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Prevalence of *Gardnerella vaginalis* in HR HPV-positive women and its association with squamous intraepithelial lesions

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Abstract

Background The human papillomavirus (HPV) is the most common sexually transmitted infection (STI) worldwide. Only a small percentage of high-risk (HR) HPV infections progress to cervical precancer and cancer. Recent research indicates the potential association between the variation of vaginal microbiota and the acquisition and persistence of human papillomavirus (HPV) infection. However, the association of STIs with HPV cervical infection and cervicovaginal lesions has not yet been fully elucidated. The aim of this study was to assess how *G. vaginalis* is associated with HPV infection and cervical dysplasia, and the beneficial role of *Lactobacillus*.

Methods A total of 723 women aged between 17 and 79 years were studied. A complete cervico-vaginal swab for a cervico-vaginal cytological examination (Pap test) and the simultaneous identification, by Real-Time PCR, of the presence of HR-HPV and other STI pathogens, was conducted.

Results The HPV positive women were 37.2%, with a prevalence significantly different in the various age groups. The most frequent genotype was HPV-16, while *G. vaginalis* was found in 369 women, in which 51.8% of cases occurred together with HPV-HR, highlighting a significant association between the two infections ($p < 0.001$). Moreover, a marked reduction in HR-HPV infection was observed in the presence of *Lactobacillus*. ($p < 0.001$). All-grade lesions were significantly associated with *G. vaginalis* and detected in 65.4% of *G. vaginalis*-positive samples ($p < 0.01$).

Conclusions The results of this study strengthen the hypothesis of the association between HPV infections and microbiota in cervical lesions.

Clinical trial number Not applicable.

Keywords HPV genotypes, *Gardnerella vaginalis*, Cervical cancer, Real-time PCR, Pap test

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Background

HPV (human papillomavirus) is a common sexually transmitted virus [1]that causes about 4.5% of all cancers in women and men worldwide [2]. In particular, the virus is the leading cause of cervical cancer, which is the fourth most common cancer in women [3], with about 660 000 new cases and around 350 000 deaths in 2022 worldwide [4], and is also responsible for a high proportion of anal, penile, vaginal, vulvar, and oropharyngeal cancers (5). In the European continent, about 2.5% of cancers are attributable to HPV [6], with about 58,000 new cervical cancer cases diagnosed annually [5]. In Italy, current estimates indicate that every year, 3152 women are diagnosed with cervical cancer, and 1011 die from the disease [7].

The peak prevalence of infection is between the ages of 15 and 26 and around 45 years. It has been shown that the persistence of HPV infection increases with age, and its incidence decreases with age [8]. Most deaths due to cervical cancer occur in low-and middle-income countries with limited access to screening and health care [9], such as in sub-Saharan Africa, Latin America and the Caribbean, Eastern Europe, and South-East Asia. Furthermore, some epidemiological reports indicate that the vaginal microbiota of women varies by race and region [10, 11]. HPVs include a group of more than 200 related viruses, and can be classified into two types based on their carcinogenic potential: low-risk (LR-HPV) and high-risk (HR-HPV). At present, the HR-HPV types are identified as 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, 73, and 82 [12, 13]. Of HR-HPV, types 16 and 18 are responsible for about 70% of all cervical cancer cases worldwide.

Though the majority of HPV-infected women can control and eliminate the virus within a few months, 5% to 10% of infections with HR-HPV types can persist and culminate in cervical precancerous lesions and eventual cervical cancer 15–20 years after HPV infection [14–18].

At present, it is still unclear why some hr HPV infections resolve clinically while others persist and cause dysplasia and even cervical cancer, but one of the determining elements is possibly cervicovaginal microecology [19–22]. However, the role of the local microbiome in the natural cause of HPV infection is still very debated in many studies [23].

Several studies have reported a close association between the female cervical and vaginal microbiota and human papillomavirus (HPV) infection, cervical intraepithelial neoplasia (CIN), and the development of cervical cancer [24–26], even if the mechanisms involved are poorly understood. Although the vaginal microbiota is composed of a diverse range of microorganisms, *Lactobacillus* species dominate the vaginal microbiota of most healthy women [27], which create a low pH environment protecting against both exogenous bacteria and viruses [28, 15]. Still, when bacterial vaginosis is present, the replacement of beneficial Lactobacillus with anaerobic and facultative anaerobic bacteria may occur. Regarding these, the anaerobic *Gardnerella* spp. was present in nearly all cases [29, 30], and *G. vaginalis* was found to be implicated with higher frequency and severity of disease, potentially resulting in precancerous and cancerous cervical lesions [31]. However, the precise impact of a single bacterial species is tough to unravel, and conclusive evidence for each bacterial species is not yet well established.

