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HPV prevalence and type-distribution in cervical cancer and premalignant lesions of the cervix: A population-based study from Northern Ireland

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3 cervix: a population-based study from Northern Ireland.

4 **RUNNING HEAD:** HPV genotype prevalence in cervical cancer

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27 **ABSTRACT:**

28 **Introduction:** Assessment of Human papillomavirus (HPV) prevalence and genotype distribution is
29 important for monitoring the impact of prophylactic HPV vaccination. This study aimed to
30 demonstrate the HPV genotypes predominating in pre-malignant and cervical cancers in Northern
31 Ireland (NI) before the vaccination campaign has effect.

32 **Methods:** Formalin fixed paraffin embedded tissue blocks from 2,303 women aged 16-93 years
33 throughout NI were collated between April 2011 and February 2013. HPV DNA was amplified by PCR
34 and HPV genotyping undertaken using the Roche® linear array detection kit.

35 **Results:** In total, 1,241 out of 1,830 eligible samples (68.0%) tested positive for HPV, with the
36 majority of these [1,181/1,830 (64.5%)] having high-risk (HR) HPV infection; 37.4% were positive for
37 HPV-16 (n=684) and 5.1% for HPV-18 (n=93). HPV type-specific prevalence was 48.1%, 65.9%, 81.3%,
38 92.2% and 64.3% among cervical intraepithelial neoplasias (CIN) grades I-III, squamous cell
39 carcinomas (SCC) and adenocarcinoma (AC) cases, respectively. Most SCC cases (81.3%) had only one
40 HPV genotype detected and almost a third (32.0%) of all cervical pathologies were HPV negative
41 including 51.9% of CIN I (n=283), 34.1% CIN II (n=145), 18.7% of CIN III (n=146), 7.8% of SCC (n=5)
42 and 35.7% of AC (n=5) cases.

43 **Conclusions:** This study provides important baseline data for monitoring the effect of HPV
44 vaccination in NI and for comparison with other UK regions. The coverage of other HR-HPV
45 genotypes apart from 16 and 18, including HPV-45, 31, 39 and 52, and the potential for cross
46 protection, should be considered when considering future polyvalent vaccines.

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48 **Keywords:** human papillomavirus, cervical cancer, cervical intraepithelial neoplasia, frequency,
49 population-based.

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53 **INTRODUCTION**

54 Annually 3,000 women are diagnosed with, and almost 1,000 die from cervical cancer in the United
55 Kingdom (UK), making it the 12th most common cancer diagnosis and 17th most common cancer
56 death [Cancer Research UK, 2014]. Two main subtypes exist, squamous cell carcinoma (SCC) and
57 adenocarcinoma (AC). Persistent infection with high-risk (HR) oncogenic human papillomavirus
58 (HPV) family *alpha-Papillomaviridae* is a necessary, but insufficient cause, for invasive cervical cancer
59 [IARC Working Group on the Evaluation of Carcinogenic Risks to Humans., 2012]; with HPV-16 and
60 HPV-18, the most common oncogenic types, contributing to around 70% of all cases worldwide
61 [WHO/ICO Information Centre on HPV and Cervical Cancer, 2010].

62
63 HPV is highly sexually transmissible with 80% of women expected to have a HPV infection at some
64 point during their sexually active life [Alexander *et al*, 2012]. Many women are repeatedly infected,
65 with most infections occurring in those aged under 25 years [de Sanjosé *et al*, 2007; Smith *et al*,
66 2008; Anderson *et al*, 2013]. However, despite frequent exposure to HPV, development of cervical
67 cancer is uncommon with most HPV infections being transient and spontaneously regressing within
68 two years with no residual abnormality [Castellsagué, 2008]. In some cases HPV infection may
69 persist [Trottier *et al*, 2009], with some HPV genotypes more persistent than others [Louvanto *et al*,
70 2010]. Recent studies indicate a higher proportion of CIN II and CIN III lesions progressing to invasive
71 cancer in HPV-16 positive lesions, often within as little as 10-20 years [Jaisamrarn *et al*, 2013; Vink *et*
72 *al*, 2013; Wentzensen *et al*, 2013]. Many aspects of the natural history of HPV infection remain to be
73 fully elucidated, including the probable existence of latent infections and potential reactivation.

74
75 Although HPV-16, 18, 31, 52 and 58 are cited among the top 10 most common HPV genotypes
76 worldwide [Bruni *et al*, 2010], there is significant variation in the reported prevalence of HR-HPV
77 genotypes between countries. We previously reported that the prevalence of any HPV infection in
78 Northern Ireland (NI) women having a pap smear was 17.1%, ranging from 42.5% in those aged 20-

79 24 years to 4.2% in women aged 60-64 years; HPV prevalence was lower in women with normal
80 cytology at 13.2% increasing in those with cervical disease [Anderson *et al*, 2013]. However, this
81 study was underpowered to detect the prevalence of HPV genotypes in high-grade cervical
82 abnormalities (n=72).

83

84 The aim of the present study was therefore to provide an estimate of the background prevalence of
85 HPV infection and age-specific HPV type distribution, and to highlight the HPV genotypes most
86 frequently present in cervical cancer tissue and precursor lesions, before the HPV vaccination
87 programme alters the distribution of HPV infection in NI. Of note, unpublished data from the present
88 study has been recently incorporated into a pooled analysis of the HPV type-specific prevalence in
89 invasive cervical cancers in the UK [Mesher *et al*, 2014] where the overall prevalence of any HR-HPV
90 was found to be 95.8%; data that will be valuable in future HPV vaccine monitoring and effectiveness
91 studies. More detailed data on the prevalence of all detected HPV genotypes stratified by five year
92 age-bands, inclusive of CIN I-III and cervical pathologies are reported herein.

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105 **METHODS**

106 ***Sample selection***

107 Prospectively derived formalin fixed paraffin embedded (FFPE) cervical tissue blocks (processed at all
108 four pathology laboratories in NI) pertaining to routine diagnostic samples collected between April
109 2011 and February 2013 from all women attending for cervical screening across NI (population-
110 based cohort), and which were determined to have cervical abnormalities, were eligible for
111 inclusion. No age or histological restrictions were applied to the cohort during specimen collection.

112

113 Through data supplied by the Public Health Agency Northern Ireland, we estimate that only a small
114 proportion of samples may have been derived from previously HPV vaccinated women (5.9%), thus
115 the majority of samples included in this study are from HPV unvaccinated women. Following routine
116 examination of the biopsy in each reporting laboratory, consultant pathologists selected the most
117 appropriate FFPE block containing the highest grade of disease reported. The selected cervical
118 tissue blocks were sent to the Department of Cellular & Molecular Pathology, Northern Health &
119 Social Care Trust for HPV determination. For the purposes of the present study, analysis was
120 restricted to samples identified as cervical intraepithelial neoplasia (CIN) grades I, II and III, SCC and
121 AC. On reception of the FFPE tissue blocks, pathology records were accessed and clinical pathological
122 information including patient age, sample type and histology, were extracted. A unique study
123 number was then assigned to each case and the link to the original case broken, ensuring
124 confidentiality and complete anonymisation of the results. Ethical approval for the study was
125 granted from the Office of Research Ethics Committees NI (ORECNI ref: 08/NIIR02/104).

