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Meta-analysis shows that prevalence of Epstein-Barr virus-positive gastric cancer differs based on sex and anatomic location

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Abstract

Background & Aims—Epstein-Barr virus (EBV) has been causally associated with cancer; some gastric carcinomas have a monoclonal EBV genome in every cancer cell, indicating that they arose from a single infected progenitor cell. However, the proportion of EBV-positive gastric carcinomas is uncertain and the etiological significance is unknown.

Methods—We conducted a meta-analysis of 70 studies including 15,952 cases of gastric cancer assessed by *in situ* hybridization for EBV-encoded small RNA.

Results—The pooled prevalence estimate of EBV-positivity was 8.7% (95% CI: 7.5, 10.0) overall, with a two-fold difference by sex: 11.1% (95% CI: 8.7, 14.1) of gastric cancer cases in males vs. 5.2% (95% CI: 3.6, 7.4) of cases in females. Tumors arising in the gastric cardia (13.6%) or corpus (13.1%) were more than twice as likely to be EBV-positive as those in the antrum (5.2%; $p<0.01$ for both comparisons). EBV-prevalence was four times higher (35.1%) for tumors in post-surgical gastric stump/remnants. Over 90% of lymphoepithelioma-like carcinomas were EBV-positive but only 15 studies reported any cases of this type; prevalence did not significantly differ between the more common diffuse (7.6%) and intestinal (9.5%) histologies. EBV-prevalence was similar in cases from Asia (8.3%), Europe (9.2%), and the Americas (9.9%).

Conclusions—EBV-positive gastric cancers greatly differ from other gastric carcinomas based on sex, anatomic subsite, and surgically disrupted anatomy, indicating that it is a distinct etiologic entity. Epidemiologic studies comparing EBV-positive and -negative gastric cancers are warranted to investigate EBV's role in gastric carcinogenesis.

Keywords

Epstein-Barr virus; gastric cancer; meta-analysis; prevalence

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Introduction

Despite its decline in incidence during the 20th century, gastric cancer (GC) remains the fourth most commonly diagnosed cancer and the second leading cause of cancer-related mortality worldwide[1]. Risk of gastric cancer is now believed to be modulated by a complex interaction between *Helicobacter pylori* (*H. pylori*) and a myriad of human genetic polymorphisms, as well as a number of other environmental and lifestyle factors[2–4].

Epstein-Barr Virus (EBV) is a ubiquitous gamma-1 herpes virus usually acquired during childhood via salivary transmission which establishes a life-long persistent infection of B-cells in over 90% of adults[5]. EBV is an established cause of Burkitt lymphoma, sinonasal angiocentric T-cell lymphoma, immunosuppression-related lymphoma, Hodgkin's lymphoma and nasopharyngeal carcinoma [6]. The oncogenic effects of the virus are likely exerted via the expression of EBV nuclear antigens (EBNAs) and latent membrane proteins (LMPs) which interact with a number of tumor suppressor genes and signaling pathways[7–10].

EBV is known to be present in a small percentage of gastric carcinomas; estimates vary widely but EBV-positive GC has been reported to constitute between 2 and 16% of cases[11]. In EBV-positive cases, virtually 100% of the carcinoma cells contain EBV nucleic acid sequences[12], and the EBV terminal repeat sequences are always uniform[13–15]. These observations imply that the tumor arose from a single EBV infected cell and that the EBV genome was retained during malignant transformation and proliferation. Moreover, EBV is routinely detected in an uncommon histologic entity, undifferentiated lymphoepithelioma-like gastric carcinoma (also known as medullary carcinoma), the microscopic appearance of which resembles nasopharyngeal lymphoepithelioma[16, 17].

Recent reviews[18, 19] have qualitatively described some of the epidemiological and clinicopathological features of Epstein-Barr virus associated GC. However, to date, there has not been a formal overview of published prevalence estimates. We therefore undertook a rigorous meta-analysis of papers demonstrating EBV tumor positivity using the demonstrated gold standard (*in situ* hybridization). This type of formal meta-analysis technique using a random effects model allowed our prevalence estimate to include consideration of within and between study variation in estimating the overall prevalence of EBV-positive GC and assessing variation by regional, clinical and tumor characteristics.

Material and Methods

We used PubMed® software tosearch Medline (U.S. National Library of Medicine, Bethesda, MD) using the following search terms: "Epstein Barr Virus AND gastric cancer", "EBV and gastric cancer", "Epstein Barr Virus AND stomach cancer", "EBV AND stomach cancer" for studies listed on or before September 30th 2008. Eligibility criteria for inclusion were: (i) studies must have ascertained EBV status of gastric tumor tissue using EBER *in situ* hybridization (the accepted gold standard in determining EBV-positivity in tumor tissue) and (ii) studies had to report prevalence of EBV-positivity in unselected GC cases, or provide enough information to calculate this estimate.

