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Transition from opportunistic cytological to organized screening program with DNA-HPV testing detected prevalent cervical cancers 10 years in advance

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Cervical cancer screening in Brazil is opportunistic, based on cytology and offered for women aged 25–64 years, with low coverage (30%) and 70% of cancer diagnoses done in advanced stages, without impact on mortality. The current study reports 5-year first-round results of a population-based DNA-HPV testing screening program in a Brazilian city, which intended to be a model for transition to a more efficient program. Program flowchart is simple and current, indicating repetition of a negative test after five years. The first-round (October 2017-September 2022) screened 20,551 women by DNA-HPV testing with 58.7% coverage and 99.4% compliance with the program's targeted age range. Coverage increases to 77.8% when excluding the 'pandemic period'. The DNA-HPV testing was 87.2% negative with 6.2% colposcopy referrals and 84.8% colposcopies performed. A total of 258 high-grade precursor lesions and 29 cervical cancers (mean age = 41.4 years, 83% Stage I) were detected. As a reference, 41,387 cytology tests from the previous program (2012–2016) detected 36 cervical cancers (mean age = 52.0 years, p = 0.0005), with 67% in advanced stages (p < 0.0001). Organizing cervical cancer screening using DNA-HPV testing demonstrated good coverage, high age and colposcopy compliance, and detection of more precancerous lesions and cervical cancers 10 years in advance.

Globally, annual deaths from cervical cancer are high, especially in low- and middle-income countries[1]. In Brazil, 17,010 new cervical cancer cases are expected in 2023 with 6606 deaths, or one death every 80 minutes[2]. Population-based screening is thus essential to reduce mortality. The Brazilian cervical cancer screening program began in 1984 and was based on conventional cytology (Pap-test)[3]. The latest National Cancer Institute (INCA) guideline (2016), recommends Pap-test screening every 3 years in women aged 25–64 years[4]. The public health system (*Sistema Unico de Saude*, SUS) offers free testing, diagnosis, and treatment to women with a cervix who are sexually active, including transsexual men and non-transsexual binary individuals designated women at birth[4]. Despite these national program and strategic initiatives, coverage remains low, and cervical cancer incidence and mortality rates have remained relatively stable for decades[2].

The primary factor impeding progress may be the opportunistic pattern of Brazil's current screening program, in which testing is conducted on demand. Due to excess cytology performed outside the official guidelines and low effective coverage, 60–70% of cervical cancers are diagnosed in advanced stages[5,6].

In the 1990s, some European countries transitioned from opportunistic to organized screening programs, resulting in sustainable reductions in cervical cancer mortality[7,8]. Successful screening programs must be well-organized, inform the population, achieve good coverage, ensure access to diagnosis and treatment, and maintain guideline-compliant quality and continuous monitoring[3].

Cervical cancer is thought to be caused by persistent infection with high-risk human papillomaviruses (hr-HPVs), primarily HPV-16 and HPV-18[9]. Several studies demonstrated the superiority of population-based screening using primary DNA-HPV testing[10,11]. In 2013, the World Health Organization (WHO) recommended replacing cytology-based careening with HPV test-based screening.

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We initiated a population-based demonstration project in 2017 to identify indicators related to epidemiologic, cost–benefit ratios, and life-gain factors to support transition of the current Brazilian opportunistic cytology-based screening strategy to an organized program based on HPV testing[12] Previous results demonstrated the cost-effectiveness and compliance with program guidelines of our approach. After 30 months, increases were observed in the rates of early cancer diagnosis and identification of cervical precursor lesions[13–15].

Here, we report the 5-year first-round performance of the screening program in terms of detecting precancerous lesions and cervical cancers.

Results

In the first round, the PREVENTIVO program screened 20 551 women by DNA-HPV testing, with increasing population coverage until the COVID-19 pandemic started in March 2020, achieving 58.7% (20,551/35,000) coverage of the target population. Excluding the 17 months related to the 'pandemic waves period', the coverage was 77.8% (19,144/24606). The impact of the pandemic waves is shown in Fig. 1. Considering the pandemic period, the period from April 2020 to July 2022, only a limited number (n = 1286) of screening tests were performed. From August 2021 onward, when primary healthcare facilities began returning to normal activity, the number of tests increased; however, testing did not reach the previous number. Comparing the 6 months before the pandemic with the 6 months after the pandemic's third wave 'ended', the average number of the tests performed per month was 396 and 320, respectively.

