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Digital Cytology Combined With Artificial Intelligence Compared to Conventional Microscopy for Anal Cytology: A Preliminary Study

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ABSTRACT

Introduction: Recent studies have shown that digital cytology (DC) coupled with artificial intelligence (AI) algorithms is a valid approach to the diagnosis of cervico-vaginal lesions using liquid-based cytology (LBC). We evaluated the use of these methods for anal LBC specimens.

Methods: A series of 124 anal LBC slides previously diagnosed by conventional microscopy (CC) were reviewed with a DC/AI system that generated a gallery of images. Diagnoses based on the selected images, according to the 2014 Bethesda System for Reporting Cervical Cytology, were compared to CC.

Results: Overall, CC and DC/AI approaches detected a similar number of abnormal (ASC-US+) cases (63 and 62 cases, respectively). We observed an exact concordance between CC and DC in 70 (57.9%) cases, corresponding to a moderate agreement between the two approaches ($\kappa = 0.41$, $p < 0.001$). A moderate agreement ($\kappa = 0.48$, $p < 0.001$) was also found when positive cases were stratified into 'low-grade' (ASC-US, LSIL) and 'high-grade' lesions (ASC-H, HSIL). The DC/AI system detected more cases of higher severity (ASC-H, HSIL: 9 and 2 cases, respectively) than CC (3 cases classified as HSIL).

Conclusions: The number of ASC-US+ cases detected by both systems was similar. The DC/AI system detected more cases of higher severity compared to the CC.

1 | Introduction

Although rare in the general population, the incidence of anal cancer has been steadily increasing in specific groups, such as people living with human immunodeficiency virus (HIV), men who have sex with men (MSM), women with previous genital human papillomavirus (HPV)-related diseases and non-HIV immunosuppressed individuals [1]. The increase in the incidence of anal cancer is very likely related to a rise in sexual exposure to HPV and persistent infection by the virus [2]. Indeed, the principal histological type of anal cancer—squamous cell carcinoma (SCC)—is highly associated with HPV (almost 90% of the cases), mostly the HPV16 subtype. Moreover, the HPV16 positivity increases according to the severity of the anal lesion, from precursor lesions (termed anal intraepithelial neoplasia (AIN), grades 1–3) to cancer, independently of sex and HIV status [2, 3].

The screening of anal precancerous lesions would focus on the population of 'high risk' to develop cancer: persons who are HIV-positive (especially MSM); individuals with a history of solid organ transplantation (especially those of more than 10 years of transplant); and females with precancerous lesions and cancer of the vulva [1]. Different approaches are available

for the early detection of anal cancer including digital anorectal examination, cytology, high-risk HPV (HR-HPV) testing and high-resolution anoscopy (HRA) [4]. While there are no consensus guidelines for anal cancer screening, cytology has been frequently recommended by several medical societies to diagnose anal precancerous lesions and cancer [3, 5].

The accuracy of anal cytology for the detection of high-grade AIN or cancer is variable according to the target population [6, 7]. A systematic review and meta-analysis study revealed that in the group of MSM, the sensitivity of anal cytology for those who were HIV-positive was 80.8%; contrasting with a sensitivity of 'only' 43.5% for those who were HIV-negative [3]. According to the same study, HR-HPV testing is sensitive but less specific than cytology, especially when there is a high prevalence of HPV in the target population, such as in the HIV-positive MSM (sensitivity of 95.4% and specificity of 23.8%).

The major limitation of anal cytology is the relative lack of specificity. Indeed, several studies have shown a poor concordance between the cytological and histological grades of anal lesions [8–10]. Cytology usually underestimates the grade of a lesion. In fact, the presence of low-grade cytological abnormalities does not rule out the risk of a high-grade AIN on histology [8, 9]. In general, the detection of any abnormality in cytology is followed by HRA allowing the performance of biopsies for the histological diagnosis in the presence of suspect anal lesions. However, HRA is a very specific technique that requires expertise and is available only at limited healthcare centres. In contrast, cytology is less invasive and easier to perform by trained professionals. In the appropriate clinical context (targeting the population of 'high risk' to develop anal cancer), cytology is a suitable technique for the screening of anal lesions [4, 5].

The process of glass slides digitisation by scanners, known as whole slide imaging (WSI), generates virtual (digital) slides with high resolution. WSI for histological sections has been used for education, teleconsultation (second opinion diagnoses) as well as primary routine diagnoses. Usually, histological sections have a uniform thickness, making them easier to scan when compared to cytological specimens that have variable thickness, three-dimensional cell clusters and not infrequently obscuring material. New technical advances in scanners have improved scanning at multiple focal planes (z-stacking), allowing the production of digital slides with optimal resolution, which makes this technology suitable also for cytological specimens. Indeed, an interobserver diagnostic concordance of 84.1% was reported in a systematic review of several studies that compared WSI and conventional microscopy in the field of cytopathology [11]. Recently, digital cytology systems based on artificial intelligence (AI) algorithms have been investigated for the screening of cervico-vaginal cytology. In general, these studies showed higher sensitivity and non-inferior performance when compared to conventional microscopy [12–14].

