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CANCER EPIDEMIOLOGY

Detection of high-grade cervical disease among women referred directly to colposcopy following a positive HPV screening test varies with age and cytology findings

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Novelty and impact (75 words max):

This report describes the first follow-up colposcopy findings in Australia's new human papillomavirus (HPV)-based cervical screening program. Nearly one in four women referred directly for colposcopy under a risk-based algorithm, had an underlying histological high-grade abnormality (HGA). Detection of HGA increased with increasing grade of reflex cytology, but there was a substantial

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reduction in the detection of HGA with increasing age. Further refinement of the algorithm to account for age may be warranted.

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ABBREVIATIONS

AC	Adenocarcinoma
AIS	Adenocarcinoma in situ
ASC-H	Atypical squamous cells cannot exclude high grade squamous intraepithelial lesion
ASC-US	Atypical squamous cells of undetermined significance
HGA	Histological high-grade abnormality
HPV	Human papilloma virus
HSIL	High-grade squamous intraepithelial lesion
LBC	Liquid based cytology
LGA	Histological low-grade abnormality
LSIL	Low-grade squamous intraepithelial lesion
NATA	National Association of Testing Authorities, Australia
RCPA	The Royal College of Pathologists of Australasia
SCC	Squamous cell carcinoma

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ABSTRACT (unstructured 250 words)

Australia's new HPV-based cervical screening program is based on an algorithm that incorporates reflex cytology to guide decisions about further follow-up with colposcopy and, if indicated, biopsy. We reviewed results for 2,300 women referred directly for colposcopy following their first positive HPV screening test, to determine the proportion that had underlying histological high-grade abnormality (HGA). Overall, HGA was detected in 24.3% of women. Among HPV16/18 positive women, 18.0% had HGA, increasing from 6.6% among women with negative cytology to 79.7% among women with high-grade squamous lesion or worse, or any glandular lesion on cytology (HSIL+; p-trend<0.001). For this latter group, the proportion with HGA was higher among HPV16/18 positive women than among those positive for other oncogenic types (68.8%) (p=0.029). Among women with ASC-H cytology, 51.8% had HGA, with no difference between HPV groups (p=0.314). In analyses by age-groups, detection of HGA was highest, at 36.4%, among women younger than 35 years, then decreased significantly to 5.9%, among women aged 65–74 years (p-trend<0.001). The relationship of decreasing HGA detection with increasing age was strong for women with negative cytology, and those with ASC-H cytology (p-trend<0.001 for each). For women with HSIL+ cytology, detection of HGA was high and stable, regardless of age (p-trend=0.211). This report describes the first follow-up colposcopy findings in Australia's new HPV-based cervical screening program. The results demonstrate the additional value of reflex cytology in managing HPV positive women and suggest that further refinement of the risk-based algorithm to account for age may be warranted.

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INTRODUCTION

In December 2017, the Australian National Cervical Screening Program (NCSP) underwent major changes, switching from biennial cytological testing of women aged 18 to 69 years to five yearly primary human papillomavirus (HPV) nucleic acid amplification testing of women aged 25 to 74 years[1]. These changes were informed by a detailed review (referred to as 'Renewal' of the NCSP), which took account of limitations of cytology and advances in HPV detection technology, as well as Australia's highly successful National HPV Vaccination Program, which has led to substantial reductions in the incidence of cervical abnormality among vaccine-eligible women[2]. The cytology-based primary screening program had been successful in reducing the incidence and mortality from invasive cervical cancer by more than 50% following introduction in 1991, but rates have since plateaued[3].

Testing for the presence of oncogenic HPV has been shown to be more sensitive, but less specific than cytology for detecting underlying disease[4]. To improve the specificity of the test, screening in Australia's renewed program includes genotyping for the most oncogenic HPV types, 16 and 18, and reflex liquid-based cytology (LBC) following detection of any oncogenic type[1]. The nature and timing of subsequent investigations is managed according to a woman's risk of having or developing significant cervical abnormality (low, intermediate, or higher risk) as indicated by the screening test result (See Figure 1)[1]. Among screen positive women, this risk-based approach offers the opportunity to identify, and directly refer for colposcopy, women deemed at greatest risk of underlying disease, while delaying further, and potentially unnecessary, investigations among those in whom a substantial proportion of HPV infection is likely to clear.

