Prevalence of HER2 overexpression and amplification in uterine cervical cancer: a systematic review and a meta-analysis.

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Abstract

Background

The reported rates of HER2 positivity in uterine cervical cancer (CC) range from 0–87%. The organization of clinical studies of HER2 targeting agents in CC requires a better knowledge of HER2 positivity prevalence. We aimed to provide precise estimates of HER2 overexpression and amplification in CC.

Methods

We conducted a PRISMA compliant meta-analytic systematic review. We searched electronic databases for articles reporting the proportion of HER2 positivity by immunohistochemistry or in situ hybridization in CC. We performed descriptive analyses of all 65 included studies. Of these, we selected 26 studies that used standardized American Society of Clinical Oncology / College of American Pathologists (ASCO/CAP) Guidelines compliant methodology. We conducted several meta-analyses of proportions to estimate the pooled prevalence of HER2 positivity and subgroup analyses using geographic region, histology, tumor stage, primary antibody brand, study size, and publication year as moderators.

Results

The estimated pooled prevalence of HER2 overexpression was 5.7% (CI 95%: 1.5–11.7%) in ASCO/CAP compliant studies and 27.0%, (CI 95%: 19.9–34.8%) in ASCO/CAP non-compliant ones, $p < 0.001$. The estimated pooled prevalence of HER2 amplification was 1.2% (CI 95%: 0.0–5.8%) and 24.9% (CI 95%: 12.6–39.6%), respectively, $p = 0.004$. No other factor was significantly associated with HER2 positivity rates.

Conclusion

A small proportion of CC is expected to be HER2 positive. Variations in previously reported HER2 positivity rates are mainly related to methodological issues.

Background

Uterine cervical cancer (CC) is a significant global public health problem.(1)

Despite advances in the prevention and treatment of locoregional stages and improvements in care delivery, the burden of patients with advanced disease is still high, especially in developing countries.(2, 3) Currently, the available standard treatment options for progressive, recurrent, and metastatic disease are limited highlighting the need to develop new therapeutics.(4–6)

The enhanced signaling via the HER2 receptor plays a crucial role in the cellular transformation, carcinogenesis, and maintenance of malignant phenotype and is considered an ideal target for antitumor treatments.(7) It may occur due to an activating mutation, amplification of the *HER2* gene, or either because of overexpression of the HER2 protein.

HER2 gene somatic mutations in CC are shown to be a promising actionable target for specific inhibitors in preclinical models and a clinical study. They are beyond the scope of this work.(8, 9) On the other hand, several drugs targeting *HER2* amplification and overexpression are available. Trastuzumab, pertuzumab, trastuzumab emtansine, lapatinib, and other agents have demonstrated clinical efficacy against *HER2* positive breast, esophageal/gastric, and serous endometrial cancers becoming current therapeutic standards in these conditions and now are under investigation in a continuously expanding set of solid tumors.(10–14) However, to our knowledge, no clinical trial assessing *HER2* amplification/overexpression targeting drugs in CC has been published to date. The organization and planning of this kind of study would require a better knowledge about *HER2* positivity prevalence in CC.

Standards of the accurate evaluation of *HER2* status and the proper definition of *HER2* positivity evolved through time and vary across tumor types. In early clinical trials of trastuzumab for breast cancer, *HER2* status was determined by immunohistochemistry (IHC), and the score 3 + corresponded to “more than moderate”, i.e. strong, staining of the “entire tumor-cell membrane... in more than 10 percent of tumor cells.”(15) *HER2* gene amplification, defined as a *HER2* gene to chromosome 17 (*HER2* /CEP17) ratio of at least 2.0 by Fluorescent In Situ Hybridization (FISH), was also a part of the positivity definition endorsed by the US Food and Drug Administration.(16) In 2007, the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) released guidelines, that defined as positive those cases with > 30 % of invasive tumor cells with a uniform intense membrane staining by IHC or *HER2* /CEN17 of > 2.2 for dual probes or > 6 *HER2* gene copies for single probes by FISH. The updated 2013 ASCO/CAP recommendations returned the IHC positivity threshold to a 10% cut point and changed In Situ Hybridization (ISH) grading criteria.(17, 18) It was estimated that these changes in the scoring system would lead to a reclassification of only 7.7% of patients scored by FISH and 3.7% of patients graded by IHC. (16, 17) The 2018 focused update of the ASCO/CAP Guidelines addressed some infrequent clinical situations and allowed primary ISH.(19) A slightly different IHC 3 + definition is used for gastric cancer, as apical membranous staining is not required.(20)

Since 1990, many studies assessed *HER2* receptor status in CC. The reported proportion of *HER2* positive tumors oscillated in a wide range from 0 to 87%.(21–26) As far as we know, factors accounting for such a disparity have not been formally assessed and hitherto no systematic review on the topic has been published. As guidelines for *HER2* assessment in cervical cancer have not been developed, some studies utilized one of the existing ASCO/CAP guidelines for breast and gastroesophageal cancers as a reference point.

