Microcolposcopy in the diagnostic evaluation of abnormal cervical cytology: when and why to do it

A. Lukic[1], *M.D., Ph.D.*; S. Iannaccio[1], *M.D.*; M. Di Properzio[1], *M.D.*; E. Carico[2], *M.D.*; A. Camboni[3], *M.D.*; A. Vecchione[4], *M.D.*; M. Moscarini[1], *M.D.*

[1]Department of Gynecological Sciences, Perinatology and Child Health, [2]Cytopathology Unit, [3]Histopathology Unit, University of Rome "Sapienza", [4]G. Pascale National Cancer Institute, Naples (Italy)

Summary

Microcolposcopy is an *in vivo c*ytological examination of the uterine cervix allowing the localization of exoendocervical precancerous lesions. The aim of this study was to assess the diagnostic reliability of microcolposcopy by means of correlation with histology, colposcopy and Pap test results. For the study, 256 patients with abnormal Pap test results were selected and subjected to colposcopy and microcolposcopy with the aim of evaluating the presence of any intraepithelial lesions. One hundred and nine of these patients were subjected to a biopsy. Colposcopy, histology and cytology results were compared with those obtained by microcolposcopy. In low-grade squamous intraepithelial lesion (LSIL) cytology cases, the percentage agreement on lesion grade between Pap test and microcolposcopy results was 74%, while in high-grade squamous intraepithelial lesion (HSIL) cytology cases, it was equal to 80%. The comparison between colposcopy and microcolposcopy showed a level of agreement of 72% for lower grades and 68% for higher grades. Finally, histology was in agreement with microcolposcopy in 73% of cervical intraepithelial grade 1 neoplasia (CIN 1) cases and reached 71% for CIN 2-3. Microcolposcopy proved to be accurate with regard to the diagnosis of lesion grade, and showed to be definitive in patients where cytology was positive for HPV infection and colposcopy was not able to identify any lesions.

Key words: Pap test; Abnormal cervical cytology; Colposcopy; Microcolposcopy; Squamous intraepithelial lesions.

Introduction

Microcolposcopy is a rarely used diagnostic technique, introduced in 1981 by Hamou, who invented the microcolpohysteroscope [1]. This device allows magnification of up to 150 times for performing in vivo cytological examinations of the uterine cervix. It is possible to obtain a panoramic view to study tissue structure, and a contact microscopic view to evaluate individual cellular components. Lugol's solution (2%) and Waterman blue are used to stain the squamous epithelium during the examination [2].

Since the 1980s, various authors have conducted studies in which this method was compared with both histology and colposcopy results. In the literature, the reported microcolposcopy-histology level of agreement varies between 74.4% [3], and 88.5% [4], to even 93% [5]. In another study, the level of agreement was 78.7% although in 47.5% of cases, colposcopy was judged unsatisfactory and, of these, 51.7% were better studied using microcolposcopy [6]. In other studies, it has been claimed that in 46% of cases, colposcopy highlighted aceto-white areas that, when subjected to biopsy, showed no intraepithelial lesions [7]. It has also been reported that colposcopy failed to diagnose any lesions in 7.1% of patients with positive Pap test results [8].

Indeed, it is well known that colposcopy has certain limitations, including not being able to satisfactorily examine the cervical canal and not being able to identify the squamocolumnar junction (SCJ) in certain cases, above all in menopausal women, where the junction starts towards the inside of the canal [9]. It is precisely in such circumstances where colposcopy is lacking, that microcolposcopy can make a valid contribution to diagnosis.

However, microcolposcopy also has limitations in terms of diagnostic accuracy: in the literature, there are reports of false-negative percentages of 5.6% [5] and lesion grade identification error percentages of 21.4%, with 4.6% grade overestimation and 16.8% underestimation [3]. On the other hand, the false-positive percentage was reported as 11.3% in two cases of acute cervicitis, classified as severe dysplasia by microcolposcopy, and in one case of basal cell hyperplasia, classified as moderate dysplasia [4].

The main limitation of microcolposcopy is the "superficiality" of observation, i.e., that it is impossible to examine glandular crypts. In spite of this, studies agree on the efficiency of microcolposcopy for examinations of the cervical canal, deemed superior to endocervical curettage, especially in cases where the colpocytology is in doubt (abnormal cytology, negative or unsatisfactory colposcopy) [5].

Thus, it is believed that the main indications for conducting a microcolposcopy examination are: unsatisfactory colposcopy examination (SCJ not visualized), discrepancies between the cytology and colposcopy reports, topography of the lesions extending into the endocervical canal (microcolposcopy map), in order to perform a "personalized" cone biopsy, and finally, post-cone follow-up.

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The aim of this study was to assess the diagnostic reliability of microcolposcopy by means of correlation with histology, colposcopy and Pap test results.