The results of the tests used in both screening program (DNA-HPV-based and conventional cytology) are summarized in Table 1. Negative DNA-HPV test results were observed in 87.2% of women (*vs.* 98.6% in the cytology program, $p < 0.0001$). Overall, 3.5% tested positive for HPV-16 and/or HPV-18, and 9.3% tested positive for one of the 12 other hr-HPVs and performed a reflex cytology. Reflex cytology results were negative in seven of each 10 cytology tests, who were recommended to return after 12 months to repeat the DNA-HPV test. The colposcopy referral rate was 6.2% for DNA-HPV testing, a rate that was 4.4 times higher than that of the previous cytology program (1.41%, 584/41,383, $p < 0.0001$).

The target age compliance for the first round of the DNA-HPV test program was 99.4%, with 122 tests (0.6%) performed in women aged 20–24 years and 122 tests (0.6%) in women ≥ 65 years old (considered an 'exit of screening' test). In the previous 60 months of the cytology program, 16.4% of tests (6797/41,383) were performed in women < 25 years old (6.3% performed in women up to 19 years old; Table 2).

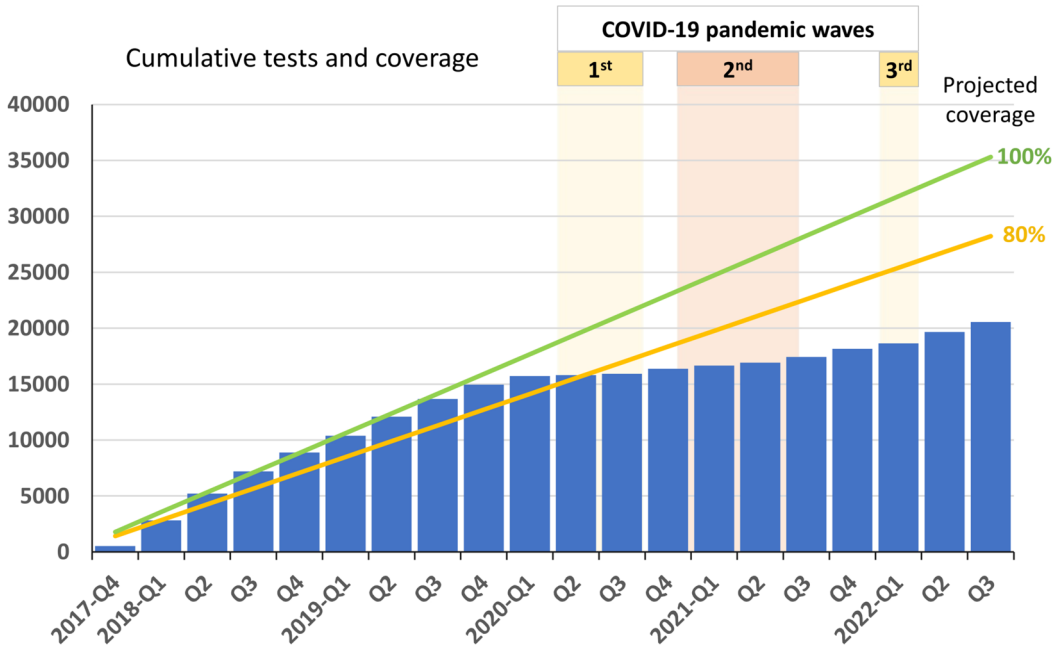
Colposcopy compliance was high in the PREVENTIVO program, with a diagnosis reached in 84.8% (1073/1266) of cases referred for colposcopy. For the cytology program, data were available for only 37.2% (217/584) of the colposcopies indicated (Table 1).