126

127 ***Specimen processing***

128 FFPE tissue blocks underwent tissue microtomy and were processed under molecular pathology
129 conditions, to reduce the risk of cross contamination. Tissue lysis was performed using the QIAGEN
130 ATL (EDTA and 10% sodium dodecyl sulphate) buffer (P/N 19076, Qiagen, Crawly, West Sussex, UK),

131 in the presence of 20µl of proteinase K (P/N 19131, Qiagen, Crawley, West Sussex, UK). The tissue
132 was incubated at 56°C ± 2°C for 24 hours with occasional vortex mixing during this incubation
133 period. During post enzymatic incubation, the lysate was added to processing tubes and was
134 subjected to automated DNA extraction on the Roche COBAS® 480 X module as per manufactures
135 protocols. Extracted DNA was eluted into buffer EB and stored at -20°C until amplification. Detection
136 of HPV genotypes were undertaken using a modified Roche Linear Array HPV genotyping test (LA
137 HPV GT P/N:04391853 190) and the Linear Array detection kit (LA DK P/N: 03378179 190, Roche
138 Molecular Systems, Inc. Branchburg, NJ, USA). The modification from manufactures protocol
139 consisted of the addition of a compound into the HPV MasterMix, to allow for compatibility with the
140 DNA extracted from the COBAS® 480 X module. Extracted DNA was quantified via NanoDrop
141 spectrophotometry with all samples standardised to a concentration of 24ng/ul; the optimum level
142 determined in-house to allow for successful HPV detection in the background of human DNA and
143 reduce the number of test failures due to an “overloaded” PCR reaction. Of note, the Roche linear
144 array uses multiple type (mixed) probes to detect DNA from HPV-52 which limits the assay’s ability
145 to discriminate HPV-52 status in the presence of HPV 33, 35 and/or 58 infections. The HPV-52
146 genotype was thus derived as positive if co-infection of HPV 33, 35 and 58 was not present in the
147 sample. Cervical SCC samples that tested HPV negative were revisited, DNA isolated and retested to
148 ensure the result. Samples were considered HPV negative only when a linear array negative result
149 was obtained and confirmed by repeat testing.

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151 ***Specimen Reporting***

152 The term HR-HPV is used to describe a number of HPV genotypes that have been shown to be
153 associated with an increased risk of cervical cancer and include: HPV-16, 18, 31, 33, 35, 39, 45, 51,
154 52, 56, 58, 59 (carcinogenic), 68 (probably carcinogenic) and 66 (possibly carcinogenic) based on the
155 World Health Organization (WHO) HPV categorizations [Cogliano *et al*, 2005]. The term ‘low-risk

156 HPV' covers all other HPV genotypes detected by the Roche HPV linear array genotyping test. All
157 HPV types identified by the Roche linear array are shown in Table 3.

158

159 ***Statistical analysis***

160 The overall prevalence, genotype and age-specific HPV prevalence among pre-malignant lesions (CIN
161 I-III) and cervical cancers (SSC and AC) were estimated for all samples. The frequency of those testing
162 positive for HPV-16 and HPV-18 with or without other HR-HPV genotypes was investigated. Women
163 within the study were stratified into five year age bands, this was further classified into three age
164 groups for the following reasons; under 25 years (outside the age range for the current NI Cervical
165 Screening Programme, but current at the time of our previous prevalence study in the screened
166 population); 25 to 34 years (reported to have a higher transient HPV infection risk with no significant
167 underlying disease [Khan *et al*, 2005]), over 35 years (this group of HR-HPV positive is said to have a
168 higher chance of significant pathological outcome [Khan *et al*, 2005]). STATA IC/11.2 (StataCorp,
169 College Station, TX, USA) was used for all analyses.

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182 **RESULTS**

183 In total, 2,303 samples were received between April 2011 and February 2013. Figure 1 is a flow
184 diagram providing justification for the selection of study samples. Exclusions included 58 samples
185 that were not tested due to overrepresentation in the low-grade dyskaryosis groups, a further 200
186 were excluded at the post-analytical phase of the study due to insufficient tissue remaining for
187 molecular analysis. 218 samples were also excluded as they were not SCC, AC or CIN I-III pathologies.
188 A total of 1,827 eligible samples were included for analysis, the majority of which were acquired
189 through large loop excisions of the transformation zone (64.8%) or punch biopsies (34.2%).

190

191 **Prevalence of HPV infection:**

192 In total, 1,243/1,827 samples (68.0%) tested positive for HPV, with the majority 1,183/1,827 (64.8%)
193 having HR-HPV infection. A total of 584/1,827 (32.0%) of samples were HPV negative, 37.4% were
194 positive for HPV-16 (n=684) and 5.1% for HPV-18 DNA (n=93). Figure 2 outlines the overall HPV
195 genotype profile among the cohort and HPV multiplicity. The five most common HPV genotypes
196 detected across all cervical pathologies examined were HPV-16 (n=684), HPV-31 (n=150), HPV-52
197 (n=125), HPV-18 (n=93) and HPV-33 (n=91) (Figure 2).

198

199 **HPV type-specific prevalence:**

200 The majority of samples were from CIN III cases (42.6%). HPV DNA was detected in 262/545 (48.1%)
201 of CIN I samples, 280/425 (65.9%) of CIN II lesions, 633/779 (81.3%) of CIN III specimens, 59/64
202 (92.2%) of SCCs and 9/14 (64.3%) of AC samples. Table 1 details the number of HPV genotypes
203 detected by pathological subtype. Half of all samples (49.7%) had only one HPV genotype detected
204 (n=908). Multiple HPV genotypes (>1) were more common in CIN I-III lesions than other cervical
205 pathologies. Among SCC specimens, 7.8% had no HPV DNA detected and most (81.3%) had only 1
206 HPV genotype persisting, Table 1. HPV-16 and/or HPV-18 DNA was present in the majority of SCC
207 (82.8%) and AC (64.3%) cases and over half (56.2%) of CIN III samples, Table 2. Other HR HPV

208 genotypes were more prevalent in CIN I-III pathologies, and present in less than 10% of SCCs and AC
209 cases. LR-HPV genotypes were most common in lower grade cervical lesions and absent in cervical
210 cancer specimens, Table 2. Almost a third (32.0%) of all pathologies were HPV negative. The
211 distribution of HPV genotypes detected within each cervical pathology is detailed in Table 3. The
212 current HPV vaccination genotypes (HR-HPV 16 and or HR-HPV 18) were found in at least 82.8% of
213 women with SCC of the cervix. For cervical SCC, HPV-16 (n=51/64), HPV-18 (n=3/64), HPV-45
214 (n=3/64) and HPV-52 (n=5/64) made up 96% of the HPV positive samples. HPV-16 was the most
215 common HPV detected across all cervical pathologies, except for AC where HPV-18 predominated
216 with HPV-16, HPV-51 and HPV-54 making up the remaining HPV genotypes present, Table 3.

217

218 **Age-specific prevalence for HPV infection:**

219 The pathological distribution of samples by five-year age group is shown in Table 4. The mean age of
220 women included in the study was 32 years (range 16-93 years, standard deviation (SD) 9.4 years) and
221 significantly differed between histopathological groups (P <0.001). In total, 375 samples were from
222 women aged 24 years and under, 887 from women aged 25 to 34 years and 565 samples from those
223 aged 35 years and over. CIN I was more common in younger women and CIN II and CIN III most
224 common in those aged 25-29 years. SCC was most common in women aged 35-39 years, Table 4.

225

226 The number of HPV genotypes decreased with increasing age, with just over half (51.0%) of all HPV
227 infections found in tissue from women aged between 25-29 years, Table 1. Most tissue samples had
228 a single HPV genotype and this was more common in those aged over 60 years. Five or more HPV
229 genotypes were detected in some women but this was largely limited to those aged under the age of
230 40 years, Table 1. 41.7% of women with cervical pathology had HPV-16 or HPV-18 detected, and the
231 highest proportion HPV 16/18 positive were over the age of 65 years, Table 2. Other HR-HPV
232 genotypes were more common in younger women, particularly those aged 35-39 years. LR-HPV

233 genotypes were most common in those aged 55-59 years, with women over the age of 60 years
234 having no LR-HPV genotypes detected, Table 2.

