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Circulating antibodies against Epstein-Barr virus (EBV) and p53 in EBV-positive and -negative gastric cancer

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Abstract

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Analysis and interpretation of data: MCC, AM, TW, MS, MP, CSR

Drafting of the manuscript: MCC, CSR

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Study supervision: MP, CSR

Conflicts of interest: none reported

Background: Epstein-Barr virus (EBV)-positive gastric cancers (GC) have clinicopathologic differences from EBV-negative tumors and lack *TP53* mutation. Serological profiles may inform viral contribution to carcinogenesis.

Methods: We compared humoral responses of EBV-positive (n=67) and -negative (n=137) GC patients from the International EBV-Gastric Cancer Consortium. Serum antibodies against four EBV proteins, nuclear (EBNA), viral capsid (VCA), early-diffuse (EA-D) and Zta replication activator (ZEBRA), and to p53 were assessed by multiplex assays. Odds ratios (OR) of antibody level tertiles (T1-T3) were adjusted by logistic regression. We also conducted a meta-analysis of reported anti-p53 seropositivity in GC.

Results: Consistent with EBV's ubiquity, 99% of patients were seropositive for anti-EBNA and 98% for anti-VCA, without difference by tumor EBV status. Seropositivity varied between patients with EBV-positive and -negative tumors for anti-EA-D (97% *vs.* 67%, respectively, p<0.001) and anti-ZEBRA (97% *vs.* 85%, respectively, p=0.009). Adjusted ORs (*vs.* T1) for patients with EBV-positive *vs.* -negative tumors were significantly elevated for higher antibodies against EBNA (2.6 for T2 and 13 for T3), VCA (1.8 for T2 and 2.4 for T3), EA-D (6.0 for T2 and 44 for T3), and ZEBRA (4.6 for T2 and 12 for T3). Antibodies to p53 were inversely associated with EBV positivity (3% *vs.* 15%; adjusted OR=0.16, p=0.021). Anti-p53 prevalence from the literature was 15%.

Conclusions: These serological patterns suggest viral reactivation in EBV-positive cancers and identify variation of p53 seropositivity by subtype.

Impact: Anti-EBV and anti-p53 antibodies are differentially associated with tumor EBV positivity. Serology may identify EBV-positive GC for targeted therapies.

Keywords

EBV; EBNA; EAD; gastric cancer; meta-analysis; p53; serology; VCA; ZEBRA

INTRODUCTION

Chronic *Helicobacter pylori* infection is the primary cause of gastric cancer (1), the third leading cause of cancer death worldwide (2). Epstein-Barr virus (EBV) is also implicated in gastric carcinogenesis, as about 9% of gastric tumors harbor monoclonal viral episomes (3). EBV-positive gastric tumors have demographic and clinicopathologic differences from EBV-negative tumors. Tumor EBV positivity is increased with male sex, smoking, non-antral localization and post-gastrectomy (3, 4). In addition, patients with EBV-positive gastric tumors have better overall survival as compared to those with EBV-negative tumors (5). The Cancer Genome Atlas project (6) identified EBV-positive tumors as one of four molecular subtypes of gastric cancer. EBV-positive tumors are characterized by recurrent *PIK3CA* mutation, absence of *TP53* mutation, *JAK2* amplification and extreme DNA hypermethylation. EBV-positive gastric cancer is classically considered to exhibit type I viral latency with EBV protein expression largely restricted to EBNA-1. However, several lytic proteins and transcripts have been also found (BZLF1, BcLF1, BLLF1, BHRF1, BRLF1, BMRF1) in EBV-positive tumors (7).

EBV infection occurs ubiquitously in the world's population. Primary infection is followed by lifelong persistence of proviral DNA in B-lymphocytes, recognized by a restricted humoral response (8). EBV infection may reactivate from latency by switching to the lytic replication cycle. Circulating viral particles trigger immunological response, generating additional anti-EBV antibodies. Following primary infection, antibodies to viral capsid antigen (VCA) are produced within a few days and peak after 3 weeks, then subsequently decline but persist for life. Antibodies to EBV nuclear antigen (EBNA) are not seen during acute infection, but develop 2–4 months afterwards and persist as markers of exposure. Antibody titers against early antigen (EA) rise on primary infection and in pathological states of EBV reactivation (8). EA consists of two components, diffuse (D) and restricted (R). Antibodies to EA-D show a transient rise during the acute phase and are generally undetectable 6 months later. Antibodies to EA-R follow the disappearance of anti-EA-D and are detectable for up to 2 years (9). ZEBRA (BamH1 Z encoded replication activator) expression coincides with the switch of EBV from the latent to lytic cycle (10). Therefore, elevated levels of anti-ZEBRA antibodies may serve as an indicator of recent EBV reactivation.

EBV reactivation from latency is a postulated mechanism for viral presentation to epithelial cells, triggering cellular replication and potential for transformation in EBV-positive malignancies (11). A deeper understanding of the humoral response to chronic EBV infection may shed light on the biological mechanisms for development of EBV-positive gastric cancer. We therefore examined the association of serum EBV antibody levels with tumor EBV status using samples from the United States (US) National Cancer Institute (NCI)'s International EBV-Gastric Cancer Consortium.(5) Given that EBV-positive tumors rarely carry mutation of *TP53*, we also evaluated the association between p53 antibodies and tumor EBV positivity. Finally, we conducted a meta-analysis of published studies assessing the prevalence of anti-p53 antibodies in patients with gastric cancer.