Materials and Methods

Twohundred and fifty-six microcolposcopy examinations were requested for patients with abnormal Pap test results in the period between January 2005 and July 2006 in our colposcopy and lower genital tract pathology unit of the Department of Gynecological Sciences, Perinatology and Child Health, University of Rome "Sapienza".

Microcolposcopy examinations were conducted within our facility by a single operator, using the Hamou I microcolpohysteroscope in panoramic view and contact mode, and using 2% Lugol's and Waterman blue as stains. Microcolposcopy examinations were reported using the following terminology: viral cytopathic effects (VCE) for cellular alterations compatible with human papillomavirus (HPV) infection, for low-grade (LG) lesions and for high-grade (HG) lesions. In addition, the presence of any mature or immature metaplasia and keratosis was always recorded.

All Pap tests were reported in accordance with the 2001 Bethesda System [10].

Colposcopy examinations, conducted in all cases, were reported in accordance with SICPCV (Società Italiana di Colposcopia e Patologia Cervico-Vaginale - the Italian Colposcopy and Cervico-Vaginal Pathology Society) criteria [9], using the colposcopy report form comprising the abbreviations TA1 for a grade 1 abnormal transformation and TA2 for a grade 2 abnormal transformation. The term "unsatisfactory colposcopy" was used for all colposcopy examination cases that were non-diagnostic at the time of evaluation, and thus referred for subsequent examination.

Targeted biopsy was conducted in cases deemed indicated. At the time of data evaluation, 109 patients had been subjected to targeted biopsy. Histology reports were classified using the CIN (cervical intraepithelial neoplasia) nomenclature, introduced by Richart [11].

Results from microcolposcopy examinations were compared with colposcopy, cytology and histology results, and the percentage agreement and Pearson index calculated.

Results

Overall, 17.6% (45/256) of the cytology examinations showed evidence of atypical squamous cells of undetermined significance (ASCUS), 2.3% atypical squamous cells (ASC) (6/256), 56.3% (144/256) low-grade squamous intraepithelial lesions (LSIL), 17.2% (44/256) high-grade (H)SIL, 6.6% (17/256) atypical glandular cells (AGC) (Table 1), and finally there was a single case with high-grade atypical glandular cells (AGC-H).

Table 1. — *Pap test frequency.*

	Frequency	Percentage
ASCUS	45	17.6
ASC-H	6	2.3
LSIL	144	56.3
HSIL	44	17.2
AGC	17	6.6
Total	256	100.0

ASCUS: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells cannot exclude HSIL; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; AGC: atypical glandular cells.

All patients enrolled with positive Pap tests underwent second level colposcopy examinations and microcolposcopy (MCH). Over 30% of the examinations conducted were non-diagnostic or negative (Tables 2 and 3).

Table 2. — *Colposcopy frequency.*

	Frequency	Percentage
Unsatisfactory	93	36.3
TA1	107	41.8
TA2	56	21.9
Total	256	100.0

TA1: abnormal transformation zone (grade 1); TA2: abnormal transformation zone (grade 2).

Table 3. — *Microcolposcopy frequency.*

	Frequency	Percentage
HG	40	15.6
LG	46	18.0
Negative	86	33.6
VCE	84	32.8
Total	256	100.0

HG: high-grade lesion; LG: low-grade lesion; VCE: viral cytopathic effect.

From data reported subsequently, it emerged that MCH and Pap tests, both cytological examinations, showed an interesting level of agreement.

Among the total number of patients classified as HSIL, the MCH (HG) results agreed with the cytology results in 80% of cases (35/44), while among the low cytological grades (LSIL) the corresponding MCH results (VCE and LG) were observed in 74% of cases (107/144) (Table 4a). This level of agreement is increased if just the histologically confirmed cases are considered: 33/39 (85%) for high grades and 48/56 (86%) for low grades (Table 4b).

Table 4a. — *Microcolposcopy and Pap test level of agreement for the entire patient sample.*

		MCH				Total
		Negative	VCE	LG	HG	
Pap	AGC	16	1	0	0	17
	ASCUS	33	11	5	2	51
Test	HSIL	3	2	4	35	44
	LSIL	34	70	37	3	144
Total		86	84	46	40	256

MCH: microcolposcopy; ASCUS: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells can not exclude HSIL; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; AGC: atypical glandular cells; HG: high-grade lesion; LG: low-grade lesion; VCE: viral cytopathic effect.

Table 4b. — *Agreement between microcolposcopy and Pap test results from the sample of patients subjected to biopsy.*

		MCH				Total
		Negative	VCE	LG	HG	
Pap	AGC	7	0	0	0	7
	ASCUS	2	2	1	2	7
Test	LSIL	5	25	23	3	56
	HSIL	1	1	4	33	39
Total		15	28	28	38	109

MCH: microcolposcopy; ASCUS: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells can not exclude HSIL; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; AGC: atypical glandular cells; HG: high-grade lesion; LG: low-grade lesion; VCE: viral cytopathic effect.