We previously reported that 2.6% of 156,683 women aged 25–74 years screened by a large private pathology provider in the first six months of the program were classified as higher risk and referred for colposcopy[5]. More recently these findings have been replicated in national data, with 2.5% of primary screening tests in 2018 across Australia classified as higher risk[3]. These referral rates are substantially higher than those based on primary cytology[5, 6]. While increases in colposcopy referrals were anticipated due to the higher sensitivity of a screening test based on HPV, data from the program on the detection of histological abnormality following colposcopy, have not so far been available. Furthermore, anecdotal clinical observations have emerged from colposcopy clinics,

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regarding the lack of recognisable abnormalities at colposcopy in older women but empirical evidence to support these reports is lacking[7]. We therefore undertook an analysis of results from 2300 women referred for colposcopy to determine the proportion that had underlying histological high-grade abnormality including cancer (HGA), and examined the relationship to age, HPV type and reflex cytology results.

MATERIALS AND METHODS

The study sample was drawn from women who underwent HPV testing for cervical screening by medical practitioners who referred specimens to a single, large general pathology laboratory in Sydney between 1 December 2017 and 31 May 2018[5]. The laboratory has an in-house follow-up system for colposcopy and histology results, using standardised form letters which are sent to all referring doctors in the state of New South Wales, except for areas outside the follow-up network. Eligible for inclusion in the current study were all women whose HPV screening resulted in a classification of higher risk (See Figure 1), and who were therefore referred for colposcopy. Letters were sent in May 2019, 12 months following the issuing of the last of the higher risk reports and returned over the subsequent months.

As previously reported HPV testing was performed on the Cobas 6800 platform (Roche Diagnostics, Indianapolis,IN, USA)[5]. Cytology was reported using ThinPrep Imaging System (TIS, Cytyc Corp, Malborough, MA), as per The Bethesda System for Reporting Cervical Cytology 2014[8]. Histology results were reported, based on the Lower Anogenital Squamous Terminology, with the addition of glandular categories of adenocarcinoma in situ and adenocarcinoma[9].

All follow-up reports received from doctors were categorised by the highest level of investigation, either colposcopy alone, or colposcopy with biopsy and histology. The findings were then grouped into three categories according to clinical significance, as follows: normal, referred to colposcopy alone, that was either normal or had low-grade features, or normal colposcopy followed by negative histology on biopsy; ‘low grade abnormality’ (LGA) meant a histology result report of low-grade squamous lesion (LSIL); ‘high grade abnormality’ (HGA) indicated a histology result of high-grade squamous lesion (HSIL), adenocarcinoma in situ (AIS), adenocarcinoma (AC) or squamous cell carcinoma (SCC).

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The primary study outcome was detection of HGA that was confirmed on histology, expressed as a percentage of all higher risk women for whom follow-up was available. For the primary analyses, women with normal colposcopy were included in the denominator. A sensitivity analysis limited to women with biopsy and histology results (i.e. excluding the normal colposcopy group from the denominator) was also performed. We calculated these percentages overall, and by 10-year age groups, dividing HPV types into HPV16/18 or other oncogenic HPV types (not 16/18), and reflex cytology results were classified as: negative; atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion (ASC-US/LSIL); atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H); high-grade squamous intraepithelial lesion or worse (HSIL+), which included SCC and mixed HSIL and AIS, and any glandular lesions. This latter group included atypical endocervical cells; atypical endocervical cells, favour neoplastic; adenocarcinoma in situ and adenocarcinoma. We used x^2 (chi-square) test for trend to examine the association between proportion of HGA across age-groups and reflex cytology categories. Data analysis were performed using STATA version 15 (Stata Corporation, College Station, Tx, USA).

The study was approved by the Royal Women's Hospital Human Research and Ethics Committees (18/46). As the study design was a retrospective analysis based on de-identified data, approval was obtained without a requirement of individual informed consent.

