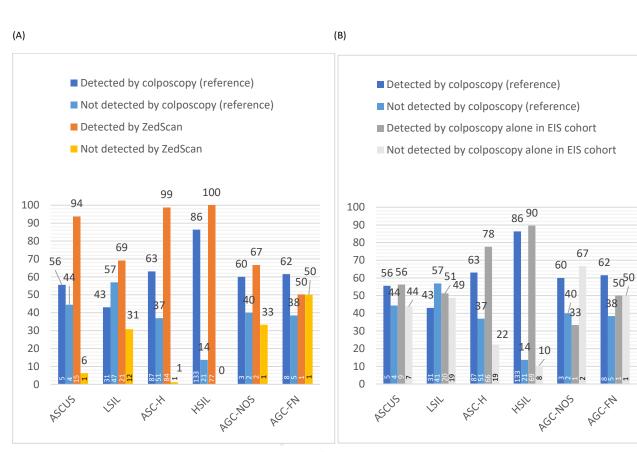
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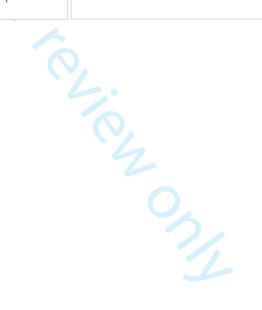
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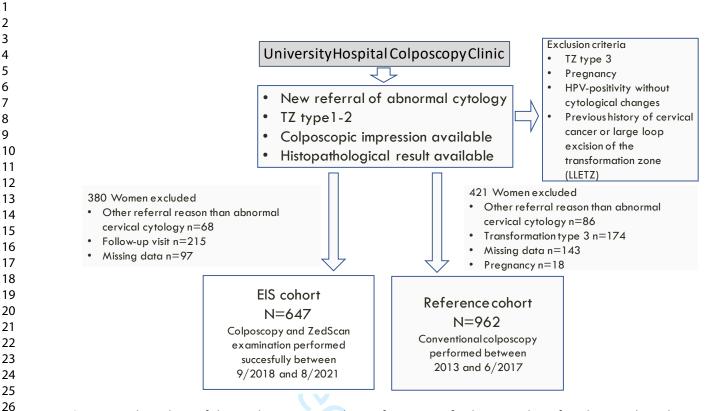


Figure S1. Flow-chart of the study comparing the performance of colposcopy by referral cervical cytology in two cohorts with and without electrical impedance spectroscopy as an adjunctive technology.

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Table S1. Sensitivity, specificity, positive and negative predictive value of the electrical impedance spectroscopy (EIS) cohort and the reference cohort for the detection of CIN2+ lesions within

different cervical cytology by TZ type and age group.