In our case histories 109 colposcopy-guided targeted biopsies were conducted.

The MCH patterns classified as LG or VCE in 73% (18+15/45) of cases correspond to CIN1 histology, while those classified as HG to MCH in 71% (7+25/17+28) of cases correspond to CIN2 or CIN3 (Table 5).

Table 5. — *Absolute microcolposcopy and histology frequency.*

		Histology				Total
		Negative	CIN1	CIN2	CIN3	
MCH	Negative	6	7	2	0	15
	VCE	6	18	4	0	28
	LG	6	15	4	3	28
	HG	1	5	7	25	38
Total		19	45	17	28	109

MCH: microcolposcopy; HG: high-grade lesion; LG: low-grade lesion; VCE: viral cytopathic effect; CIN1: cervical intraepithelial neoplasia grade 1; CIN2: cervical intraepithelial neoplasia grade 2; CIN3: cervical intraepithelial neoplasia grade 3.

Colposcopy results classified as TA1 in 80% (36/45) of cases correspond to CIN1 histology. The observation of grade 2 abnormal transformation zone (TA2) by colposcopy in 68% (11+20/17+28) of cases corresponds to CIN2 or CIN3 (Table 6). The greater diagnostic "accuracy" becomes evident when considering just the cases of CIN3 biopsy, where the percentage agreement was 89% (25/28) for microcolposcopy and 71% (20/28) for colposcopy (Tables 5, 6 and 7).

MCH examination in nine positive histology cases, seven of which CIN1 and two CIN2, showed no signs of any neoplastic intraepithelial lesions (Table 5). The level of agreement between colposcopy and MCH was 72% for low-grade lesions (TA1 vs VCE and LG) and 68% for the high-grade (TA2 vs HG) lesions (Table 8).

Table 6. — *Absolute colposcopy and histology frequency.*

		Histology				Total
		Negative	CIN1	CIN2	CIN3	
Colposcopy	TA1	15	36	6	8	65
	TA2	4	9	11	20	44
Total		19	45	17	28	109

TA1: abnormal transformation zone grade 1; TA2: abnormal transformation zone grade 2; CIN1: cervical intraepithelial neoplasia grade 1; CIN2: cervical intraepithelial neoplasia grade 2; CIN3: cervical intraepithelial neoplasia grade 3.

Table 7. — *Absolute colposcopy and histology frequency.*

Histology			MCH				Total
			Negative	VCE	LG	HG	
Negative	Colposcopy	TA1	4	6	5	0	15
		TA2	2	0	1	1	4
	Total		6	6	6	1	19
CIN1	Colposcopy	TA1	5	17	13	1	36
		TA2	2	1	2	4	9
	Total		7	18	15	5	45
CIN2	Colposcopy	TA1	1	2	3	0	6
		TA2	1	2	1	7	11
	Total		2	4	4	7	17
CIN3	Colposcopy	TA1			1	7	8
		TA2			2	18	20
	Total				3	25	28

MCH: microcolposcopy; HG: high-grade lesion; LG: low-grade lesion; VCE: viral cytopathic effect; TA1: abnormal transformation zone grade 1; TA2: abnormal transformation zone grade 2; CIN1: cervical intraepithelial neoplasia grade 1; CIN2: cervical intraepithelial neoplasia grade 2; CIN3: cervical intraepithelial neoplasia grade 3.

Table 8. — *Agreement between colposcopy and microcolposcopy on degree of histologically conformed lesions.*

	Colposcopy	MCH	%
Level of agreement among the lower grades	47	65	72.3
Level of agreement among the higher grades	30	44	68.2

MCH: Microcolposcopy.

Analysis of the correlation between histological tests on biopsy samples and the other three variables (cytology, colposcopy and microcolposcopy) in the 109 cases subjected to biopsy in the sample population considered shows an intermediate level of positive correlation (Table 9).

Table 9. — *Analysis of the correlation between histology, cytology, microcolposcopy and colposcopy in the 109 cases subjected to biopsy.*

	Pearson correlation
Pap test	0.453
Colposcopy	0.447
MCH	0.623

MCH: Microcolposcopy.

The value of the correlation is an index comprised of between -1 and 1, hence, there is a moderate positive correlation between the pairs of examinations considered, with rather modest differences.

From the evaluation of the paired correlations, the highest value was observed with the MCH examination.

Discussion

In comparison to the literature, the overall percentage agreements between microcolposcopy and histology (73% for the higher grades, with a peak value of 89% for CIN3 cases, and 71% for the lower grades) were in accordance with the data from the literature [3-6].

