EJGO European Journal of Gynaecological Oncology

Review Article

Human papillomavirus prevalence and breast carcinogenesis: a systematic review and meta-analysis of published literature

K. Favere[1], S. Menon[2], K. Valentine[2], J. Bogers[3,4,5], D. Vanden Broeck[3,4,5]

[1]Faculty of Medicine and Health Sciences, Ghent University, Ghent
[2]International Centre for Reproductive Health, Ghent University, Ghent
[3]Applied Molecular Biology Research Group, University of Antwerp, Antwerp; [4]AML-Molecular Diagnostics, Antwerp
[5]International Centre for Reproductive Health, Ghent University, Ghent (Belgium)

Summary

Purpose of investigation: The association between human papillomavirus (HPV) infection and breast cancer remains inconclusive as detection rates of high-risk HPV in breast cancer samples are extremely variable. A meta-analysis was conducted to determine the prevalence of HPV in breast neoplasms, with emphasis on genotype distribution. *Materials and Methods:* A systematic literature search of MEDLINE, The Cochrane Library, and ISI Web of Science databases was conducted, ending in August 2016. A meta-analysis was performed applying the random-effects model. Sub-analyses allowed to estimate the impact of different variables on the pooled prevalence. *Results:* Forty studies, representing 4762 breast cancer cases, were included. The pooled prevalence of HPV in breast cancer tissue was 20% (95% confidence interval (CI) [12%;29%]). HPV prevalence in breast neoplasms varied by publication period, continental region, HPV primer design, and HPV oncogenic features. Continental region of origin determined the prevailing genotype. *Conclusion:* The high prevalence of HPV in breast cancer supports the hypothesis that HPV infection is involved in breast carcinogenesis.

Key words: Breast neoplasms; Review; Meta-analysis; Epidemiology; Etiology; Papillomaviridae.

Introduction

Breast cancer is the most common female malignancy in both the developing and developed world [1]. In 2012, approximately 1.7 million people were diagnosed with breast cancer worldwide [2]. In that same year, over 500,000 women died from breast cancer, representing the second leading cause of female cancer death [1-3]. Development of an integrative breast cancer therapy together with increased public awareness about early detection has led to a good overall prognosis [4]. The global burden of breast cancer in women, measured by incidence, mortality, and economic costs, is nevertheless substantial and on the rise [5].

The multifactorial etiology of breast cancer remains poorly understood [6]. Various risk factors such as age, geographic location, hormone levels, genetic predisposition, smoking, and alcohol consumption are known to modulate the development of breast cancer [3]. Recently, viruses, including the human papillomaviruses (HPV), the mouse mammary tumour virus (MMTV), and the Epstein-Barr virus [EBV), have been increasingly implicated in the pathogenesis of breast cancer [7, 8].

Band *et al.* were the first to suggest a causal relationship between breast cancer and HPV by demonstrating immortalisation of normal human mammary epithelial cells by high-risk (HR) HPV-16 and -18 genomes [9]. These findings were further confirmed by Wazer *et al.*[10]. Di Lonardo *et al.* were the first to determine HPV prevalence in breast cancer cases. Their polymerase chain reaction (PCR)-based results supported a potential relation by reporting HPV-16 deoxyribonucleic acid (DNA) in 29.4% of the 17 breast cancer cases [11]. Subsequently, a growing number of research groups studied HPV prevalence, but the published percentages differed widely. However, an increasing number of researchers failed to demonstrate HPV presence in breast carcinoma tissue, despite using identical detection techniques, such as broad-spectrum PCR methods [8, 12-15]. In addition, in the past decade, several case-control studies reported considerable prevalence rates of HPV in control specimens, although a recent meta-analysis concluded a statistically significant pooled odds ratio of 4.02 [4, 16-18]. The latter statistical significant result indicates that breast cancer tissue is four times more likely to contain HPV material opposed to non-malignant breast ma-

Revised manuscript accepted for publication January 19, 2017

Eur. J. Gynaecol. Oncol. - ISSN: 0392-2936
XXXIX, n. 2, 2018
doi: 10.12892/ejgo4068.2018

7847050 Canada Inc.
www.irog.net

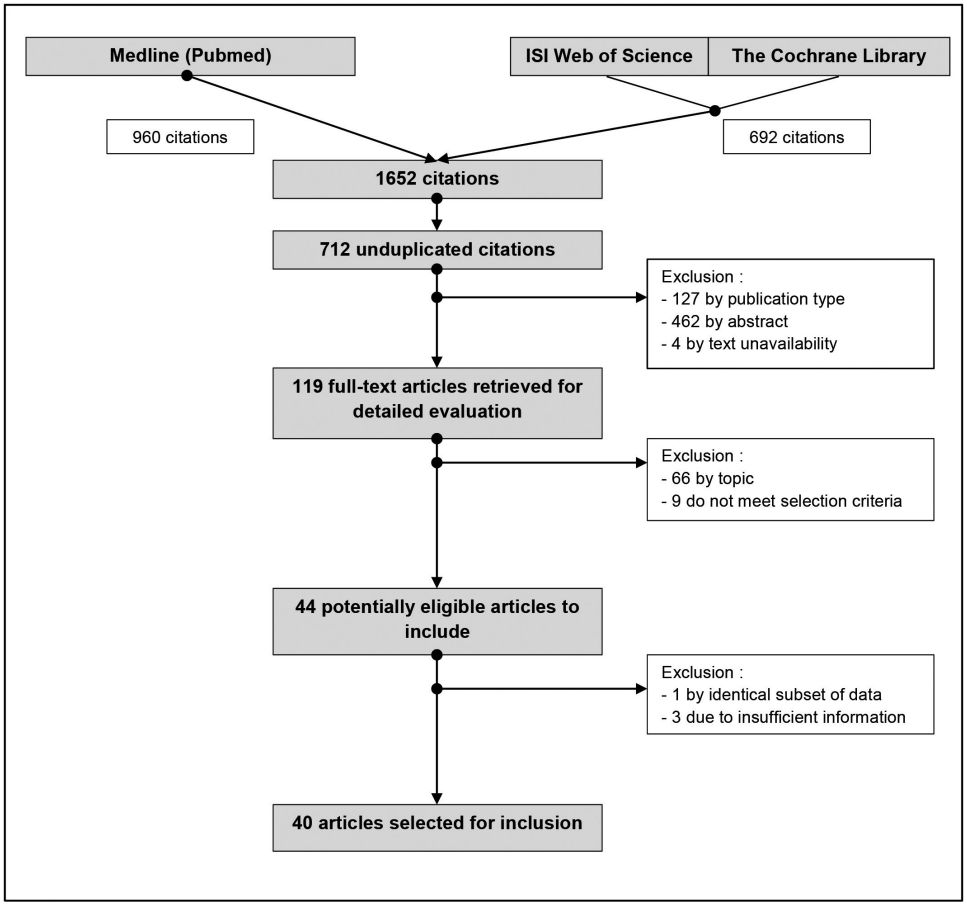

Figure 1. — Flow-chart of article selection for inclusion in meta-analysis HPV prevalence – breast cancer.

terial. The recent observation that the viral load in breast cancer is extremely low compared to the cervical cancer viral load, again contributed to the uncertainty about a proposed causal relationship [19]. The possible influence of contamination is another important factor not yet fully understood. An Indian study used fine needle aspiration (FNA) to avoid contamination by surrounding tissues and reported absence of HPV in the examined breast biopsy samples [14]. On the contrary, Lawson *et al.* recently reported the presence of biologically active HR-HPVs in breast cancer material, using methods insusceptible to contamination [20].

As the relationship between breast cancer and HPV remains a topic of debate, the purpose of this meta-analysis is to combine published information on HPV prevalence in breast carcinoma cases, and the factors relating to it, in order to give insight in the relation between mammary carcinogenesis and HPV infection. Further elaboration of the HPV infection theory could lead to a new paradigm in the prevention and treatment of breast cancer.

Materials and Methods

Relevant studies on the association between breast cancer and HPV infection were identified through an extensive search of MEDLINE, The Cochrane Library, and ISI Web of Science. In MEDLINE, the following key words were applied: “HPV”, “human papillomavirus”, and “breast cancer”. Secondary, the following medical subject headings (MeSH) terms were used: “papillomaviridae”, and “breast neoplasms”. These search queries yielded 960 citations in total.