The purpose of this study was to retrospectively investigate if AI algorithms coupled with digital cytology imaging could improve the detection of anal precancerous lesions and cancer compared to conventional light microscopic cytology.

2 | Material and Methods

This is a descriptive, retrospective and non-interventional study that included 124 consecutive anal cytology specimens routinely evaluated from January to December 2022. The samples were collected from 117 patients that had been followed up for established anal cancer or precancerous lesions or that were in their first cytological screening for anal cancer. The studied population included 103 (88.0%) men and 14 (12.0%) women, with a mean age of 45.2 years (range: 7–72 years old). In the course of 2022, the majority of the patients (111, 94.9%) had only one anal cytology sample, five patients had two samples (4.3%) and one patient had three samples (0.9%). The current study has no impact on the clinical management of each patient because all the original diagnoses were completed and reported in 2022. Cytological correlation with histology of anal biopsies was not performed in this study.

The anal samples were collected in ThinPrep Pap test (HOLOGIC Inc., USA) vials containing a methanol-based fixative (PreservCyt, HOLOGIC Inc., USA). Liquid-based cytology (LBC) preparations were made onto ThinPrep glass slides by an auto-loader processor (ThinPrep 5000 Autoloader, HOLOGIC Inc., USA). The LBC slides were stained with a modified Papanicolaou technique using an automated slide stainer (Tissue-Tek Prisma, SAKURA. Japan).

From January to December 2022, the anal LBC glass slides were routinely analysed and reported by two pathologists (RG or CR) in the context of routine diagnostic workup using a conventional light microscopy (Leica DM2000 LED, Leica Microsystems). This corresponds to the original diagnosis, which was considered the 'ground-truth' diagnosis and referred to in this study as the conventional cytology (CC). The LBC glass slides were retrieved from the archive, anonymised and digitised with a scanner (Genius Digital Imager, HOLOGIC Inc., USA), resulting in the WSIs that are referred to in the current study as digital cytology (DC). The scanned slides were analysed through a software (Genius Cervical AI, HOLOGIC Inc., USA) resulting in a gallery of images that were displayed on the working review station (Genius Review Station, HOLOGIC Inc., USA) on a high-resolution monitor screen (MDPC-8127, 27″ 8MP, BARCO).

For each case, the working review station shows a gallery of 30 tiles distributed in five rows of six images each. The whole digitised slide image is also displayed by the system (Figure 1). Briefly, the first and the second rows show the images of abnormal cells (when present) suggesting low-grade and high-grade squamous lesions, respectively. The third row shows mainly a mixture of normal, reactive or abnormal squamous cells mixed with inflammatory cells. The fourth row presents usually the images of glandular cells, abnormal or not, and the fifth row is designed to show the presence of germs among epithelial and inflammatory cells.

The evaluation of DC started in 2023, meaning a washout period of at least 8 months compared to CC (cases evaluated between January and December 2022). Only one of the authors (RG) interpreted the DC images. This author is familiar with the digital system: The system has been in use since 2022 for the routine