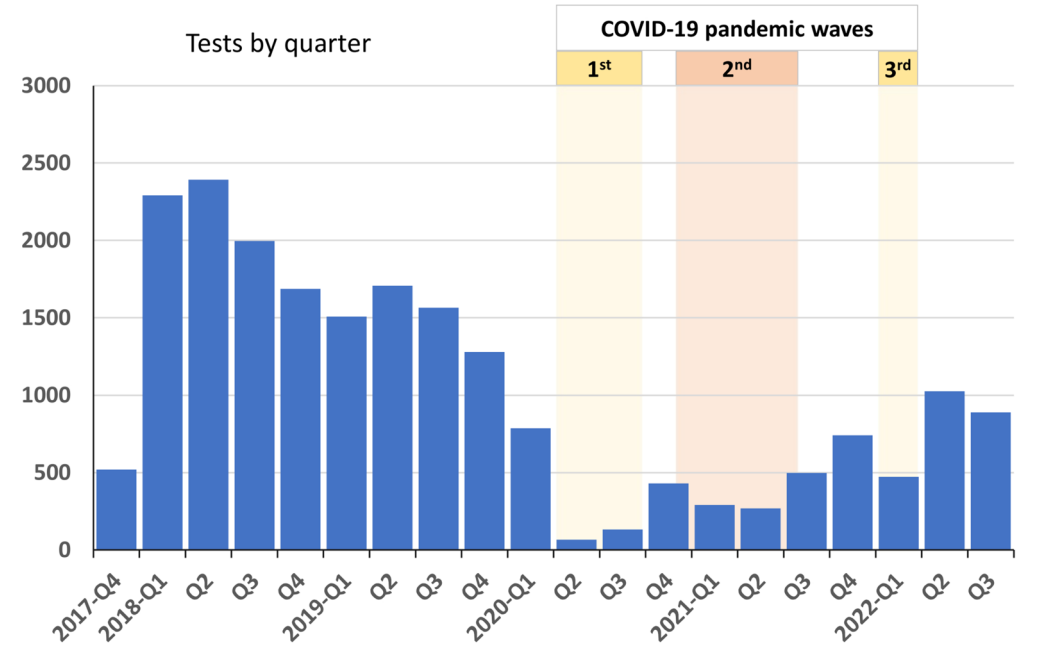
The new screening program detected twice as many significant precancerous lesions (113 CIN2, 137 CIN3, and 4 adenocarcinomas in situ—[AIS]) than the previous cytology program (36 CIN2 and 90 CIN3, $p < 0.0001$). The detection rate for CIN2 was 6.1 and for CIN3/AIS 3.1 favored the HPV testing program. Additionally, the new program showed an LSIL detection rate of 10 times more (n = 231 *vs.* n = 46 detections in the cytology program; $p < 0.0001$; Table 1).

Table 2 summarizes the proportion of screening tests, colposcopy referral rates, and final diagnosis by age group. Considering the target age group of 25–64 years, the programs performed a similar proportion of tests by age group. The odds ratio for colposcopy referral for all age groups tested was significantly higher (4–5 times) with the DNA-HPV test program than the cytology screening program. The indication for colposcopy declined by age group in both programs. For the younger age group (25–29 years), the DNA-HPV test program exhibited a colposcopy referral rate of 12.3% (338/2742), detecting 65 HSIL (including two AIS) and two cervical cancer (Stage IA1, microinvasive, Table 3) cases. In the cytology program, the colposcopy referral rate for the same age group was 2.4% (97/4012) with 26 HSIL and no cervical cancer cases detected. No significant differences in the proportion of HSIL detection were observed in the DNA-HPV test program for the age groups 25–29 years versus 30–39 years (2.4% *vs.* 1.9% CIN2/3 [$p = 0.1877$], respectively; and 1.1% *vs.* 1.2% CIN3, respectively [$p = 0.7759$]).

Considering screening tests performed outside the target age group, a total of 122 DNA-HPV tests performed in the 20–24 years age group exhibited a low rate of HSIL detection (3.3%, 4/122), and no HSIL cases were detected in 122 women aged ≥ 65 years. In the previous cytology program, by comparison, the HSIL detection rate in the 20–24 years of age was 0.4% (15/4192). The HSIL detection rate in women aged ≥ 65 years was 0.02% (2/2492), with seven cervical cancer cases detected, all of which were symptomatic and in an advanced stage (two Stage II, three Stage III, and three Stage IV).

A detailed analysis of the cervical cancer cases diagnosed in both programs is shown in Table 3. A total of 29 cases were detected in the DNA-HPV program (mean age 41.4 years), and 36 cases were detected in the cytology program (mean age 52 years, $p = 0.0005$). Glandular-type cancers accounted for 28% of cases detected in the DNA-HPV test program and 19% of cases detected in the cytology program ($p = 0.7369$). A total of 83% of cancers detected in the DNA-HPV program were FIGO Stage I, and cases with FIGO Stage II-IV predominated in the cytology program, at 67% ($p < 0.0001$). The DNA-HPV test detected 62% (18/29) of microinvasive cancers (FIGO Stage IA), compared to only 14% (5/36) detected in the cytology program. A review of the status of cancer cases in the DNA-HPV testing program revealed that one 48-year-old woman died 6 months after diagnosis (Squamous cell carcinoma, Stage IIIB), and 10 deaths were recorded in the previous cytology program, all of which involved Stage III-IV cancers in women with a mean age of 58 years.