The Cochrane Library and ISI Web of Science databases were searched using the keywords “breast”, “cancer”, and “HPV”. This search produced 692 citations.

Studies addressing the relationship between breast cancer and HPV were reviewed and evaluated critically for predefined eligibility criteria. Two authors (K.F. and D.V.B.) independently performed data retrieval and reached consensus on all items. The literature search comprised all literature until August 2016, with no publication starting-date limitation. Reference lists of relevant papers, including reviews and meta-analyses, were examined to identify other relevant articles. The ‘Related Citations’ tool of PubMed was applied whenever a suitable article was included. The 11 articles concerning HPV prevalence retained in the meta-analysis conducted by Li *et al*., were independently selected for use in this meta-analysis [21]. Figure 1 summarises the study selection process. This systematic review and meta-analysis was conducted in accordance with the ‘Meta-analysis Of Observational Studies in Epidemiology’ (MOOSE) guidelines and ‘Pre-

Figure 2. — MOOSE checklist for meta-analyses of observational studies.

Item No	Recommendation	Reported on Page No
Reporting of background should include		
1	Problem definition	5-6
2	Hypothesis statement	6
3	Description of study outcome(s)	5-6
4	Type of exposure or intervention used	N/A
5	Type of study designs used	5-6
6	Study population	5-6
Reporting of search strategy should include		
7	Qualifications of searchers (eg, librarians and investigators)	7
8	Search strategy, including time period included in the synthesis and key words	7
9	Effort to include all available studies, including contact with authors	7
10	Databases and registries searched	7
11	Search software used, name and version, including special features used (eg, explosion)	7
12	Use of hand searching (eg, reference lists of obtained articles)	7
13	List of citations located and those excluded, including justification	figure 1
14	Method of addressing articles published in languages other than English	7
15	Method of handling abstracts and unpublished studies	7
16	Description of any contact with authors	N/A
Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	7
18	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	7-9
19	Documentation of how data were classified and coded (eg, multiple raters, blinding and interrater reliability)	7-9
20	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	N/A
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	N/A
22	Assessment of heterogeneity	9
23	Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	9
24	Provision of appropriate tables and graphics	25-29
Reporting of results should include		
25	Graphic summarizing individual study estimates and overall estimate	figure 2
26	Table giving descriptive information for each study included	table 1
27	Results of sensitivity testing (eg, subgroup analysis)	table 3
28	Indication of statistical uncertainty of findings	figure 2, table 3
Reporting of discussion should include		
29	Quantitative assessment of bias (eg, publication bias)	N/A
30	Justification for exclusion (eg, exclusion of non-English language citations)	7-8
31	Assessment of quality of included studies	10-11, 14-16
Reporting of conclusions should include		
32	Consideration of alternative explanations for observed results	13-17
33	Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	18
34	Guidelines for future research	18
35	Disclosure of funding source	1

From: Stroup DF, Berlin JA, Morton SC, et al, for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group.Meta-analysis of Observational Studies in Epidemiology. A Proposal for Reporting. JAMA. 2000;283(15):2008-2012. doi: 10.1001/jama.283.15.2008.
Transcribed from the original paper within the NEUROSURGERY® Editorial Office, Atlanta, GA, United Sates. August 2012.

Figure 3. — Prisma checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5-6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7-8
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7, figure 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7-8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7-8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8-9
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	N/A
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Prevalence
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	13-17
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	N/A
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	figure 2, table 2,3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-12
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10-12, table 3
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	13-17
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	18
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

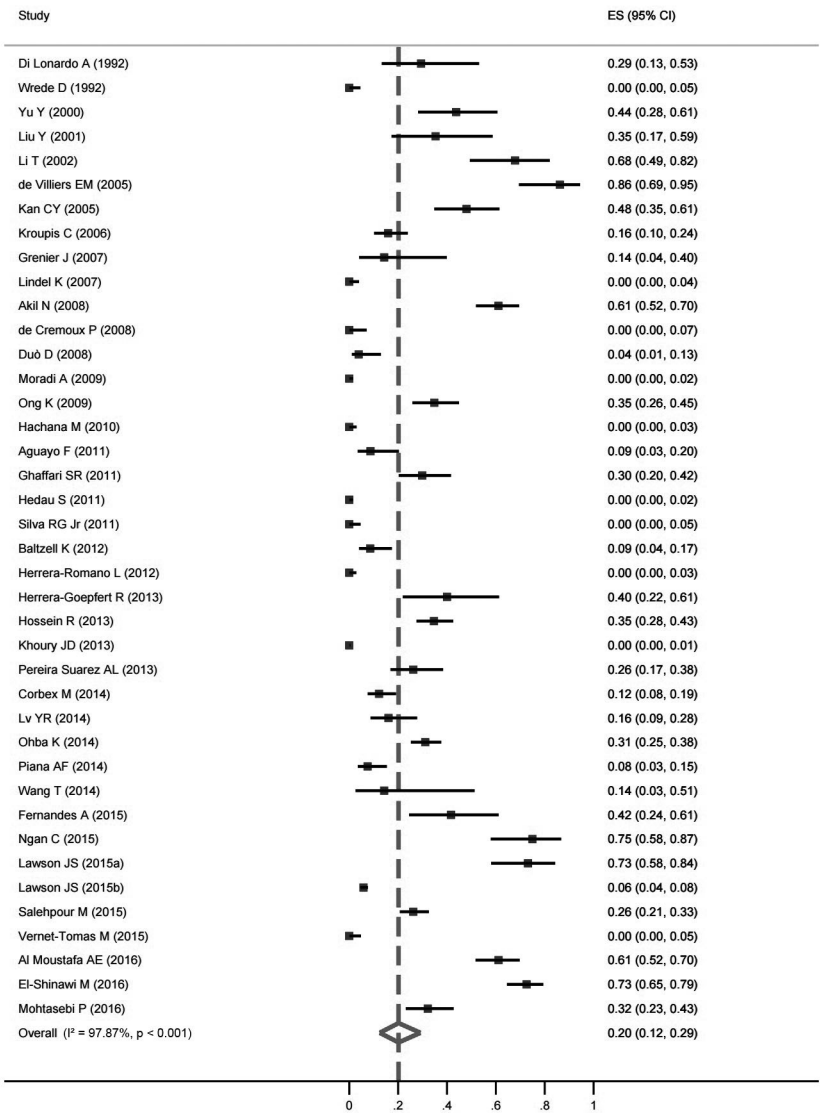

Figure 4. — a) Forest plot of prevalence estimates of HPV in breast cancer tissue.
Studies are identified by references. Each study is represented by a black square and a horizontal line, which corresponds to the prevalence and 95% confidence interval (CI) respectively. The area of the black square reflects the weight of the study in the meta-analysis. The rhombus depicts the pooled prevalence. The vertical black line corresponds to the zero prevalence rate.

ferred Reporting Items for Systematic reviews and Meta-Analyses' (PRISMA) statement [22, 23] (see MOOSE and PRISMA checklist) (Figures 2 and 3).

Studies were limited to those written in English and French. This meta-analysis was restricted to original articles, excluding case reports, reviews, and editorials. Conference abstracts and other unpublished articles were excluded, as these could not be systematically reviewed and data could not be verified.

This meta-analysis was limited to cross-sectional and cohort studies dealing with clinical breast cancer. If (subsets of) data were published in different articles, only the study with the largest sample size was included [24, 25]. Research studies focusing solely on breast neoplasm with unusual histopathology, such as lymphoepithelioma-like carcinoma or Paget's disease, were excluded [26, 27]. However, the 14 squamous cell carcinomas studied by Grenier *et al.*, demonstrating patients characteristics comparable to patients with invasive ductal carcinoma, were included [28]. Special study populations, for instance adolescents or women with a history of cervical cancer or high grade cervical intraepithelial neoplasia, were also excluded from analysis [29-32]. The subset of 38 patients with an anamnestic family history of breast cancer, as reported by Mohtasebi *et al.*, were not excluded from analysis [33]. Eligible studies were included when necessary information could be retrieved; no criteria were imposed on patient number, nor on presentation of results.