Considering the importance of better understanding the relationship between the presence of *G. vaginalis* and the outcome of HR-HPV infection, in this study, we evaluated the association of the coinfection of *G. vaginalis* and HR-HPV with cervical cytomorphological lesions, and the protective role of *Lactobacillus*.

Methods

Study population

This study included 723 female subjects aged between 17 and 79 years, residents of the province of Pesaro-Urbino (PU, Italy), who, in the period between January 2022 and December 2024, at the Biolab Clinical Analysis Laboratory in Vallefoglia (PU, Italy), requested either spontaneously or at the recommendation of their general doctor or gynaecologist, the execution of a complete cervicovaginal swab. In women of childbearing age, cervical sampling was performed at least five days after the end of the menstrual cycle and no more than five days before the expected start date, and at least two days after the last sexual intercourse. Women with bacterial vaginosis (BV) (both symptomatic and asymptomatic) were not excluded from the study. Each woman participating in the study completed an appropriate medical history sheet, indicating any symptoms (vulvar itching/burning, leukorrhea). The study complied with the principles of the Declaration of Helsinki and European data protection regulations (Regulation 2016/679). Informed consent was obtained from participants, and the study received approval from the Marche territorial ethics committee with a favorable opinion on 12/12/2024 Prot. 2024 242.

Cytology and biomolecular analysis for HPV and vaginosis bacteria

The collection for the cervicovaginal swab and the Pap test was performed by appropriately trained collectors: gynecologists or obstetricians. The swabs were stored in the refrigerator, while conventional Pap tests and thin prep tests were stored at room temperature. The execution of a complete cervico-vaginal swab would allow for a cervico-vaginal cytological examination (Pap test) and the simultaneous identification, by Real-Time PCR, of the presence of HR-HPV, *C. trachomatis*, *N. gonorrhoeae*, *G.*

vaginalis, *T. vaginalis*, *M.hominis*, *M. genitalium*, *U. urealyticum*, *and U. parvum*.

The Real-Time PCR performed the biomolecular analyses used the instruments QIAsymphony SP/AS and Rotor Gene Q Mdx 5plex HRM, both from QIAGEN.

The kit used for the biomolecular analysis of HPV was the GeneNavTM HPV One qPCR Kit (GenomeMe-CANADA), which is used in combination with the QIAsymphony DSP Virus/Pathogen Midi kit through the use of the QIAsymphony SP module for DNA purification and extraction, with the QIAsymphony AS module for setting the PCR dosage. This in vitro diagnostic kit allows for the specific detection and discrimination between HPV 16, HPV 18 and the non-specific pooled detection of the other 12 high-risk HPV genotypes (HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 66 and HPV 68). An internal β-Actin control is also used in the GeneNav™ HPV One qPCR Kit to evaluate the extraction quality of the samples and ensure the reliability of the HPV detection results. The primers used in the amplification are universal oligonucleotides designed on the genomic region encoding the E6/E7 proteins.

All the 723 cervico-vaginal swab samples were also searched for the presence of *C. trachomatis*, *N. gonorrhoeae*, *G. vaginalis*, *T. vaginalis*, *M. hominis*, *M. genitalium*, *U. urealyticum*, *and U. parvum.* The DNA extraction phase was performed in a single session using the QIAsymphony SP instrument. Subsequently, following the automatic transfer to the QIAsymphony AS module, it was possible to set the PCR assay. At this stage, different diagnostic kits were used for the various pathogens. The REALQUALITY RQ-SevenSTI kit from AB Analitica allowed for the identification of seven pathogens using multiplex Real-time PCR: *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *M. hominis*, *T. vaginalis*, *U. urealyticum*, *and U. parvum.* Finally, DNA amplification was performed using the Rotor-Gene Q. The *Gardnerella vaginalis*/*Lactobacillus* RealTM Quant kit from Sacace Biotechnologies was used to identify *G. vaginalis* and *Lactobacillus*.