Test (n)	13 144	2373	200		733		474	1917
Month average	571	396	33		92		158	320

Fig. 1. Cumulative number of HPV screening tests performed in the first round (60 months) of the program (bars; total tests = 20,551), including the COVID-19 pandemic period. Population considered: 35,000 women using the public health system aged 25–64 years from the official 2020 population estimate[23,24]. The first-round coverage was 58.7% (20,551/35,000). For reference, lines represent the projected cumulative population coverage for 5 years: 80% coverage (orange line) and 100% coverage (green line). The monthly mean of number HPV tests performed during the COVID-19 pandemic waves in Brazil were 33 tests/month in April-September 2020, 92 tests/month in December 2020-July 2021, and 158 tests/month in January-March 2022). Six months before and after the pandemic, the average tests/month was 396 (November 2019-February 2020) and 320 (April-September 2022). Excluding 17 months related to the 'pandemic waves period', the coverage increased to 77.8% (19,144/24,817).

Outcome	Screening program DNA-HPV test October 2017-September 2022 (n = 20,551)		Outcome	Screening program Cytology test January 2012-December 2016 (n = 41,383)		d	
	n	%		n	%	Rate[#]	*P-value**
Test result			Test result				
Negative	17,925	*87.2*	*Negative*	40,799	*98.6*	–	< 0.0001
HPV16 + [α]	506	*2.5*	*ASC-US*	364	*0.9*	–	
HPV18 + [α]	174	*0.9*	*LSIL (CIN1)*	115	*0.3*	–	
HPV16 + and 18 + [α]	33	*0.2*	*HSIL (CIN2)*	53	*0.1*	–	
12OT hr-HPV + and cytology +	554	*2.7*	*HSIL (CIN3)*	37	*0.1*	–	
12OT hr-HPV + and cytology(-)	1352	*6.6*	*AGC and AIS*	7	*0.02*	–	
			Cancer invasive	8	*0.02*		
Colposcopy referral	1266	*6.2*	Colposcopy referral[β]	584	*1.4*	***4.4***	< 0.0001
Diagnosis completed[ε]	1073	*84.8*	Diagnosis completed[ε]	217	*37.2*	–	
Negative	559	*2.7*	*Negative*[γ]	9	0.02	–	
LSIL	231	*1.1*	*LSIL*	46	*0.1*	***10****.2*	< 0.0001
HSIL (CIN2)	113	*0.6*	*HSIL (CIN2)*	36	*0.1*	***6****.1*	< 0.0001
HSIL (CIN3)[δ]	141	*0.7*	*HSIL (CIN3)*	90	*0.2*	***3****.1*	< 0.0001
Cancer	29	*0.14*	*Cancer*	36	*0.09*	***1.6***	0.0504

Table 1. Outcomes from the 5 year first round of the screening program based on the DNA-HPV test and from the previous opportunistic screening test using conventional cytology. DNA-HPV test program considered colposcopies performed until May 2023. *CIN* cervical intraepithelial neoplasia, *12OT hr-HPV* 12 other high-risk human papillomaviruses (types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), *ASC-US* atypical squamous cell of undetermined significance, *LSIL-HSIL* low-grade or high-grade squamous intraepithelial lesions, AGC atypical glandular cells. *Chi-squared test. [#]Rate: HPV test/Cytology test. [α]HPV-16 and/or -18 positive ± 12OT hr-HPV positive. [β]Colposcopy indicated if two consecutive abnormal cytology tests in six-12 month intervals for ASC-US and in six months for LSIL[4]. [ε]Diagnosis completed: worse histologic result by biopsy or excision of the transformation zone. [γ]Negative in cytology program: nine cases biopsy negative, 359 cases with missing information (if colposcopy negative without biopsy or colposcopy not done), and eight cases 'unknown' (four dropped out and four with missing biopsy result). [δ]HSIL (CIN3): four AIS cases added in the HPV test program.

Discussion

The 5 year first round of the PREVENTIVO screening program utilizing primary DNA-HPV testing showed high coverage of the target group (25–64 years old), even considering the COVID-19 pandemic. Age compliance was nearly 100%, with a higher rate of precancerous lesion detection, potentially enabling diagnosis of prevalent cervical cancers as much as 10 years in advance and at an early stage.