				EIS co	hort (n=647	7)						Reference	ce cohort	(n=962)		
		Colpo+ZS ¹			Colpo+ZS ³					Colpo ²			Colpo ⁴			
	CIN2+/n	CIN2+	Sensitivity	<cin n<="" th=""><th><cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th><th>CIN2+/n</th><th>CIN2+</th><th>Sensitivity</th><th><cin2 n<="" th=""><th><cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th></cin2<></th></cin2></th></cin2<></th></cin>	<cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th><th>CIN2+/n</th><th>CIN2+</th><th>Sensitivity</th><th><cin2 n<="" th=""><th><cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th></cin2<></th></cin2></th></cin2<>	Specificity	PPV	NPV	CIN2+/n	CIN2+	Sensitivity	<cin2 n<="" th=""><th><cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th></cin2<></th></cin2>	<cin2< th=""><th>Specificity</th><th>PPV</th><th>NPV</th></cin2<>	Specificity	PPV	NPV
ASC-US	16/94	15	94(70-100)	78/94	37	47(36-59)	27(16-40)	97(86-100)	9/99	5	56(21-86)	90/99	87	97(91-99)	63(25-92)	96(89-99
TZ1	11/66	10	91(59-100)	55/66	24	44(30-58)	24(12-40)	96(80-100)	7/57	3	43(10-82)	50/57	47	94(84-99)	50(12-88)	92(81-98
TZ2	5/28	5	100(48-100)	23/28	13	57(35-77)	33(12-62)	100(75-100)	2/42	2	100(16-100)	40/42	40	100(91-100)	100(16-100)	100(91-10
<30 y	6/28	5	83(36-100)	22/28	12	55(32-76)	33(12-62)	92(64-100)	1/43	0	0(0-98)	42/43	39	93(81-99)	0(0-71)	98(87-10
30-44 y	10/52	10	100(69-100)	42/52	21	50(34-66)	32(17-51)	100(84-100)	7/28	4	57(18-90)	21/28	21	100(84-100)	100(40-100)	88(68-97
>45 y	0/14	0	0	14/14	4	29	0	100	1/28	1	100(3-100)	27/28	27	100(87-100)	100(3-100)	100(87-10
LSIL	39/236	30	77(61-89)	197/236	82	42(35-49)	21(14-28)	90(82-95)	72/381	31	43(31-55)	309/381	285	92(89-95)	56(42-70)	87(83-91
TZ1	26/157	20	77(56-91)	131/157	53	41(32-49)	20(13-30)	90(79-96)	53/235	23	43(30-58)	182/235	169	93(88-96)	64(46-79)	85(79-90
TZ2	13/79	10	77(46-95)	66/79	29	44(32-57)	21(11-36)	91(75-98)	19/146	8	42(20-67)	127/146	116	91(85-96)	42(20-67)	91(85-96
<30 y	4/39	1	25(1-81)	35/39	21	60(42-76)	7(0-32)	88(68-97)	17/79	9	53(28-77)	62/79	54	87(76-94)	53(28-77)	87(76-94
30-44 y	28/153	24	86(67-96)	125/153	46	37(28-46)	23(16-33)	92(81-98)	46/224	19	41(27-57)	178/224	166	93(89-97)	61(42-78)	86(80-91
>45 y	7/44	5	71(29-96)	37/44	15	41(25-58)	19(6-38)	88(64-99)	9/78	3	33(8-70)	69/78	65	94(86-98)	43(10-82)	92(83-97
ASC-H	85/192	84	99(94-100)	107/192	12	11(6-19)	47(39-55)	92(64-100)	138/237	87	63(54-71)	99/237	64	65(54-74)	71(62-79)	56(46-65
TZ1	57/134	56	98(91-100)	77/134	6	8(3-16)	44(35-53)	86(42-100)	100/167	64	64(54-73)	67/167	42	63(50-74)	72(61-81)	54(42-65
TZ2	28/58	28	100(88-100)	30/58	6	20(8-39)	54(40-68)	100(54-100)	38/70	23	61(43-76)	32/70	22	69(50-84)	70(51-84)	60(42-7
<30 y	24/72	24	100(86-100)	48/72	5	10(4-23)	36(25-49)	100(48-100)	57/90	34	60(46-72)	33/90	18	55(36-72)	69(55-82)	44(29-60
30-44 y	46/90	45	98(89-100)	44/90	4	9(3-22)	53(42-64)	80(28-100)	67/120	45	67(55-78)	53/120	37	70(56-82)	74(61-84)	63(49-75
>45 y	15/30	15	100(78-100)	15/30	3	20(4-48)	56(35-75)	100(29-100)	14/27	8	57(29-82)	13/27	9	69(39-91)	67(35-90)	60(32-84
HSIL	77/94	77	100(95-100)	17/94	1	6(0-29)	83(74-90)	100(3-100)	154/200	133	86(80-91)	46/200	21	46(31-61)	84(78-90)	50(34-6
TZ1	58/67	58	100	9/67	0	0	87	0	104/131	88	85(76-91)	27/131	15	56(35-75)	88(80-94)	48(30-6
TZ2	19/27	19	100(82-100)	8/27	1	13(0-53)	73(52-88)	100(3-100)	50/69	45	90(78-97)	19/69	6	32(13-57)	78(65-88)	55(23-8
<30 y	25/31	25	100	6/31	0	0	81	0	54/75	48	89(77-96)	21/75	10	48(26-70)	81(69-90)	63(35-8
30-44 y	45/54	45	100(92-100)	9/54	1	11(0-48)	85(72-93)	100(3-100)	84/102	72	86(76-92)	18/102	9	50(26-74)	89(80-95)	43(22-6
>45 y	7/9	7	100	2/9	0	0	78	0	16/23	13	81(54-96)	7/23	2	29(4-71)	72(47-90)	40(5-85