Statistical analysis

Differences in the HR-HPV infection and cytomorphological lesions, considering the co-infection with G. vaginalis or Lactobacillus spp., were evaluated using the chi-square test and odds ratio. Spearman's rho coefficients were calculated to find correlations between cytomorphological lesion levels and HR-HPV, *G. vaginalis*, and presence of *Lactobacillus*.

Results

A total of 723 women (age range 17–79 years) were included in this study and tested for positivity to HR-HPV and other sexually transmitted diseases (STDs) during the period January 2022 and December 2024. A total of 269 women (37.2%) tested positive for HR-HPV, with a prevalence significantly different in the various age groups ($p < 0.001$). The highest prevalence was found in the 31–35-year-old group (62%), followed by the age group of 26–30 years (50%), and the group 20–25 (42%), whereas a very low prevalence was observed in women born before 1962 (Fig. 1). The most frequent genotype detected in the sample was HPV-16 (56 times), followed by types 18 and 45, which were isolated 33 times. The infections with other HPV types (genotype HR: 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68) occurred in 180 cases. In this study, the presence of other infections of the genital tract was also evaluated. In particular, *G. vaginalis* was found in 369 women, *U. parvum* in 75, *M. hominis* in 58, *U. urealyticum* in 28, *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* in fewer than 5 cases. Co-infection with two or more species was also observed. One hundred and nine women with G. vaginalis were always in co-infection with other STD pathogens, whereas *G. vaginalis* was absent in the coinfections detected in 94 women. The concomitant infections of HR-HPV with *G. vaginalis* were found in 191 women, representing 51.8% of all *G. vaginalis*-positive samples, and in 78 women infected with HR-HPV alone, representing 21.7% of all *G. vaginalis*-negative samples, highlighting a significant association between the two infections ($p < 0.001$)(Tables 1 and 2). The odds ratio (OR) of having HR-HPV positivity in the presence of *G. vaginalis* was 3.797 (Table 1).

A negative correlation between *G. vaginalis* and *Lactobacillus* was found (Table 2). Moreover, a marked reduction in HR-HPV infection was observed in the presence of *Lactobacillus*. ($p < 0.001$) (Tables 1 and 2*)*; 108 (24.2%) and 161 (58.1%) of *Lactobacillus* positive and *Lactobacillus* negative samples, respectively, were found positive for HR-HPV (Table 1). The odds ratio (OR) of having HR-HPV positivity in the presence of *Lactobacillus* was 0.230 (Table 1).

In 60 samples, HR-HPV types were simultaneously present with other STD pathogens ($p > 0.05$). The infection with *G. vaginalis* varied significantly ($p < 0.05$) with age and was higher in the 31–35 years old group (90%) and in the 41–45 years old group (78%) (Fig. 1). Furthermore, the presence of cytomorphological lesions was evaluated in women positive for HR-HPV infection. A total of 163 women were found to be positive for cytomorphological lesions. Interestingly, all-grade lesions were significantly associated with *G. vaginalis* (Table 2) and detected in 65.4% (125/191) of *G. vaginalis*-positive samples (Table 3; $p < 0.01$). The odds ratio (OR) of having