These remarkable results were obtained in a population with access to the opportunistic screening program based on conventional cytology. The achievements of the present DNA-HPV test reflect an organized program implementing a more accurate test combined with higher coverage and high proportion of adequate follow-up of women who tested positive.

Proper organization of a cervical cancer screening program is crucial to reducing mortality[3]. Cytology-based screening demands a high degree of quality control and is not cost-effective if it does not have adequate coverage[16]. The fragile framework of health systems in low- and middle-income countries, where the incidence of cervical cancer is high, makes overcoming these barriers challenging[17].

In planning the new program, the first obstacle to overcome was the culture of annual screening tests in the previous cytology-based program. The switch to DNA-HPV testing enabled the implementation of new practices. Another advance was the implementation of a single flowchart for women aged 25–29 years and those aged ≥ 30 years. Indaiatuba City was chosen because it already had a structured healthcare system in which all care units were networked, and a centralized digital system to control entrance into the screening program and monitor follow-up of abnormal test results was developed. All healthcare professionals were trained to follow the flowchart, and various strategies for providing guidance to patients regarding periodicity and actions to identify candidates for screening were added.

The present first-round analysis revealed high age compliance and sustained coverage, even considering the negative effects of the COVID-19 pandemic. The rapid recovery in the number of tests performed after the pandemic suggests well-targeted and well-coordinated action. Before the new program was initiated, Indaiatuba City performed 11,000 cytology tests annually, with an estimated coverage of 33%.

Although the new program demonstrated significant gains, 4.4 times more colposcopies were performed compared with the previous opportunistic screening program. Though expected, part of this increase can be attributed to expanded coverage. Therefore, it will be necessary to plan how to manage the number of colposcopy professionals and update their training[3]. Another challenge facing colposcopy professionals was the need to

Age group (years)	Screening program																
	DNA-HPV test									Cytology							
	Women screened		Colposcopy referral		Colposcopy done		Final diagnosis (n)			Women screened		Colposcopy referral[#]		Final diagnosis (n)			Colposcopy referral OR (95% CI)	
	n	(%)	n	(%)	n	(%)	CIN1	CIN2	CIN3	n	(%)	n	(%)	CIN1	CIN2	CIN3		
Up to 19	*0*	*(0)*	–	–						2605	*(6.3)*	83	*(3.2)*	5	7	3	–	
20–24	122	*(0.6)*	19	*(15.6)*	17	89.5	3	1	3	4192	*(10.1)*	101	*(2.4)*	6	2	13	7.5	[4.41—12.67]
25–29	2742	*(13.3)*	338	*(12.3)*	274	81.1	64	34	31*	4012	*(9.7)*	97	*(2.4)*	8	9	17	5.7	[4.50—7.15]
30–39	5489	*(26.7)*	416	*(7.6)*	351	84.4	79	40	66*	8437	*(20.4)*	125	*(1.5)*	10	7	34	5.5	[4.45—6.68]
40–49	5583	*(27.2)*	280	*(5.0)*	244	87.1	55	26	26*	8706	*(21.0)*	104	*(1.2)*	5	6	10	4.4	[3.48—5.48]
50–64	6493	*(31.6)*	208	*(3.2)*	182	87.5	29	12	15	10,939	*(26.4)*	68	*(0.6)*	3	3	11	5.3	[4.02—6.99]
≥65	122	*(0.6)*	5	*(4.1)*	5	100.0	1	0	0	2492	*(6.0)*	6	*(0.2)*	3	1	1	17.7	[5.33—58.86]
Total	***20,551***	***(100.0)***	***1266***	***(6.16)***	***1073***	***84.8***	***231***	***113***	***141***	***41,383***	***(100.0)***	***584***	***(1.41)***	***46***[α]	***36***[α]	***90***[α]	***4.6***	***[4.15—5.07]***

Table 2. Distribution of women screened, colposcopy referrals, and colposcopies performed, with final diagnosis by age group and screening program. Final diagnoses: worse histologic result by biopsy or excision of the transformation zone. DNA-HPV test program considered all colposcopies performed through May 2023. Cytology program: total colposcopies done unknown; information available only for cases with histologic results (missing information regarding negative colposcopy results, without biopsy). ***CIN*** cervical intraepithelial neoplasia. [#]Colposcopy indicated if two consecutive abnormal cytology tests with ASC-US in six-12 month intervals or LSIL in 6 months.[4] *Cases of adenocarcinoma in situ (AIS) added: two in the 25–29 years old and one each in the 30–39 and 40–49 years old groups. [α]Counting missing age information for 6 CIN1, 1 CIN2, and 1 CIN3 cases. **OR (95%CI)** odds ratios (95% CIs) for colposcopy referral by DNA-HPV testing, with $p < 0.0001$ for all age groups.