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259 **DISCUSSION:**

260 This is the largest population-based study investigating the HPV genotype distribution in women
261 with CIN I-III and cervical SCC and AC in NI. The overall prevalence of any HPV in SCC samples was
262 92.2% and was 68.0% across all cervical samples investigated. HR-HPV was detected in 64.8% of all
263 samples with HPV-16 being the most common HPV genotype identified, which is consistent with that
264 described elsewhere in CIN cases across Europe in countries such as Spain, France and Germany
265 [García-Espinosa *et al*, 2012; Monsonogo *et al*, 2012; de Jonge *et al*, 2013; Leinonen *et al*, 2013;
266 Rössler *et al*, 2013] and in invasive disease internationally [Bosch *et al*, 2008; de Sanjose *et al*, 2010;
267 Li *et al*, 2011]. As previously reported the prevalence of HPV increased through CIN I-III lesions in NI
268 [Anderson *et al*, 2013] from 48.1% in CIN I to 81.3% in CIN III cases. Most HPV positive samples had
269 HR-HPV genotypes with HPV-16 and/or HPV-18 present in 20.6%, 35.3% and 56.2% of CIN I-III cases
270 respectively.

271

272 In an examination of FFPE tissue from more than 6,000 women from 17 European countries using
273 the SPF10-LiPA25 assay, including data from neighbouring Scotland, Ireland and Wales, Tjalma *et al*
274 found HPV-16 was the most frequent HPV type detected in both CIN and invasive cervical cancer
275 [Tjalma *et al*, 2013]. HPV-16 and/ or HPV-18 prevalence (among HPV positive cases) was reported as
276 45.8% in CIN II and 67.3% in CIN III cases [Tjalma *et al*, 2013], slightly higher than the current study.
277 The authors reported HPV-31, 33, 35, 51, 52, 58 and 68 as the most frequently detected genotypes
278 in women with high-grade CIN lesions. Apart from HPV-16, we similarly found that HPV-18, 31, 33,
279 45, 51 and 52 were the most common genotypes identified in high grade lesions. Building on
280 previous meta-analyses of type-specific HPV prevalence worldwide by specific grades of cervical
281 disease [Clifford *et al*, 2003, 2005; Smith *et al*, 2007; Li *et al*, 2011] Guan *et al* in a further meta-
282 analysis of 423 studies (144 of which were from Europe) using PCR assays based on various primers
283 among cell or biopsy/tissue cervical diagnoses also showed an increasing HPV prevalence with
284 increasing severity of cervical disease, with HPV-16 the most frequently detected HR-HPV type in all

285 grades [Guan *et al*, 2012]. The prevalence (including HPV negative cases in the denominator) of HPV-
286 16 was 27.6%, 39.8% and 58.2% and HPV-18 prevalence 9.0%, 10.0% and 7.4% in CIN I to CIN III
287 cases respectively [Guan *et al*, 2012], again higher than the estimates reported in the present study,
288 variation which may have arisen from methodological differences in HPV detection techniques. The
289 majority of SCCs (92.2%) in the present study were HPV positive, this is in accordance with the
290 proportions of invasive cervical cancers (the majority of which were SCCs) testing HPV positive in
291 other countries of the UK [Mesher *et al*, 2014], Sweden, Spain Italy and eastern Europe [Du *et al*,
292 2011; Alemany *et al*, 2012; Giorgi Rossi *et al*, 2012; Guan *et al*, 2012; Piana *et al*, 2013; Pista *et al*,
293 2013; Škamperle *et al*, 2013; Tjalma *et al*, 2013; Kjær *et al*, 2014; Simanaviciene *et al*, 2014].

294

295 There were only 14 cases of AC included in the current investigation, and therefore our study is likely
296 underpowered to investigate HPV prevalence in this subgroup. Of note however, HPV-18 was the
297 most common HPV type in AC cases with a prevalence of 42.9% in the current study, akin to a recent
298 Scottish estimate of 44.0% (among HPV positive cases) [Powell *et al*, 2013] which examined HPV
299 DNA in FFPE tissue detected using the SPF10-DEIA/LiPA25-PCR assay. The prevalence of HPV-16
300 and/or HPV-18 was 64.3%, lower than reported in an English multi-site investigation of HPV DNA in
301 cervical cytology and cervical cancer biopsies using the Roche Linear array typing system (81.9%,
302 among HPV positive cases) [Howell-Jones *et al*, 2010] and among AC cases from other European
303 studies (94.6%) [Tjalma *et al*, 2013]; perhaps due to a lower HPV-18 rate.

304

305 The number of HPV genotypes detected in the current study varied across pathological grade, with
306 the lowest percentage of single genotypes (31.0%) in CIN I lesions and the highest proportion
307 (81.3%) in cervical SCCs, indicative that a single HPV genotype remains persistent in patients that
308 progress. Although HPV persistence has been shown to vary by geographical region, HPV-16, 18, 31,
309 33, 45, 52 and 58 are the most persistent HPV genotypes reported among women with invasive
310 cervical cancer [Bernard *et al*, 2013; Rositch *et al*, 2013]. The most frequent single genotypes in the

311 current investigation in SCCs were HPV 16, 18, 45, 31, 39 and HPV 52. Notably, this profile includes
312 several HPV genotypes which are not currently incorporated in the present HPV vaccine programme.
313 These should be considered in the next generation of anti-HPV vaccines. In line with the UK HPV
314 vaccination programme, in September 2012 NI began using the quadrivalent vaccine Gardasil®
315 (Gardasil, Sanofi Pasteur MSD), with a relatively high uptake of 86.8% for all three doses in girls aged
316 12-13 years [HSC Public Health Agency Northern Ireland, 2014]. Markov modelling has shown that
317 the UK HPV vaccination programme would require 80% uptake to have a reduction of 66% in the
318 prevalence of high-grade precancerous lesions and a 76% reduction in cervical cancer deaths [Kohli
319 *et al*, 2007]. Therefore the HPV genotypes covered by the current HPV vaccination programme will
320 lower the prevalence of HPV 16/18, as has already been evidenced in Scotland [Kavanagh *et al*,
321 2014], and should prevent the majority of cervical cancers in NI. The formerly used bivalent vaccine
322 (Cervarix®) offers some cross-protection for HPV genotypes of the A7 species including HR HPV-31,
323 33 and 45 [Paavonen *et al*, 2009; Malagón *et al*, 2012; De Vincenzo *et al*, 2013; Verdenius *et al*,
324 2013]. The potential effects of vaccine cross protection against other oncogenic non-target
325 genotypes should also be considered when conducting future cost-benefit analyses.

326

327 The principal strength of this study is its size and population-based design. The study was able to
328 report the identification of high and low-risk HPV genotypes as well as the prevalence of multiple
329 HPV infections. When comparing the HPV prevalence between countries it is important to consider
330 that variations in HPV positivity may be explained by differences in the quality and type of samples
331 analyzed (biopsies, surgical specimens or fresh tissue), as well as the methods of HPV detection and
332 assessment.

333

334 While it is frequently reported that 99.7% of cervical SCCs are HPV positive [Walboomers *et al*,
335 1999], women have been shown to have cervical disease without testing HPV positive using current
336 methods. In a pooled analysis of 3 large Italian case series using three different PCR methods, 24

337 (4.2%) of 574 invasive cervical cancers were found to be 'true' HPV negative cases [Giorgi Rossi *et al*,
338 2012]. Similarly, Tjalma *et al* in large-scale data from 17 European countries found that 8.2% of
339 invasive cervical cancers were HPV negative [Tjalma *et al*, 2013]. HPV-negative carcinoma, if it exists
340 at all, is likely to be rare. In the present study 10/78 invasive cervical cancers (12.8%) tested HPV
341 negative. Although other aetiologic factors, such as mutations within the p53 gene, may explain
342 some HPV negative cases [Fogel *et al*, 1995] the potential for false negative results arising from
343 differences in analytic sensitivity for different HPV types particularly in the presence of multiple
344 infections, low titer or copy number of HPV DNA and inadequacy of the specimen should be
345 carefully considered. For instance, HPV genotyping analysis in the present study was undertaken
346 using the Roche linear array detection kit. Importantly, this assay uses PGMY L1 consensus primers
347 to amplify a 450-bp fragment. As it is well known that formaldehyde fixation provides low yields of
348 extractable DNA due to protein cross-linking and strand cleavage, such a large amplicon size
349 provides the potential for false negative results on FFPE tissue. Whilst the detection of HPV in fresh
350 frozen tissue appears superior [Odida *et al*, 2010], this would have been difficult to acquire and
351 process in a large population-based study such as this.