EIS: electrical impedance spectroscopy; CIN: cervical intraepithelial neoplasia; TZ: transformation zone; PPV: positive predictive value; NPV: negative predictive value; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells that cannot exclude HSIL; AGC-NOS: atypical glandular cells not otherwise specified; AGC-FN: atypical glandular cells that favor neoplasia; LLETZ: large loop excision of the transformation zone; HG: high grade; LG: low grade

¹Colposcopic impression and/or ZedScan result of CIN2+ of histologically confirmed CIN2+ cases.

²Colposcopic impression of CIN2+ of histologically confirmed CIN2+ cases.

³Colposcopic impression and ZedScan result less than CIN2 of histologically confirmed cases <CIN2.

⁴Colposcopic impression less than CIN2 of histologically confirmed cases <CIN2

ASC-US

LSIL

ASC-H

HSIL

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Table S2. Average number of biopsies by cytology in the electrical impedance spectroscopy (EIS) cohort and in the reference cohort.

ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous

Reference

cohort

2.3

2.2

2.7

2.8

intraepithelial lesion; ASC-H: atypical squamous cells that cannot exclude HSIL

Average number of biopsies

EIS cohort

1.7

1.8

2.0

2.3

Table S3. Sensitivity, specificity, negative and positive predictive value of the electrical impedance spectroscopy (EIS) cohort and the reference cohort for the detection of CIN2+ lesions by cervical cytology, TZ type and age group.