A total of 407 papers were identified and their titles and abstracts reviewed for relevance. 157 papers were discounted as irrelevant and 64 as duplications from a single population already represented. 28 papers were excluded due to patient selection (thereby making calculation of true prevalence impossible), 17 had not used EBER *in situ* hybridization, 19 were in languages other than English and 56 were found to be review articles. Thus, 70 papers met the inclusion criteria and were abstracted for prevalence data. Of the 63 studies which were included in the analyses of adenocarcinoma (defined from here onwards as

primary GC tumors which are not stump/remnant cancers), 12 studies also included lymphoepithelioma-like gastric carcinoma tumors and 4 included stump/remnant cancers. In addition, 3 studies were included that exclusively described lymphoepithelioma-like gastric carcinoma as were 5 describing stump/remnant cancers only. Separate analyses were conducted for gastric adenocarcinoma (63 studies), lymphoepithelioma-like gastric carcinoma tumors (15 studies) and stump/remnant cancers (9 studies). One publication clearly differentiated between ethnic Japanese and non-Japanese GC cases in Brazil[20] and is, therefore, included in the meta-analysis of gastric adenocarcinoma as two separate studies.

The following data were abstracted as available: first author, year of publication, sample size, EBV prevalence (or EBV-positive cases), sex, country of origin, regional group (Asia, Europe, Americas), histologic type (Laurén classification[21]) and tumor anatomic subsite (cardia, middle/corpus or antrum).

Statistical analysis

Meta-analyses were performed with Stata version 10 (StataCorp, College Station, TX), using the "metan" command[22]. Summary estimates (% prevalence), standard errors and 95% confidence intervals (CIs) were calculated, using the Wilson method[23], for each study. As the meta-analysis technique assumes normally distributed data, we logarithmically transformed all prevalence estimates[24], which necessitated adding a correction factor of 0.5 to both numerator and denominator[25] for reported prevalence of 0.

We first computed pooled summary estimates using the Mantel-Haenszel method assuming a fixed effects model[26]. However, as we found significant heterogeneity in prevalence estimates across studies, we also employed the random effect model of DerSimonian and Laird[27] and focus on those results in our presentation. Heterogeneity was described using the I^2 statistic, that represents the approximate proportion of total variability in point estimates that can be attributed to heterogeneity[28]:

$$I^2 = \frac{\tau^2}{\tau^2 + \sigma^2},$$

where σ^2 denotes the within-study variance and τ^2 denotes the between-studies variance component.

Meta-regression models were estimated using the "metareg" command in Stata *v*10.1, to analyze associations of EBV prevalence in GC with national incidence rates, study size and study quality. Incidence rates of GC among males were obtained from GLOBOCAN estimates for individual countries[29]; national incidence was treated both as a continuous variable and as a categorical variable, comparing countries in the top quintile (>21.7 cases per 100,000 population) to all other countries[1]. Study size was categorized according to whether the prevalence estimate was based on more than or less than 100 GC cases. Although we have no direct measure of 'quality' across reports we calculated a surrogate measure based on the number of variables (0–3) included among the following: (i) sex, (ii) anatomic subsite and (iii) histologic type.

Meta-analytic assumptions were assessed with Egger's test ("metabias") of funnel plot asymmetry (publication bias). This test identified no evidence of publication bias ($P = 0.49$). The influence of individual studies on the summary effect estimate was analyzed using the using the "metainf" command[30], which graphically compares meta-analytic estimates computed by omitting each study in turn. None of the included studies appeared to dominate the overall meta-analysis.

Results

A total of 70 studies were chosen for inclusion in the meta-analysis; these represented hospital cancer case series, together reporting a grand total of 15,952 GC cases. The earliest study was published in 1992[15] and the most recent studies in 2008[31, 32], the largest study included 2966 GC cases[33] and the smallest, 19 cases[34]. The majority of the 70 studies included originated in Asia (45/70), with a similar number of studies from Europe (12/70) and America (13/70). Of the 70 studies included, 47 provided information on patients' sex.