352

353 In conclusion, HPV-16 was identified as the main HPV genotype associated with cervical disease in
354 NI, contributing to around 83.0% of the cervical SCCs investigated. Provided there is sustained high
355 HPV vaccine coverage in NI, the current HPV vaccination programme should prevent the majority of
356 cervical cancers but coverage of other HR-HPV genotypes with high prevalence and oncogenic
357 potential including HPV-31, 39, 45 and 52 and the potential influence of cross protection, should be
358 considered in any future polyvalent vaccines.

359

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368 **FIGURE LEGENDS:**

369 **Figure 1:** Flowchart of sample selection and eligibility

370 **Figure 2:** Overall HPV genotype profile denoting the prevalence of single and multiple HPV infections

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372 **REFERENCES:**

373

374 Alemany L, Pérez C, Tous S, Llombart-Bosch A, Lloveras B, Lerma E, Guarch R, Andújar M, Pelayo A,
375 Alejo M, Ordi J, Klaustermeier J, Velasco J, Guimerà N, Clavero O, Castellsagué X, Quint W, Muñoz N,
376 Bosch FX, de Sanjosé S (2012) Human papillomavirus genotype distribution in cervical cancer cases in
377 Spain. Implications for prevention. *Gynecol Oncol* 124: 512–517 doi:10.1016/j.ygyno.2011.11.024.

378 Alexander K, Daley AM, Dempsey AF (2012) Rationale for reducing the spread of human
379 papillomavirus in adolescents: strategies to improve outcomes (CME multimedia activity). *J Adolesc*
380 *Health* 50: IBC doi:10.1016/j.jadohealth.2011.10.014.

381 Anderson L, O'Rorke M, Jamison J, Wilson R, Gavin A (2013) Prevalence of human papillomavirus in
382 women attending cervical screening in the UK and Ireland: new data from northern Ireland and a
383 systematic review and meta-analysis. *J Med Virol* 85: 295–308 doi:10.1002/jmv.23459.

384 Bernard E, Pons-Salort M, Favre M, Heard I, Delarocque-Astagneau E, Guillemot D, Thiébaud ACM
385 (2013) Comparing human papillomavirus prevalences in women with normal cytology or invasive
386 cervical cancer to rank genotypes according to their oncogenic potential: a meta-analysis of
387 observational studies. *BMC Infect Dis* 13: 373 doi:10.1186/1471-2334-13-373.

388 Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, Tortolero-Luna G, Kjaer SK,
389 Muñoz N (2008) Epidemiology and natural history of human papillomavirus infections and type-
390 specific implications in cervical neoplasia. *Vaccine* 26 Suppl 1: K1–K16
391 doi:10.1016/j.vaccine.2008.05.064.

392 Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S (2010) Cervical human
393 papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological
394 findings. *J Infect Dis* 202: 1789–1799 doi:10.1086/657321.

395 Cancer Research UK (2014) Cervical cancer [http://www.cancerresearchuk.org/cancer-](http://www.cancerresearchuk.org/cancer-info/cancerstats/types/cervix)
396 [info/cancerstats/types/cervix](http://www.cancerresearchuk.org/cancer-info/cancerstats/types/cervix).

397 Castellsagué X (2008) Natural history and epidemiology of HPV infection and cervical cancer. *Gynecol*

398 *Oncol* 110: S4–S7 doi:10.1016/j.ygyno.2008.07.045.

399 Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM (2005) Human papillomavirus
400 genotype distribution in low-grade cervical lesions: comparison by geographic region and with
401 cervical cancer. *Cancer Epidemiol Biomarkers Prev* 14: 1157–1164 doi:10.1158/1055-9965.EPI-04-
402 0812.

403 Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S (2003) Human papillomavirus types in
404 invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer* 88: 63–73
405 doi:10.1038/sj.bjc.6600688.

406 Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, Ghissassi F El (2005) Carcinogenicity of human
407 papillomaviruses. *Lancet Oncol* 6: 204 doi:10.1016/S1470-2045(05)70086-3.

408 Du J, Näsman A, Carlson JW, Ramqvist T, Dalianis T (2011) Prevalence of human papillomavirus (HPV)
409 types in cervical cancer 2003–2008 in Stockholm, Sweden, before public HPV vaccination. *Acta Oncol*
410 50: 1215–1219 doi:10.3109/0284186X.2011.584556.

411 Fogel S, Ahomadegbe J, Lebihan M, Barrois M, Riou G (1995) Characterization of a new p53-mutated
412 and hpv-negative human squamous-cell cervical carcinoma-derived cell-line. *Int J Oncol* 6: 681–686.

413 García-Espinosa B, Moro-Rodríguez E, Alvarez-Fernández E (2012) Genotype distribution of human
414 papillomavirus (HPV) in histological sections of cervical intraepithelial neoplasia and invasive cervical
415 carcinoma in Madrid, Spain. *BMC Cancer* 12: 533 doi:10.1186/1471-2407-12-533.

416 Giorgi Rossi P, Sideri M, Carozzi FM, Vocaturo A, Buonaguro FM, Tornesello ML, Burrioni E, Mariani L,
417 Boveri S, Zaffina LM, Chini F (2012) HPV type distribution in invasive cervical cancers in Italy: pooled
418 analysis of three large studies. *Infect Agent Cancer* 7: 26 doi:10.1186/1750-9378-7-26.

419 Guan P, Howell-Jones R, Li N, Bruni L, de Sanjosé S, Franceschi S, Clifford GM (2012) Human
420 papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to
421 cancer. *Int J Cancer* 131: 2349–2359 doi:10.1002/ijc.27485.

422 Howell-Jones R, Bailey A, Beddows S, Sargent A, de Silva N, Wilson G, Anton J, Nichols T, Soldan K,
423 Kitchener H (2010) Multi-site study of HPV type-specific prevalence in women with cervical cancer,
424 intraepithelial neoplasia and normal cytology, in England. *Br J Cancer* 103: 209–216
425 doi:10.1038/sj.bjc.6605747.

426 HSC Public Health Agency Northern Ireland (2014) Annual HPV vaccine coverage in Northern Ireland:
427 2012–13 <http://www.publichealthagency.org/sites/default/files/directorates/files/2012-2013.pdf>
428 (accessed: 20/11/2014).

429 IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2012) A Review of Human
430 Carcinogens. vol 100B. Biological Agents (Lyon).

431 Jaisamrarn U, Castellsagué X, Garland SM, Naud P, Palmroth J, Del Rosario-Raymundo MR, Wheeler
432 CM, Salmerón J, Chow S-N, Apter D, Teixeira JC, Skinner SR, Hedrick J, Szarewski A, Romanowski B,
433 Aoki FY, Schwarz TF, Poppe WAJ, Bosch FX, de Carvalho NS, Germar MJ, Peters K, Paavonen J,
434 Bozonnet M-C, Descamps D, Struyf F, Dubin GO, Rosillon D, Baril L (2013) Natural history of
435 progression of HPV infection to cervical lesion or clearance: analysis of the control arm of the large,

436 randomised PATRICIA study. *PLoS One* 8: e79260 doi:10.1371/journal.pone.0079260.

437 de Jonge M, Busecke G, Heinecke A, Bettendorf O (2013) Human papillomavirus genotype
438 distribution in cytologically screened women from northwest Germany. *Acta Cytol* 57: 591–598
439 doi:10.1159/000355099.

440 Kavanagh K, Pollock KGJ, Potts A, Love J, Cuschieri K, Cubie H, Robertson C, Donaghy M (2014)
441 Introduction and sustained high coverage of the HPV bivalent vaccine leads to a reduction in
442 prevalence of HPV 16/18 and closely related HPV types. *Br J Cancer* 110: 2804–2811
443 doi:10.1038/bjc.2014.198.

444 Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, Rush BB, Glass AG, Schiffman M
445 (2005) The elevated 10-year risk of cervical precancer and cancer in women with human
446 papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical
447 practice. *J Natl Cancer Inst* 97: 1072–1079 doi:10.1093/jnci/dji187.