RESULTS

Of the 4006 women whose HPV tests were classified as higher risk during the study period[5], 978 (24.4%) were seen in practices outside the follow-up network and 14 were tests wrongly classified as screening at the time of reporting. For the remaining 3014 women, results of colposcopy, histology or both were available for 2300 (76.3%). Providers did not respond to requests for follow-up information for 684 (22.7%), 21 (0.7%) women did not undergo colposcopy examination at the time of visit, and nine (0.3%) had colposcopy but no indication of the clinical impression and accompanied by an unreadable biopsy. The median age was 42 (IQR 33–54) among both the group with follow-up information and the group without (p=0.509). Women with follow-up information were slightly more likely than those without to have ASC-H or higher on reflex cytology (29.0% versus 24.9% respectively, p=0.004), but the proportion of women with HPV16/18 (85.0% and 87.0% respectively)

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and other oncogenic HPV types (15.0% and 13.0% respectively) did not differ between the groups (p=0.08).

Among the 2,300 women with follow-up results (Table 1 and Supplementary Table 1), 748 (32.5%), had a negative or low-grade colposcopy, 557 (24.2%) had a negative biopsy, 436 (19.0%) had LGA, and 559 (24.3%) had HGA. Most HGA was HSIL (n=518); with much fewer cases of mixed AIS/HSIL (n=16), AIS (n=13), AC (n=5) and SCC (n=7) (Supplementary Table 1).

Among HPV16/18 positive women, 18.0% overall had HGA. As shown in Table 2, the percentage with HGA increased with grade of cytology, from 6.6% among cytology negative women to 79.7% among women with HSIL+ abnormality on cytology (p-trend<0.001). Among women positive for other oncogenic HPV types (not 16/18) and HSIL+ abnormality on cytology, the percentage with HGA was 68.8% (compared to 79.7% in the HPV16/18 positive group; p=0.029). The percentage of HGA among women with ASC-H cytology was 51.8%, with no differences by HPV group (p=0.314) (Table 2).

In further analyses by age (Table 3 and Figure 2), detection of HGA was highest, at 36.4%, among women younger than 35 years, then decreased significantly to 5.9%, among women aged 65–74 years (p-trend<0.001). The relationship of decreasing detection of HGA with higher age was strong for women with negative cytology (<0.001), and those with ASC-H cytology (p<0.001). In contrast, among women with HSIL+ abnormality on cytology, detection of HGA was unaffected by age and consistently high, increasing from 70.3% among women younger than 35 years, to 83.3% among women aged 55–64 years, then decreased to 66.7% at 65–74 years (p-trend=0.211). In a sensitivity analysis limited to women with biopsy and histology results, identical age effects to that seen in the overall study population were observed (Supplementary Table 2).

Of the 12 cancers, 10 (83.3%) were in women with HSIL+ abnormality on cytology, one was in a woman with ASC-US cytology, and one had negative cytology. Of 34 histological glandular lesions, all but one (97.1%) were among women in the HPV16/18 group and seven (21.2%) were among women with <ASC-H cytology. The single non-HPV16/18 lesion was a mixed lesion including AIS and HSIL (Supplementary table 1).

DISCUSSION

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This is the first report of histological outcomes in Australia's newly introduced HPV-based cervical screening program. Among 2300 women referred directly for colposcopy under the risk-based algorithm, we found that nearly one in four (24%), had HGA, including two cancers among women with negative or low-grade cytology. Stratification based on the findings of reflex cytology significantly increased the predictive value of HPV, demonstrating the usefulness of this method in managing HPV positive women. We observed a substantial reduction in histological high-grade outcomes with increasing age, a finding which suggests that further refinement of the risk-based algorithm to account for age may be needed.

The overall percentage of women with HGA after a positive HPV test was 24%, which is well above the threshold at which colposcopy referral is recommended in some settings[10]. In European guidelines, for instance, 10% risk of CIN3 is considered sufficient for colposcopy referral. Screening for cervical precancer using HPV has been consistently shown to be more sensitive than cytology[11, 12]. The specificity of HPV testing is much lower however, leading to concerns about the potential over-investigation of clinically unimportant HPV infections with associated increases in cost of the program and morbidity of the screened women[1]. A clear aim of the new program was to move to a risk-based management algorithm that is informed by both partial genotyping and reflex cytology, to increase the specificity of the HPV test. The direct referral of all HPV16 or 18 positive women is underpinned by the higher cancer potential of these genotypes, and their lower population-level prevalence among young women due to vaccination[2, 13]. Among women positive for other oncogenic HPV types, the ASC-H or higher cytology threshold intends to triage those at highest risk while avoiding unnecessary colposcopy referrals among those in whom infections are likely to be transient[1]. A number of different algorithms and management pathways are currently under consideration or in use in different countries[14, 15]. The high predictive value reported in our study supports the value of this approach in the Australian setting.