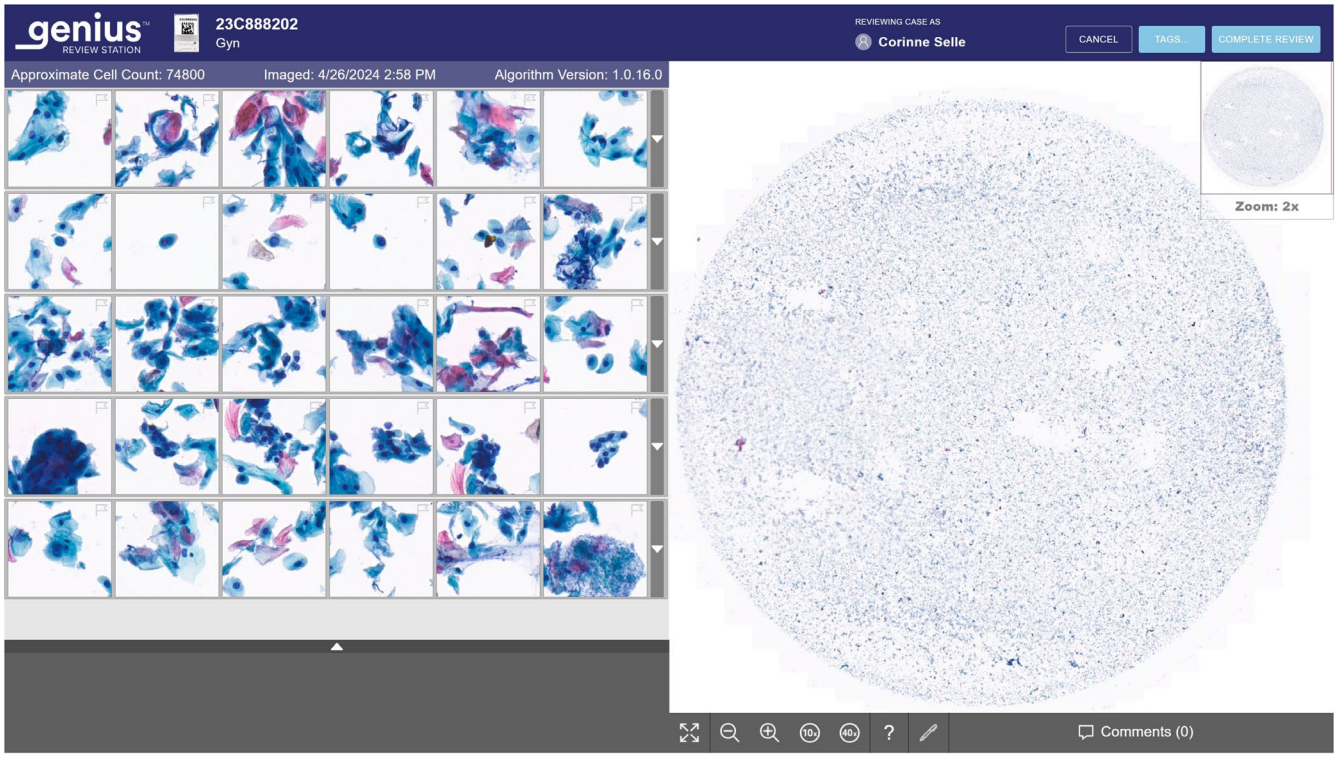

FIGURE 1 | This is an example of the working review station of a case showing a gallery of 30 tiles (5 rows of 6 images each) on the left and the whole slide image on the right.

diagnosis of cervico-vaginal cytology. Both CC and DC diagnoses were reported according to the third edition of the Bethesda System for Reporting Cervical Cytology [15] which contains a comprehensive chapter dedicated to the morphological criteria and diagnostic terminology for anal cytology. Indeed, the cytology of anal lesions follows the same diagnostic terminology of the cervico-vaginal cytology: unsatisfactory for evaluation (US); negative for intraepithelial lesion or malignancy (NILM); atypical squamous cells of undetermined signification (ASC-US); low-grade squamous intraepithelial lesion (LSIL); high-grade squamous intraepithelial lesion (HSIL); and atypical squamous cells cannot exclude HSIL (ASC-H).

All diagnoses were input in MS Excel. Data were categorised to determine the proportion rate of concordance between CC and DC. Cohen's kappa coefficient (κ) was calculated to evaluate CC and DC concordances using the statistical package R, version 4.3 [16]. The κ values were interpreted to represent the following levels of agreement: poor if less than 0.00; slight, 0.00–0.20; fair, 0.21–0.40; moderate, 0.41–0.60; substantial, 0.61–0.80; and almost perfect, 0.81–1.00 [17]. The results of the statistical analyses were displayed in tables.

3 | Results

From the 124 anal LBC slides, the majority (121, 97.6%) were successfully digitised, resulting in a gallery of images on the working review station. Three slides (2.4%) failed digitalisation; these cases were excluded and only the 121 digitised cases were considered for the analysis and final results. In general, the working review station displayed the abnormal cells (low and high-grade lesions) correctly at the expected rows (Figures 2 and 3).

A similar distribution of diagnosis according to the type of examination (CC and DC) was found for the 121 cases (Table 1 and Figure 4). Nine cases each (7.4%) were classified as unsatisfactory for evaluation by CC and DC. Forty-nine (40.5%) and 50 (41.3%) cases were interpreted as NILM by CC and DC, respectively. Generally, the distribution of cases with cytological abnormalities (ASC-US or worse: 'ASC-US+') was similar: 63 cases (52.1%) for CC and 62 (51.2%) cases for DC. However, the two types of examination (CC and DC) classified the cases differentially according to the severity of cytological abnormality (Figure 5). Indeed, CC classified 60 cases as low-grade lesions, including 36 cases (29.8%) of ASC-US and 24 cases (19.8%) of LSIL, and only three cases (2.5%) as high-grade lesions (all HSIL). In contrast, DC classified 51 cases as low-grade lesions, comprising 30 cases (24.8%) of ASC-US and 21 cases (14.7%) of LSIL, and 11 as high-grade lesions, including nine cases of ASC-H (7.4%) and two cases of HSIL (1.7%).

An exact concordant cytological diagnosis between CC and DC was found for 70 cases (57.9%) with a moderate agreement ($\kappa = 0.41$, $p < 0.001$) according to the Cohen's kappa statistic (Table 2). In a further analysis, 11 cases classified as unsatisfactory for evaluation (US) by CC and/or DC were excluded, resulting in 110 cases corresponding to the negative (NILM) and positive (ASC-US or worse) cytological diagnoses. Furthermore, the positive cases were stratified into 'low-grade' (ASC-US, LSIL) and 'high-grade' lesions (ASC-H, HSIL). This analysis showed an exact concordance rate of 70.9% and a moderate agreement ($\kappa = 0.48$, $p < 0.001$) between CC and DC (Table 3).