For each study, following data were extracted: first author's name, year of publication, country of origin, study design, number of cases enrolled, study population, age range and/or mean age of patients, test sample source, histological classification of examined breast cancer tissue, type of study material, method of HPV detection, PCR primers, HPV types tested, number of HPV-positive cases, and HPV prevalence (overall and type-specific if mentioned). All HPV genotypes were recorded. Co-infections were separated into constituent genotypes. Accordingly, type-specific prevalence rates represent both single and multiple HPV infections.

Publication calendar period was dichotomised into '1992-2008' and '2009-2016'. Countries were divided into six continental regions according to the United Nations classification [34].

Carcinomas were categorised into six groups: invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), ductal carcinoma *in situ* (DCIS), mucinous carcinoma, medullary carcinoma

and other (including rare histological types like papillary, tubular, squamous cell (SCC), and mixed carcinomas).
HPV was detected by means of different detection techniques, summarised per study in Table 1 [8, 11, 13, 15, 34-65].

Koilocytosis was not considered specific enough as indicator of HPV infection. PCR primers were grouped into either 'type-specific', 'broad spectrum' or 'combined usage of both primer types'. Type-specific primers target DNA sequences unique to a particular genotype. Broad spectrum primers are directed at relatively conserved regions of the HPV genome and consequently permit detection of a broad array of genotypes. The classification proposed by Muñoz *et al.* was used to categorise the HPV genotypes into three groups according to their oncogenic potential. The aforementioned classification covers 30 genotypes, dividing them into high-risk (HR), probable high-risk (pHR), and low-risk (LR) types [35]. A register of the included studies and a summary of the retrieved information are given in Table 1 [8, 11, 13, 15, 34-65].

Table 1. — *Characteristics of the selected studies included in the meta-analysis HPV prevalence - breast cancer.*

Lead author	Year of publication	Nation	Sample size	No. positive cases (%)	Sample source	Age range/mean age (years)	HPV detection method
Di Lonardo [11]	1992	Italy	17	5 (29.41%)	PET	-	PCR + ISH
Wrede [13]	1992	UK	80	0 (0.00%)	Fresh tissue	29 - 76/54	PCR
Yu [25]	2000	China	32	14 (43.75%)	PET	40 - 82	PCR + Southern blot
Liu [65]	2001	USA	17	6 (35.29%)	Frozen tissue	-	PCR + Dot plot + sequencing
Li [64]	2002	China	28	19 (67.86%)	PET	-	PCR + ISH
de Villiers [63]	2005	Germany	29	25 (86.21%)	PET	30 - 88	PCR + ISH + sequencing
Kan [62]	2005	Australia	50	24 (48.00%)	Previously extracted DNA & FFT	-	PCR + sequencing
Kroupis [61]	2006	Greece	107	17 (15.89%)	Frozen tissue	35- 63	PCR + RFLP
Grenier [28]	2007	France	14	2 (14.29%)	PET	31 - 86/55	PCR
Lindel [15]	2007	Germany	92	0 (0.00%)	PET	-	PCR
Akil [59]	2008	Syria	113	69 (61.06%)	PET	26 - 66	PCR + LPA + IHC
de Cremoux [8]	2008	France	50	0 (0.00%)	Fresh tissue	37 - 92/54.54	PCR
Duò [60]	2008	Italy	52	2 (3.85%)	PET	36 - 71	PCR + LPA
Moradi [46]	2009	Iran	231	0 (0.00%)	-	20-84	PCR
Ong [58]	2009	Singapore	92	32 (34.78%)	PET	19 - 86	PCR + sequencing
Hachana [57]	2010	Tunesia	123	0 (0.00%)	Frozen tissue	31 - 87/49.3	PCR + ISH
Aguayo [55]	2011	Chile	46	4 (8.70%)	PET	48 - 69	PCR + LPA
Ghaffari [56]	2011	Iran	67	20 (29.85%)	Frozen tissue	-	PCR
Hedau [54]	2011	France	228	0 (0.00%)	Frozen tissue	25 - 80	PCR
Silva [7]	2011	Brasil	79	0 (0.00%)	PET	23 - 87	PCR + electrophoresis
Baltzell [53]	2012	USA	70	6 (8.57%)	PET	32 - 77/54	IS-PCR + ISH
Herrera-Romano [52]	2012	Mexico	128	0 (0.00%)	PET & 10 fresh samples	30 - 81/55	PCR
Herrera-Goepfert [50]	2013	Mexico	20	8 (40.00%)	PET	24 - 73/49	PCR
Hossein [49]	2013	Iran	150	52 (34.67%)	PET	20 - 80	PCR
Khoury [51]	2013	USA	750	0 (0.00%)	RNA sequence data	-	RNA sequencing
Pereira Suarez [48]	2013	Argentina	61	16 (26.23%)	FFT	35-92	PCR + sequencing
Corbex [44]	2014	Algeria	123	15 (12.20%)	PET	-	TS-MPG
Lv [45]	2014	China	56	9 (16.07%)	-	-	Hybrid capture II
Ohba [42]	2014	Singapore	209	65 (31.10%)	FFT	-	TOSHIBA DNA chip
Piana [41]	2014	Italy	80	6 (7.50%)	PET	60.3	LPA
Wang [43]	2014	China	7	1 (14.29%)	Frozen tissue	37-85	Capture + MPS
Fernandes [47]	2015	Venezuela	24	10 (41.67%)	Fresh tissue	37-84/56.75	LPA
Ngan [40]	2015	Australia	32	24 (75.00%)	PET	34-83	PCR + IHC + sequencing
Lawson [34]	2015	Australia	41	30 (73.17%)	PET	56.1	PCR + IHC + sequencing
Lawson [34]	2015	Australia	855	50 (5.85%)	TCGA data	31-84	NGS
Salehpour [35.]	2015	Iran	206	54 (26.21%)	PET	24-74	PCR
Vernet-Tomas [39]	2015	Spain	76	0 (0.00%)	PET	28-98/61.5 (median)	PCR + DEIA + LPA
Al Moustafa [37]	2016	Syria	108	66 (61.11%)	PET	-	PCR + IHC
El-Shinawi [36]	2016	Egypt	135	98 (72.59%)	Fresh tissue	27-78	PCR + sequencing
Mohtasebi [33]	2016	Iran	84	27 (32.14%)	PET	25-80	PCR

Abbreviations: DEIA = DNA enzyme immunoassay, FFT=fresh frozen tissue. IHC = immunohistochemistry, ISH = in situ hybridization, IS-PCR = in situ polymerase chain reaction, LPA = line probe assay, MPS = massive parallel sequencing, NGS = next-generation sequencing, PCR = polymerase chain reaction, PET = paraffin-embedded tissue, RFLP = restriction fragment length polymorphism, RNA = ribonucleic acid, TCGA = the cancer genome atlas, TS-MPG = type-specific multiplex genotyping.

Summary estimates were derived using a random-effects model to account for the between-study heterogeneity, expected when studies are conducted in different continents with different subjects. Prevalence rates were presented with 95% CI.

Heterogeneity was assessed using the I-squared (I^2) test, which describes the percentage of variation across studies that is due to heterogeneity rather than chance and judged considerable if ≥ 75% [66].

Subgroup analyses were performed to find the possible source of statistical heterogeneity among studies. These were carried out according to the continental region in which the study was conducted, the time period during which it was conducted ('1992-2008' or '2009-2016'), the type of primer used, and the carcinogenic potential of the HPV genotypes.

The total number of HPV-positive cases was used as denominator to calculate the genotype-specific prevalence rates. Accordingly, the pooled prevalence of the different HPV risk groups (HR, pHR and LR) was calculated by dividing the number of infections caused by genotypes belonging to the risk group by the total number of cases where genotypes were reported.

All statistical analyses were performed with STATA, version 13.1. Metaprop was employed in prevalence studies to deal with binomial data. Metaprop is a Stata module to perform fixed and random effects meta-analysis of proportions [67].

Results

Initial search gave rise to 712 unduplicated articles. Primary exclusion was based on publication type, abstract, and text availability. One hundred nineteen articles were retained. More profound evaluation of the whole article led to the exclusion of 75 studies. Eventually 44 articles were considered eligible for inclusion and statistical analysis. The last step of exclusion was based on information availability and duplication of study data.