There is ongoing debate about the optimum triage strategy among HPV positive women[16]. Most HPV screening programs use cytology as a triage test and our results suggest that high-quality cytology provides excellent stratification of risk among 'higher risk' women[15]. After a cytology prediction of HSIL, 75% of women had HGA. In accord with the increased neoplastic potential of HPV16 and 18, this was higher among the HPV16/18 positive than among other oncogenic (non16/18) positive (80%

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vs 70%). After ASC-H cytology, detection of HGA was significantly lower than for HSIL cytology, but showed no variation by HPV type. If the cytology were negative or low-grade (LSIL or ASC-US), an outcome of HGA was significantly less likely. This gradation confirms the added value of cytology in the context of an underlying positive HPV result. The performance of cytology can vary widely in different settings however and there may be situations where other biomarkers are needed[15]. As such, a number of molecular tests are being evaluated as triage tests, including p16/Ki67 dual staining, host and viral methylation markers and HPV E6 protein and these may prove useful when the cytology infrastructure is not reliable[15].

We observed considerable reduction in the detection of HGA with increasing age across all categories of cytology with the exception of HSIL for which the percentage was robust across all age groups. These findings are consistent with data from some maturing clinical trials which show a decreased risk of HSIL with increasing age, particularly in HPV positive women with negative cytology[17, 18]. The reason for this age effect is not known as most primary screening trials and natural history studies have been in women under the age of 40[19]. HPV positivity without visible cervical disease could reflect age-related biological changes in the location of the cervical transformation zone making the cervix less susceptible to new infection, or making small lesions more difficult to detect during colposcopy[20, 21]. It could also reflect reactivation of latent infection causing mostly low-level productive infection. The concept of latency has often been discussed by microbiologists[22], but the significance of viral re-activation in the context of screening and the risk of cervical cancer is poorly understood. Nevertheless, these observations are particularly relevant in the Australian program as the exit age was extended to 75 years and all higher risk women are referred for colposcopy. If these results can be confirmed with Australia-wide data, further risk-stratification of women using age and reflex cytology result may be required. For example, using the European guidelines recommendation of 10% as the risk threshold above which colposcopy should follow, HPV16/18 positive women over the age of 35 with negative cytology and women over 55 with ASC-US or LSIL cytology could potentially avoid immediate colposcopy and instead be triaged to early repeat HPV testing.

This study has limitations. The results presented reflect the risk of HGA at a single time point. Longer-term follow-up data on disease detection in screen positive women, and data from the

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second round of screening among negative women, will contribute important additional information on the performance of the new screening algorithm, and particularly on negative predictive value of the algorithm. Furthermore, information on screening histories of the 12 detected cervical cancers was not available for this report but is of particular interest in future analyses. Another limitation is that for a small proportion of women (n=55), the transformation zone could not be visualised during colposcopy. In these cases, a lesion may have been present, but was not visible resulting in a potentially false negative colposcopy. Also, the sample sizes for some categories of cytology and age groups were small and larger numbers are needed to confirm the observed patterns. Finally, follow-up results were available for 2300 of the 4006 women (57%) described in our first study, which may have introduced unknown bias in the analysis[5]. National data are needed to confirm the generalisability of these findings to all women classified as higher risk by the new algorithm. Furthermore, it is difficult to compare these findings with other data because of the unique nature of the Australian program and management algorithms, and the changing epidemiology of HPV infection due to high and widespread vaccination[5, 23]. A major strength is that these results represent real world experience as part of Australia's new national cervical cancer screening program. The colposcopy and laboratory testing have all been performed by clinicians and laboratories licensed to do this testing and management, operating under national laboratory accreditation guidelines, as well as colposcopy quality guidelines and national standardised management algorithms[22, 24].

In conclusion, data describing the first cross-sectional findings on the performance of Australia's newly introduced HPV-based cervical screening program showed a high predictive value of HPV testing for detecting cervical high-grade disease among women referred directly for colposcopy. The predictive value of HPV testing showed a significant variation in age, particularly among women over the age of 55 which warrants further monitoring. In the Australian setting, cytology triage is an appropriate method in managing positive women.