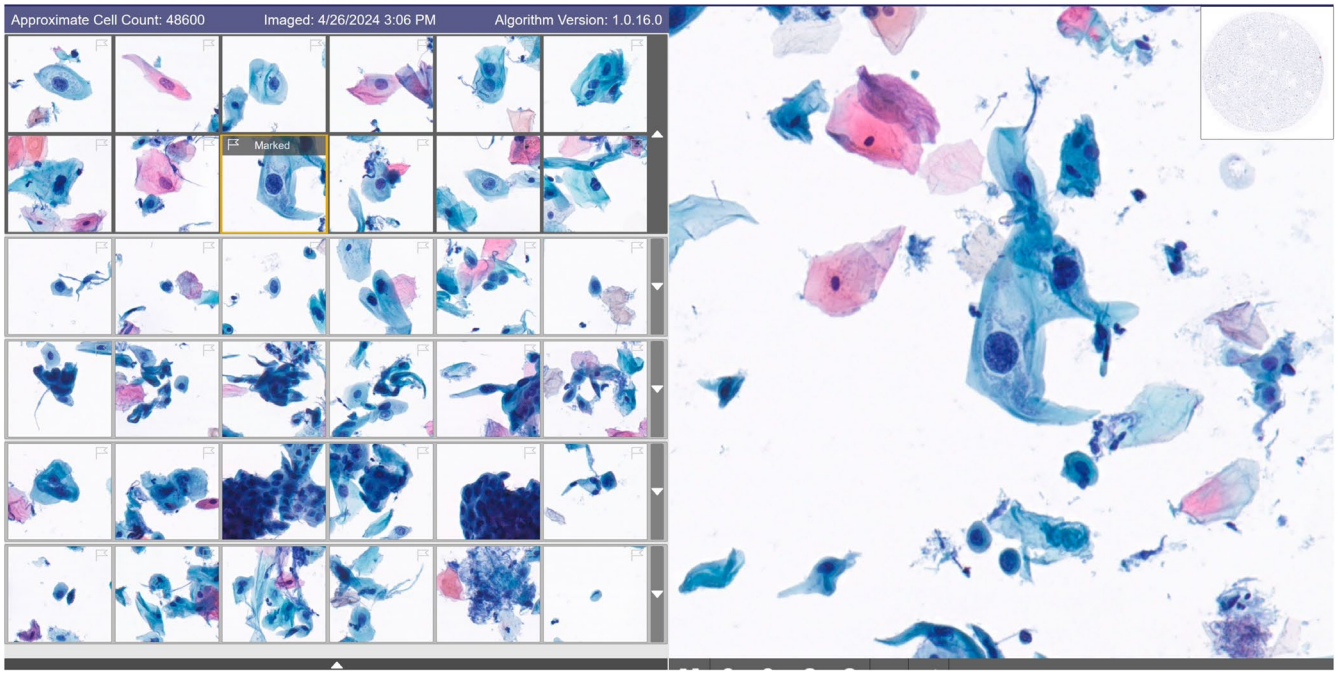

FIGURE 2 | This is an example of a case of low-grade squamous intraepithelial lesion (LSIL) showing the image of an abnormal mature squamous cell selected by the system and displayed in the row of 'low-grade lesions' on the working review station.