Cervical cancer diagnoses	DNA-HPV test October 2017-September 2022 (n = 20,551)	Cytology test January 2012-December 2016 (n = 41,387)	*P-value*
Detection (n, %)	29 (0.14%)	36 (0.09%)	0.0504*
Age (mean year)	41.4y	52.0y	0.0005[#]
Age group (n, %)			
25–29 years	2 (7%)	0	
30–39 years	8 (28%)	12 (33%)	
40–49 years	15 (51%)	4 (11%)	
50–64 years	4 (14%)	13 (36%)	
≥65 years	0	7 (20%)	
Histology (n, %)			0.7369**
Squamous cell	21 (72%)	29 (81%)	
Adenocarcinoma	6 (21%)	5 (14%)	
Adenosquamous	2 (7%)	2 (5%)	
Stage[α] (n, %)			< 0.0001**
IA (microinvasive)	18 (62%)	5 (14%)	
IB	6 (21%)	7 (19%)	
II-III	5 (17%)	20 (56%)	
IV	0 (0%)	4 (11%)	

Table 3. Cervical cancer cases detected in a 60-month period according to the DNA-HPV test or conventional cytology screening program. *Chi-squared test; **Fisher's exact test; [#]*t*-test. [α]Cancer staging according to FIGO[28].

perform colposcopies without cytology information when HPV-16 and/or -18 were present. The 84.8% colposcopy compliance rate was a program milestone, higher than the 74.6% rate obtained in a similar implementation screening study performed in Argentina (2012–2014) involving 49,000 HPV tests[18].

Detecting CIN2/3 is the primary goal of screening programs[3,10,19]. The PREVENTIVO program detected an impressive number of 254 HSIL compared with 125 cases of HSIL detected in the previous cytology program.

Possible explanations include the higher sensitivity of the HPV-DNA test[11,19], the expanded population coverage, high adherence to the program's flowchart, and the high colposcopy compliance rate. However, this achievement was accompanied by 231 cases of LSIL (CIN1) transient lesions, which overloaded the system. The integration of additional tests in the DNA-HPV program could improve discrimination in the future.

Another significant gain associated with the improvement in screening was the early detection of prevalent cervical cancers, including asymptomatic cancers. The downstaging observed in the first round of the DNA-HPV testing program was significant. There was a remarkable shift from 67% of cancers being detected in advanced stages (FIGO Stages II-IV) in the previous cytology program to 62% of microinvasive cancers (FIGO Stage IA) being detected in the new program. The association between the 10-year drop in mean age and cancer staging inversion observed in the new program means a significant increase in anticipated diagnoses. These findings indicate that more lives and resources will be saved[13].

A challenge addressed by the new program was how to manage women in the 25- to 29-years-old group, as the recommendation was to begin DNA-HPV testing after the age of 30 years. Considering the programmatic issues of maintaining the health structure for cytology screening to a specific subgroup of targeted women, such as the difficulty in controlling actions on the front and the possibility of carrying out two tests, leading to a risk of confusion and loss of economic performance of the program, it was decided to remove all cytology testing from primary healthcare facilities and offer DNA-HPV tests to women 25–29 years old. The HPV-16 and -18 genotyping compensated, in part, for the low specificity of the HPV test in women < 30 years old.

As expected, the 25–29 years age group had more colposcopy referrals, but comparison with the cytology program revealed an odds ratio similar to the 30–39 years age group. A total of 2.4% of detected lesions (65/2742) were HSIL (CIN2/3) in the 25–29 years age group, similar to the 30–39 years age group (1.9%, 106/5489, $p = 0.1877$). Additionally, the DNA-HPV program detected two cancer cases (both microinvasive) in the younger group.

As a geographically large country, Brazil has regions with diverse levels of development. The Brazilian Ministry of Health is concerned about the cervical cancer situation and offers sufficient cytology testing to reach 80% coverage. However, actual coverage does not exceed 30%; thus, cervical cancer mortality rates have remained stable for decades[2,5,20]. Additionally, the first girls vaccinated against HPV in the national program started in 2014 will be screened in 2025. Therefore, 2025 is considered the target for implementing organized screening programs involving primary DNA-HPV testing[21,22]. The present study describes a path to transition programs tested under real-world conditions within the Brazilian public health system, and for the ongoing second round, a reduction in colposcopy referrals and cervical cancer detection are expected, thereby reducing the needs for diagnosis, treatment, and costs of the program.