Of the 63 studies of primary non-remnant GC, 43 included information on patients' sex. 31 studies included information on histological type and 20 had information on anatomic subsite. Of the 12 studies that included both lymphoepithelioma-like gastric carcinoma and adenocarcinoma, lymphoepithelioma-like gastric carcinoma cases comprised between 0.9%[34] and 15%[35] of the respective series. Only 3 of the lymphoepithelioma-like gastric carcinoma studies included information on sex [36–38]. Of the 5 studies describing both gastric stump/remnant cancer and adenocarcinoma, gastric stump/remnant cancer comprised between 2%[39] and 25%[40] of the series. Five of the gastric stump/remnant studies included information on sex[40–44]. Together, the 70 studies reported a grand total of 15,952 GC cases.

Gastric adenocarcinoma meta-analysis

Figure 1 shows EBV prevalence and 95% CI estimates from individual studies based on the random effects model. The pooled prevalence of EBV-positive GC, as a proportion of gastric adenocarcinoma was 8.7% (95% CI: 7.5%, 10.0%). EBV prevalence was similar in cases from each of the three geographic regions: 9.9% (95% CI: 7.9%, 12.3%) for cases from America, 8.3% (95% CI: 6.9%, 9.9%) for Asian cases, and 9.2% (95% CI: 5.4%, 15.7%) for European cases (Figure 1).

There was a two-fold difference in the proportion of EBV-positive GC among cases in males compared to cases in females, with a prevalence of 11.1% (95% CI: 8.7%, 14.1%) in male GC cases compared to 5.2% (95% CI: 3.6%, 7.4%) in female cases (Figure 2). Notably, nine studies[31, 45–52] reported no cases of EBV-positive GC in females, whereas only a single study reported no cases among males[34].

Of gastric tumors from the antrum, 5.2% (95% CI: 3.8, 7.0) were EBV-positive, which was significantly lower than the proportion for either cardia (13.6%; 95% CI: 9.9, 18.7) or middle/corpus (13.1%; 95% CI: 10.4, 16.5) stomach tumors. In contrast, there was no statistically significant difference in the proportion EBV-positive for tumors of intestinal (9.5%; 95% CI: 7.2, 12.5) relative to diffuse (7.6%; 95% CI: 5.7, 10.3) histology.

Prevalence of EBV-positivity was similar for cases from high GC incidence countries (8.6; 95% CI: 7.2, 10.4) compared to all others (8.7; 95% CI: 6.9, 11.0). Analyzed as a continuous variable, there was no significant correlation between EBV-prevalence and national incidence ($r = 0.08$; $p=0.51$).

Heterogeneity was notably high in that the overall I^2 for the meta-analysis was 84% ($\tau^2 = 0.24$; $p < 0.001$). A number of variables (distribution of cases by sex, anatomic subsite and histologic type, national incidence rates, regional group and year of publication) were alternately added to the meta-regression model to examine the extent to which one or more of these covariates might explain the heterogeneity between studies. Size of study was significantly associated with EBV-positive GC prevalence so that for each ten-fold increase in the total number of GC cases, the prevalence of EBV decreased by 0.6% (95% CI: 0.5, 0.8). When sex, histological type, and study size were included together, I^2 was reduced to 63%. The number of "quality" variables reported was also associated with heterogeneity in

that studies providing prevalence estimates alone (and none of the 3 quality variables) had an I^2 of 89% ($\tau^2 = 0.43$) whereas studies which also included sex, anatomic subsite and histologic type had an I^2 of 56% ($\tau^2 = 0.08$).

Lymphoepithelioma-like gastric carcinoma

The pooled prevalence of EBV-positivity among lymphoepithelioma-like gastric carcinoma cases was 90.5% (95% CI: 85.7%, 95.5%; Figure 3). Prevalence estimates did not differ significantly by regional group: 88.9% (95% CI: 64.2%, 100%) for American cases, 90.3% (95% CI: 85.2%, 95.6%) for Asian cases and 75.7% (95% CI: 53.0%, 100%) for cases from Europe. There was very little heterogeneity across the 15 lymphoepithelioma-like gastric carcinoma studies ($I^2 = 0.0\%$, p=0.61).

Gastric stump/remnant cancer

The pooled prevalence of EBV-positivity among stump/remnant cancers was 35.1% (95% CI: 24.5, 50.2; Figure 4). From the 5 studies[40–43, 53] which also listed information on sex, the prevalence among stump/remnant cancers in males was estimated at 40% (± 7.44%). 3 studies included cases in females[40, 41, 43] where the EBV-positive cancer prevalence was 17% (± 9.6%). These studies were markedly heterogeneous ($I^2 = 99.5\%$, $p < 0.001$).