448 Kjær SK, Munk C, Junge J, Iftner T (2014) Carcinogenic HPV prevalence and age-specific type
449 distribution in 40,382 women with normal cervical cytology, ASCUS/LSIL, HSIL, or cervical cancer:
450 what is the potential for prevention? *Cancer Causes Control* 25: 179–189 doi:10.1007/s10552-013-
451 0320-z.

452 Kohli M, Ferko N, Martin A, Franco EL, Jenkins D, Gallivan S, Sherlaw-Johnson C, Drummond M
453 (2007) Estimating the long-term impact of a prophylactic human papillomavirus 16/18 vaccine on
454 the burden of cervical cancer in the UK. *Br J Cancer* 96: 143–150 doi:10.1038/sj.bjc.6603501.

455 Leinonen MK, Anttila A, Malila N, Dillner J, Forslund O, Nieminen P (2013) Type- and age-specific
456 distribution of human papillomavirus in women attending cervical cancer screening in Finland. *Br J*
457 *Cancer* 109: 2941–2950 doi:10.1038/bjc.2013.647.

458 Li N, Franceschi S, Howell-Jones R, Snijders PJF, Clifford GM (2011) Human papillomavirus type
459 distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region,
460 histological type and year of publication. *Int J Cancer* 128: 927–935 doi:10.1002/ijc.25396.

461 Louvanto K, Rintala MA, Syrjänen KJ, Grénman SE, Syrjänen SM (2010) Genotype-specific persistence
462 of genital human papillomavirus (HPV) infections in women followed for 6 years in the Finnish Family
463 HPV Study. *J Infect Dis* 202: 436–444 doi:10.1086/653826.

464 Malagón T, Drolet M, Boily M-C, Franco EL, Jit M, Brisson J, Brisson M (2012) Cross-protective
465 efficacy of two human papillomavirus vaccines: a systematic review and meta-analysis. *Lancet Infect*
466 *Dis* 12: 781–789 doi:10.1016/S1473-3099(12)70187-1.

467 Mesher D, Cuschieri K, Hibbitts S, Jamison J, Sargent A, Pollock KG, Powell N, Wilson R, McCall F,
468 Fiander A, Soldan K (2014) Type-specific HPV prevalence in invasive cervical cancer in the UK prior to
469 national HPV immunisation programme: baseline for monitoring the effects of immunisation. *J Clin*
470 *Pathol* jclinpath – 2014–202681 – doi:10.1136/jclinpath-2014-202681.

471 Monsonego J, Zerat L, Syrjänen K, Zerat J-C, Smith JS, Halfon P (2012) Prevalence of type-specific
472 human papillomavirus infection among women in France: Implications for screening, vaccination,
473 and a future generation of multivalent HPV vaccines. *Vaccine* 30: 5215–5221

474 doi:10.1016/j.vaccine.2012.06.013.

475 Odida M, de Sanjose S, Sandin S, Quiros B, Alemany L, Lloveras B, Quint W, Kleter B, Alejo M, van
476 Doorn L-J, Weiderpass E (2010) Comparison of human papillomavirus detection between freshly
477 frozen tissue and paraffin embedded tissue of invasive cervical cancer. *Infect Agent Cancer* 5: 15
478 doi:10.1186/1750-9378-5-15.

479 Paavonen J, Naud P, Salmerón J, Wheeler CM, Chow S-N, Apter D, Kitchener H, Castellsague X,
480 Teixeira JC, Skinner SR, Hedrick J, Jaisamrarn U, Limson G, Garland S, Szarewski A, Romanowski B,
481 Aoki FY, Schwarz TF, Poppe WAJ, Bosch FX, Jenkins D, Hardt K, Zahaf T, Descamps D, Struyf F,
482 Lehtinen M, Dubin G (2009) Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine
483 against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of
484 a double-blind, randomised study in young women. *Lancet* 374: 301–314 doi:10.1016/S0140-
485 6736(09)61248-4.

486 Piana A, Sotgiu G, Cocuzza C, Musumeci R, Marras V, Pischedda S, Deidda S, Muresu E, Castiglia P
487 (2013) High HPV-51 prevalence in invasive cervical cancers: results of a pre-immunization survey in
488 North Sardinia, Italy. *PLoS One* 8: e63395 doi:10.1371/journal.pone.0063395.

489 Pista A, de Oliveira CF, Lopes C, Cunha MJ (2013) Human papillomavirus type distribution in cervical
490 intraepithelial neoplasia grade 2/3 and cervical cancer in Portugal: a CLEOPATRE II Study. *Int J*
491 *Gynecol Cancer* 23: 500–506 doi:10.1097/IGC.0b013e318280f26e.

492 Powell N, Cuschieri K, Cubie H, Hibbitts S, Rosillon D, De Souza SC, Molijn A, Quint W, Holl K, Fiander
493 A (2013) Cervical cancers associated with human papillomavirus types 16, 18 and 45 are diagnosed
494 in younger women than cancers associated with other types: a cross-sectional observational study in
495 Wales and Scotland (UK). *J Clin Virol* 58: 571–574 doi:10.1016/j.jcv.2013.08.020.

496 Rositch AF, Koshiol J, Hudgens MG, Razzaghi H, Backes DM, Pimenta JM, Franco EL, Poole C, Smith JS
497 (2013) Patterns of persistent genital human papillomavirus infection among women worldwide: a
498 literature review and meta-analysis. *Int J Cancer* 133: 1271–1285 doi:10.1002/ijc.27828.

499 Rössler L, Reich O, Horvat R, de Souza SC, Holl K, Joura EA (2013) Human papillomavirus in high-
500 grade cervical lesions: Austrian data of a European multicentre study. *Wien Klin Wochenschr* 125:
501 591–599 doi:10.1007/s00508-013-0403-6.

502 de Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N, Bosch FX (2007) Worldwide
503 prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal
504 cytology: a meta-analysis. *Lancet Infect Dis* 7: 453–459 doi:10.1016/S1473-3099(07)70158-5.

505 de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, Tous S, Felix A, Bravo
506 LE, Shin H-R, Vallejos CS, de Ruiz PA, Lima MA, Guimera N, Clavero O, Alejo M, Llombart-Bosch A,
507 Cheng-Yang C, Tatti SA, Kasamatsu E, Iljazovic E, Odida M, Prado R, Seoud M, Grce M, Usubutun A,
508 Jain A, Suarez GAH, Lombardi LE, Banjo A, Menéndez C, Domingo EJ, Velasco J, Nessa A, Chichareon
509 SCB, Qiao YL, Lerma E, Garland SM, Sasagawa T, Ferrera A, Hammouda D, Mariani L, Pelayo A,
510 Steiner I, Oliva E, Meijer CJ, Al-Jassar WF, Cruz E, Wright TC, Puras A, Llave CL, Tzardi M, Agorastos T,
511 Garcia-Barriola V, Clavel C, Ordi J, Andújar M, Castellsagué X, Sánchez GI, Nowakowski AM, Bornstein
512 J, Muñoz N, Bosch FX (2010) Human papillomavirus genotype attribution in invasive cervical cancer:

513 a retrospective cross-sectional worldwide study. *Lancet Oncol* 11: 1048–1056 doi:10.1016/S1470-
514 2045(10)70230-8.

515 Simanaviciene V, Gudleviciene Z, Pependikyte V, Dekaminaviciute D, Stumbryte A, Rubinaite V,
516 Zvirbliene A (2014) Studies on the prevalence of oncogenic HPV types among Lithuanian women
517 with cervical pathology. *J Med Virol* doi:10.1002/jmv.24073.

518 Škamperle M, Kocjan BJ, Maver PJ, Seme K, Poljak M (2013) Human papillomavirus (HPV) prevalence
519 and HPV type distribution in cervical, vulvar, and anal cancers in central and eastern Europe. *Acta*
520 *dermatovenerologica Alpina, Pannonica, Adriat* 22: 1–5.