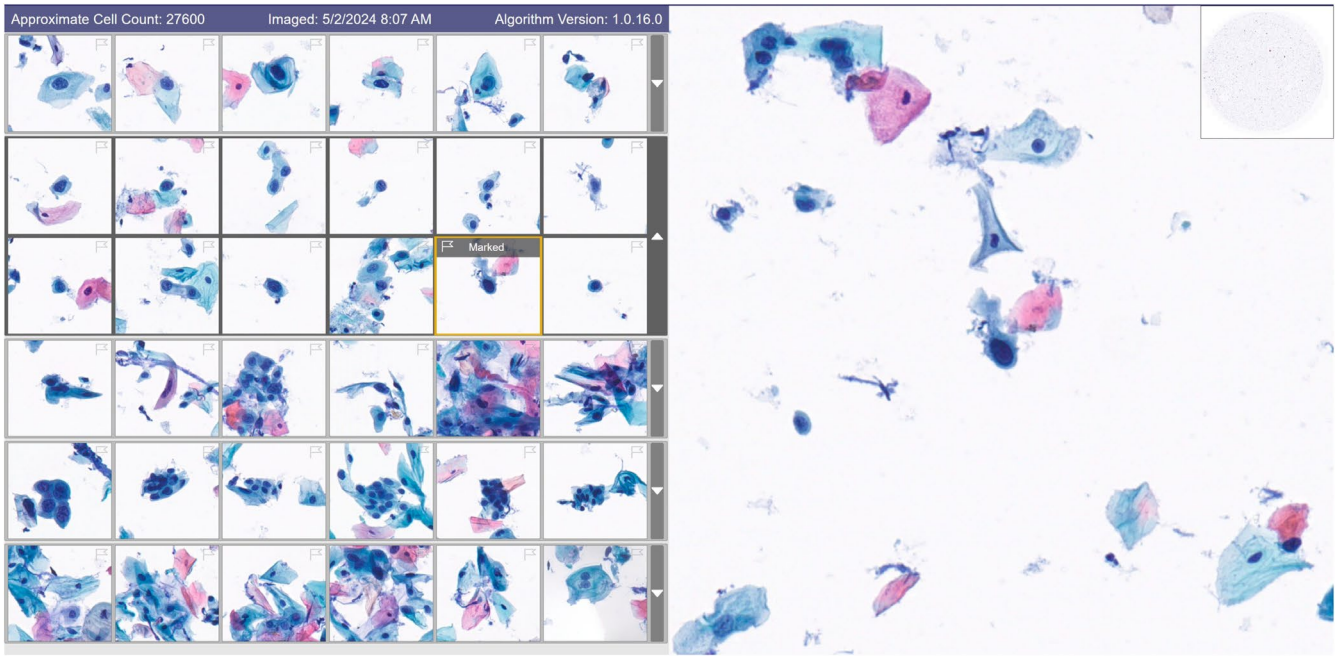

FIGURE 3 | This is an example of a case of high-grade squamous intraepithelial lesion (HSIL) showing an image of an abnormal small and immature squamous cell selected by the system and displayed in the row of 'high-grade lesions' on the working review station.

Cytological diagnostic discordances between CC and DC were found in 51 (42.1%) of the 121 cases (Table 4). In comparison to CC and excluding the four cases with an unsatisfactory result (US), 'undercall' and 'overcall' DC diagnoses were found in 22 and 25 cases, respectively. Considering these data, we described the pair of CC/DC (and vice versa) disagreements. In four (7.8%) cases, the disagreements involved the adequacy of cytological samples, that i, the presence of a diagnosis of unsatisfactory for evaluation: US/NILM (3, 5.9%) and US/ASC-US (1, 2.0%). The remaining 47 (92.2%) cases showed the following discordances between CC and DC (from the most frequent to the less frequent CC/DC pair and vice versa): NILM and ASC-US (17, 33.3%); ASC-US and LSIL (15, 29.4%); NILM and LSIL (5, 9.8%); ASC-US and ASC-H (5, 9.8%); NILM and ASC-H (2, 3.9%); LSIL and ASC-H (2, 3.9%); and LSIL and HSIL (1, 2.0%).

4 | Discussion

In this study, we investigated the role of DC on the diagnosis of anal specimens using the Genius Digital Imager system (HOLOGIC Inc., USA). In general, a quite similar distribution of cytological diagnoses was found for the 121 successfully digitised cases according to the type of examination (DC and CC). An exact concordance rate of 57.9% and a moderate agreement ($\kappa = 0.41$) between DC and CC was shown for specific cytological diagnoses. The concordance rate for a dichotomy diagnostic category (negative vs. positive) was 70.9% with a moderate agreement ($\kappa = 0.48$). Interestingly, DC classified more cases as 'severe lesions' (ASC-H and HSIL) compared to CC.

Some studies investigated the interobserver variation in the interpretation of anal cytology by CM, and, in general, moderate to good concordance rates were found [18, 19]. In a study that enrolled HIV-infected MSM, the comparison of cytological interpretation of 339 anal samples between two cytopathologists showed an overall concordance of 66% and moderate ($\kappa = 0.54$) to good (linear-weighted $\kappa = 0.69$) agreement [20]. A recent analysis of 713 anal cytological specimens from a population of HIV-negative MSM demonstrated higher diagnostic concordance among the cytopathologists of the study: a concordance rate of 93.3% and excellent agreement according to the kappa statistic ($\kappa = 0.82$) for a dichotomous classification (NILM vs. ASC-US+) [21].

TABLE 1 | Distribution of diagnoses according to conventional (CC) cytology and digital cytology (DC).

Diagnostic categories	CC	DC
US	9 (7.4%)	9 (7.4%)
NILM	49 (40.5%)	50 (41.3%)
ASC-US	36 (29.8%)	30 (24.8%)
LSIL	24 (19.8%)	21 (17.4%)
ASC-H	0 (0.0%)	9 (7.4%)
HSIL	3 (2.5%)	2 (1.7%)
Total	121 (100.0%)	121 (100%)

Note: The diagnostic categories were defined according to The Bethesda System for Reporting Cervical Cytology as follows: ASC-H, atypical squamous cells cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined signification; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; US, unsatisfactory for evaluation.