Discussion

We conducted a formal meta-analysis of the published literature to estimate the prevalence of EBV-positivity in GC. The overall pooled prevalence was estimated at 8.7% (95% CI: 7.5%, 10.0%) and was four times higher in gastric stump/remnant cancers than in primary non-remnant GC. Male GC patients were twice as likely to have EBV-positive tumors as female patients and antral tumors were half as likely to be EBV-positive as tumors from other sub-sites. In contrast, we found no quantitative evidence of regional variation, which had been previously assumed to be important[18,19], and no significant difference between intestinal and diffuse histologies. As has been reported previously, over 90% of lymphoepithelioma-like gastric carcinoma cancers were EBV-positive.

Heterogeneity across studies for the overall meta-analysis was substantial. We investigated possible sources for this heterogeneity using meta-regression methods; however, most of the residual heterogeneity was not explained by measured covariates. Laboratory methodology should not have been a large source of variation since we included only studies using EBER *in situ* hybridization on paraffin embedded tissue, however, minor differences in technical quality cannot be ruled out.

The 70 studies provided sparse patient and clinical data and it is possible that the observed heterogeneity might relate to such factors. For example, associations with age have been reported previously observed although results have been discordant: some studies find an age-dependent decrease in EBV-positivity rates[54, 55] and others an age-dependent increase in rates[36]. If there is an age association overall as well as variation between studies in patient age, this factor might explain some of the residual heterogeneity, however, patient age was generally not reported.

Our calculated prevalence estimate of 8% in gastric carcinomas contrasts sharply with the consistent absence of gastric EBV infection in non-cancer control conditions. In two studies with 16%[15] and 13%[56] EBV prevalence in gastric carcinomas, gastric biopsies from patients with Barrett's esophagitis[15], gastric ulcer disease[15] and healthy control subjects[15, 56] were uniformly EBV-negative (odds ratios: incalculable). These early and definitive studies associated gastric EBV infection only with the presence of malignancy. Normal gastric

tissue surrounding neoplastia is also EBV-negative[39, 42, 50, 51] further demonstrating the specificity of EBV infection.

Contrary to previous suggestions in the literature, we found no evidence that background incidence of GC is associated with rates of EBV-positivity in gastric adenocarcinoma. However, there are more reports and more cases in the published literature from Asia than from other regions of the world. Since regions with particularly high risk of GC, outside of Asia, are underrepresented in the literature, our analysis may not provide a complete picture.

To date, there is little evidence of interaction or antagonism of EBV with *H. pylori,* the agent most strongly implicated in gastric carcinogenesis[48, 57]. However, some *in vitro* experimental data suggests that *H. pylori*-associated monochloramine may induce EBV lytic conversion in gastric epithelium latently infected with EBV[58]. Results of previous clinical studies have demonstrated no correlation between *H. pylori* and EBV infections, with *H. pylori* equally likely regardless of EBV status [48, 59, 60]. EBV may be acting as a co-factor in *H. pylori* related gastric carcinogenesis, contributing to the likelihood of malignant transformation. Importantly, the relative predilection of EBV-positive tumors for the non-antral stomach may be analogous to the stronger association of *H. pylori* with non-cardia cancer, implying distinctive etiologies for gastric carcinogenesis at different subsites[61].

EBV infection in GC seems to be associated with increased inflammation, as seen in lymphoepithelioma-like gastric carcinoma with its the high-degree of lymphocytic infiltrate. The monoclonal nature of infection of tumor cells[14] necessarily implies that the clonal progenitor cell was EBV positive. Thus EBV must have been present at tumor inception regardless of whether the onset of inflammation was earlier or later. Furthermore, although it has been demonstrated that EBV is not present in normal epithelium adjacent to EBV-positive GC[37], it is unknown whether EBV is associated with inflammation in pre-cancerous states, such as gastritis[62, 63]. Even so, EBV positivity of inflammatory infiltrate would not demonstrate causality, since recruited lymphocytes may be coincidentally EBV-positive.

The mechanism of entry of EBV into gastric epithelial cells is not understood. At initial infection of B cells, EBV binds via CD21 receptors; however, whether CD21 is expressed on epithelial cells and/or carcinoma tissue has not been definitively determined[64]. CD21 independent mechanisms of EBV transfer have also been proposed, including direct cell-to-cell contact with virus-infected B cells and IgA mediated transport[64]. Interestingly, frequent salty food intake and occupational exposure to wood dust and/or iron filings have been associated with EBV-positive GC[18, 65], which has been interpreted as indicating that mechanical injury to gastric epithelia increases susceptibility to EBV infection.