Our objectives were, hence, to estimate the pooled prevalence of the *HER2* amplification and overexpression in patients with invasive CC, globally and according to the *HER2* scoring system and positivity definition using meta-analysis to increase the statistical precision. Also, we aimed to determine, if *HER2* status varies across different histologic subtypes and clinical stages trying to identify the subset of patients with a higher proportion of *HER2* positivity and to explore associations between *HER2* positivity and demographic, clinicopathologic, and assay-related variables.

Our research questions were: a) how common is HER2 overexpression/amplification in patients with invasive CC? b) Is the proportion of HER2 positive CC the same in studies that used ASCO/CAP compliant methodology and those that used other methods? and c) Is there a relationship between HER2 pooled prevalence and relevant subgroups like histologic subtype, World Health Organization (WHO) geographic region, tumor stage, primary antibody brand, and publication year?

Methods

The present study's protocol was registered in the University of York's PROSPERO International prospective database of systematic reviews, under ID: CRD42018096078 on June 11, 2018.(27)

Eligible studies were those of any epidemiologic design and year of publication, published in any language, reporting on the overexpression and/or amplification of HER2 in CC by IHC or ISH in patients with histopathologically confirmed invasive CC, regardless of age, ethnicity, or geographic region. We allowed the inclusion of microinvasive carcinomas and did not impose restrictions regarding the treatment used before tissue collection.

We excluded narrative reviews, comments, letters, editorials, case reports, and studies assessing *in vitro* and *in vivo* models. Also, we excluded studies with less than ten patients, those reporting HER2 status in preinvasive epithelial cervical lesions, lymphoid neoplasms, and melanoma of the uterine cervix, studies missing relevant data, and those publications whose full text was unavailable to access.

If a study used IHC for HER2 protein overexpression followed by a non-ISH method for HER2 amplification assessment, only data on IHC were included in the review.

Search strategies for the identification of studies and data sources

We searched Medline, EMBASE, LILACS, Cochrane and Google Scholar search engines with no language and date restrictions (up to December 22, 2020) using the following syntax: (Receptor, ErbB-2[Mesh] OR ErbB-2[tiab] OR CD340[tiab] OR Proto-Oncogene Protein*[tiab] OR HER-2[tiab] OR Neu Receptor*[tiab]) AND (Uterine Cervical Neoplasms[Mesh] OR Cervical Neoplas*[tiab] OR Cervical Cancer[tiab] OR Cervical Tumor*[tiab] OR Cervical Carcinom*[tiab] OR Cervix Neoplas*[tiab] OR Cervix Cancer[tiab] OR Cervix Tumor*[tiab] or Cervix Carcinom*[tiab] OR Cervical Adenocarcinom*[tiab] OR Cervix Adenocarcinom*[tiab] OR Cervical Intraepithelial Neoplasia[Mesh] OR Cervical Intraepithelial[tiab] OR Cervix Intraepithelial[tiab]). We translated it into the different databases, accordingly. We searched lists of references from relevant primary studies, reviews, and key journals for additional studies. Likewise, we explored books and grey literature, master/doctoral theses, and meeting procedures (see Additional file1 for details).

Data management

We used Cochrane's web-based systematic review data management Covidence software for handling the initial phases of this review.(28) If a study report's duplication were the concern, we kept the one with better

methodological quality, and/or larger size and/or longer follow-up, as judged by the whole team of investigators in consensus.

Study selection and data collection

After the initial screening of titles and abstracts, the second round of screening by full-text was performed according to the aforementioned eligibility criteria. Selected papers were qualitatively described. For the quantitative synthesis, we considered only studies that used a methodology compliant with ASCO/CAP guidelines. Each step of the study selection and data extraction process has been done by at least two independent reviewers (BI, SS, EA, and AG). Disagreements, if detected, were referred to a third author or solved by consensus of the whole team. If additional information to resolve questions about eligibility was required, authors of articles were contacted by email. Reasons for exclusion of all the ineligible studies were recorded. The study flowchart is shown in Figure 1.