In contrast to the above studies, we did not perform intra- or interobserver comparisons. Indeed, we analysed the diagnostic concordance between two different approaches: CC and DC. The digital images were interpreted by only one of the investigators of this paper (RG) and CC evaluation had been routinely performed by two investigators (RG and CRC) to establish a diagnosis for clinical management, namely the original cytological diagnosis (the 'ground-truth' diagnosis in this study). For the one who evaluated DC cases (RG), the washout period was at least 8 months, which is long enough to avoid biases regarding the awareness of CC diagnoses.

As expected, the majority of diagnostic discordances between CC and DC involved the ASC-US category. In fact, from the 51 discordant cases of our study, 37 (72.5%) were related to this category, as shown by the CC/DC pairs: NILM/ASC-US (17, 33.3%); ASC-US/LSIL (15, 29.4%); and ASC-US/ASC-H (5, 9.8%). Lytwyn et al. [19], showed a slight agreement for classifying the cytological specimens as ASC-US among the four investigators of the study, finding a κ score equal to only 0.12. In contrast, a surprisingly high interobserver diagnostic concordance rate (76.9%) for the ASC-US category was found in the study of Benevolo et al. [21]. Although not concerning anal specimens, a web-based interobserver study to evaluate a set of cervico-vaginal cytology images from the Bethesda Atlas 2014 (the Bethesda interobserver reproducibility study-2, or BIRST-2) showed that the concordance rate with an 'expert panel' interpretation was 62% for the ASC-US category [22].

Several studies that compared DC with CC have been published. A systematic review of 19 publications about the comparison between WSI and conventional microscopy diagnoses in the field of cytopathology revealed a mean intra-observer

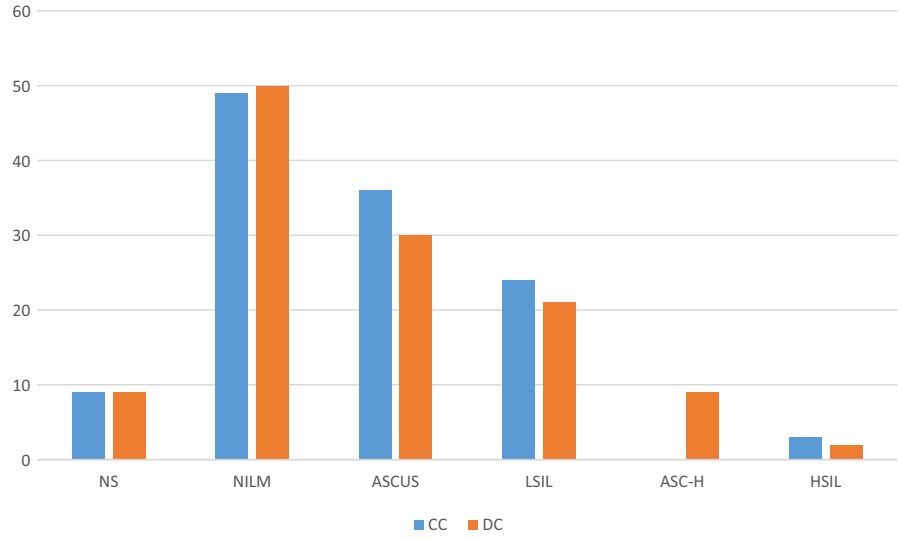

FIGURE 4 | Histogram of the distribution of diagnoses according to the type of examination—CC and DC.

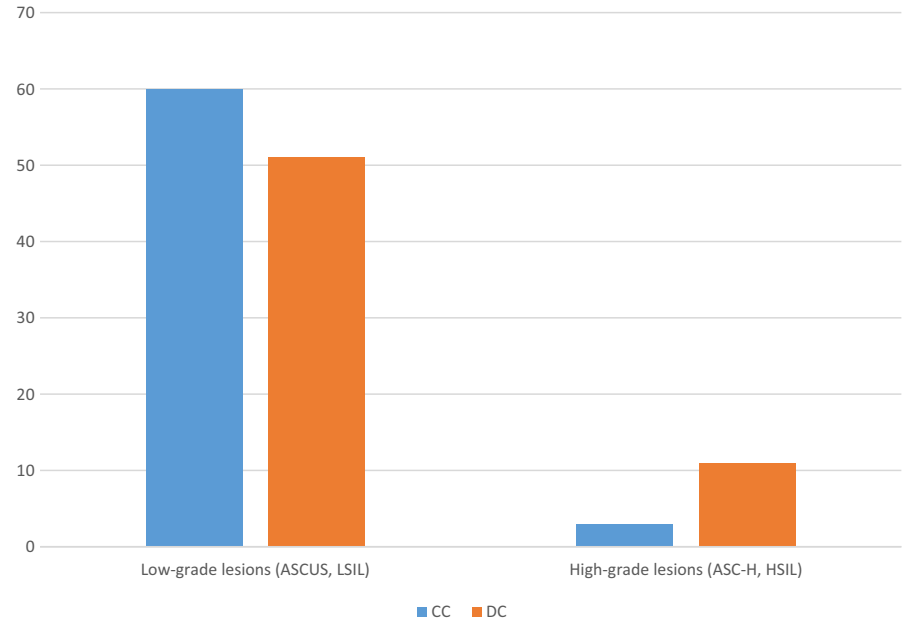

FIGURE 5 | Histogram of the distribution of low-grade (atypical squamous cells of undetermined significance, ASC-US; low-grade squamous intraepithelial lesion, LSIL) and high-grade lesions (atypical squamous cells cannot exclude HSIL, ASC-H; high-grade squamous intraepithelial lesion HSIL), according to the type of examination—CC and DC.