The relatively high proportion of GC stump/remnant cancers which are EBV-positive is thought to result from bile reflux. As well as directly damaging the mucosal barrier[66] prolonged exposure to bile acids and pancreatic juices may mediate EBV entry into normally non-susceptible epithelial cells, for instance by inducing the fusion with infected B cells[41]. Interestingly, very high prevalence of EBER expression has been reported in both ulcerative colitis and Crohn's disease[67], which suggests that epithelial damage more generally is associated with increased vulnerability to EBV colonization.

Likewise, the etiologic role of the virus in gastric carcinogenesis remains largely unknown. EBV-positive GC exhibits the EBV latency I pattern also seen in Burkitt lymphoma, in contrast to nasopharyngeal carcinoma and Hodgkin's lymphoma which both express latency II pattern[16]. The absence of the known transforming proteins LMP1 and EBNA2 brings into question the oncogenic potential of the latency I pattern EBV. However, the viral genes that are expressed in EBV-positive GC also display carcinogenic activities: EBER-1 and -2 have been shown to upregulate insulin-like growth factor expression *in vitro*, BARF1 can

transform rodent fibroblasts *in vitro* and is known to increase the bcl-1 to bax ratio which promotes cell survival, and LMP2A has been found to confer resistance to apoptosis[68].

Data on lymphoepithelioma-like gastric carcinoma were only reported in a limited number of studies. The uneven reporting across studies and the absence of standardized criteria for its histologic appearance may indicate that GC cases classified as lymphoepithelioma-like gastric carcinoma by some pathologists are classified as 'carcinoma with heavy lymphocytic infiltrate' by others. This speculation and the finding that lymphoepithelioma-like gastric carcinoma is >90% EBV-positive has led us to question the separate classification of lymphoepithelioma-like gastric carcinoma, for which the extra-ordinary lymphocytic infiltrate might merely indicate one extreme of host response to an EBV-positive tumor.

Worldwide, men are twice as likely as women to develop GC[69], which the established risk factors for GC (*H. pylori* and smoking) do not fully explain this sex difference in incidence rates[70]. Given this sex difference in incidence rates overall, the two-fold sex difference in EBV-positivity implies that the incidence of EBV-positive GC is four times higher in males than females. The reasons for this large sex difference are, as yet, unknown but may relate to differing exposure to lifestyle or occupational factors risk factors for EBV-positive GC[18]. Alternatively intrinsic biological and/or hormonal factors, might also be responsible[71]. Interestingly, other EBV associated cancers including Hodgkin's lymphoma, Burkitt lymphoma and nasopharyngeal carcinoma are two to three times as common in men[72–75]. The "immunocompetence" hypothesis has been put forward as one possible explanation for sex difference in these and other cancers relating to infection, based on data suggesting that testosterone causes immunosuppression[76]. The hypothesis holds that men are thus more susceptible to infections and their sequelae which increase morbidity and mortality throughout all ages of life[77, 78].

EBV-positive GC is a distinct entity accounting for 8.7% of GC worldwide. Considering the worldwide burden of GC, the paucity of data regarding the significance of EBV-positivity is remarkable. Further epidemiologic studies are warranted to determine the role of EBV with respect to etiology, treatment and prognosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

EBV	Epstein-Barr virus
GC	gastric adenocarcinoma
EBNAs	EBV nuclear antigens
LMPs	latent membrane proteins