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CONFLICTS OF INTEREST

DAM has received grants from Seqirus, and non-financial support and honoraria (donated to her institute) from MSD, outside the submitted work. SMG has received grants from Merck, BioCSL, unrelated to the work described in this article; she has delivered lectures and received speaking fees from MSD performed in her personal time. All other authors declare no potential conflicts of interest.

DATA ACCESSIBILITY

Access to the data is only available upon request by contacting the corresponding author, and is subject to additional Human Research and Ethics Committee approval.

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RESULTS

Table 1. Summary of colposcopy results among 2,300 women classified as higher risk at their first HPV-based cervical screening test

Highest level of investigations	Colposcopy result	n (%)
Colposcopy, no biopsy	Normal impression	622 (27.0%)
	Normal impression (TZ3)	55 (2.4%)
	Low-grade impression	71 (3.1%)
Colposcopy, with biopsy	Negative biopsy	557 (24.2%)
	Low-grade abnormality[1]	436 (19.0%)
	High-grade abnormality[2]	559 (24.3%)

[1] Includes LSIL on histology; [2] Includes HSIL (n=518), mixed AIS and HSIL (n=16), AIS (n=13), AC (n=5) and SCC (n=7) on histology.

Abbreviations: HPV: Human papillomavirus; TZ3: Transformation zone not visualised; LSIL: Low-grade squamous intraepithelial lesion; AIS: Adenocarcinoma in situ; HSIL: High-grade squamous intraepithelial lesion; AC: Adenocarcinoma; SCC: Squamous cell carcinoma.

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Table 2. Detection of histological high-grade abnormality (HGA), by HPV and reflex cytology results, among 2300 women classified as higher risk at their first HPV-based cervical screening test

HPV result	Reflex LBC	N	HGA (n)	HGA (%)
HPV16/18 (n=1954)[1]	Negative	1197	79	6.6
	ASC-US/LSIL	415	58	14.0
	ASC-H	150	73	48.7
	HSIL+	172	137	79.7
Other oncogenic (not 16/18) (n=345)	ASC-H	207	112	54.1
	HSIL+	138	95	68.8
Any oncogenic (n=2300)[2]	ASC-H	357	185	51.8
	HSIL+	311	233	74.9
	Unsatisfactory	21	4	19.1

1: All reflex LBC results of ≤LSIL were among women positive for HPV16/18; 2: One woman with HSIL reflex LBC was positive for other oncogenic HPV types, but the HPV16/18 result was invalid.
Abbreviations: HPV: Human papillomavirus; ASC-US: Atypical squamous cells of undetermined significance; LSIL: Low-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; HSIL+: includes high-grade squamous intraepithelial lesion (HSIL); squamous cell carcinoma, mixed HSIL and adenocarcinoma in situ, and any glandular lesions; HGA including colposcopy with biopsy and histology result of high-grade squamous lesion, adenocarcinoma in situ, adenocarcinoma or squamous cell carcinoma.

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Table 3. Age-specific detection of histologically confirmed high-grade abnormality (HGA), overall and by reflex cytology result, among 2300 women classified as higher risk at their first HPV-based cervical screening test

	N	25–34 years		35–44 years		45–54 years		55–64 years		65–74 years		p-trend
		n	HGA, n (%)	n	HGA, n (%)	n	HGA, n (%)	n	HGA, n (%)	n	HGA, n (%)	
Overall[1]	2300	698	254 (36.4)	593	175 (29.5)	468	85 (18.2)	370	35 (9.5)	171	10 (5.9)	<0.001
Negative[2]	1196	247	30 (12.2)	296	24 (8.1)	264	14 (5.3)	265	9 (3.4)	124	2 (1.6)	<0.001
ASC-US/LSIL[2]	415	140	22 (15.7)	103	18 (17.5)	99	13 (13.1)	57	4 (7.0)	16	1 (6.3)	0.073
ASC-H	357	163	99 (60.7)	90	52 (57.8)	59	24 (40.7)	23	7 (30.4)	22	3 (13.6)	<0.001
HSIL+	311	145	102 (70.3)	101	80 (79.2)	41	32 (78.1)	18	15 (83.3)	6	4 (66.7)	0.211

1: Includes 21 women with unsatisfactory reflex cytology results at the primary screening visit; 2: All reflex LBC results of ≤LSIL were among women positive for HPV16/18.