					EIS cohort	(n=647)			<u> </u>				Ref	erence coh	ort (n=96	52)		
			Colpo+ZS			Colpo +ZS						Colpo			Colpo			
	CIN2+/n	CIN2%	CIN2+1	Sensitivity	<cin2 n<="" th=""><th><cin2<sup>3</cin2<sup></th><th>Specificity</th><th>PPV</th><th>NPV</th><th>CIN2+/n</th><th>CIN2%</th><th>CIN2+²</th><th>Sensitivity</th><th><cin2 n<="" th=""><th><cin2<sup>4</cin2<sup></th><th>Specificity</th><th>PPV</th><th>NPV</th></cin2></th></cin2>	<cin2<sup>3</cin2<sup>	Specificity	PPV	NPV	CIN2+/n	CIN2%	CIN2+ ²	Sensitivity	<cin2 n<="" th=""><th><cin2<sup>4</cin2<sup></th><th>Specificity</th><th>PPV</th><th>NPV</th></cin2>	<cin2<sup>4</cin2<sup>	Specificity	PPV	NPV
ASC-US	16/94	17.0	15	94(70-100)	78/94	37	47(36-59)	27(16-40)	97(86-100)	9/99	9.1	5	56(21-86)	90/99	87	97(91-99)	63(25-92)	96(89-
LSIL	39/236	16.5	30	77(61-89)	197/236	82	42(35-49)	21(14-28)	90(82-95)	72/381	18.9	31	43(31-55)	309/381	285	92(89-95)	56(42-70)	87(83-
ASC-H	85/192	44.3	84	99(94-100)	107/192	12	11(6-19)	47(39-55)	92(64-100)	138/237	58.2	87	63(54-71)	99/237	64	65(54-74)	71(62-79)	56(46-
HSIL	77/94	81.9	77	100(95-100)	17/94	1	6(0-29)	83(74-90)	100(3-100)	154/200	77.0	133	86(80-91)	46/200	21	46(31-61)	84(78-90)	50(34-
AGC-NOS	3/28	10.7	2	67(9-99)	25/28	11	44(24-65)	13(2-38)	92(62-100)	5/28	17.9	3	60(15-95)	23/28	22	96(78-100)	75(19-99)	92(73-
AGC-FN	2/3	66.7	1	50(1-99)	1/3	1	100(3-100)	100(3-100)	50(1-99)	13/17	76.5	8	62(32-86)	4/17	1	25(1-81)	73(39-94)	17(0-6
ALL	222/647	34.3	209	94(90-97)	425/647	144	34(29-39)	43(38-47)	92(86-96)	391/962	40.6	267	68(63-73)	571/962	480	84(81-87)	75(70-79)	80(76-
TZ1	156/446	35.0	146	94(89-97)	290/446	90	31(26-37)	42(37-48)	90(82-95)	279/620	45.0	187	67(61-73)	341/620	287	84(80-88)	78(72-83)	76(71-
TZ2	66/201	32.8	63	96(87-99)	135/201	54	40(32-49)	44(36-52)	95(85-99)	112/342	32.7	80	71(62-80)	230/342	193	84(79-88)	68(59-77)	86(81-
<30 y	60/175	34.3	56	93(84-98)	115/175	40	35(26-44)	43(34-52)	91(78-98)	134/295	45.4	96	72(63-79)	161/295	124	77(70-83)	72(64-80)	77(69-
30-44 y	131/366	35.8	124	95(89-98)	235/366	78	33(27-40)	44(38-50)	92(84-97)	211/495	42.6	144	68(62-75)	284/495	244	86(81-90)	78(72-84)	79(74
>45 y	31/106	29.2	29	94(79-99)	75/106	26	35(24-47)	37(27-49)	93(77-99)	46/172	26.7	27	59(43-73)	126/172	112	89(82-94)	66(49-80)	86(78-
HG cytology	164/289	56.7	162	99(96-100)	125/289	14	11(6-18)	59(53-65)	88(62-98)	305/454	67.2	228	75(70-80)	149/454	86	58(49-66)	78(73-83)	53(45
LG cytology	58/358	16.2	47	81(69-90)	300/358	130	43(38-49)	22(16-28)	92(87-96)	86/508	16.9	39	45(35-57)	422/508	394	93(91-96)	58(46-70)	89(86
ASC-H, HSIL	162/286	56.6	161	99(97-100)	124/286	13	11(6-17)	59(53-65)	93(66-100)	292/437	66.8	220	75(70-80)	145/437	85	59(50-67)	79(73-83)	54(46
ASC-US,LSIL	55/330	16.7	45	82(69-91)	275/330	119	43(37-49)	22(17-29)	92(86-96)	81/480	16.9	36	44(33-56)	399/480	372	93(90-96)	57(44-70)	89(86
Glandular	5/31	16.1	3	60(15-95)	26/31	12	46(27-67)	18(4-43)	86(57-98)	18/45	40.0	11	61(36-83)	27/45	23	85(66-96)	73(45-92)	77(58
1 biopsy	11/165	6.7	7	64(31-89)	154/165	78	51(43-59)	8(4-17)	95(88-99)	14/109	12.8	5	36(13-65)	95/109	94	99(94-100)	83(36-100)	91(84
2 biopsies	78/263	29.7	70	90(81-96)	185/263	43	23(17-30)	33(27-40)	84(71-93)	112/420	26.7	66	59(49-68)	308/420	276	90(86-93)	67(57-77)	86(81
≥3 biopsies	43/84	51.2	43	100	41/84	0	0	51	0	168/305	55.1	113	67(60-74)	137/305	92	67(59-75)	72(64-78)	63(54
LLETZ	90/113	79.6	89	99(94-100)	23/113	1	4(0-22)	80(72-87)	50(1-99)	97/118	82.2	83	86(77-92)	21/118	8	38(18-62)	87(78-93)	36(17

EIS: electrical impedance spectroscopy; CIN: cervical intraepithelial neoplasia; TZ: transformation zone; PPV: positive predictive value; NPV: negative predictive value; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells that cannot exclude HSIL; AGC-NOS: atypical glandular cells not

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 ³Colposcopic impression and ZedScan result less than CIN2 of histologically confirmed cases <CIN2.