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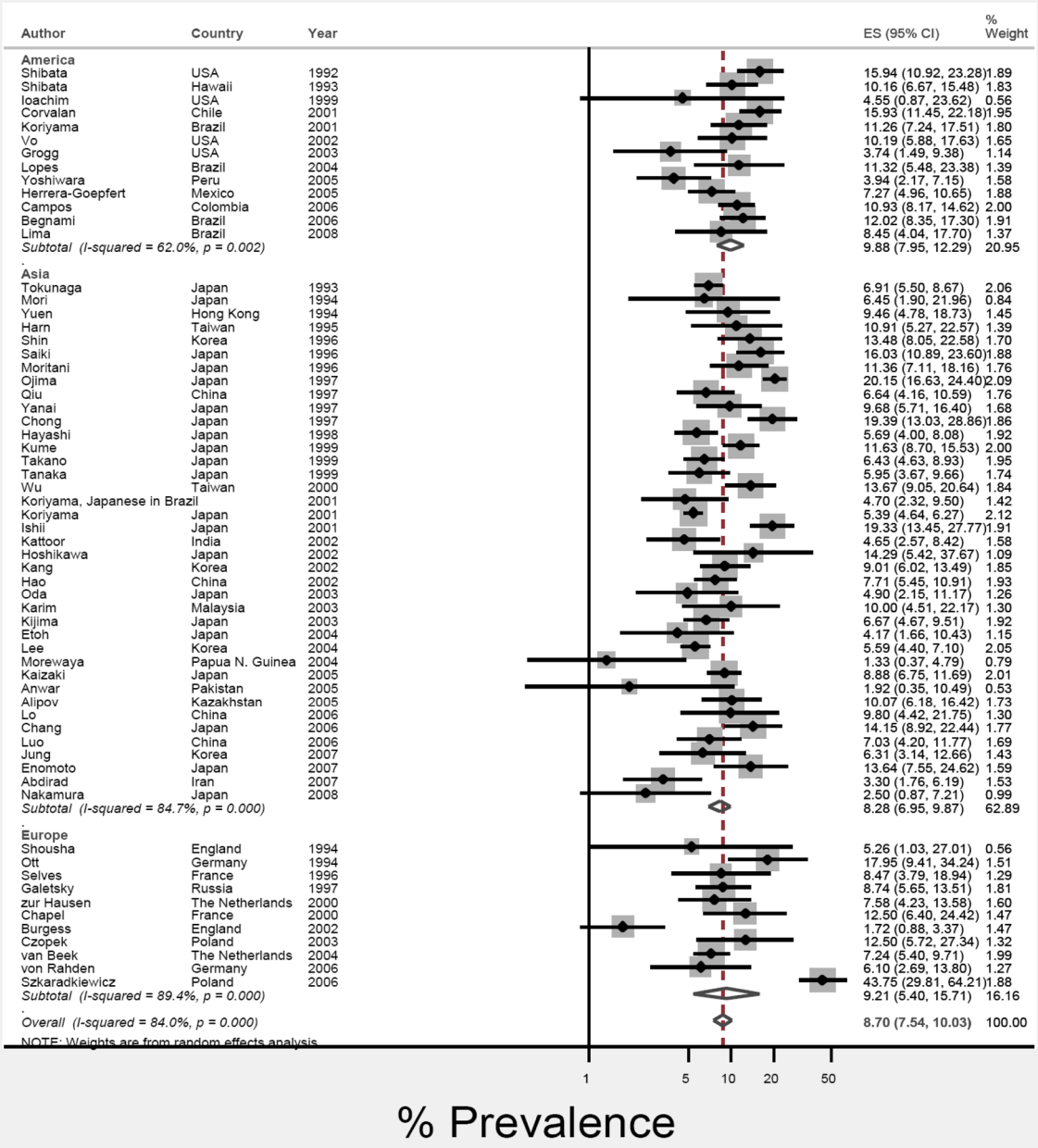

Figure 1.

Forest plot (random-effects model) of prevalence of EBV-positivity in gastric cancer cases by region of residence, in order of year of publication. Rhomboids indicate pooled prevalence for each region and in all studies.