Another point to be considered is the non-negligible number of cancers detected at age 65 or older, related to a deficiency in the previous cytology program, as previously reported for the same region[6]. In our evaluation, there were seven cases reported for the previous program with cytology, all symptomatic. Although a DNA-HPV test at the exit of the screening was offered to women aged 65 or older, there were no significant lesions detected in 122 women tested. Therefore, in the second round, a campaign is planned to call and test older women aiming at the detection of cancers prevalent in this group.

The main strength of this demonstration study is that it describes, for the first time, the steps to transition the screening programs to a more-targeted organization under real-world scenarios within the Brazilian public health system. The results obtained through the application of a single flowchart are striking and support the modernization of the current Brazilian program. The results could serve as a reference for other countries in similar situations.

Although the PREVENTIVO program was open to all women of target age, the main limitation was the inability to include users of private clinics, which encompasses approximately 50% of the population. Furthermore, replicating this program will require a greater investment in the acquisition of HPV tests and establishing consistent and accessible population records on dedicated digital platforms to successfully run and manage the program. Some data from the previous cytology program may be less reliable, which is expected considering its limited organization, and the results presented were used for reference only and should thus be considered with caution.

In summary, the implementation of an organized screening program for cervical cancer prevention via DNA-HPV testing in a Brazilian city demonstrated good coverage, overcoming the effects of the COVID-19 pandemic and achieving high age and colposcopy compliance in a real-world scenario. The program provided notably increased precancerous lesions detection, potentially enabling cervical cancer diagnosis 10 years in advance, with a high proportion of cancers detected at an early stage. The program results should be reproducible in similar settings in which the burden of this preventable cancer is high.

Methods

This population-based demonstration study evaluated the "PREVENTIVO" program (PREvention of HPV Viruses in ENTire Indaiatuba by Vaccination and Organization of the screening) cervical cancer screening program, which was initiated in October 2017 in Indaiatuba City, Sao Paulo State, Brazil. The program targeted women 25–64 years old for screening via primary DNA-HPV testing. Briefly, the inclusion criteria considered women in the targeted age group (women over 64 years were permitted for a single exit test by personal request) who have started sexual activity and are physically well enough to undergo a pelvic exam. The entrance should be postponed if any of the following conditions are met: 40 days postpartum period, abundant genital bleeding, cervicovaginal infection, and having undergone the last cervical cytology in the last 12 months. The exclusion criteria considered women who have already undergone DNA-HPV testing for different medical purposes

except screening, with a previous diagnosis of the cervical lesion followed in specialized clinics, and women who underwent a total hysterectomy.

The study considered information derived from the first round of the new program, from October 2017 to September 2022, including the COVID-19 pandemic period. The program protocol was previously reported[12].

The program targeted women utilizing their municipal public health system, which corresponded to 50% of the total targeted population of 70 573 (estimated correct for 2020)[23,24]. Therefore, 35,000 women constituted the target population for coverage calculation[24] No consistent information was available regarding HPV vaccination status among the screened women. However, the Brazilian National Vaccination Program, started in 2014, offers vaccination free of charge to girls 9–14 years old. These girls, who were vaccinated in 2014, will reach the screening age in 2025; therefore, we assumed that nearly 100% of women screened in the new program were unvaccinated.

The COVID-19 pandemic impacted the study. In Brazil, to control COVID-19, the government recommended to stay at home on March 20, 2020, and screening activities associated with this study were suspended. In July 2020, the Ministry of Health recommended a gradual return to normal activities[25]. In Sao Paulo State, the pandemic evolved in three 'waves': April-September 2020, December 2020 to July 2021, and January-March 2022[26]. Additionally, we considered the 6-month period from November 2019 to February 2020 as 'before' the pandemic and the 6-month period of April to September 2022 as 'after' the pandemic.

The first-round evaluation focused on coverage, age and colposcopy compliance, COVID-19 pandemic impact, and diagnosis of cervical lesions. The program's performance among women 25–29 years old was also evaluated.

HPV test selection

The Cobas® HPV test (Roche Molecular Systems, Pleasanton, CA, USA) was used for screening in the study. This test was previously validated for screening programs in large clinical trials[10,19]. The Cobas HPV test simultaneously provides individual results regarding the highest-risk genotypes (HPV-16 and HPV-18) and aggregates results for the 12 other hr-HPV genotypes (types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).