We extracted information on a pre-piloted spreadsheet. This comprised geographic location, study design, patients' age, tumor stage, histology, sample and assay characteristics including brands and clones of primary antibodies and probes, as well as criteria used by authors of included studies for the definition of HER2 positivity. The full-length list of extracted variables is available in Additional file 2.

Risk of bias assessment

We used the checklists of the National Institutes of Health Study Quality Assessment Tools for observational studies.⁽²⁹⁾ The methodology used for determining HER2 positivity was classified as ASCO/CAP compliant if the scoring system and positivity definition used in the study matched those made explicit in any American Society of Clinical Oncology / College of American Pathologists ASCO/CAP guidelines for HER2 testing (2007, 2013, or 2018) for either breast or gastric cancer regardless of the year of study publication.^(18, 20, 30)

If a study had an ASCO/CAP compatible scoring system, but a different positivity definition (for example, both 2+ and 3+ were considered positive) and provided the data on the proportion of 3+ positive cases separately, it was also classified as ASCO/CAP compliant. In such situations, only the number of 3+ positive cases was used to calculate the proportion of the HER2 positive tumors.

We hypothesized that the departure from ASCO/CAP standards might introduce bias, so when assessing the domain "outcomes measurements", ASCO/CAP compliant studies were classified as "low risk" of bias and *vice versa*. While studies that have reported the prevalence of HER2 overexpression/amplification using any definition and cut-off values for HER2 positivity were eligible for qualitative synthesis, we restricted the quantitative synthesis only to those ASCO/CAP compliant. The same rule was not applied in the case of the domain "outcome assessment by two independent pathologists" since there was no statistically significant difference in the estimated pooled HER2 positivity prevalence between studies classified as low and high risk of bias in this domain, $p = 0.81$.

For publication bias appraisal, we graphed funnel plots (Additional files 3A, 3B, 3C) and reported Egger's test.

Adenocarcinomas, adeno-squamous, glassy cell, clear cell, neuroendocrine and adenoid cystic carcinomas were grouped as non-squamous.

Statistical analyses

We transformed proportions using the variance-stabilizing double arcsine square root Freeman-Tukey procedure. The between-study variance was computed by the Der Simonian and Laird method.

For moderator analyses, we have chosen a mixed-effect approach assuming a common between-study variance component.(31) For continuous moderators, a meta-regression technique was used to describe the associations with the outcome variable HER2 positivity. Higgin's I^2 was used for assessing heterogeneity alongside a visual inspection of the Forest plot and R-squared values for the proportion of between-study variance that can be explained by each moderator. To identify outliers and influential studies we screened for studentized residuals absolute z-values close to or larger than 2. Leave-one-out analyses and Baujat plots were performed.(31) Statistical analyses were conducted with "meta" and "metafor" packages under R 3.6.3 or a later version.(32) Subgroup analyses were pre-specified in the study protocol for variables ASCO/CAP compliance and histology and exploratory for other moderators. All tests were done at 0.05 alpha level.

Results

Study selection

After removing the duplicates, we screened 651 documents, 649 of which came from electronic databases, and two from grey literature (Figure 1).(33, 34) Of them, 167 were assessed for eligibility in full-text in English, German, Chinese, Russian, and Turkish languages with the aid of the Google Translator tool, and finally, 65 were included in the review, as described. The main reasons for the exclusion were the inability to locate the article/unavailability of its full-text version (31), wrong outcomes (20), and duplicated reports (14).

Study Characteristics

Included studies were published between 1990 and 2020, 64 of them in English and one in Russian.(35) Twenty-six studies were case series, 26 were retrospective cohorts, six were cross-sectional studies, three were case-control studies, three were prospective cohorts, and one study was a mixed cohort (see Additional file 4).

As for their region of origin, 22 studies were from Europe, 18 were from Asia Pacific Region, 14 were from the Americas, ten were from South-Eastern Asia, and one study was from Eastern Mediterranean WHO Region (Additional file 5).

Outcomes, Assays, and Measurements

The 65 studies included in the review (N = 5237) were classified into three overlapping categories (Fig. 2, Table 1). The first subset comprised 62 studies that reported on the prevalence of HER2 overexpression by IHC (N = 5076). Fifty-four of them (N = 4399) reported on IHC only. In the remaining eight studies (N = 677) ISH was also performed in addition to IHC (N = 406).