In total, 40 cross-sectional studies with different study populations and using different detection techniques were included [7, 8, 11, 13, 15, 20, 24, 28, 33, 36-65]. This resulted in 4,762 cases of breast carcinoma in total. The contribution of each study ranged from seven to 855 breast cancer cases [20, 43]. The studies were published between 1992 and 2016. The majority (60%) of the studies were published after 2010, indicating the increasing interest in the subject. Six continental regions (Africa, Asia, Europe, North America, Latin America, and Oceania) and 20 countries were represented. Asia (29.04%) represented the largest share of the breast carcinoma cases. Oceania (20.54%), North America (17.58%), and Europe (17.32%) yielded a comparable number of cases. A minority of the study population originated from Africa (8.00%) and Latin America (7.52%).

The quality of data reporting concerning patient characteristics differed considerably. Eleven of the included studies did not mention the age of the cases [11, 15, 37, 42, 44, 45, 51, 56, 62, 64, 65] The majority of the other studies reported solely the age range, sometimes combined with the mean age. The largest proportion (33.85%) of these cases was histologically classified as IDC, followed by ILC (2.22%) and DCIS (1.26%) as second and third most frequent type. Sixteen studies did however not address the histological types. Twenty-three (57.5%) of the studies used paraffin-embedded tissue [7, 11, 15, 20, 25, 28, 33, 37-41, 44, 49, 50, 52, 53, 55, 58-60, 63, 64] Two studies did not clearly report the type of tissue material studied [45, 46].

Different combinations of detection methods were applied throughout the studies. Thirty-two (80%) of the studies used PCR as a diagnostic technique. Eleven studies (27.5%) made use of sequencing to accurately detect the different genotypes. Other common detection techniques included immunohistochemistry (IHC), in situ hybridisation (ISH), line probe assay (LPA), and Southern blot. Before 2001, only types-specific primers were used for PCR-based HPV detection. Afterwards, both type-specific and broad spectrum primers were employed, as well as the combination of the two types. Twelve of the 14 studies that only used broad-spectrum primers as PCR detection technique, specifically targeted the L1 region of the HPV genome [15, 20, 28, 39, 40, 46, 48, 55, 58, 60, 61, 63]. One of these 14 studies used primers only amplifying the E6-E7 genes [37]. One study targeted both the L1 and E6-E7 region [65]. Five studies did not report the genotypes of the detected HPV viruses [28, 37, 42, 45, 63]. Forty-five genotypes were detected. Twelve were classified as HR, one as pHR and two as LR.

Characteristics of the studies included can be found in Table 1. To strengthen the reliability of the pooled analysis, raw data were systematically retrieved.

The reported prevalence ranged from 0.00% to 86.21% [65]. There was no HPV-positivity in ten of the 40 studies included [7, 8, 13, 15, 39, 46, 51, 52, 54, 57]. Pooled analysis led to an overall prevalence of 20% (95% confidence interval (CI) [12%; 29%]). A large proportion of the variation could not be explained by chance as $I^2 = 97.87\%$ ($p < 0.001$). The reported prevalence across the different studies is shown in the forest plot displayed in Figure 4.

The pooled prevalence varied according to the time period of publication. The reported rate was highest during the period 1992-2008 with a pooled prevalence of 25% (95% CI [9%; 46%]). The studies published between 2009 and 2016 had a lower pooled prevalence of 18% (95% CI [10%; 28%]. The I^2 values for the time periods 1992-2008 and 2009-2016 were 96.71% and 98.14%, respectively.

Considering continental region of origin, the pooled prevalence of Africa, Asia, Europe, Latin America, North America, and Oceania was 21% (95% CI [0%; 75%]), 33% (95% CI [19%; 48%]), 7% (95% CI [1%; 18%]), 13% (95% CI [1%; 33%]), 8% (95% CI [0%; 33%]), and 48% (95% CI [8%; 90%]), respectively. Subgroup analysis demonstrated significant heterogeneity for all continental regions ($I^2 = 97.87\%$).

PCR primer type had a minor influence on the pooled prevalence. HPV prevalence detected by type-specific and

broad-spectrum primers was 23% (95% CI [7%; 45%]) and 19% (95% CI [9%; 30%]), respectively.

The pooled prevalence of the HR-HPVs and pHR-HPVs combined was significantly higher opposed to the LR-HPVs. The respective values were 63% (95% CI [60%; 67%]) and 11% (95% CI [9%; 14%]). HPV-18 was the most frequently detected HPV type with a pooled prevalence of 35% (95% CI [30%; 39%]). HPV-16 was the second most frequent genotype with a pooled prevalence of 18% (95% CI [15%; 22%]). I^2 was nevertheless considerably high (97.2%). Geographical location determined the predominant HR-HPV types detected. In alphabetical order of continental regions, the prevailing types were HPV-16, HPV-33, HPV-16, HPV-16, HPV-16, and HPV-18, respectively. Genotypes HPV-45, -58, and -59 were detected solely in Europe. HPV-51 was only reported by one Venezuelan study. Table 2 gives an overview of the pooled prevalence rate per genotype and the predominant genotypes per continent.

Discussion

To the best of the present authors knowledge, this systematic review and meta-analysis is the most comprehensive evaluation to date of published literature concerning the prevalence of HPV, and more specifically genotypes. The focus of this study is, in other words, the burden of HPV in breast cancer. In contrast to two recent meta-analyses on the subject, the authors only included prevalence studies to calculate pooled prevalence rates, hereby excluding case-control studies. In addition, the exclusion criteria were rigid in order to limit the heterogeneity between studies populations. Nevertheless, an unprecedented number of 4,762 cases were reached, nearly doubling and quadrupling the pooled study population of the aforementioned meta-analyses [21, 68].

The overall prevalence of 20% is significantly lower when compared to previous reports, which also used case-control studies to calculate prevalence rates. Li *et al.* recorded a prevalence of 24.49% (95% CI [22.07%; 27.05%]), pooling 1,184 cases of breast cancer [21]. The meta-analysis conducted by Simões *et al.* compromised 1,932 samples with a prevalence rate of 23% (95% CI [21.20%; 24,80%]) [69]. Zhou *et al.* included 2,569 breast cancer cases and reported a pooled prevalence of 30.30% (95% CI [22.30; 38.40]). The latter authors did however not include studies with a zero prevalence rate [68].

Myriad arguments have been brought forward to explain the disparity among prevalence rates in published literature, and by extension between meta-analyses. To begin with, the characteristics of the study population vary across different studies. Important variables are country of origin (as HPV prevalence is potentially related to sexual behaviour, environmental factors, socioeconomic status and genetic background), age, tumour characteristics, and past medical history. In addition, there is large variation in tissue processing and preservation, detection methods, and laboratory protocol.

Although the prevalence was considerably lower in recently published studies compared to early publications, this difference was not statistically significant. This finding is in accordance with the publication by Zhou *et al*. There are several possible explanations for this observation. Firstly, the present results argue that type-specific primers, predominantly used in the early publications, show a higher prevalence compared to broad-spectrum primers. Other meta-analyses reported an identical comparison [21, 68]. On the contrary, in the case of cervical cancer, an inverse relationship has been observed between primer design and HPV prevalence [21]. The rationale behind the lower prevalence rates in case of broad-spectrum primers is discussed further on in this paper. Secondly, it is well-known that early reports were of poor methodological quality as sample sizes were limited and detection methods were unstandardised [18, 68]. This lack of international standards for HPV assays and experienced laboratory staff remains problematic to date [70].

The observed prevalence of female genital HPV infection is highly dependent on the age category of the studied population [71-73]. Although the poor data reporting by the majority of studies prohibited the present authors from examining this relationship, previous literature reports clearly suggest an association.