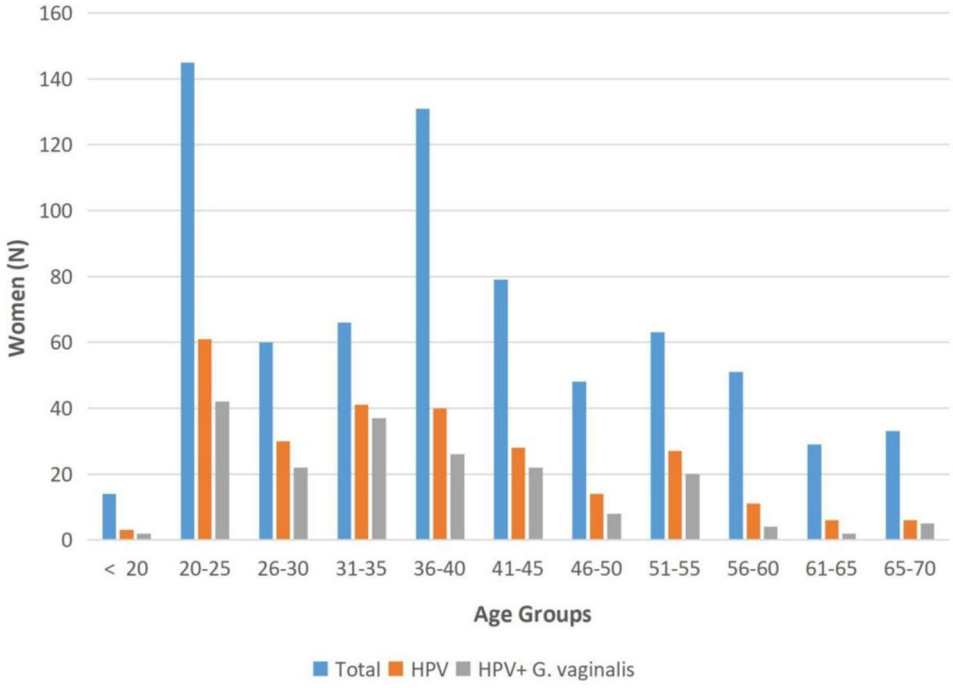

Fig. 1 Prevalence of HR-HPV infection and HR-HPV and *G. vaginalis* coinfection among women by age groups

Table 1 HR-HPV positivity related to *G. vaginalis* and *Lactobacillus*

	HR-HPV		*p*[a]	OR (95% CI)
	Negative	Positive		
G. vaginalis				
Negative	276	78		
Positive	178	191	*<0.001*	3.797 (2.747–5.249)
Lactobacillus				
Negative	116	161		
Positive	338	108	*<0.001*	0.230 (0.167–0.318)

[a] chi-squared test

Table 2 Spearman's correlation coefficients between HR-HPV, cytomorphological lesions, *G. vaginalis*, and *Lactobacillus*

	HR-HPV	Cytomorphological lesions *	G. vaginalis	Lactobacillus
HR-HPV	1,000			
Cytomorphological lesions *	0,550*	1,000		
G. vaginalis	0,307*	0,254*	1,000	
Lactobacillus	-0,378*	-0,290*	-0,654*	1,000

*$p < 0.0001$

all-grade lesions in the presence of *G. vaginalis* was 1.994 (Table 3). Considering low-grade lesions (LL), the low-grade squamous intraepithelial lesions (L-SIL) and the Atypical Squamous Cells of Undetermined Significance (ASCU-S) were evidenced in 134 (82.2% of total lesions) participants; concerning the high-grade lesions (HL), high-grade squamous intraepithelial lesion (H-SIL) and Atypical squamous cells - cannot exclude H-SIL (ASC-H) were revealed in 29 women (17.8% of total lesions) (Table 3). Comparing the data of the lesions with the presence of *G. vaginalis* reveals that in the coinfection, low-grade lesions were observed in 99 cases (79.2%), compared to 35 (92.1%) in the absence of *G. vaginalis*, and that 26 (20.8%) cases of high-grade lesions were observed in the presence of *G. vaginalis*, compared to only 3 (7.9%) cases when *G. vaginalis* was absent ($p = 0.07$). The odds ratio (OR) of having high-grade lesions in the presence of *G. vaginalis* was 3.064 (Table 3).

Moreover, a negative correlation between all-grade cytomorphological lesions and *Lactobacillus* positivity was found (Table 2). Lesions were found in 67.7% (109/161) of negative and 50% (54/108) of positive *Lactobacillus* samples. The odds ratio (OR) of having all-grade lesions in the presence of *Lactobacillus* spp. was 0.477 (Table 3). No significant differences were found between *Lactobacillus* spp. and cytomorphological lesion grade. Considering *G. vaginalis*-positive samples only, we did not find significant differences in the presence of cytomorphological lesions between *Lactobacillus* positive and -negative samples (not shown). However, while not significant, a smaller number of high-level grade lesions ($n = 3$; 12%) were found in *Lactobacillus* positive samples compared to *Lactobacillus* negative ones ($n = 23$; 23%). Finally, a significant difference in cervical cytomorphological lesions ($p < 0.05$) has been found in the age groups:

Table 3 Cytomorphological lesion presence and level in HR-HPV positive samples related to *G. vaginalis* and *Lactobacillus*

	Cytomorphological lesions [a]			p^b	OR (95% CI)	Cytomorphological lesions [a]			p^b	OR (95% CI)
	Total	Negative	Positive			Total	+ (L-SIL, ASC-US)	++ (H-SIL, ASC-H)		
Total	269	106 (39.4)	163 (60.6)			163	134 (82.2)	29 (17.8)		
G. vaginalis										
Negative	78	40 (51.3)	38 (48.7)			38	35 (92.1)	3 (7.9)		
Positive	191	66 (34.6)	125 (65.4)	*< 0.01*	1.994 (1.168–3.403)	125	99 (79.2)	26 (20.8)	*= 0.07*	3.064 (0.873–10.755)
Lactobacillus										
Negative	161	52 (32.3)	109 (67.7)			109	86 (78.9)	23 (21.1)		
Positive	108	54 (50.0)	54 (50.0)	*< 0.01*	0.477 (0.289–0.788)	54	48 (88.9)	6 (11.1)	*ns*	0.467 (0.178–1.227)

[a] n; percentage with respect to "total" columns in brackets

[b] chi-squared test

90% from the 51–55 years old group to 47% in the 20–25 year old group (Fig. 1).

Discussion

Cervical cancer is one of the most prevalent and fatal malignancies affecting women, and studies have confirmed that persistent high-risk HPV infection is the leading cause of pre-cancerous lesions and, eventually, cervical cancer in females [32, 33].

In this study, we explored the vaginal microbiota of HR-HPV-infected women and evaluated the presence of clinical cervical lesions using the Pap test. We initially focused on *G. vaginalis*, since previous studies have identified this bacterium as a key biomarker directly [34, 35] or indirectly [36] associated with CIN2+, and available evidence demonstrates an association between *Gardnerella* spp. and persistent HR-HPV infection and the progression of cervical lesions [36, 37]. Furthermore, other studies reported that women with persistent HPV16 and HPV18 infections have increased baseline loads of *G. vaginalis* [38], suggesting that this bacterium was the key component of anaerobic diversity responsible for symptoms found in BV patients [39, 40].

In our study, the overall HR-HPV prevalence in the population attending the north of the Marche region diagnostic laboratory was 37.2% (269/723), a value slightly higher than that reported in other studies [41, 42]. The HPV-16 was the most prevalent detected genotype, followed by HPV-18, while in 180 samples, one of the other HR HPV (31,33,36,39,45,51,52,56,58,66,68) was isolated; these results are in agreement with other reports [42–44]. *G. vaginalis* was isolated in 51.8% of samples, and vaginal microbiota was dominated by *Lactobacillus*.

The possible role of some specific bacterial species of the genital tract microbiota in the etiology of endometrial cancer was reported for the first time by Marina Walther et al. in 2016 [45]. Today, however, the influence of local microbiota in the natural course of HPV infection is highly debated in many studies [46] as the vaginal ecosystem is a very heterogeneous microbial site where a number of both beneficial bacteria as well as opportunistic pathogens reside and proliferate.

More frequently (71%), *G. vaginalis* was isolated together HR-HPV, suggesting a significant association of the two species with cervical vaginal infection, in agreement with other studies [43].

Our findings highlighted an increased case of a cervical squamous epithelial lesion in the coinfection of HR-HPV and *G. vaginalis*, in agreement with [47, 48]. These results would suggest an increased risk of cervical cancer in women coinfected with HR-HPV and *G. vaginalis*. Conversely, no significant associations were found with other STIs (*U. parvum*, *M. hominis*, *U. urealyticum*, *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*). On the contrary, there are studies that suggest that there is a connection between HPV infection and other STI agents [49–51].

The higher risk of HR-HPV infection in the presence of *G. vaginalis* can be attributed to the expression of several virulent factors. Among these, sialidase is an enzyme that breaks down cervical and vaginal mucus [40], and vaginolysin, which causes the lysis of vaginal epithelial cells, is a major pathogenic trait of *Gardnerella* spp. Furthermore, sialidase can break down vaginal mucins, decreasing the viscosity of local secretion, which makes pathogens more susceptible to infection [52]. *G. vaginalis* can also induce biofilm formation [41]. Other authors suggest that the association between HPV and *G. vaginalis* may be tied to the ability of *Gardnerella* to be immunosuppressive in the cervicovaginal region [53].