Table 1. Main characteristics of included studies.

Author	Year of publication	Country	Histology	Method Used	ASCO/CAP compliance	N Analyzed IHC	% 3+ IHC	N Aalized by ISH	% Positive by ISH
Shi(36)	2020	China	Ns	IHC,FISH	Yes	209	18.2	209	6.2
Varshney(47)	2020	India	Sq, Ns	IHC	Yes	38	21.1	0	
Wong(62)	2020	China	Ns	IHC	Yes	14	21.4	0	
Nakamura(45)	2019	Japan	Ns	IHC, DISH	Yes	13	0	4	25.0**
Rahmani(69)	2018	Sudan	Sq, Ns	IHC	No	65	43.1	0	
Kumari Mitra(34)	2018	India	Sq, Ns	IHC	Yes	30	10	0	
Bajpai(50)	2017	India	Sq, Ns	IHC	Yes	43	4.7	0	
Halle(51)	2017	Norway	Sq, Ns	IHC	Yes	292	20.8	0	
Martinho(70)	2017	Brazil	Sq, Ns	IHC	No	170	53.5	0	
Ueda(23)	2017	Japan	Ns	IHC	No	43	20.9	0	
Xiang(44)	2017	China	NA	IHC, FISH	Yes	157	2.5	8	25.0**
Carleton(48)	2016	UK	Ns	IHC	Yes	26	3.8	0	
Sarwade(33)	2016	India	Sq, Ns	IHC	Yes	41	7.3	0	
Nimisha Sharma(52)	2016	India	Sq, Ns	IHC	Yes	25	4.0	0	
Fukazawa(71)	2014	Brazil	Sq	IHC	No	179	16.2	0	
Nishio(53)	2014	Japan	Sq, Ns	IHC	Yes	204	4.9	0	
Vosmik(54)	2014	Czech Rep	Sq	IHC	Yes	70	0	0	
Barbu(49)	2013	Romania	Ns	IHC	Yes	13	23.1	0	
Conesa-Zamora(38)	2013	Spain	Sq	IHC, FISH	Yes	32	3.1	32	0
Khalimbekova(35)	2013	Russia	Clear cell	IHC	Yes	14	0	0	
Ueno(43)	2013	Japan	Clear cell	IHC, FISH	Yes	13	23.1	8	12.5
Sukpan(72)	2011	Thailand	NE	IHC	No	100	2.0	0	
Perez - Regadera(73)	2010	Spain	Sq, Ns	IHC	No	136	23.5	0	
Gupta(55)	2009	India	Sq, Ns	IHC	Yes	65	27.6	0	
Lesnikova(39)	2009	Denmark	Sq, Ns	IHC, CISH	Yes	136	0.7	136	3.7
Yamashita(74)	2009	Japan	Sq	IHC	No	57	24	0	
Shen(56)	2008	China	Sq	IHC	Yes	53	0	0	
Carreras(75)	2007	Spain	Sq	IHC	No	10	50	0	
Panek(58)	2007	Poland	Sq, Ns	IHC	Yes	298	7.8	0	
Protrka(76)	2007	Serbia	Sq	IHC	No	13	46.2	0	
Fuchs(46)	2007	Germany	Sq	IHC, FISH	No	78	ND	ND	21.8*
Califano(77)	2006	Italy	Sq, Ns	IHC	No	65	0	0	
Kuroda(25)	2006	Japan	Glassy cell	IHC	No	11	45.4	0	
Ravazoula(78)	2006	Greece	Sq	IHC	No	42	19.0	0	
Kim(57)	2005	Korea	Sq, Ns	IHC	Yes	258	0.4	0	