TABLE 2 | Number of cases according to diagnosis and type of examination—CC and DC.

	DC						
CC	**US**	**NILM**	**ASC-US**	**LSIL**	**ASC-H**	**HSIL**	**Total (CC)**
US	7	2	—	—	—	—	9
NILM	1	36	7	3	2	—	49
ASC-US	1	10	14	6	5	—	36
LSIL	—	2	9	11	2	—	24
ASC-H	—	—	—	—	—	—	0
HSIL	—	—	—	1	—	2	3
Total (DC)	9	50	30	21	9	2	121

Note: Exact concordance rate: 57.9%. Kappa = 0.41 ($p < 0.001$). The diagnostic categories were defined according to The Bethesda System for Reporting Cervical Cytology, as follows: ASC-H, atypical squamous cells cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined signification; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; US, unsatisfactory for evaluation.

concordance rate of 92.5% and a substantial agreement according to the kappa statistic ($\kappa = 0.66$) [11]. Interobserver comparisons with the original diagnosis were also assessed by the authors, corresponding to a mean concordance rate of 84.1% and a substantial agreement ($\kappa = 0.69$) across the studies. The mean concordance rate was slightly higher (88.2%) when only the studies that evaluated cervico-vaginal specimens were considered [11].

With the purpose of demonstrating that WSI was non-inferior to conventional microscopy for the diagnosis in the field of gynaecological cytology, Bongaerts et al. [23], evaluated two large series (cohorts) of cervical LBC specimens: comparison between original diagnoses ('first reading') and re-examination ('second reading') of glass slides by conventional microscopy (first cohort with 500 cases); comparison between WSI and conventional microscopy diagnoses (second cohort with 505 cases). Similar results were found for both cohorts: The overall concordance rates for the first and the second cohorts were 97.8% and 95.3%, respectively [23].

More recent studies investigated and validated the diagnostic performance of cervico-vaginal cytology screening using automated systems based on fully digitised LBC slides by rapid scanners and AI algorithm approaches for the selection of individual abnormal cells. These studies compared the new generation systems to the traditional automated screening methods, which select and display a limited number of abnormal cells that require the revision of the glass slide by a cytotechnician operating a conventional microscopy to establish a final diagnosis.

TABLE 3 | Number of cases with negative cytological diagnosis (NILM) and positive cytological diagnosis (low-grade and high-grade lesions) and type of examination—CC and DC.

	DC			
CC	**NILM**	**Low-grade lesions**	**High-grade lesions**	**Total (CC)**
NILM	36	10	2	48
Low-grade lesions	12	40	7	59
High-grade lesions	—	1	2	3
Total (DC)	48	51	11	110

Note: Exact concordance rate: 70.9%. Kappa = 0.48 ($p < 0.001$). 'Low-grade lesions' correspond to the cytological diagnoses of ASC-US and LSIL; 'High-grade lesions' correspond to the cytological diagnoses of ASC-H and HSIL; and NILM corresponds a cytological diagnosis negative for intraepithelial lesion or malignancy, according to the diagnostic terminology of The Bethesda System for Reporting Cervical Cytology.

TABLE 4 | Number of discordant cases according to diagnosis by type of examination—CC and DC and number of under- and overcall cases by DC.

CC	**DC**	***N***	**Undercall by DC**	**Overcall by DC**
US	NILM	2	—	—
NILM	US	1	—	—
NILM	ASC-US	7	—	7
NILM	LSIL	3	—	3
NILM	ASC-H	2	—	2
ASC-US	US	1	—	—
ASC-US	NILM	10	10	—
ASC-US	LSIL	6	—	6
ASC-US	ASC-H	5	—	5
LSIL	NILM	2	2	—
LSIL	ASC-US	9	9	—
LSIL	ASC-H	2	—	2
HSIL	LSIL	1	1	—
Total		51	22	25

Note: The diagnostic categories were defined according to The Bethesda System for Reporting Cervical Cytology, as follows: ASC-H, atypical squamous cells cannot exclude HSIL; ASC-US, atypical squamous cells of undetermined signification; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; US, unsatisfactory for evaluation.

One of those systems, the CytoProcessor (DATEXIM, Caen, France), was evaluated in a study that included 1352 cervico-vaginal LBC specimens [12]. According to the authors, the new system demonstrated a significantly higher sensitivity for the detection of squamous lesions (ASCUS/LSIL or more severe lesions) in comparison with the traditional computer-assisted screening method (ThinPrep Imaging System (TIS), HOLOGIC Inc., USA). Moreover, the false-negative rate, that would potentially have a clinical impact on the follow-up of patients, was statistically significantly lower for the new system [12].