From the percentage agreements between colposcopy and histology on the one hand, and microcolposcopy and histology on the other, improved diagnostic accuracy may be seen for the microcolposcopic examination of higher grade lesions with respect to colposcopy, while the opposite situation is true for the lower grades, for which colposcopy remains, in our case study, more predictive.

From the Pearson index values it may be claimed that none of the examinations (Pap test, colposcopy and microcolposcopy) alone can identify the grade of lesions diagnosed by histological examination.

One point worthy of note is the distribution of results conditioned by the CIN3 biopsy category, where MCH shows a higher tendency for correct identification of the seriousness of the lesion compared to colposcopy (25/28 vs 20/28) (Tables 5 and 6).

From analysis of the absolute frequency results (Tables 6, 7 and 8) it is possible to extrapolate the main contribution of colposcopy examinations. Indeed, this method is more reliable compared to MCH in negative result histology cases, since MCH classified a total of nine examinations in the CIN1 (7 cases) and CIN2 (2 cases) categories as "false-negatives". Microcolposcopic false-negatives are also reported in the literature (5.6%) [5, 6]. This result can be explained by colposcopy-guided targeted biopsy sampling in all cases and the deep intraglandular localization of certain endocervical lesions, which not even microcolposcopy is capable of detecting.

The negative histology results in 19 cases of lesions detected by colposcopy, 15 of which were grade 1 abnormal transformations, confirms the low specificity of this examination technique (Table 6). Thus, it is possible to hypothesize an error in the targeted biopsy site, both in terms of grade of lesion and site of the same, or in the histological interpretation of certain borderline situations, such as chronic cervicitis or immature metaplasia, the latter often being indistinguishable from a low-grade abnormal transformation. Indeed, the literature reports false-positive colposcopy cases, where biopsy analysis of aceto-white areas did not correspond to histologically detectable lesions [7, 14].

MCH overestimated lesion grade in five CIN1 cases and underestimated it in eight CIN2 cases and three CIN3 cases, data most likely correlated with biopsy sampling conducted in all cases with the aid of colposcopy and not MCH. Indeed, the literature reports percentages of 4.6% for overestimation and 16.8% for underestimation [3].

It should be noted that the greatest percentage of histology-microcolposcopy disagreement was observed in CIN2 biopsy cases, where the outcome of microcolposcopy examinations showed evidence of LG lesions in 8/15 subjects with positive MCH results (Table 5). This disagreement may be partly explained by the incompletely defined natural history of this clinical phenomenon, which can represent an intermediate condition between moderate dysplasia (CIN1) and severe dysplasia (CIN3), frequently much closer to a low-grade lesion [13].

In negative biopsy cases, the lack of agreement between histology and MCH diagnoses was much more evident. Indeed, a microcolposcopic "false-positive" number equal to 13/19 was recorded (Table 5). However, it is evident that all the cases, with the exception of one, involved low-grade microcolposcopic lesions. It is essential to once more remember that biopsies are always conducted with the aid of colposcopy, which can be less accurate in identifying clinical situations of VCE (Table 7).

With regard to the level of agreement between colposcopy and microcolposcopy in terms of the grade of lesion identified, it was 72% for low-grade (TA1 vs VCE and LG) and 68% for high-grade (TA2 vs HG) lesions (Table 8).

Conclusion

Microcolposcopy demonstrated its usefulness in the biological characterization of preneoplastic lesions in patients with abnormal cytology results, particularly in HSIL and LSIL cytology cases. The method proved to be sufficiently accurate in relation to the diagnosis of lesion grade. Furthermore, in cases where cytology was positive for HPV infection and colposcopy did not identify any lesions, MCH was definitive in identifying microscopic VCE phenomena, not always identifiable with a pathognomonic colposcopy pattern.

Having thoroughly considered the results, it may be deduced from the sample that microcolposcopy cannot be used in place of colposcopy (due to the presence of "false negatives"), but is capable of providing additional information on "grade" of the lesion (with greater accuracy for higher lesion grades).

Thus, it may be concluded that microcolposcopy is a useful diagnostic tool, to be used in a manner complimentary to colposcopy. From the results that emerged from our study, and in agreement with the literature (15-20), its main indications in the pretreatment examination of cervical lesions remain as follows: unsatisfactory colposcopy due to SCJ not visualized or endocervical, the pre-surgical topographic localization of exoendocervical lesions, and early HPV infections not yet clinically identifiable by colposcopy examination.

On the other hand, the main limitation of the method remains the number of "false-negatives" in cases of glandular localization of lesions.

In consideration of these results, greater knowledge and more widespread use of the method, for the purpose of improving the diagnosis and treatment of intraepithelial lesions, would seem desirable.

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Address reprint requests to:
A. LUKIC, M.D.
Via Michele Amari, 47
00179 Roma (Italy)
e-mail: luki.anki@tin.it