Tangjitgamol(79)	2005	USA	NE	IHC	No	24	0	0	
Chavez - Blanco(37)	2004	Mexico	Sq, Ns	IHC, FISH	Yes	35	2.9	4	0
Graflund(80)	2004	Sweden	Sq, Ns	IHC	No	172	5.2	0	
Rosty(41)	2004	France	Sq, Ns	IHC, FISH	No	82	2.4	5	0
Bellone(59)	2003	USA	ND	IHC	Yes	10	20.0	0	
Dellas(61)	2003	Switzerland	Ns	IHC	Yes	22	0	0	
Heller(60)	2003	USA	Sq	IHC	Yes	24	0	0	
Niibe(81)	2003	Japan	Sq	IHC	No	21	42.8	0	
Kedzia(82)	2002	Poland	Sq	IHC	No	47	4.3	0	
Lee(83)	2002	Korea	Ns	IHC	No	37	29.7	0	
Bhadauria(84)	2001	India	Sq	IHC	No	50	26.0	0	
Leung (26)	2001	China	Ns	IHC	No	78	87.2	0	
Ngan(85)	2001	China	Sq	IHC	No	101	19.8**	0	
Straughn(86)	2001	USA	NE	IHC	No	16	0	0	
Chang(87)	1999	China	Sq	IHC	No	56	46.4	0	
Kersemakers(88)	1999	Netherlands	Sq, Ns	IHC	No	132	9.1	0	
Laksmi(89)	1999	India	Sq	IHC	No	166	34.9	0	
Mark(40)	1999	USA	Sq, Ns	FISH	No	0		23	8.7
Nevin(90)	1999	UK	Sq, Ns	IHC	No	126	38.1	0	
Nishioka(91)	1999	UK	Sq, Ns	IHC	No	107	32.7	0	
Sharma(42)	1999	India	Sq	FISH	No	0		60	36.6
Mandai(92)	1997	Japan	Sq, Ns	IHC	No	88	38.6	0	
Ndubisi(93)	1997	USA	Sq, Ns	IHC	No	150	22.7	0	
Kristensen(94)	1996	Norway	Glassy cell	IHC	No	132	12.1	0	
Nakano(95)	1996	Japan	Sq	IHC	No	52	46.2	0	
Costa(96)	1995	USA	Ns	IHC	No	82	39.0	0	
Kihana(97)	1994	Japan	Ns	IHC	No	44	25.0	11	
Oka(98)	1994	Japan	Sq, Ns	IHC	No	192	19.3	0	
Hale(99)	1992	UK	Sq, Ns	IHC	No	62	38.7	0	
Berchuck(100)	1990	USA	Sq, Ns	IHC	No	33	9.1	0	

*Among all included patients. **Among cases 2+ by immunohistochemistry.

Abbreviations: USA = The United States of America, Czech Rep = The Czech Republic, UK = The United Kingdom of Great Britain and Northern Ireland, Sq = squamous, Nsq = non-squamous, NE = neuroendocrine, IHC = immunohistochemistry, FISH = fluorescence in situ hybridization, CISH = chromogenic in situ hybridization, DISH = dual in situ hybridization.

The second subset encompassed ten studies that reported the rates of HER2 amplification by ISH (N = 489).(36–45) Eight of them, besides, determined the HER2 overexpression by IHC and two studies (N = 83) used ISH as the only method.(40, 42) Among eight studies that reported results of both IHC and ISH, six

studies (N = 394)(36–39, 41, 43) performed ISH in patients not previously selected by IHC and two studies (N = 170)(44, 45) only in those patients with equivocal results (2+) of IHC testing.

In the third subset, there was only one study (N = 78).(46) It reported the prevalence of the HER2 positivity by IHC tiebreaking the equivocal cases (2+) by ISH, but positivity rates by each method were not provided separately (Fig. 2). No study performed IHC in patients pre-selected by ISH.

Of 11 studies that assessed HER2 positivity using ISH, nine performed FISH, one study chromogenic ISH (CISH), and the other one dual ISH (DISH) technique (Additional file 6).

Of 62 studies reporting on HER2 positivity prevalence by IHC, 26 (41.9%) used the positivity definition and grading criteria compatible with ASCO/CAP 2007, 2013, or 2018 guidelines and have been classified as ASCO/CAP compliant, while the remaining 36 (58.1%) studies as ASCO/CAP non-compliant. (Fig. 3A, Table 1). Among ASCO/CAP compliant studies, five used the 30% positivity cut-off (ASCO/CAP 2007)(35, 43, 47–49) and 19 studies the 10% cut off (ASCO/CAP 2013 or 2018).(33, 34, 36, 37, 39, 44, 45, 50–62)

Nine out of 11 studies that used ISH followed ASCO/CAP compliant or slightly more stringent positivity criteria (see Additional file 6).

Subjects

The subjects' median age was 49.0 years, (interquartile range [IQR] 45.0 – 51.0). Data on the tumor stage was available from 56 studies. Five studies included women with stage I only, 11 studies with stages I and II, two studies with stage III only. The remaining studies contained a mixture of several stages. Median HPV positivity rate was 76.5%, (IQR: 57.3% to 90.3%). For more detailed information on patients' clinical features see Additional file 7.