A meta-analysis on cervical HPV prevalence among one million women with normal cytological findings recorded a pooled prevalence of 19.20% (95% CI [18.90%; 19.60%]) in the age group under 25 years of age. This percentage showed a linear decrease with advancing age, reaching 10% in the age group of 55-64 years (95% CI [9.8%; 10.1%]) [73]. Furthermore, several studies reported that patients with HPV positive breast cancers are significantly younger at age of diagnosis when compared to women with HPV negative breast carcinomas [74]. Other researchers doubt these age differences [31, 74, 75] Assuming an association between breast cancer HPV positivity and age, selection of an older study population could explain very low prevalence or absence of HPV in breast cancer as reported by several study groups. One theory states that women who have HPV-associated cervical pathology and who later develop HPV positive breast cancer at younger age, may have sexually transmitted HPV [76]. Sexual activity could thus be a risk factor of HPV positive breast cancer. The two common theories concerning the route by which HPV reaches the breast are both consistent with the sexual origin of the mammary HPV infections (see Supplemental Digital Content 1). The ‘systemic path theory’ claims that HPV uses hematogenic and/or lymphatic transfer. The ‘mechanical path theory’, on the other hand, states that the virus is scrubbed through the skin. The latter hypothesis implies an external route through sexual

Table 2. — *Pooled prevalence of the four most frequently detected high-risk HPV genotypes across the different continental regions. The authors used the breast cancer cases with HPV as denominator and the specific HPV genotypes as nominator. This may have inadvertently introduced bias if the cases were selected in a different way. Values in brackets indicate the 95% confidence interval.*

Genotype	Africa	Asia	Europe	North America	Latin America	Oceania	Pooled prevalence	No. of cases
HPV-16	51% (0.44-0.57)	25% (0.19-0.31)	11%(0.07-0.16)	2%(0.01-0.05)	7% (0.04-0.11)	5%(0.02-0.09)	33%(0.21-0.44)	210
HPV-18	-	32%(0.24-0.42)	-	1%(0.00-0.05)	3%(0.01-0.08)	64%(0.55.5-0.73)	44%(0.22-0.65)	109
HPV-31	15% (0.03-0.38)	75% (0.51-0.91)	5%(0.00-0.25)	-	-	5%(0.00-0.25)	5%(0.02-0.08)	20
HPV-33	-	97% (0.90-0.99)	-	-	4%(0.01-0.10)	-	28%(0.24-0.31)	85

Table 3. — *HPV prevalence in breast neoplasms stratified by subgroup. Values in brackets indicate the 95% confidence interval.*

Variable	No. of studies	No. of cases	HPV prevalence (%) (95% CI)
Publication period			
1992-2008	13	681	25% (0.09;0.45)
2009-2016	27	4081	18% (0.10;0.28)
Continental region of origin			
Africa	3	381	21% (0.00;0.75)
Asia	13	1383	33% (0.19;0.48)
Europe	11	825	7% (0.01;0.18)
North America	3	837	8% (0.00;0.33)
Latin America	6	358	13% (0.01;0.33)
Oceania	4	978	48% (0.08;0.90)
PCR primer type			
Type-specific	10	750	23% (0.07;0.45)
Broad-spectrum	15	998	19% (0.09;0.30)
HPV oncogenic potential			
LR	10	89	11% (0.09;0.14)
pHR+HR	23	491	63% (0.60;0.67)

Abbreviations: CI = confidence interval, HR = high-risk, LR = low-risk, PCR = polymerase chain reaction, pHR = potential high-risk.

practices and/or transmission by hand from the female perineum to the breast [62]. One could thus argue that the mammary HPV prevalence is a reflection of the cervical cancer burden. This hypothesis is supported by a high grade of genotype correspondence between breast cancer samples and high grade cervical intraepithelial neoplasia/cervical cancer samples in the same patients [30-32]. In the light of the two common theories of mammary HPV infection, prophylactic HPV vaccines for cervical cancer could also prohibit breast cancer development.

The influence of sample material on HPV prevalence was not repeated as this relationship has already been thoroughly explored in current literature. The use of paraffin-embedded tissue has become highly contested because of its proneness to measurement errors [18]. The significantly higher prevalence rates observed in paraffin-embedded tissue compared to fresh frozen tissue are illogical because of several reasons and must thus be seriously questioned. Firstly, sample fixation and processing are potential causes of virion destruction. In addition, long-standing tissue preservation can impede HPV detection [70]. Substantial contamination has been demonstrated and could account for the unexpectedly high prevalence rates [16, 18, 21].

The choice of PCR primer pairs influences both the spectrum of genotypes detected as the clinical relevance of the reported HPV positivity. The justifiable focus on HR-HPVs explains the substantial prevalence rate of this section of the genotype spectrum. As mentioned before, type-specific primers have higher HPV detection rates compared to broad-spectrum primers. In addition, also consensus primers differ concerning detection capability [56]. As stated above, 12 studies used L1 broad-spectrum primers in isolation to detect HPV. It is, however, well-known that the L1/E1 sequences, in contrast to E6 and E7 regions, are lost during integration of the viral DNA into the host genome. This process of integration is considered crucial in malignant transformation caused by HPV [70]. This limitation of certain primers is a valid explanation for the lower detection rates observed when using L1 or E1 consensus primers.

The value of proven HPV presence is at least questionable. Co-existence cannot be confused with a causal relationship. The HPV infection could for example have occurred after carcinoma development as opposed to prior to the carcinogenesis. Hossein *et al.* described absence of functional E6/E7 messenger ribonucleic acid (mRNA) production of HR-HPVs [49]. This finding, together with the extremely low viral load (over 4,000 times lower than cervical cancer), raised doubt about the assumption that HPV is the driving force of tumour development [19]. The low viral loads are, moreover, considered the explanation for the low sensitivity of non-PCR based methods. However, the recent study by Lawson *et al.* demonstrated both transcription of HPV DNA to RNA and expression of HPV E7 proteins, strongly suggesting biological activity [20]. In addition, a single copy of viral DNA would suffice to promote carcinogenesis (see Supplemental Digital Content 2) [77].

The continental region of origin seems to be related to both HPV prevalence as well as prevailing genotypes. Overall, HPV-18 was the most prevalent genotype. The majority of breast cancers has a glandular origin and are there-

fore termed adenocarcinomas. As HPV-18 has a tropism for glandular epithelial cells (opposed to squamous epithelium), this observation is to be expected [21, 78].
Kan *et al.* suggested that HPV-18 is the predominant genotype in Caucasian populations, whereas HPV-33 would mainly appear in Asian subjects [62]. Several authors shared a similar opinion [20, 21, 24]. The present results support this assertion with high prevalences of HPV-31 and HPV-33 (both alpha-9 genotypes) in the Asian continent [79]. The other continental regions were characterised by HPV-16 or -18 predominance.

The present results should be interpreted with caution because of lack in information availability on the one hand and limitations inherent to the study design on the other hand. Age and estrogen receptor status are suspected to influence the prevalence, but could not be explored due to poor information reporting. An important observation of this meta-analysis was the substantial between-study heterogeneity, reflected in the high I^2 values. However, given the fact that the included studies took place in different settings, applying different inclusion and exclusion criteria, such considerable heterogeneity was to be expected, as in other studies estimating pooled prevalence rates. The high value of heterogeneity persisted even within the subgroups the present authors have investigated, including publication period and continental region, and must therefore be attributed to factors that have not been explored. The present positive results cannot prove causality, but merely indicate the burden of HPV in breast cancer.

Supplemental digital content 1: Route of mammary HPV infection

There are two common theories in the international literature concerning the route by which HPV reaches the breast. The 'systemic path theory' claims that HPV uses hematogenic and/or lymphatic transfer. This theory is supported by several observations. Firstly, the HPV virus is present in unexpected sites like Hodgkin's lymphomas and bronchopulmonary cancer [80]. Secondly, the presence of HPV has been identified in peripheral blood mononuclear cells (PBMCs) of patients with urogenital HPV infections and in the sera of patients with HPV-associated head and neck squamous cell carcinoma [81, 82]. PBMCs can also harbour HPV in circumstances unrelated to malignant lesions [83]. Thirdly, multiple patients with cervical intraepithelial neoplasia (CIN) have serum antibodies to a linear epitope on the major coat protein (L1) of HPV-16. This is at least partially consistent with viremia. Lastly, Hennig *et al.* reported that all HPV-16 positive breast carcinomas, as second primary cancer, were HPV-16 positive in their corresponding CIN III [31]. Analogously, an Austrian study examined the axillary lymph nodes of patients with both HPV positive cervical and breast cancer and detected the same HPV genotype in the nodes [30].