Our findings also highlighted a significant correlation between decreased *Lactobacillus* spp and increased *G. vaginalis* infection. These results are in agreement with [54], which pointed out a correlation between decreased Lactobacillus and increased *Gardnerella* presence with HPV-induced cervical cancer. In most women, the vaginal microbiota consists mainly of *Lactobacillus* species, which creates a low pH environment protecting against both exogenous bacteria and viruses [28], and reduces the risk of progression of HR-HPV infection [55]. A microbiota characterized by a low presence of Lactobacillus spp.

(especially L. crispatus) reduces lactic acid production and increases vaginal pH, creating an environment favorable to HPV infection and its persistence. Bacteria such as *G. vaginalis* or *Atopobium vaginae* are often associated with this risk factor. Conversely, HPV infection can alter the cervicovaginal microenvironment, triggering changes in the microbiota and promoting dysbiosis that facilitates progression to high-grade lesions or cancer [56].

The beneficial action of *Lactobacillus* can be due to lactate production that increases the viscosity of cervical mucus that traps the viral particles and inhibits the access of papillomavirus to basal keratinocytes, assuming an essential role in maintaining the cervical epithelial barrier [55]; other antimicrobial substance produced by *Lactobacillus* that play essential roles in host defense are bacteriocins, lactic acid and hydrogen peroxide (9). In our study, the beneficial action of *Lactobacillus* was also shown in the slight reduction of cytomorphological lesions although this effect is not significant.

Our diagnostic approach does not differentiate between *Lactobacillus* species with markedly different—and in some cases opposing—clinical implications. For example, *L. crispatus* is strongly associated with vaginal health and reduced HPV persistence, whereas *L. iners* and *L. jeseniiis* are frequently observed in transitional or dysbiotic states and may coexist with BV and HPV infection [55]. Thus, even though our results would suggest a beneficial role of *Lactobacillus* in the prevention of HPV infection, this hypothesis needs to be further investigated at the species level. Finally, the prevalence of HR-HPV, the prevalence of *G. vaginalis* infection, and cervical cytomorphological lesions varied significantly with the women's age.

These results showed the importance of simultaneous diagnosis of sexually transmitted infections in the genital tract, contributing to the growing body of evidence on the vaginal microbiome's role in cervical health, and can be useful in preventing adverse clinical outcomes of uterine cancer.

However, our study is not without limitations. Firstly, the RealTM Quant kit test used in this study is a method that non differentia between *Lactobacillus* species with different clinical implications, this limits the biological interpretability of the findings. Secondly, we have not utilized 16 S and WGS for the identification of the taxa of *G. vaginalis*.

Conclusion

In conclusion, the results of this work strengthen the hypothesis of the link between cervical lesions and vaginal microbiota, that is emerging as a biomarker for predicting disease outcome, as well as the monitoring of some bacterial species, in particular *G. vaginalis* and *Lactobacillus*, can be potentially helpful for diagnosis and risk stratification.

Abbreviations

HPV	Human papillomavirus
HR-HP	High-risk humano papillomavirus
Real-time PCR	Real-time Polymerase chain reaction
CIN	Cervical intraepithelial neoplasia
HSIL	High-grade squamous intraepithelial lesion
LSIL	Low-grade squamous intraepithelial lesion
ASC-US	Atypical squamous cells of unknown significance
ASC-H	Atypical squamous cells of cannot exclude high-grade squamous intraepithelial lesion

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Author contributions

Angela Serafini: Methodology, Investigation, Formal analysis, Data curation. Giuditta Fiorella Schiavano: Writing-original draft; Writing – review & editing, Conceptualization, Validation. Mauro De Santis: Writing-original draft; Data curation. Michele De Nictolis: Validation. Giorgio Brandi: Supervision, Conceptualization, Project administration, Writing-original draft; Writing – review & editing.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to partecipate

The study complied with the principles of the Declaration of Helsinki and European data protection regulations (Regulation 2016/679). Informed consent was obtained from participants, and the study received approval from the Marche territorial ethics committee with a favorable opinion on 12/12/2024 Prot. 2024 242.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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