Tumors and samples

In 14 studies (28.6%) the tissue for analyses was obtained by biopsy, in 25 (51.0%) by surgery, and the remaining ten studies (20.4%) used samples from either biopsy or surgery combined in varied proportions. Of them, the primary tumor was the exclusive sampling site in 41 (91.1%) of studies. In the residuary 4 (8.9%) studies, aside from the primary, the samples from nodes or either distant or local recurrences were included. No study provided sampling-to-fixation and fixation-to-assay times.

Data on histologic subtypes were available from 63 (96.9%) of studies. Eighteen studies (28.6%) included patients with squamous carcinoma, 18 (28.6%) non-squamous histology, and 27 (42.9%) studies with both.

Risk of bias assessment.

The risk of bias appraisal in studies is summarized in Additional file 8. In the tool utilized, the two domains of the potential bias most frequently involved were "outcomes measurements"(60%) and "outcome assessment by two independent pathologists" (75%).

The funnel plots for publication bias assessment are shown in Additional files 3A, 3B, and 3C. No significant asymmetry has been detected neither in the entire set of included studies (IHC, $p = 0.769$; ISH $p = 0.543$) nor in the subset of ASCO/CAP compliant ones, $p = 0.936$, suggesting the absence of substantial bias.

Immunohistochemistry

Overall, the estimated pooled prevalence of HER2 overexpression was 17.0% (95% confidence interval [95%CI]: 11.7% to 23.0%), $I^2 = 96\%$ (Additional file 9).

If only studies that used an ASCO/CAP compliant IHC method (26 studies, $N=2135$) were included, under the random-effects model the estimated pooled prevalence of HER2 overexpression was 5.7%, (CI 95%: 1.5% to 11.7%), $I^2 = 87\%$ (Figure 3A).[\(33-39, 43-45, 47-62\)](#) In the subset of studies judged ASCO/CAP non-compliant (36 studies, $N=2941$), the estimated pooled prevalence of HER2 overexpression was 27.0%, (CI 95%: 19.8% to 34.8%), $I^2 = 96\%$ (Figure 3A). In the mixed-effects model, the difference between these ASCO/CAP compliant and non-compliant subgroups was statistically significant ($p < 0.001$). The amount of heterogeneity accounted for (R^2) has been estimated at 26.8%. Thus, for further analyses, we only considered the subgroup of ASCO/CAP compliant studies. As there was no statistically significant difference in the pooled prevalence of HER2 overexpression between subsets of studies that used ASCO/CAP 2007 and 2013/2018 cut-offs, $p = 0.11$ (not shown), we pooled them together for subsequent analyses.

As significant heterogeneity in the ASCO/CAP compliant subgroup persisted, we conducted influence and moderator analyses. Although two studies have been identified as outliers, they were not judged influential cases since their removal neither significantly shifted the summary proportion nor markedly reduced the heterogeneity (Additional file 10).[\(51, 55\)](#)

Moderator analyses

In the subgroup of the squamous histology (12 studies, $N=1018$) the estimated pooled prevalence of HER2 overexpression was 4.1% (CI 95%: 0.6% to 9.8 %), $I^2 = 91\%$, while in the non-squamous carcinoma studies (15 studies, $N=467$) it was 10.3% (CI 95%: 3.6 to 19.2 %), $I^2 = 73\%$. The differences were not statistically significant neither if all the studies were considered, $p = 0.054$, $R^2 = 11\%$, (Figure 4A), nor if the analysis was restrained to only those studies that included both histologic subtypes, $p = 0.12$, $R^2 = 5.6\%$ (Figure 4B).

No statistically significant relationship has been observed between pooled HER2 overexpression rate and predictor variables geographic region ($p = 0.40$), primary antibody brand ($p = 0.051$), year of study publication ($p = 0.067$), study size ($p = 0.871$), and the proportion of the HPV positive tumors ($p = 0.842$). See Figures 5 - 7.