The 'mechanical path theory' states that the virus is scrubbed through the skin. It is suggested that HPV infects the epithelium of nipple and areola and subsequently transfers in a retrograde fashion via the nipple, areola, lactiferous ducts, and sinuses [63]. The fact that HPV is a family of epitheliotropic viruses, and thus requires a habitat of differentiating squamous epithelium for their life cycle, makes this theory more plausible [83]. Hence, this hypothesis implies an external route through sexual practices and/or transmission by hand from the female perineum to the breast [62].

Supplemental digital content 2: Low HPV viral load

The viral load in breast cancer seems to be over 4,000 times lower than the viral load in cervical cancer [19]. This makes the HPV viral load in breast cancer a problematic issue when considering the sensitivity of certain detection techniques. A Japanese study, applying real-time polymerase chain reaction, estimated the mean viral load at 5.4 copies per 10^4 cells [77]. This phenomenon of extreme low viral loads has also been reported in other non-genital carcinomas like lung, esophageal, and head-and-neck cancers [57]. If the viral deoxyribonucleic acid (DNA) is integrated into the host genome, low viral loads are sufficient to be a factor in carcinogenesis. However, if assumed that one copy per cell suffices, a mean viral load below this threshold makes a causal role of HPV in breast cancer less likely.

Breast cancer is considered a monoclonal proliferation. Once integrated, the viral genome is unlikely to disappear during cancer replication. Khan *et al.* reported that all of the detected HPV-16 in their studied breast cancer tissue, was integrated. Therefore, if causally related, at least one copy of HPV DNA is expected in every carcinoma cell [77]. The 'hit and run virus' phenomenon, originally proposed by Skinner, attempts to explain that the observed viral load may be below one copy per cell. According to this theory, once genomic aberrations are attained, the virus itself does no longer need to be present. This implicates that loss of the virus during cell division can lead to a virally transformed cell, without viral DNA present [84, 85]. However, as previously mentioned, viral loss is considered an uncommon event.

Conclusion

This meta-analysis reports a pooled prevalence of HPV in 20% (95% CI [12%; 29%]) of the breast cancer tissue samples, supporting the hypothesis that HPV is involved in breast carcinogenesis. The presented results are complementary to the meta-analysis on case-control studies performed by Bae *et al*. [18]. The present results allow to estimate the burden of (HR)-HPV in breast cancer, whereas the pooled odds ratio gives insight into the associated risk. Given the high burden of breast cancer, further research on

the significance of viral presence in this disease is crucial. If further research indicates that HPV has an impact on any step of the multistep process of breast cancer development, prophylaxis and anti-viral treatment will become essential for prevention and therapy.

Acknowledgements

The authors would like to thank the authors of the original studies included in this meta-analysis.

References

[1] Benson J.R., Jatoi I.: "The global breast cancer burden". *Future Oncol.*, 2012, *8*, 697.
[2] Cancer Research U.K.: "Worldwide cancer incidence statistics 2012" Available at: http://www.cancerresearchuk.org/cancer-info/cancerstats/world/incidence/#Common.
[3] Dumitrescu R.G., Cotarla I.: "Understanding breast cancer risk — where do we stand in 2005?". *J. Cell. Mol. Med.*, 2005, *9*, 208.
[4] Liang W., Wang J., Wang C., Lv Y., Gao H., Zhang K., et al.: "Detection of high-risk human papillomaviruses in fresh breast cancer samples using the hybrid capture 2 assay". *J. Med. Virol.*, 2013, *85*, 2087.
[5] Coughlin S.S., Ekwueme D.U.: "Breast cancer as a global health concern". *Cancer Epidemiol.*, 2009, *33*, 315.
[6] Mou X., Chen L., Liu F., Shen Y., Wang H., Li Y., *et al.*: "Low prevalence of human papillomavirus (HPV) in Chinese patients with breast cancer". *J. Int. Med. Res.*, 2011, *39*, 1636.
[7] Silva R.G., Jr., da Silva B.B.: "No evidence for an association of human papillomavirus and breast carcinoma". *Breast Cancer Res. Treat.*, 2011, *125*, 261.
[8] de Cremoux P., Thioux M., Lebigot I., Sigal-Zafrani B., Salmon R., Sastre-Garau X., *et al.*: "No evidence of human papillomavirus DNA sequences in invasive breast carcinoma". *Breast Cancer Res. Treat.*, 2008, *109*, 55.
[9] Band V., Zajchowski D., Kulesa V., Sager R.: "Human papilloma virus DNAs immortalize normal human mammary epithelial cells and reduce their growth factor requirements". *Proc. Natl. Acad. Sci. U.S.A.*, 1990, *87*, 463.
[10] Wazer D.E., Liu X.L., Chu Q., Gao Q., Band V.: "Immortalization of distinct human mammary epithelial cell types by human papilloma virus 16 E6 or E7". *Proc. Natl. Acad. Sci. U.S.A.*, 1995, *92*, 3687.
[11] Di Lonardo A., Venuti A., Marcante M.L.: "Human papillomavirus in breast cancer". *Breast Cancer Res. Treat.*, 1992, *21*, 95.
[12] Bratthauer G.L., Tavassoli F.A., O'Leary T.J.: "Etiology of breast carcinoma: no apparent role for papillomavirus types 6/11/16/18". *Pathol. Res. Pract.*, 1992, *188*, 384.
[13] Wrede D., Luqmani Y.A., Coombes R.C., Vousden K.H.: "Absence of HPV 16 and 18 DNA in breast cancer". *Br. J. Cancer*, 1992, *65*, 891.
[14] Gopalkrishna V., Singh U.R., Sodhani P., Sharma J.K., Hedau S.T., Mandal A.K., *et al.*: "Absence of human papillomavirus DNA in breast cancer as revealed by polymerase chain reaction". *Breast Cancer Res. Treat.*, 1996, *39*, 197.
[15] Lindel K., Forster A., Altermatt H.J., Greiner R., Gruber G.: "Breast cancer and human papillomavirus (HPV) infection: no evidence of a viral etiology in a group of Swiss women". *Breast*, 2007, *16*, 172.
[16] Antonsson A., Spurr T.P., Chen A.C., Francis G.D., McMillan N.A., Saunders N.A., *et al.*: "High prevalence of human papillomaviruses in fresh frozen breast cancer samples". *J. Med. Virol.*, 2011, *83*, 2157.
[17] Gumus M., Yumuk P.F., Salepci T., Aliustaoglu M., Dane F., Ekenel M., et al.: "HPV DNA frequency and subset analysis in human breast cancer patients' normal and tumoral tissue samples". *J. Exp. Clin. Cancer Res.*, 2006, *25*, 515.
[18] Bae J.M., Kim E.H.: "Human papillomavirus infection and risk of breast cancer: a meta-analysis of case-control studies". *Infect. Agent. Cancer*, 2016, *11*, 14.
[19] Lawson J.S., Glenn W.K., Heng B., Ye Y., Tran B., Lutze-Mann L., et al.: "Koilocytes indicate a role for human papilloma virus in breast cancer". *Br. J. Cancer.*, 2009, *101*, 1351.
[20] Lawson J.S., Glenn W.K., Salyakina D., Delprado W., Clay R., Antonsson A., *et al.*: "Human Papilloma Viruses and Breast Cancer". *Front. Oncol.*, 2015, *5*, 277.
[21] Li N., Bi X., Zhang Y., Zhao P., Zheng T., Dai M.: "Human papillomavirus infection and sporadic breast carcinoma risk: a meta-analysis". *Breast Cancer Res. Treat.*, 2011, *126*, 515.
[22] Stroup D.F., Berlin J.A., Morton S.C., Olkin I., Williamson G.D., Rennie D., *et al.*: "Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group". *JAMA*, 2000, *283*, 2008.
[23] Moher D., Liberati A., Tetzlaff J., Altman D.G., Group P.: "Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement". *Ann. Intern. Med.*, 2009, *151*, 264.
[24] Yu Y., Morimoto T., Sasa M., Okazaki K., Harada Y., Fujiwara T., *et al.*: "HPV33 DNA in premalignant and malignant breast lesions in Chinese and Japanese populations". *Anticancer Res.*, 1999, *19*, 5057.
[25] Yu Y., Morimoto T., Sasa M., Okazaki K., Harada Y., Fujiwara T., et al.: "Human papillomavirus type 33 DNA in breast cancer in Chinese". *Breast Cancer*, 2000, *7*, 33.
[26] Kulka J., Kovalszky I., Svastics E., Berta M., Fule T.: "Lymphoepithelioma-like carcinoma of the breast: not Epstein-Barr virus-, but human papilloma virus-positive". *Hum. Pathol.*, 2008, *39*, 298.
[27] Czerwenka K., Heuss F., Hosmann J.W., Manavi M., Lu Y., Jelincic D., et al.: "Human papilloma virus DNA: a factor in the pathogenesis of mammary Paget's disease?" *Breast Cancer Res. Treat.*, 1996, *41*, 51.
[28] Grenier J., Soria J.C., Mathieu M.C., Andre F., Abdelmoula S., Velasco V., *et al.*: "Differential immunohistochemical and biological profile of squamous cell carcinoma of the breast". *Anticancer Res.*, 2007, *27*, 547.
[29] Aceto G.M., Solano A.R., Neuman M.I., Veschi S., Morgano A., Malatesta S., *et al.*: "High-risk human papilloma virus infection, tumor pathophenotypes, and BRCA1/2 and TP53 status in juvenile breast cancer". *Breast Cancer Res. Treat.*, 2010, *122*, 671.
[30] Widschwendter A., Brunhuber T., Wiedemair A., Mueller-Holzner E., Marth C.: "Detection of human papillomavirus DNA in breast cancer of patients with cervical cancer history". *J. Clin. Virol.*, 2004, *31*, 292.
[31] Hennig E.M., Suo Z., Thoresen S., Holm R., Kvinnsland S., Nesland J.M.: "Human papillomavirus 16 in breast cancer of women treated for high grade cervical intraepithelial neoplasia (CIN III)". *Breast Cancer Res. Treat.*, 1999, *53*, 121.
[32] Lawson J.S., Glenn W.K., Salyakina D., Clay R., Delprado W., Cheerala B., *et al.*: "Human Papilloma Virus Identification in Breast Cancer Patients with Previous Cervical Neoplasia". *Front. Oncol.*, 2015, *5*, 298.
[33] Mohtasebi P., Rassi H., Maleki F., Hajimohammadi S., Bagheri Z., Fakhar Miandoab M., et al.: "Detection of Human Papillomavirus Genotypes and Major BRCA Mutations in Familial Breast Cancer". *Monoclon. Antib. Immunodiagn. Immunother.*, 2016, *35*, 135.
[34] United Nations Statistics Division: "Composition of macro geographical (continental) regions, geographical sub-regions, and selected economic and other groupings 2012". Available at: http://unstats.un.org/unsd/methods/m49/m49regin.htm.
[35] Munoz N., Bosch F.X., de Sanjose S., Herrero R., Castellsague X., Shah K.V., *et al.*: "Epidemiologic classification of human papillomavirus types associated with cervical cancer". *N. Engl. J. Med.*, 2003, *348*, 518.
[36] El-Shinawi M., Mohamed H.T., Abdel-Fattah H.H., Ibrahim S.A., El-Halawany M.S., Nouh M.A., *et al.*: "Inflammatory and Non-inflammatory Breast Cancer: A Potential Role for Detection of Multiple Viral DNAs in Disease Progression". *Ann. Surg. Oncol.*, 2016,