521 Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM (2007) Human papillomavirus
522 type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update.
523 *Int J Cancer* 121: 621–632 doi:10.1002/ijc.22527.

524 Smith JS, Melendy A, Rana RK, Pimenta JM (2008) Age-specific prevalence of infection with human
525 papillomavirus in females: a global review. *J Adolesc Health* 43: S5–S25, S25.e1–e41
526 doi:10.1016/j.jadohealth.2008.07.009.

527 Tjalma WA, Fiander A, Reich O, Powell N, Nowakowski AM, Kirschner B, Koiss R, O’Leary J, Joura EA,
528 Rosenlund M, Colau B, Schledermann D, Kukk K, Damaskou V, Repanti M, Vladareanu R, Kolomiets L,
529 Savicheva A, Shipitsyna E, Ordi J, Molijn A, Quint W, Raillard A, Rosillon D, De Souza SC, Jenkins D,
530 Holl K (2013) Differences in human papillomavirus type distribution in high-grade cervical
531 intraepithelial neoplasia and invasive cervical cancer in Europe. *Int J Cancer* 132: 854–867
532 doi:10.1002/ijc.27713.

533 Trottier H, Mahmud SM, Lindsay L, Jenkins D, Quint W, Wieting SL, Schuind A, Franco EL (2009)
534 Persistence of an incident human papillomavirus infection and timing of cervical lesions in previously
535 unexposed young women. *Cancer Epidemiol Biomarkers Prev* 18: 854–862 doi:10.1158/1055-
536 9965.EPI-08-1012.

537 Verdenius I, Groner JA, Harper DM (2013) Cross protection against HPV might prevent type
538 replacement. *Lancet Infect Dis* 13: 195 doi:10.1016/S1473-3099(13)70024-0.

539 De Vincenzo R, Ricci C, Conte C, Scambia G (2013) HPV vaccine cross-protection: Highlights on
540 additional clinical benefit. *Gynecol Oncol* 130: 642–651 doi:10.1016/j.ygyno.2013.05.033.

541 Vink MA, Bogaards JA, van Kemenade FJ, de Melker HE, Meijer CJLM, Berkhof J (2013) Clinical
542 progression of high-grade cervical intraepithelial neoplasia: estimating the time to preclinical cervical
543 cancer from doubly censored national registry data. *Am J Epidemiol* 178: 1161–1169
544 doi:10.1093/aje/kwt077.

545 Walboomers JM, Jacobs M V, Manos MM, Bosch FX, Kummer JA, Shah K V, Snijders PJ, Peto J, Meijer
546 CJ, Muñoz N (1999) Human papillomavirus is a necessary cause of invasive cervical cancer
547 worldwide. *J Pathol* 189 : 9–12.

548 Wentzensen N, Walker J, Schiffman M, Yang HP, Zuna RE, Dunn ST, Allen RA, Zhang R, Sherman M,
549 Gold MA, Wang SS (2013) Heterogeneity of high-grade cervical intraepithelial neoplasia related to
550 HPV16: implications for natural history and management. *Int J Cancer* 132: 148–154

551 doi:10.1002/ijc.27577.

552 WHO/ICO Information Centre on HPV and Cervical Cancer (2010) Human Papillomavirus and related
553 cancers in the United Kingdom, summary report.

554

Table 1: Percentage distribution of the number of HPV genotypes detected by pathological subtype and 5-year age group.

Number of HPV Genotypes detected (% of each pathology)							
Pathology	HPV negative	1	2	3	4	5 or more	Total n (%)
CIN I	283 (51.9)	169 (31.0)	63 (11.6)	19 (3.5)	9 (1.7)	2 (0.4)	545 (100.0)
CIN II	145 (34.1)	217 (51.1)	46 (10.8)	15 (3.5)	1 (0.2)	1 (0.2)	425 (100.0)
CIN III	146 (18.7)	462 (59.3)	129 (16.6)	32 (4.1)	4 (0.5)	6 (0.8)	779 (100.0)
SCC	5 (7.8)	52 (81.3)	6 (9.4)	1 (1.6)	0 (0.0)	0 (0.0)	64 (100.0)
AC	5 (35.7)	8 (57.1)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	14 (100.0)
Age group (yrs)	HPV negative	1	2	3	4	5 or more	Total n (%)
Under 25	115 (30.7)	172 (45.9)	64 (17.1)	18 (4.8)	3 (0.8)	3 (0.8)	375 (100.0)
25-29	152 (28.7)	270 (51.0)	79 (14.9)	21 (4.0)	6 (1.1)	1 (0.2)	529 (100.0)
30-34	110 (30.7)	186 (52.0)	44 (12.3)	14 (3.9)	1 (0.3)	3 (0.8)	358 (100.0)
35-39	74 (33.3)	119 (53.6)	23 (10.4)	4 (1.8)	1 (0.5)	1 (0.5)	222 (100.0)
40-44	51 (33.8)	77 (51.0)	16 (10.6)	6 (4.0)	1 (0.7)	0 (0.0)	151 (100.0)
45-49	41 (44.6)	39 (42.4)	9 (9.8)	2 (2.2)	1 (1.1)	0 (0.0)	92 (100.0)
50-54	24 (49.0)	17 (34.7)	6 (12.2)	1 (2.0)	1 (2.0)	0 (0.0)	49 (100.0)
55-59	10 (35.7)	13 (46.4)	3 (10.7)	1 (3.6)	0 (0.0)	1 (3.6)	28 (100.0)
60-64	3 (33.3)	6 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (100.0)
65+	4 (28.6)	9 (64.3)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	14 (100.0)
TOTAL	584 (32.0)	908 (49.7)	244 (13.4)	68 (3.7)	14 (0.8)	9 (0.5)	1,827 (100.0)

CIN = Cervical Intraepithelial Neoplasia (grades I-III), SCC = Squamous cell carcinoma, AC = Adenocarcinoma

Table 2: Type of HPV genotypes detected by cervical pathology and 5-year age group.

Pathology	HPV-16 and or HPV-18	Other High-risk HPV genotypes*	Low risk HPV genotypes Only	HPV negative	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
CIN I	112 (20.6)	121 (22.2)	29 (5.3)	283 (51.9)	545 (100.0)
CIN II	150 (35.3)	110 (25.9)	20 (4.7)	145 (34.1)	425 (100.0)
CIN III	438 (56.2)	184 (23.6)	11 (1.4)	146 (18.7)	779 (100.0)
SCC	53 (82.8)	6 (9.4)	0 (0.0)	5 (7.8)	64 (100.0)
AC	9 (64.3)	1 (7.1)	0 (0.0)	5 (35.7)	14 (100.0)
Age group (yrs)					
Under 25	154 (41.1)	93 (24.8)	13 (3.5)	115 (30.7)	375 (100.0)
25-29	237 (44.8)	127 (24.0)	13 (2.5)	152 (28.7)	529 (100.0)
30-34	151 (42.2)	82 (22.9)	15 (4.2)	110 (30.7)	358 (100.0)
35-39	84 (37.8)	59 (26.6)	5 (2.3)	74 (33.3)	222 (100.0)
40-44	59 (39.1)	36 (23.8)	5 (3.3)	51 (33.8)	151 (100.0)
45-49	34 (37.0)	12 (13.0)	5 (5.4)	41 (44.6)	92 (100.0)
50-54	18 (36.7)	5 (10.2)	2 (4.1)	24 (49.0)	49 (100.0)
55-59	12 (42.9)	4 (14.3)	2 (7.1)	10 (35.7)	28 (100.0)
60-64	4 (44.4)	2 (22.2)	0 (0.0)	3 (33.3)	9 (100.0)
65+	9 (64.3)	1 (7.1)	0 (0.0)	4 (28.6)	14 (100.0)
TOTAL n (%)	762 (41.7)	421 (23.0)	60 (3.3)	584 (32.0)	1,827 (100.0)