In situ Hybridization

Of 10 studies reporting on the proportion of HER2 positivity by ISH, two were excluded from the pooled analysis as they determined HER2 in patients with equivocal (2+) results of IHC.(44, 45) In the remaining eight studies of unselected patients, the estimated pooled prevalence of HER2 amplification was 5.9% (CI 95%: 1.9% to 11.3%), $I^2 = 84\%$. (Figure 3B)

In the subset of ASCO/CAP compliant studies the estimated pooled prevalence of HER2 amplification was 1.2% (CI 95% 0.0% to 5.8%) $I^2 = 0\%$.(36-39, 41, 43) compared to 24.9% (IC 95% 12.6% to 39.6%), $I^2 = 86\%$. among ASCO/CAP non-compliant ones (40, 42) The difference was statistically significant, $p = 0.004$.

Two studies reported ISH positivity rates among HER2 2+ tumors.(44, 45). Two out of eight (25%) and one out of four (25%) patients respectively were positive by ISH.

Special histologic subtypes

Varying degrees of HER2 overexpression have been observed in most histologic subtypes, except for mesonephric and perhaps neuroendocrine carcinomas (Figure 8, Additional file 11).

Micro-invasive carcinoma

Among 103 microinvasive carcinoma samples analyzed by Kim et al. using IHC, there was no HER2 positive case.(57)

Tumor stage and HER2 positivity prevalence

A meaningful and reliable analysis of the relationship between tumor stage and HER2 positivity prevalence was not possible to undertake as most of them included a mixture of several stages in varied, often unknown proportions not always providing the mapping of HER2 status to the tumor stage of the participants.

The main findings of the review are summarized in Table 2.

Table 2
Summary of the main findings of the review.

Summary of the main findings of the review.				
Grouping	Estimated HER2 Pooled Prevalence (95% CI)	Nº of participants (studies)	P-value	Risk of bias*
All studies				
IHC all studies	17.0% (11.7–23.0)	5076 (62)	-	-
ISH all studies	5.9% (1.9–11.3)	477 (8)		-
By ASCO/CAP guidelines compliance				
IHC ASCO/CAP compliant	5.7% (1.5–11.7)	2135 (26)	< 0.001	Low
IHC ASCO/CAP non-compliant	27.0% (19.9–34.8)	2941 (36)		High
ISH ASCO/CAP compliant	1.2 (0.0-5.8)	394 (6)	0.004	Low
ISH ASCO/CAP non-compliant	24.9% (12.6–39.6)	83 (2)		High
By histologic subtype			0.054	
Squamous	4.1% (0.6–9.8)	12 (1018)		Low
Non-squamous	10.3% (3.6–19.2)	15 (467)		Low
By WHO geographic region			0.40	
Abbreviations: ASCO = American Society of Clinical Oncology, CAP = College of American Pathologists, WHO = World Health Organization, *According to the compliance with the ASCO/CAP guidelines.				

Grouping	Estimated HER2 Pooled Prevalence (95% CI)	Nº of participants (studies)	P-value	Risk of bias*
Southeast Asia	11.7 (4.3–21.8)	6 (242)		Low
Americas	3.4 (0.0-16.5)	4 (95)		Low
Europe	4.1 (0.5; 10.0)	8 (877)		Low
Pacific	4.0 (0.3; 10.2)	8 (921)		Low
Abbreviations: ASCO = American Society of Clinical Oncology, CAP = College of American Pathologists, WHO = World Health Organization, *According to the compliance with the ASCO/CAP guidelines.				

Discussion

In this work, we analyzed a large ensemble of studies. We showed that the prevalence of HER2 positivity in CC heavily depended on whether the standardized ASCO/CAP Guidelines compliant methodology was used. Based on the subset of ASCO/CAP compliant studies we estimated the pooled prevalence of HER2 overexpression at 5.7% (CI 95%: 1.5% – 11.7%) and HER2 amplification at 1.2% (CI 95%: 0.0–5.8%). According to our findings, HER2 positivity rates above 10% can hardly be expected in unselected patients. As high degrees of heterogeneity were observed, the reliance on 95% confidence intervals instead of point estimates may be more appropriate when interpreting the results of pooled analyses.

Remarkably, the rate of HER2 amplification looks relatively small if compared with data from online genomic databases. For example, in the curated datasets of non-redundant studies from the cBioPortal database, HER2 amplified tumors are 5.6% of all CC, 10.9% of cervical adenocarcinomas, and 2.8% of squamous CC.(63) The cause of this discrepancy is unclear. In CC, the concordance between HER2 amplification by ISH and DNA-sequencing techniques seems to be insufficiently studied. The same may be true regarding the concordance between IHC and ISH. In our study, although the comparison is indirect, the pooled estimated HER2 positivity prevalence seems to be lower if determined by ISH than by IHC.