23, 494.

[37] Al Moustafa A.E., Al-Antary N., Aboulkassim T., Akil N., Batist G., Yasmeen A.: "Co-prevalence of Epstein-Barr virus and high-risk human papillomaviruses in Syrian women with breast cancer". *Hum. Vaccin. Immunother.*, 2016, *12*, 1936.

[38] Salehpour M., Tayyebi Meibodi N., Teimourpour R., Ghorani-Azam A., Sepahi S., Rostami S., *et al.*: "Frequency of Human Papillomavirus Genotypes 6, 11, 16, 18 And 31 in Paraffin-Embedded Tissue Samples of Invasive Breast Carcinoma, North- East of Iran". *Iran. J. Pathol.*, 2015, *10*, 192.

[39] Vernet-Tomas M., Mena M., Alemany L., Bravo I., De Sanjose S., Nicolau P., *et al.*: "Human papillomavirus and breast cancer: no evidence of association in a Spanish set of cases". *Anticancer Res.*, 2015, *35*, 851.

[40] Ngan C., Lawson J.S., Clay R., Delprado W., Whitaker N.J., Glenn W.K.: "Early Human Papilloma Virus (HPV) Oncogenic Influences in Breast Cancer". *Breast Cancer (Auckl.)*, 2015, *9*, 93.

[41] Piana A.F., Sotgiu G., Muroni M.R., Cossu-Rocca P., Castiglia P., De Miglio M.R.: "HPV infection and triple-negative breast cancers: an Italian case-control study". *Virol. J.*, 2014, *11*, 190.

[42] Ohba K., Ichiyama K., Yajima M., Gemma N., Nikaido M., Wu Q., *et al.*: "In vivo and in vitro studies suggest a possible involvement of HPV infection in the early stage of breast carcinogenesis via APOBEC3B induction". *PLoS One*, 2014, *9*, e97787.

[43] Wang T., Zeng X., Li W., Zhu H., Wang G., Liu X., *et al.*: "Detection and analysis of human papillomavirus (HPV) DNA in breast cancer patients by an effective method of HPV capture". *PLoS One*, 2014, *9*, e90343.

[44] Corbex M., Bouzbid S., Traverse-Glehen A., Aouras H., McKay-Chopin S., Carreira C., *et al.*: "Prevalence of papillomaviruses, polyomaviruses, and herpesviruses in triple-negative and inflammatory breast tumors from algeria compared with other types of breast cancer tumors". *PLoS One*, 2014, *9*, e114559.

[45] Lv Y.R., Wang J.L., Zhang K., Gao H.D., Sun J.Z., Gong Y.Y., *et al.*: "Human Papilloma Viruses (HPVs) no Co-Existence in Breast Cancer and Cervical Cells in the Same Patient". *Chinese J. Physiol.*, 2014, *57*, 105.

[46] Moradi A.M., E.; Tabarraei, A.; Bakhshandeh Nosrat, S.; Kazemi nejad, V.; Azarhosh, R.; Alizadeh, S; Bazori, M.;. "Molecular epidemiology of Human Papillomaviruses in breast cancer, Golestan province of Iran". *Medical Laboratory Journal*, 2009, *3*.

[47] Fernandes A., Bianchi G., Feltri A.P., Perez M., Correnti M.: "Presence of human papillomavirus in breast cancer and its association with prognostic factors". *Ecancermedicalscience*, 2015, *9*, 548.

[48] Pereira Suarez A.L., Lorenzetti M.A., Gonzalez Lucano R., Cohen M., Gass H., Martinez Vazquez P., *et al.*: "Presence of human papilloma virus in a series of breast carcinoma from Argentina". *PLoS One*, 2013, *8*, e61613.

[49] Hossein R., Behzad S., Tahar M., Azadeh N.A.: "Prevalence of human papillomavirus genotypes associated with cervical and breast cancers in iran". *Monoclon. Antib. Immunodiagn. Immunother.*, 2013, *32*, 399.

[50] Herrera-Goepfert R., Vela-Chavez T., Carrillo-Garcia A., Lizano-Soberon M., Amador-Molina A., Onate-Ocana L.F., *et al.*: "High-risk human papillomavirus (HPV) DNA sequences in metaplastic breast carcinomas of Mexican women". *BMC Cancer*, 2013, *13*, 445.

[51] Khoury J.D., Tannir N.M., Williams M.D., Chen Y., Yao H., Zhang J., et al.: "Landscape of DNA virus associations across human malignant cancers: analysis of 3,775 cases using RNA-Seq". *J. Virol.*, 2013, *87*, 8916.

[52] Herrera-Romano L., Fernandez-Tamayo N., Gomez-Conde E., Reyes-Cardoso J.M., Ortiz-Gutierrez F., Ceballos G., *et al.*: "Absence of human papillomavirus sequences in epithelial breast cancer in a Mexican female population". *Med. Oncol.*, 2012, *29*, 1515.