Management flowchart

Briefly, the following clinical procedures were followed based on DNA-HPV test results:

- Negative: return after 5 years to repeat the test;
- Positive HPV-16 and/or HPV-18 test: referral for colposcopy, and if necessary, biopsy;
- Positive HPV test for the 12 other hr-HPVs: liquid-based cytology performed on the same sample (reflex test). The woman was referred for colposcopy if any abnormality observed in cytology results: atypical squamous cells of undetermined significance (ASC-US), low- or high-grade squamous intraepithelial lesions (LSIL, HSIL), suspicion of a glandular lesion, or cancer[27]. If the cytology result was negative, the woman should return after 12 months to repeat the DNA-HPV test.

According to study protocols and the centralized informatics program system of the PREVENTIVO program, a woman can be screened only once within her screening round. Women with abnormal test results or indications for colposcopy were referred to a reference outpatient clinic. Excision of the transformation zone (ETZ) was indicated when cervical biopsy resulted in a grade 2 cervical intraepithelial lesion (CIN) confirmed by $p16^{INk4a}$ immunohistochemistry positivity (Ventana Medical Systems Inc., Tucson, AZ, USA), CIN3, or suspicion of micro-invasiveness. Cases suspicious for cervical cancer or those involving a more complex clinical situation were referred to the regional gynecological cancer center at the Women's Hospital of the University of Campinas. Pathologic evaluation according to the WHO classification, radical surgery, radiotherapy, or chemotherapy to manage cervical cancers were performed at the Women's Hospital. All cases in which a colposcopy referral was submitted for evaluation and a diagnosis reached were counted in the colposcopy compliance calculation. The final diagnosis was the worst grade of the histological evaluation of tissue obtained from colposcopy-directed cervical biopsy or ETZ. Cancer cases were staged according to the FIGO system[28].

Data collection and analysis

The study considered data for women screened during the 60-month first round of the new program. All data were retrieved and generated by the health information system. The research team had access to all data and participated in quality control surveillance of the program.

As an historical reference, the study also considered data from the same city for 41 387 cytology screening tests conducted between January 2012 and December 2016 (60 months before implementation of the PREVENTIVO program). All cytology tests were processed in the Regional Cytopathology Laboratory of the University of Campinas, which processes 100% of cytology tests conducted by the public health system. Cytology results were reported according to guidelines of the Bethesda System[27]. Some women had more than one cytology tests during the period, and in cases in which there was more than one abnormal cytology test indicating colposcopy (ASC-H, HSIL, suspicion of a glandular lesion, or worse), one test was counted. Colposcopy was indicated if a cytology result was ASC-US in two consecutive tests conducted over a 6- to 12-month interval or a LSIL in a 6-month interval. As a pattern consistent with opportunistic screening programs, some follow-up data were missing due to the absence of central regulatory coordination. Identification was available only for cases with abnormal cytology, and data were only available for positive colposcopies and their subsequent procedures

(biopsy or ETZ; 217 known diagnoses among 584 colposcopy referrals). Data regarding negative colposcopy results or the total number of colposcopies performed were unavailable.

Data analyses were performed using the chi-squared teste, Fisher's test, or *t*-test with StatsDirect statistical software 3.0 (Wirral, UK, www.statsdirect.com). *P*-values < 0.05 were considered significant.

Ethical aspects

The Research Ethics Committee of the University of Campinas approved this study (number 1045580; May 1, 2015). The Committee waived the need for informed consent. All study procedures were carried out in accordance with the approved research protocol and followed the regulatory standards of the National Health Council of Brazil. In 2017, the mayor of Indaiatuba sanctioned a law instituting the DNA-HPV test as the standard for screening, replacing conventional cytology in all public health systems[29]. The research group accessed electronic data from the health information system and created a spreadsheet for statistical analyses, without identifying subjects.

Data availability

The dataset from this study will be safely stored following the principles of research ethics. Upon completion of the study, data may be made available by the corresponding author (juliotex@unicamp.br) upon request with justification.

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Author contributions
JT, DV, CC, JB, and LZ developed the research protocol. JT, CC, IP, and MD working to collect the data. JT, DV, IP, MD, and LZ coordinated the analysis and the development of this manuscript. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

Ethical approval
Some parts of this manuscript were derived from an academic master's dissertation by I. Polegatto under the tutorial of J Teixeira and submitted to the Postgraduate Program of Obstetrics and Gynecology from the University of Campinas, Brazil.

Additional information
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