A trend to higher HER2 positivity rates in non-squamous than in squamous CC has not reached statistical significance. The estimated pooled prevalence of HER2 positivity in squamous CC was above 4% in our study. Although quite low in general, it looks slightly higher than figures reported in squamous carcinomas of other primary sites.(64, 65) On the other hand, the non-squamous CC subgroup was composed of numerous, sometimes dissimilar entities (Fig. 8, Additional file 11). Although we failed to hatch a histologic

subtype(s) with a particularly high HER2 positivity among this subgroup, it does not mean that such a subset could not be found in the future.

The impossibility to thoroughly explain the heterogeneity is a significant limitation of our study. Many factors can potentially contribute to the observed heterogeneity. Disparities in HER2 expression in different histologic subtypes were discussed above.(36) An unequal racial/ethnic background could act as an effect modifier variable. Santin et al. found that serous endometrial carcinoma patients of African ancestry were more frequently HER2 positive than women of other races. (66). From this point of view, the non-statistically significant trend toward a greater prevalence of HER positivity in India observed in our study might deserve further investigation. The impact of tumor stage and sample management on the pre-analytical phase could not be assessed in this study, which is another limitation of our work. Aside from pre-analytic and assay issues, the intra-tumor HER2 heterogeneity may affect the results of HER2 testing. Both the cluster and disperse types of heterogeneity have been described in breast cancer.(17) In serous endometrial carcinoma heterogeneous HER2 protein expression was found in 31% of the cases both classified as positive and negative.(67) Other possible sources of heterogeneity not addressed by this study are patients' age and treatments before the HER2 status determination. The relationship of the HER2 prevalence with the latter could not be investigated due to the lack of data in most studies. Our study's additional limitations are incomplete retrieval of the identified research and pooling of not entirely homogeneous studies, concerning their populations and risk of bias, despite the significant effort to override these issues.

Notwithstanding the great number of published studies, more work is needed. Better standardization of the IHC procedure in CC may be desirable. Another future strategy would be to guide indications of anti-HER2 therapies by DNA sequencing methods. Preferably, future studies should investigate the concordance between HER2 positivity as determined by IHC, ISH, and next-generation sequencing, and how reliably each of them predicts clinical benefit from HER2 targeted drugs. Of note, the ASCO/CAP guidelines for breast cancer were developed largely based on the benefit of trastuzumab. If new drugs active in patients with HER2-low-expressing tumors became available, the proportion of CC patients with this potentially relevant actionable target may increase.(68)

In summary, our study's results suggest that the prevalence of HER2 positive tumors among CC is low but still meaningful, and variations in previously reported HER2 positivity rates are mainly related to methodological issues. To our knowledge, this is the first meta-analytic systematic review on the subject published until now. Data provided by us reduce the uncertainty about the expected frequency of HER2 positive CC and can help to better understand the biology of this tumor, guide decisions on the appropriateness of studies of anti-HER2 agents for CC, and be useful in their design.

Abbreviations

CC

Cervical Cancer

IHC

Immunohistochemistry

ISH

In situ Hybridization

FISH

Fluorescent ISH

CISH

Chromogenic ISH

ASCO

American Society of Clinical Oncology

CAP

College of American Pathologists

WHO

World Health Organization

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Figures

Figure 1

PRISMA diagram of the study selection process.

Figure 2

Subgroups of studies according to methods used for HER2 determination. Footnote: IHC = Immunohistochemistry, ISH = In Situ Hybridization.

Figure 3

A. Prevalence of HER2 overexpression according to compliance with ASCO/CAP guidelines. Footnote: ASCO = American Society of Clinical Oncology, CAP = College of American Pathologists. B. Prevalence of HER2 amplification according to compliance with ASCO/CAP guidelines. Footnote: ASCO = American Society of Clinical Oncology, CAP = College of American Pathologists.

Figure 4

A. Subgroup analysis by histologic subtype. All studies. B. Subgroup analysis by histologic subtype. Studies that included both squamous and non-squamous histology.

Figure 5

Subgroup analysis by World Health Organization geographic region.

Figure 6

Subgroup analysis by primary antibody brand.

Figure 7

Relationship between the year of study publication, size, and HER2 overexpression prevalence.

Figure 8

HER2 overexpression prevalence in selected histologic subtypes. Footnote: NOS = Not Otherwise Specified

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