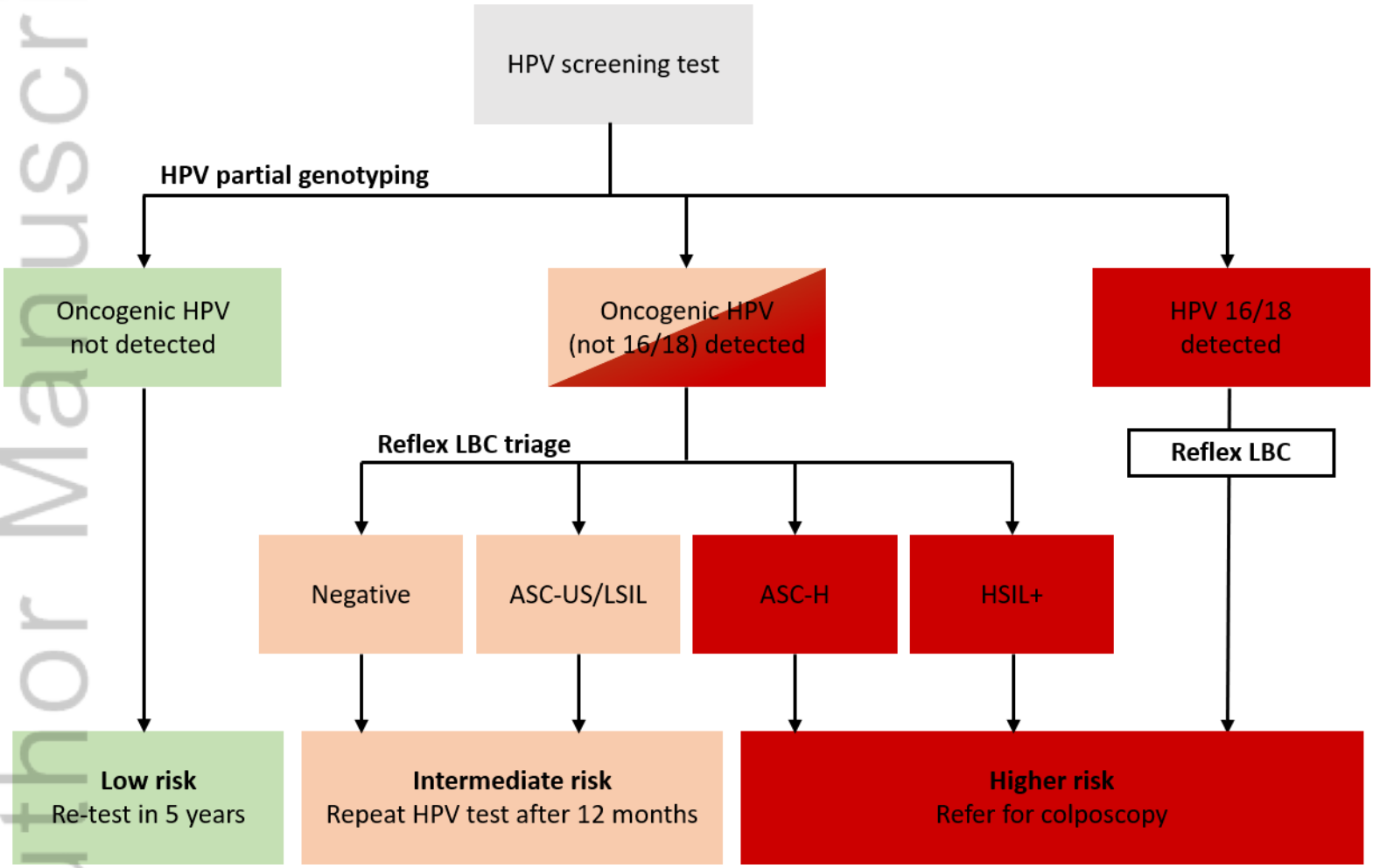
Abbreviations: HPV: Human papillomavirus; ASC-US: Atypical squamous cells of undetermined significance; LSIL: Low-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion; HSIL+: includes high-grade squamous intraepithelial lesion (HSIL); squamous cell carcinoma, mixed HSIL and adenocarcinoma in situ, and any glandular lesions; HGA including colposcopy with biopsy and histology result of high-grade squamous lesion, adenocarcinoma in situ, adenocarcinoma or squamous cell carcinoma.