⁴Colposcopic impression less than CIN2 of histologically confirmed cases <CIN2

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Table S4. Sensitivity and specificity of the electrical impedance spectroscopy cohort (EIS) and the reference cohort by cytology, TZ type and age group in identifying CIN2+, with corresponding risk differences and the risk ratios of sensitivity and specificity.

	EIS	Reference	Sens	itivity		EIS	Reference	Speci	ficity	
	Sensitivity	Sensitivity	Risk difference (95%) ¹	RR (95%) ¹	p.	Specificity	Specificity	Risk difference (95%) ¹	RR (95%) ¹	p.
SC-US	94(70-100)	56(21-86)	0.38(0.04-0.73)	1.69(0.93-3.07)	0.0219	47(36-59)	97(91-99)	-0.49(-0.610.38)	0.49(0.39-0.62)	<0.0001
SIL	77(61-89)	43(31-55)	0.34(0.16-0.51)	1.79(1.30-2.45)	0.0006	42(35-49)	92(89-95)	-0.51(-0.580.43)	0.45(0.38-0.53)	<0.0001
ASC-H	99(94-100)	63(54-71)	0.36(0.27-0.44)	1.57(1.38-1.78)	<0.0001	11(6-19)	65(54-74)	-0.53(-0.650.42)	0.17(0.10-0.30)	<0.0001
ISIL	100(95-100)	86(80-91)	0.14(0.08-0.19)	1.16(1.09-1.23)	0.0007	6(0-29)	46(31-61)	-0.40(-0.580.22)	0.13(0.02-0.89)	0.0033
GC-NOS	67(9-99)	60(15-95)	0.07(-0.62-0.75)	1.11(0.38-3.25)	0.8504	44(24-65)	96(78-100)	-0.52(-0.730.30)	0.46(0.29-0.72)	0.0001
GC-FN	50(1-99)	62(32-86)	-0.12(-0.86-0.63)	0.81(0.19-3.47)	0.7565	100(3-100)	25(1-81)	0.75(0.33-1.17)	4.0(0.73-21.84)	0.1709
ALL	94(90-97)	68(63-73)	0.26(0.20-0.31)	1.38(1.28-1.49)	<0.0001	34(29-39)	84(81-87)	-0.50(-0.560.45)	0.40(0.35-0.46)	<0.0001
Z1	94(89-97)	67(61-73)	0.27(0.20-0.33)	1.40(1.27-1.53)	<0.0001	31(26-37)	84(80-88)	-0.53(-0.600.47)	0.37(0.31-0.44)	<0.0001
722	96(87-99)	71(62-80)	0.24(0.14-0.34)	1.34(1.18-1.57)	0.0001	40(32-49)	84(79-88)	-0.44(-0.530.34)	0.48(0.38-0.59)	<0.0001
:30 y	93(84-98)	72(63-79)	0.22(0.12-0.32)	1.30(1.15-1.48)	0.0007	35(26-44)	77(70-83)	-0.42(-0.530.31)	0.45(0.35-0.59)	<0.0001
0-44 y	95(89-98)	68(62-75)	0.26(0.19-0.34)	1.39(1.25-1.53)	<0.0001	33(27-40)	86(81-90)	-0.53(-0.600.45)	0.39(0.32-0.47)	<0.0001
:45 y	94(79-99)	59(43-73)	0.35(0.18-0.52)	1.59(1.23-2.07)	0.0008	35(24-47)	89(82-94)	-0.54(-0.660.42)	0.39(0.28-0.54)	<0.0001
IG cytology	99(96-100)	75(70-80)	0.24(0.19-0.29)	1.32(1.24-1.41)	<0.0001	11(6-18)	58(49-66)	-0.47(-0.560.37)	0.19(0.12-0.32)	<0.0001
G cytology	81(69-90)	45(35-57)	0.36(0.21-0.50)	1.79(1.37-2.33)	<0.0001	43(38-49)	93(91-96)	-0.50(-0.560.44)	0.46(0.41-0.53)	<0.0001
biopsy	64(31-89)	36(13-65)	0.28(-0.10-0.66)	1.78(0.77-4.10)	0.1654	51(43-59)	99(94-100)	-0.48(-0.560.40)	0.51(0.44-0.60)	<0.0001
biopsies	90(81-96)	59(49-68)	0.31(0.19-0.42)	1.52(1.28-1.81)	<0.0001	23(17-30)	90(86-93)	-0.66(-0.730.59)	0.26(0.20-0.34)	<0.0001
3 biopsies	100	67(60-74)	0.33(0.26-0.40)	1.49(1.34-1.65)	<0.0001	0	67(59-75)	-0.67(-0.750.59)	0	<0.0001