* Including high-risk HPV genotypes other than HPV 16/18 i.e.: HPV-31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.
CIN = Cervical Intraepithelial Neoplasia (grades I-III), SCC = Squamous cell carcinoma, AC = Adenocarcinoma

Table 3: HPV genotype distribution by cervical pathology

HPV genotype	Cervical histology					Total
	AC	CIN I	CIN II	CIN III	SCC	
<i>HPV 16</i>	3 (21.4)	87 (16.0)	136 (32.0)	407 (52.3)	51 (79.7)	684 (37.4)
<i>HPV 31</i>	0 (0.0)	35 (6.4)	29 (6.8)	84 (10.8)	2 (3.1)	150 (8.2)
<i>HPV 52</i>	0 (0.0)	22 (4.0)	33 (7.8)	65 (8.3)	5 (7.8)	125 (6.8)
<i>HPV 18</i>	6 (42.9)	29 (5.1)	17 (4.0)	39 (5.0)	3 (4.7)	93 (5.1)
<i>HPV 33</i>	0 (0.0)	18 (3.3)	15 (3.5)	58 (7.5)	0 (0.0)	91 (5.0)
<i>HPV 51</i>	1 (7.1)	17 (3.1)	25 (5.9)	28 (3.6)	0 (0.0)	71 (3.9)
<i>HPV 45</i>	0 (0.0)	14 (2.6)	10 (2.4)	31 (4.0)	3 (4.7)	58 (3.2)
<i>HPV 39</i>	0 (0.0)	20 (3.7)	12 (2.8)	14 (1.8)	1 (1.6)	47 (2.6)
<i>HPV 58</i>	0 (0.0)	11 (2.0)	13 (3.1)	17 (2.2)	0 (0.0)	41 (2.2)
<i>HPV 66</i>	0 (0.0)	22 (4.0)	6 (1.4)	10 (1.3)	0 (0.0)	38 (2.1)
<i>HPV 35</i>	0 (0.0)	9 (1.7)	12 (2.8)	16 (2.1)	0 (0.0)	37 (2.0)
<i>HPV 59</i>	0 (0.0)	14 (2.6)	8 (1.9)	8 (1.0)	0 (0.0)	30 (1.6)
<i>HPV 56</i>	0 (0.0)	18 (3.3)	3 (0.7)	5 (0.6)	0 (0.0)	26 (1.4)
<i>HPV 6</i>	0 (0.0)	7 (1.3)	9 (2.1)	9 (1.2)	0 (0.0)	25 (1.4)
<i>HPV 73</i>	0 (0.0)	9 (1.7)	6 (1.4)	8 (1.0)	0 (0.0)	23 (1.3)
<i>HPV 53</i>	0 (0.0)	9 (1.7)	5 (1.2)	5 (0.6)	0 (0.0)	19 (1.0)
<i>HPV 70</i>	0 (0.0)	4 (0.7)	4 (0.9)	9 (1.2)	0 (0.0)	17 (0.9)
<i>HPV 42</i>	0 (0.0)	9 (1.7)	2 (0.5)	3 (0.4)	0 (0.0)	14 (0.8)
<i>HPV 61</i>	0 (0.0)	6 (1.1)	1 (0.2)	7 (0.9)	0 (0.0)	14 (0.8)
<i>HPV 54</i>	1 (7.1)	2 (0.4)	3 (0.7)	5 (0.8)	0 (0.0)	12 (0.7)
<i>HPV 11</i>	0 (0.0)	5 (0.9)	2 (0.5)	4 (0.5)	0 (0.0)	11 (0.6)
<i>HPV 68</i>	0 (0.0)	4 (0.7)	5 (1.2)	2 (0.3)	0 (0.0)	11 (0.6)
<i>HPV CP6108</i>	0 (0.0)	4 (0.7)	0 (0.0)	6 (0.8)	0 (0.0)	10 (0.6)
<i>HPV 82</i>	0 (0.0)	1 (0.2)	0 (0.0)	8 (1.0)	0 (0.0)	9 (0.5)
<i>HPV 62</i>	0 (0.0)	2 (0.4)	1 (0.2)	4 (0.5)	0 (0.0)	7 (0.4)
<i>HPV 84</i>	0 (0.0)	3 (0.6)	2 (0.5)	2 (0.3)	0 (0.0)	7 (0.4)
<i>HPV 81</i>	0 (0.0)	6 (1.1)	0 (0.0)	1 (0.1)	0 (0.0)	7 (0.4)
<i>HPV 69</i>	0 (0.0)	2 (0.4)	1 (0.2)	2 (0.3)	0 (0.0)	5 (0.3)
<i>HPV 55</i>	0 (0.0)	3 (0.6)	1 (0.2)	0 (0.0)	0 (0.0)	4 (0.2)
<i>HPV 83</i>	0 (0.0)	2 (0.4)	1 (0.2)	1 (0.1)	0 (0.0)	4 (0.2)
<i>HPV is39</i>	0 (0.0)	0 (0.0)	2 (0.5)	1 (0.1)	0 (0.0)	3 (0.2)
<i>HPV 67</i>	0 (0.0)	3 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.2)
<i>HPV 40</i>	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.1)	0 (0.0)	2 (0.11)
<i>HPV 26</i>	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.1)	0 (0.0)	2 (0.1)
<i>HPV 64</i>	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
<i>HPV 72</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>HPV 71</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TOTAL	11 (78.5)	400 (73.6)	364 (85.6)	861 (110.8)	65 (101.6)	1701 (93.3)*

AC = adenocarcinoma, CIN = Cervical Intraepithelial Neoplasia (grades I-III), SCC = Squamous Cell Carcinoma

* This figure does not add up to 1,243 (i.e.: the number HPV positive) as several patients may have had multiple HPV genotypes on testing

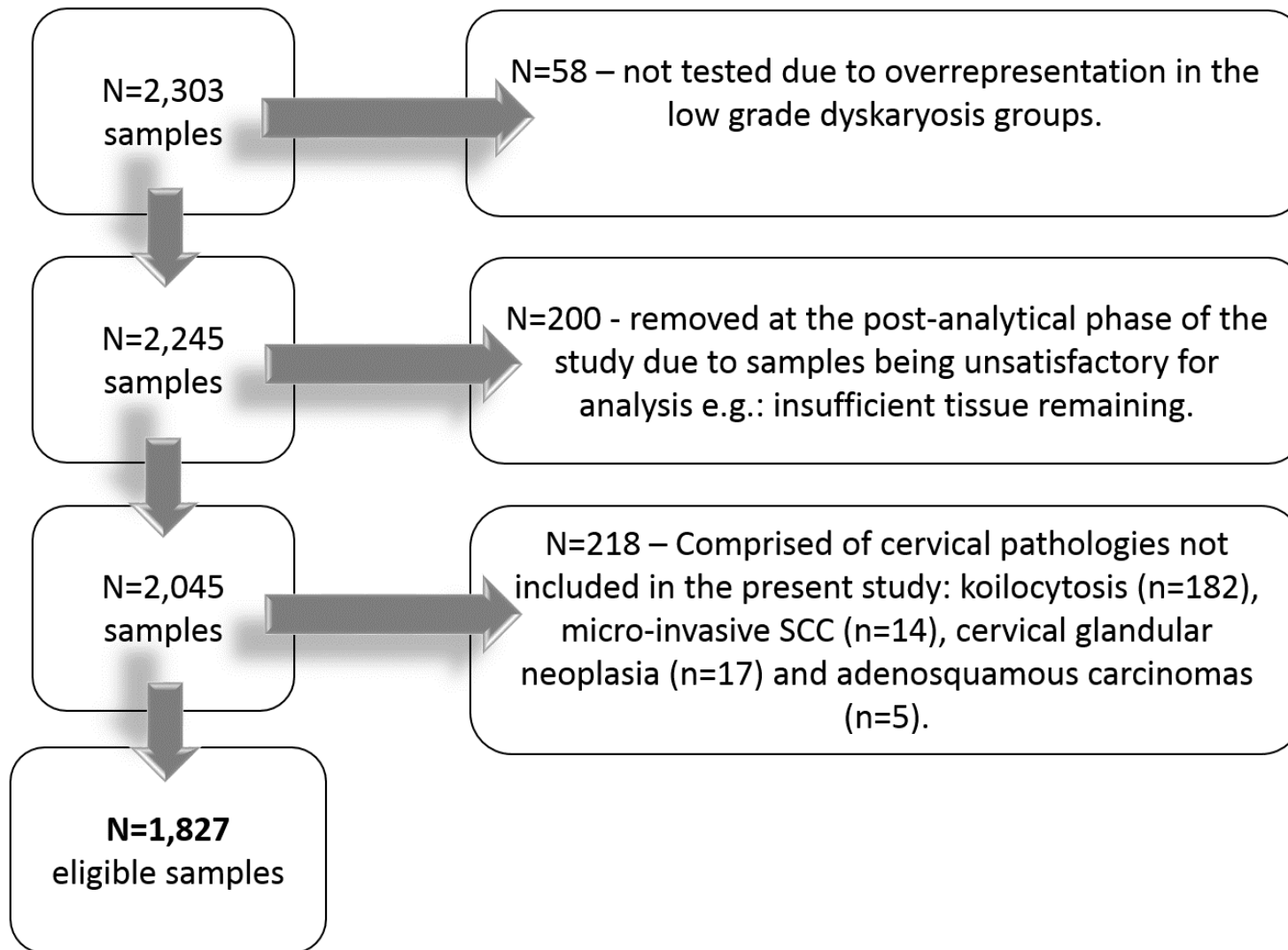
Table 4: Pathological distribution of cohort (numbers of cases) by five-year age group