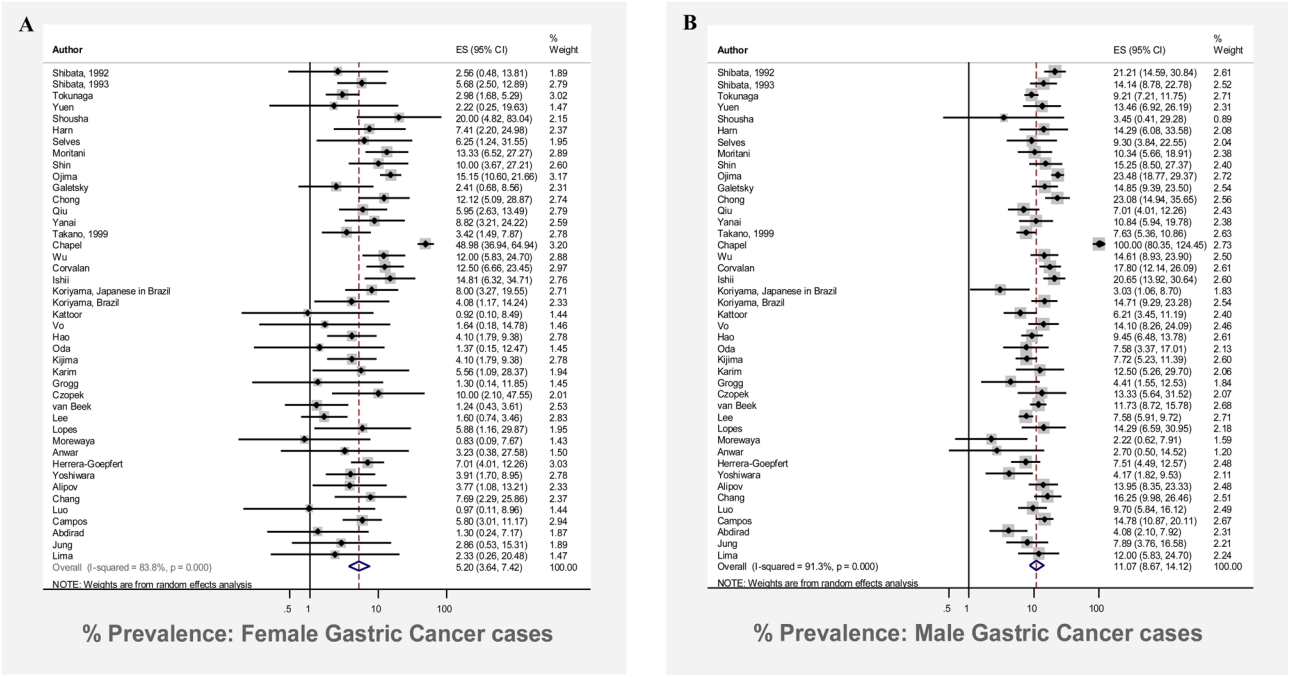

Figure 2.

Forest plot (random effects model) of prevalence of EBV-positivity in gastric cancer cases in females (Panel A) and males (Panel B), in order of year of publication. Rhomboids indicate pooled prevalence for each sex.

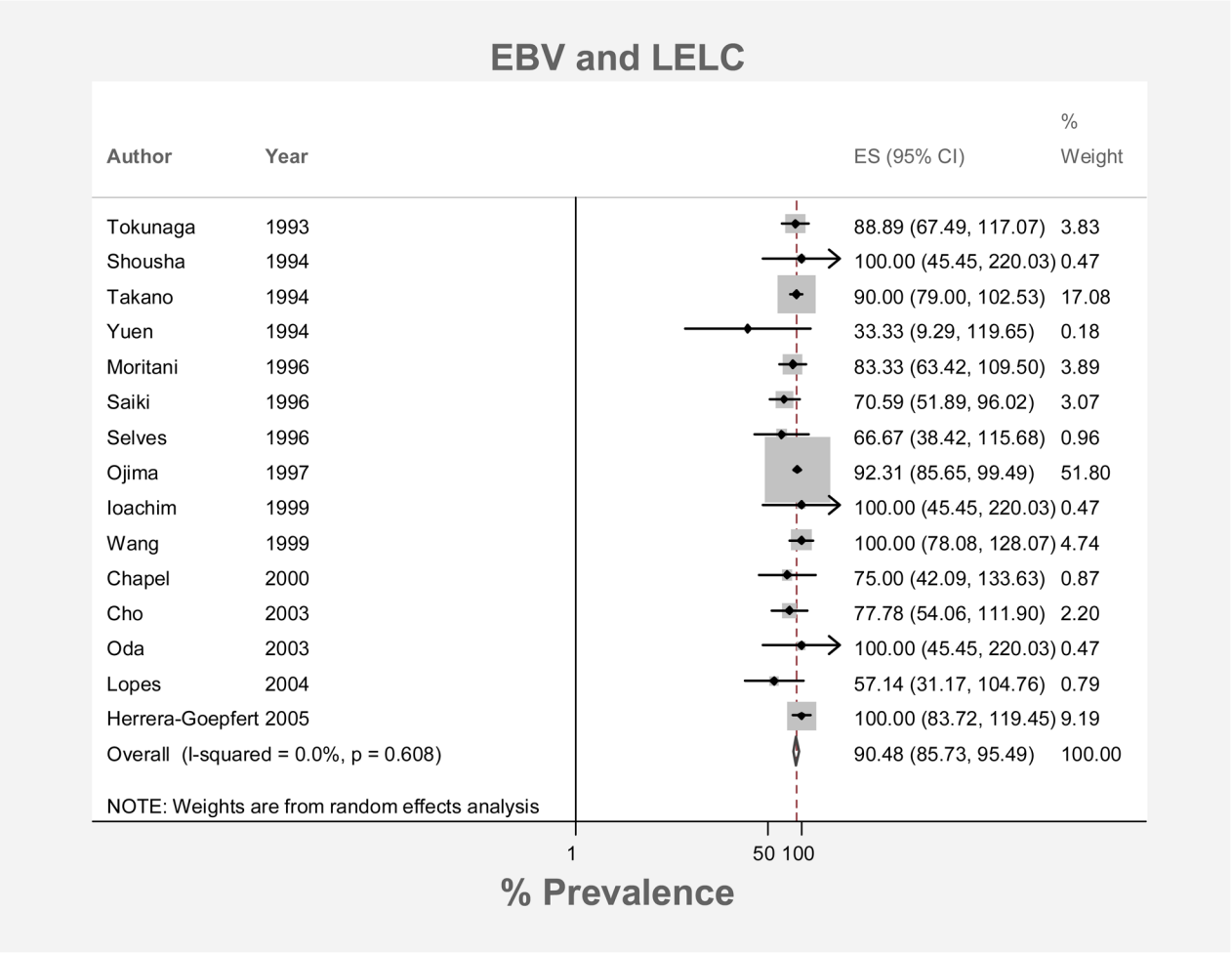

Figure 3.
Forest plot (random effects model) of prevalence of EBV positivity in gastric lymphoepithelioma-like carcinoma in order of year of publication. Rhomboid indicates pooled prevalence.