EIS: electrical impedance spectroscopy; CIN: cervical intraepithelial neoplasia; TZ: transformation zone; ASC-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells that cannot exclude HSIL; AGC-NOS: atypical glandular cells not otherwise specified; AGC-FN: atypical glandular cells that favor neoplasia; HG: high grade; LG: low grade

¹The values of risk difference >0 or the values of risk ratio >1 imply better/improved effect with ZedScan.

TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy	2
		(such as sensitivity, specificity, predictive values, or AUC)	
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions	2
		(for specific guidance, see STARD for Abstracts)	
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4-5
	4	Study objectives and hypotheses	4-5
METHODS			
Study design	5	Whether data collection was planned before the index test and reference standard	5-6
		were performed (prospective study) or after (retrospective study)	
Participants	6	Eligibility criteria	5-6
	7	On what basis potentially eligible participants were identified	5-6
		(such as symptoms, results from previous tests, inclusion in registry)	
	8	Where and when potentially eligible participants were identified (setting, location and dates)	5-6
	9	Whether participants formed a consecutive, random or convenience series	5-6
Test methods	10a	Index test, in sufficient detail to allow replication	6
	10b	Reference standard, in sufficient detail to allow replication	6
	11	Rationale for choosing the reference standard (if alternatives exist)	5-6
	12a	Definition of and rationale for test positivity cut-offs or result categories	7
		of the index test, distinguishing pre-specified from exploratory	
	12b	Definition of and rationale for test positivity cut-offs or result categories	7
		of the reference standard, distinguishing pre-specified from exploratory	
	1 3 a	Whether clinical information and reference standard results were available	6
		to the performers/readers of the index test	
	13b	Whether clinical information and index test results were available	5-6
		to the assessors of the reference standard	
Analysis	14	Methods for estimating or comparing measures of diagnostic accuracy	7
	15	How indeterminate index test or reference standard results were handled	5-6
	16	How missing data on the index test and reference standard were handled	5-6
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	7
	18	Intended sample size and how it was determined	5-6
RESULTS			
Participants	19	Flow of participants, using a diagram	Figure S1, submitted as a separate file
	20	Baseline demographic and clinical characteristics of participants	9, Table 1
	21a	Distribution of severity of disease in those with the target condition	9, Table 1
	21b	Distribution of alternative diagnoses in those without the target condition	9, Table 1
	22	Time interval and any clinical interventions between index test and reference standard	5-6
Test results	23	Cross tabulation of the index test results (or their distribution)	9,10,11 Table1-3
		by the results of the reference standard	Table S3-4
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	10,11 Table 2-3, Table S3-4
	25	Any adverse events from performing the index test or the reference standard	NA
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	13-16
	27	Implications for practice, including the intended use and clinical role of the index test	15-16

59 60



	28	Registration number and name of registry	6 ISRCTN for the reference cohort
	29	Where the full study protocol can be accessed	Submitted as a
			separate file
	30	Sources of funding and other support; role of funders	16
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STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on <u>http://www.equator-network.org/reporting-guidelines/stard.</u>