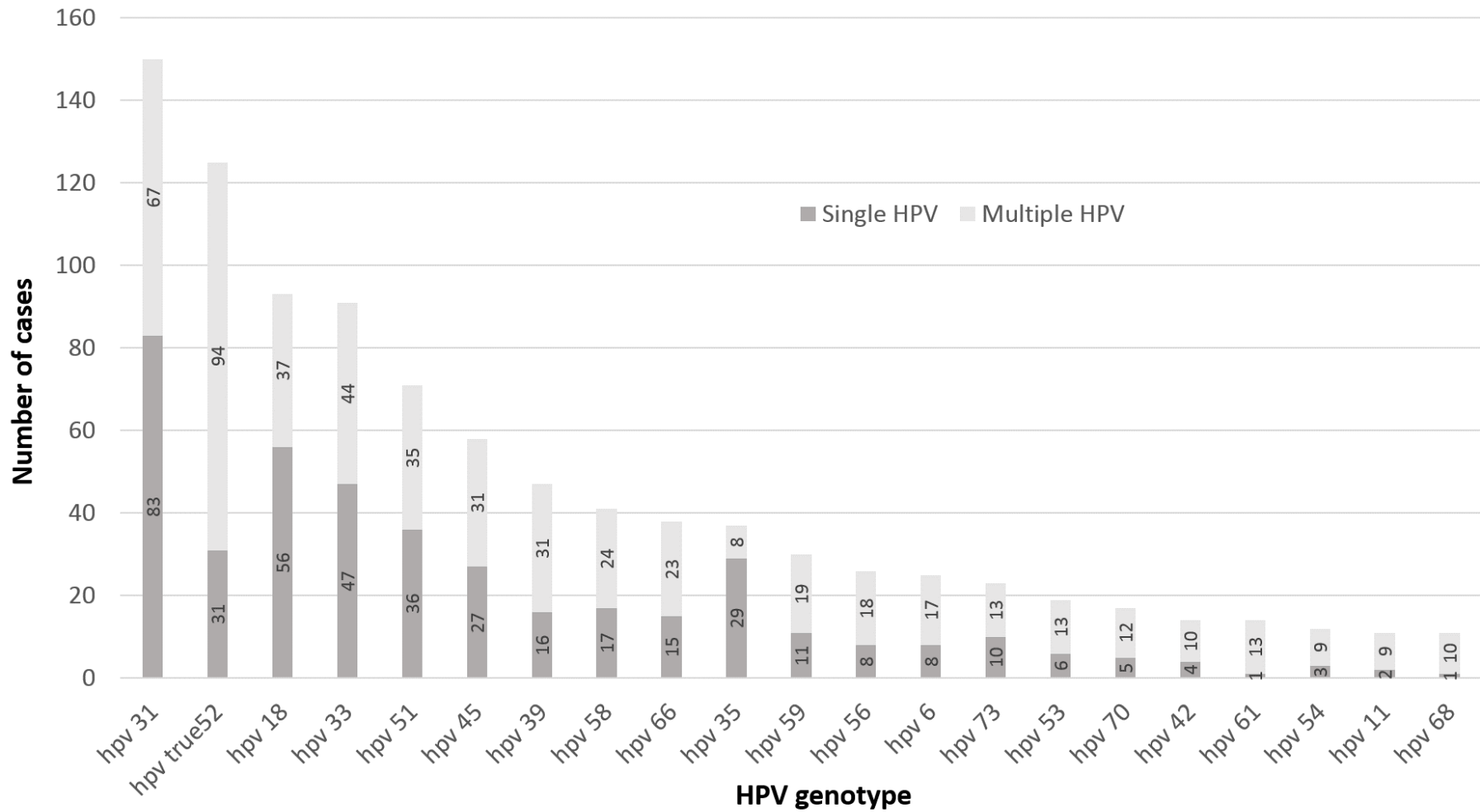
PATHOLOGY n (%)	Mean age	AGE (years)										n (%)
	years (\pm SD; range)	<25	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65+	
<i>CIN I</i>	32 (10; 16-69)	144 (26.4)	136 (25.0)	82 (15.1)	55 (10.1)	51 (9.4)	42 (7.7)	19 (3.5)	11 (2.0)	3 (0.6)	2 (0.4)	545 (100.0)
<i>CIN II</i>	32 (9; 18-80)	83 (19.5)	120 (28.2)	92 (21.7)	51 (12.0)	40 (9.4)	17 (4.0)	11 (2.6)	6 (1.4)	1 (0.2)	4 (0.9)	425 (100.0)
<i>CIN III</i>	31 (8; 18-64)	146 (18.7)	268 (34.4)	169 (21.7)	94 (12.1)	53 (6.8)	27 (3.5)	12 (1.5)	7 (0.9)	3 (0.4)	0 (0.0)	779 (100.0)
<i>SSC</i>	43 (14; 23-93)	1 (1.6)	3 (4.7)	12 (18.8)	19 (29.7)	7 (10.9)	5 (7.8)	7 (10.9)	3 (4.7)	1 (1.6)	6 (9.4)	64 (100.0)
<i>AC</i>	42 (16; 23-73)	1 (7.1)	2 (14.3)	3 (21.4)	3 (21.4)	0 (0.0)	1 (7.1)	0 (0.0)	1 (7.1)	1 (7.1)	2 (14.3)	14 (100.0)
TOTAL n (%)		375 (20.5)	529 (28.9)	358 (19.6)	222 (12.2)	151 (8.3)	92 (5.0)	49 (2.7)	28 (1.5)	9 (0.5)	14 (0.8)	1,827 (100.0)

CIN = Cervical Intraepithelial Neoplasia (grades I-III), SCC = Squamous Cell Carcinoma, AC= Adenocarcinoma

\pm SD = Standard deviation

N.B.: Please note that certain pathological groups were overrepresented in this study, so the proportions above may not be a true reflection of the distribution of cervical pathologies within each age category in Northern Ireland.





HPV genotypes omitted above (no. of single/multiple infections): HPV 16 (480/204), HPV CP6108 (1/9), HPV 82 (0/9), HPV 62 (0/7), HPV 84 (1/6), HPV 81 (3/4), HPV 69 (1/4), HPV 55 (0/4), HPV 83 (2/2), HPV IS39 (1/2), HPV 67 (2/1), HPV 40 (0/2), HPV 26 (1/1), HPV 64 (0/1), HPV 72 (0/0), HPV 71 (0/0).