Another study including 1994 cervico-vaginal LBC slides also compared the traditional automated screening method (TIS) with the Genius Digital Imager system (HOLOGIC Inc., USA) showing that the new system detected more cases of higher severity abnormalities (ASC-H and HSIL) than the traditional one [13]. Indeed, significantly higher sensitivity was shown for the digital system in identifying cases that were confirmed as high-grade cervical intraepithelial neoplasia (CIN2+, CIN3+) on histology. While the specificity and positive predictive rates were similar between the two approaches, the negative predictive value was higher for the digital system, suggesting a lower false-negative rate, comparable to the study of Crowell et al. [12].

Similar to the study from Ikenberg et al. [13], we showed that DC was capable of identifying more cases of high-grade intraepithelial squamous lesions (ASC-H and HSIL): Three cases were classified as HSIL by CC compared to nine and two cases classified by DC as ASC-H and HSIL, respectively. In their study, they found significantly different detection rates of cases with higher severity, that is, cases classified as ASC-H, atypical glandular cells, favour neoplasia (AGC-FN), HSIL and carcinoma (CA) between DC (ASC-H+, 20.6%; HSIL+, 18.0%) and the traditional computer-assisted screening method (ASC-H+, 17.5%; HSIL+, 13.6%). We have the impression that smaller or immature atypical squamous cells were more easily identified with DC because they were frequently depicted on the second row of the image gallery in the working review station, as shown in Figure 3. This could be a possible explanation for the higher detection of high-grade lesions (ASC-H, HSIL) by DC in our study.

Of course, the data from both studies are not completely exchangeable. Ikenberg et al. [13] investigated a total of 1994 cervico-vaginal digitised slides; ours comprise a small cohort of 121 LBC anal lesions. Despite the morphological similarities of cervico-vaginal and anal cytology, some differences exist between the two, as highlighted by Scholefield et al. [18]: due to keratinisation of the lower anal canal and perianal skin, anucleate squames are frequently seen in anal specimens; koilocytes are rarely found in anal samples; and anal parakeratosis favours a low-grade intraepithelial lesion because AIN are usually associated with parakeratotic cells.

Recently, a series of 320 cervico-vaginal cytology cases previously screened and diagnosed was used to perform a validation study of digital cytology using the Genius Digital Diagnostics System [14]. Residual material from the original cases was recovered to produce new slides that were digitised and reviewed by both conventional light microscopy and AI-assisted digital review. Diagnostic concordance with the original ('ground-truth') diagnosis was statistically significantly different for the AI-assisted digital evaluation (198 cases, 62.1%) compared to the light microscopic examination (178 cases, 55.8%). An exact diagnostic concordance among the two review approaches

(AI-assisted digital review and light microscopic evaluation) was found in 220 cases (69.0%) [14]. Not too far from their study, our data showed a concordance rate of 57.9%, corresponding to 70 cases with an exact diagnostic match between DC and CC.

Our study has three important limitations. First, to avoid bias in sample selection, we included consecutive cases of anal cytology that were routinely diagnosed in the entire year of 2022. This resulted in a small series of 124 cases from which 121 were successfully digitised and finally analysed. Although a potential bias, enrichment of samples with selected, non-random cases might be necessary in the context of validation studies, as demonstrated, for instance, in the study of Ikenberg et al. [13] where cases with normal (NILM) and ASC-US diagnoses were selected to complete their series of 1994 cervico-vaginal cytology specimens. Second, we did not perform an interobserver comparison because only one of the investigators (RG) evaluated the DC images. We recognise that this is a weak point of our study. In fact, a reliable analysis of diagnostic reproducibility should include interobserver comparisons between two or more investigators. Finally, histological evaluation of anal biopsies was not performed in this study. Although several studies showed a poor correlation between cytological and histological grades of anal lesions [8–10], a correlation with histology would be important to verify if DC was more accurate in the detection of high-grade lesions compared to CC.

5 | Conclusion

Our study shows that DC coupled with AI algorithms is a promising tool for the screening and diagnosis of anal pre-cancerous lesions. Although the number of ASCUS+ cases detected by both systems (DC and CC) was similar, the DC approach allowed the detection of more cases of higher severe lesions (ASC-H, HSIL) compared to CC. However, further evaluation is necessary, including the inclusion of a large number of cases, the analysis of diagnostic reproducibility between two or more pathologists and correlation with histology to determine the diagnostic accuracy of DC.

Author Contributions

Renê Gerhard: conception of the study, glass slides examination, digital images examination, data collection and data analysis, statistical analysis and manuscript writing. **Cioly Rivero Colmenarez:** glass slides examination, manuscript review. **Corinne Selle:** data collection, manuscript review. **Gaël Paul Hammer:** statistical analysis, manuscript review

Ethics Statement

This study was approved by the Service juridique, Laboratoire national de santé.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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