[53] Baltzell K., Buehring G.C., Krishnamurthy S., Kuerer H., Shen H.M., Sison J.D.: "Limited evidence of human papillomavirus in [corrected] breast tissue using molecular in situ methods". *Cancer*, 2012, *118*, 1212.

[54] Hedau S., Kumar U., Hussain S., Shukla S., Pande S., Jain N., *et al.*: "Breast cancer and human papillomavirus infection: no evidence of HPV etiology of breast cancer in Indian women". *BMC Cancer*, 2011, *11*, 27.

[55] Aguayo F., Khan N., Koriyama C., Gonzalez C., Ampuero S., Padilla O., *et al.*: "Human papillomavirus and Epstein-Barr virus infections in breast cancer from chile". *Infect. Agent. Cancer*, 2011, *6*, 7.

[56] Ghaffari S.R., Sabokbar T., Meshkat Z., Fereidooni F., Dastan J., Rafati M., *et al.*: "Tracing human papilloma virus in breast tumors of Iranian breast cancer patients". *Breast J.*, 2011, *17*, 218.

[57] Hachana M., Ziadi S., Amara K., Toumi I., Korbi S., Trimeche M.: "No evidence of human papillomavirus DNA in breast carcinoma in Tunisian patients". *Breast*, 2010, *19*, 541.

[58] Ong K., Koay E.S., Putti T.C.: "Detection of cutaneous HPV types 4 and 24 DNA sequences in breast carcinoma in Singaporean women of Asian ancestry". *Pathology*, 2009, *41*, 436.

[59] Akil N., Yasmeen A., Kassab A., Ghabreau L., Darnel A.D., Al Moustafa A.E.: "High-risk human papillomavirus infections in breast cancer in Syrian women and their association with Id-1 expression: a tissue microarray study". *Br. J. Cancer*, 2008, *99*, 404.

[60] Duo D., Ghimenti C., Migliora P., Pavanelli M.C., Mastracci L., Angeli G.: "Identification and characterization of human papillomavirus DNA sequences in Italian breast cancer patients by PCR and line probe assay reverse hybridization". *Mol. Med. Rep.*, 2008, *1*, 673.

[61] Kroupis C., Markou A., Vourlidis N., Dionyssiou-Asteriou A., Lianidou E.S.: "Presence of high-risk human papillomavirus sequences in breast cancer tissues and association with histopathological characteristics". *Clin. Biochem.*, 2006, *39*, 727.

[62] Kan C.Y., Iacopetta B.J., Lawson J.S., Whitaker N.J.: "Identification of human papillomavirus DNA gene sequences in human breast cancer". *Br. J. Cancer*, 2005, *93*, 946.

[63] de Villiers E.M., Sandstrom R.E., zur Hausen H., Buck C.E.: "Presence of papillomavirus sequences in condylomatous lesions of the mamillae and in invasive carcinoma of the breast". *Breast Cancer Res*, 2005, *7*, R1.

[64] Li T., Lu Z.M., Guo M., Wu Q.J., Chen K.N., Xing H.P., *et al.*: "p53 codon 72 polymorphism (C/G) and the risk of human papillomavirus-associated carcinomas in China". *Cancer*, 2002, *95*, 2571.

[65] Liu Y., Klimberg V.S., Andrews N.R., Hicks C.R., Peng H., Chiriva-Internati M., et al.: "Human papillomavirus DNA is present in a subset of unselected breast cancers". *J. Hum. Virol.*, 2001, *4*, 329.

[68] Zhou Y., Li J., Ji Y., Ren M., Pang B., Chu M., et al.: "Inconclusive role of human papillomavirus infection in breast cancer". *Infect. Agent. Cancer*, 2015, *10*, 36.

[66] Higgins J.P.T., Green S.: "*Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0*". The Cochrane Collaboration, 2011. Available at: http://handbook.cochrane.org.

[67] Nyaga V.N., Arbyn M., Aerts M.: "Metaprop: a Stata command to perform meta-analysis of binomial data". *Arch. Public Health*, 2014, *72*.

[69] Simoes P.W., Medeiros L.R., Pires P.D.S., Edelweiss M.I., Rosa D.D., Silva F.R., *et al.*: "Prevalence of Human Papillomavirus in Breast Cancer A Systematic Review". *International Journal of Gynecological Cancer*, 2012, *22*, 343.

[70] Wang T., Chang P., Wang L., Yao Q., Guo W., Chen J., *et al.*: "The role of human papillomavirus infection in breast cancer". *Med. Oncol.*, 2012, *29*, 48.

[71] Akarolo-Anthony S.N., Famooto A.O., Dareng E.O., Olaniyan O.B., Offiong R., Wheeler C.M., *et al.*: "Age-specific prevalence of human papilloma virus infection among Nigerian women". *BMC Public Health*, 2014, *14*, 656.

[72] Girianelli V.R., Thuler L.C., e Silva G.A.: "[Prevalence of HPV infection among women covered by the family health program in the Baixada Fluminense, Rio de Janeiro, Brazil]". *Rev. Bras. Ginecol. Obstet.*, 2010, *32*, 39.

[73] Bruni L., Diaz M., Castellsague X., Ferrer E., Bosch F.X., de Sanjose S.: "Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings".

J. Infect. Dis., 2010, *202*, 1789.
[74] Lawson JS G.W., Whitaker NJ. "Reply: Breast cancer, human papilloma virus and sexual activities". *Br. J. Cancer*, 2008, *98*, 510.
[75] Damin A.P., Karam R., Zettler C.G., Caleffi M., Alexandre C.O.: "Evidence for an association of human papillomavirus and breast carcinomas". *Breast Cancer Res. Treat.*, 2004, *84*, 131.
[76] Lawson J.S., Kan C.Y., Iacopetta B.J., Whitaker N.J.: "Are some breast cancers sexually transmitted?". *Br. J. Cancer*, 2006, *95*, 1708.
[77] Khan N.A., Castillo A., Koriyama C., Kijima Y., Umekita Y., Ohi Y., et al.: "Human papillomavirus detected in female breast carcinomas in Japan". *Br. J. Cancer*, 2008, *99*, 408.
[78] Heng B., Glenn W.K., Ye Y., Tran B., Delprado W., Lutze-Mann L., *et al.*: "Human papilloma virus is associated with breast cancer". *Br. J. Cancer*, 2009, *101*, 1345.
[79] Schiffman M., Clifford G., Buonaguro F.M.: "Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline". *Infect. Agent. Cancer*, 2009, *4*, 8.
[80] Cheng Y.W., Chiou H.L., Sheu G.T., Hsieh L.L., Chen J.T., Chen C.Y., *et al.*: "The association of human papillomavirus 16/18 infection with lung cancer among nonsmoking Taiwanese women". *Cancer Res.*, 2001, *61*, 2799.
[81] Pao C.C., Lin S.S., Lin C.Y., Maa J.S., Lai C.H., Hsieh T.T.: "Identification of human papillomavirus DNA sequences in peripheral blood mononuclear cells". *Am. J. Clin. Pathol.*, 1991, *95*, 540.
[82] Capone R.B., Pai S.I., Koch W.M., Gillison M.L., Danish H.N., Westra W.H., *et al.*: "Detection and quantitation of human papillomavirus (HPV) DNA in the sera of patients with HPV-associated head and neck squamous cell carcinoma". *Clin. Cancer Res.*, 2000, *6*, 4171.
[83] Bodaghi S., Wood L.V., Roby G., Ryder C., Steinberg S.M., Zheng Z.M.: "Could human papillomaviruses be spread through blood?". *J. Clin. Microbiol.*, 2005, *43*, 5428.
[84] Joshi D., Buehring G.C.: "Are viruses associated with human breast cancer? Scrutinizing the molecular evidence". *Breast Cancer Res. Treat.*, 2012, *135*, 1.
[85] Skinner G.R.: "Transformation of primary hamster embryo fibroblasts by type 2 simplex virus: evidence for a "hit and run" mechanism". *Br. J. Exp. Pathol.*, 1976, *57*, 361.

Corresponding Author:
D.VANDEN BROECK, MSc, PhD,
International Centre for Reproductive Health (ICRH)
Ghent University, De Pintelaan 185 P3,
9000 Ghent (Belgium)
e-mail: davy.vandenbroeck@ugent.be