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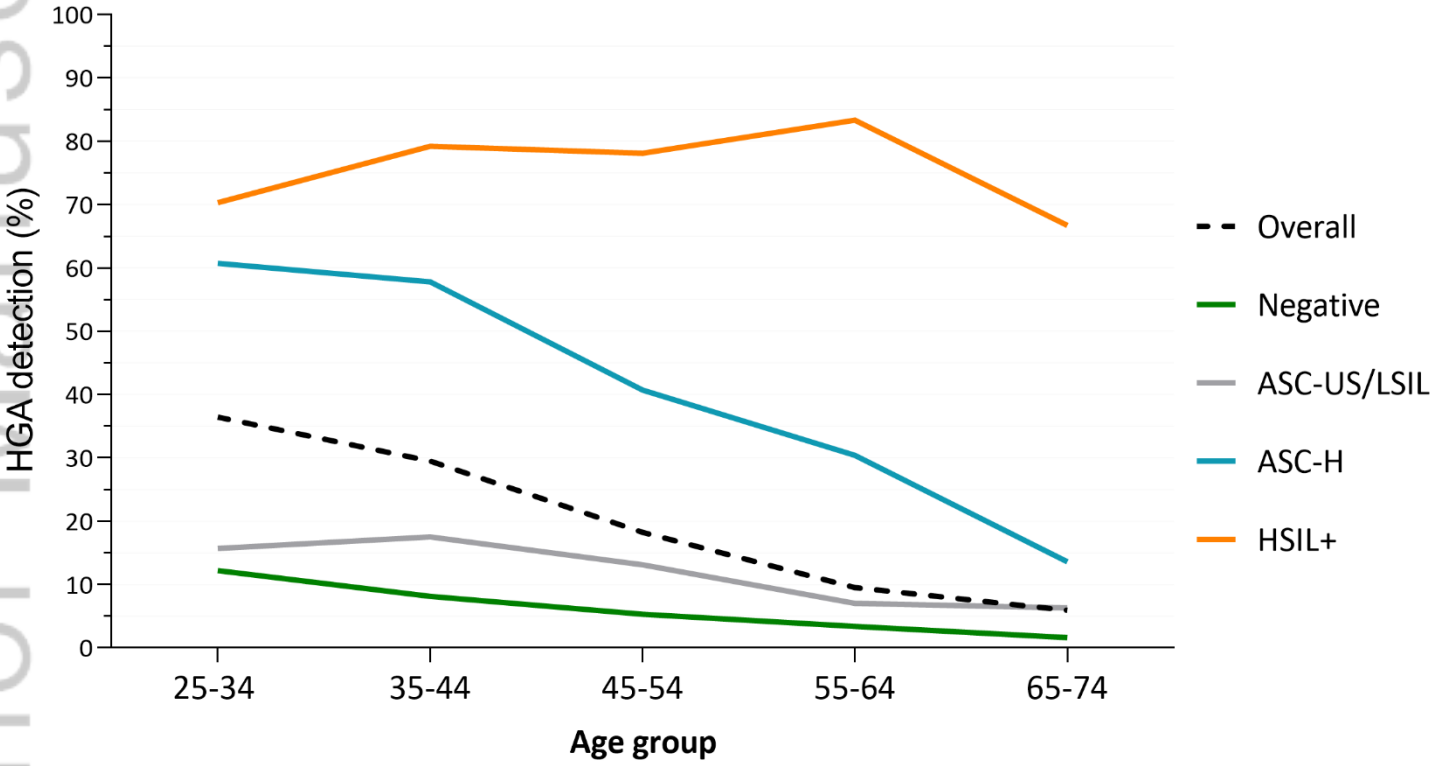
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Australia's new human papilloma virus (HPV)-based cervical screening program relies on a risk-based algorithm incorporating reflex cytology to guide decisions about follow-up colposcopy. In this report of the first follow-up colposcopy findings, nearly one in four women referred directly for colposcopy under the algorithm had an underlying histological high-grade abnormality (HGA). Detection of HGA increased with increasing grade of reflex cytology, but there was a substantial reduction in HGA detection with increasing age. The results demonstrate the additional value of reflex cytology in managing HPV-positive women and suggest that the risk-based algorithm should be further refined to account for age.

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