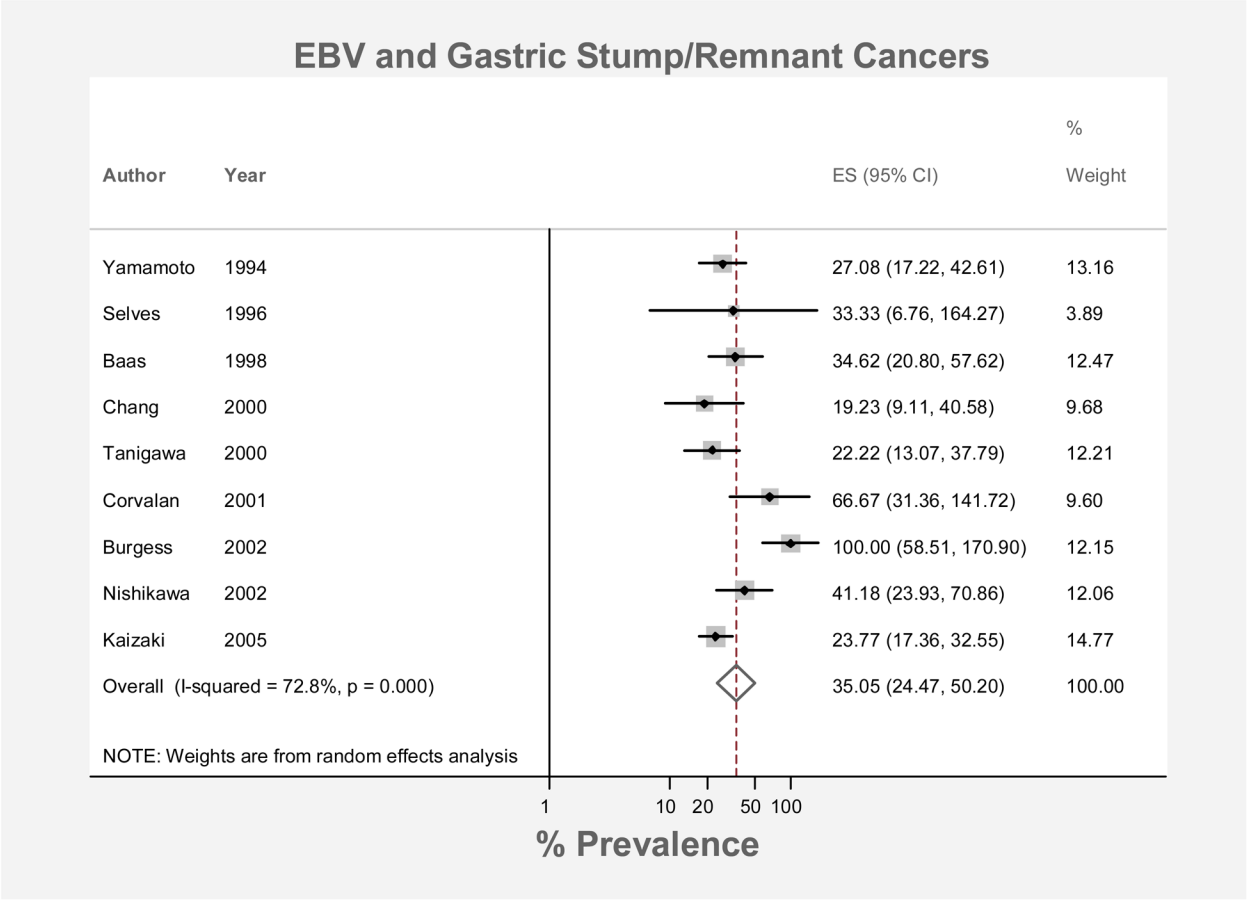

Figure 4.
Forest plot (random effects model) of prevalence of EBV positivity in gastric stump/remnant cancers in order of year of publication. Rhomboid indicates pooled prevalence.