MATERIALS AND METHODS

NCI International EBV Gastric Cancer Consortium Analysis

Study population.—Five gastric cancer case series from Korea (n=63), Japan (n=28), Poland (n=41), Mexico (n=27) and Honduras (n=45) in US NCI's International EBV-Gastric Cancer Consortium were included in this analysis. For each series, serum samples from all available EBV-positive cases and a subset of EBV-negative cases were selected, frequency matched for sex, age at diagnosis (± 5 years), anatomic subsite and year of diagnosis (± 2 years). This study comprises a total of 67 EBV-positive and 137 EBV-negative tumors. Each contributing study received local institutional review board approval, and written informed consent was obtained from all patients.

Tumor EBV status.—For all cases, the presence of EBV in cancer cells had been previously assessed by gold standard *in situ* hybridization for EBV-encoded RNA (EBER) and an RNA preservation control in paraffin embedded tissue, as previously described (4, 12, 13).

Antibody measurements.—The EBV antigens selected for analysis are representative of the different infection phases (i.e., primary infection, latency and reactivation). Immunoglobulin G (IgG) antibodies to a fragment of EBNA1 (C-terminal part AA 325–641, EBV strain B-95–8), VCA p18, full-length EA-diffuse (EA-D), ZEBRA (EBV strain M-ABA) (14), and p53 (full-length, native) (15) were measured by fluorescent bead-based multiplex serology and quantified as median fluorescence intensity (MFI). Briefly, full-length proteins or peptides were expressed in *E. coli* in fusion with an N-terminal glutathione S-transferase (GST) domain. Glutathione cross-linked to casein was covalently bound to fluorescence labeled polystyrene beads (SeroMap; Luminex), and GST-fusion proteins were affinity-purified on the beads directly. Bead types each carrying a different antigen were mixed and incubated with pretreatment sera at 1:10,000 dilutions. Antibody bound to the beads via the antigens was stained by biotinylated anti-human-Ig and streptavidin-R-phycoerythrin. Beads were examined in a Luminex 100 analyzer (Luminex, Austin, Texas, USA) that identifies the different bead types by their internal color and quantifies the antibody bound to the antigen on the different bead types via the median R-phycoerythrin fluorescence intensity of at least 100 beads of each bead type. The cutoff MFI values for seropositivity were 100 MFI for EBNA-1 and 15 for ZEBRA, EA-D, VCAp18 and p53. Serum samples were tested in one batch, using the same lot of custom reagents. Laboratory staff was blinded to tumor EBV status. In addition to the in-house controls, we inserted six coded replicates across plates. The coefficients of variation for these quality control samples were 7% for EBNA-1, 6% for EA-D, 7% for VCAp18, and 18% for ZEBRA. All six replicate pairs were reproducibly seronegative for anti-p53.

Statistical analysis.—Correlations among antibody levels were evaluated by Spearman's rank correlation. Antibody positivity in patients with EBV-positive and -negative gastric tumors was compared using the Pearson $\chi 2$ test. EBV antibody levels were also divided into tertiles based on distributions among all patients. Unconditional logistic regression models were used to estimate odds ratios (OR) with 95% confidence intervals (CI) of EBV-positive *vs.* -negative gastric cancer for seropositivity to each protein. ORs were adjusted for country, year of diagnosis (tertiles), age at diagnosis (linear), sex and anatomic subsite (cardia, noncardia, overlapping subsites or unspecified). A p-value less than 0.05 was considered statistically significant and all tests were two-sided. Statistical analyses were performed in Stata version 15 (Stata Corp, College Station, TX, USA).

Meta-analysis

Search strategy and selection criteria.—The literature database PubMed® (National Library of Medicine, Bethesda, MD, USA) was searched for observational studies evaluating prevalence of anti-p53 antibodies in patients with gastric cancer, published in any language up to April 30, 2019. The following broad search strategy was used: (stomach neoplasms AND (p53 OR anti-p53) AND (antibodies OR autoantibodies)).

Data extraction.—Two investigators (MCC and MS) independently reviewed titles and abstracts for selection of potentially relevant articles; any disagreement was resolved by consulting a third reviewer (JZ). Citations of retrieved articles were reviewed for studies that may have been missed or absent from the database query. The following information was

abstracted from each selected article: first author, year of publication, study location (country), year of sample collection, participant age (range or mean) and sex (proportion of males), number of gastric cancer cases, prevalence of anti-p53, and method of antibody assessment.

Statistical analysis.—We used random effects models (16) to summarize prevalences of anti-p53 antibodies. Between-study heterogeneity was assessed for statistical significance using the Q test and quantified with the I^2 statistic as low (<25%), moderate (25%−50%) or high (>50%) (17). Meta-analyses were performed with Stata version 15 (StataCorp, College Station, TX, USA) using the macro metaprop (18). A p-value less than 0.05 was considered statistically significant and tests were two-sided.

RESULTS

NCI International EBV Gastric Cancer Consortium Analysis

All pair-wise correlations among the four anti-EBV antibodies were statistically significant in the combined group of patients. The correlation coefficients ranged from 0.6 (anti-ZEBRA *vs.* anti-EA-D) to 0.3.

Ninety-nine percent of patients were seropositive for anti-EBNA and 98% for anti-VCA, without difference by tumor EBV status. Seropositivity varied between patients with EBV-positive and -negative tumors for anti-EA-D (97% *vs.* 67%, respectively, p<0.001) and anti-ZEBRA (97% *vs.* 85%, respectively, p=0.009). In analyses based on tertiles, each viral antibody was associated with tumor EBV positivity (Figure 1). Adjusted ORs for EBV-positive *vs.* -negative tumors were significantly elevated for patients with higher levels (*vs.* T1) of antibodies against EBNA (13.0 for T3), VCA (2.4 for T3), EA-D (6.1 for T2 and 44.5 for T3), and ZEBRA (4.6 for T2 and 12.4 for T3).

Antibodies to p53 were detected in 11% of the gastric cancer patients overall, including 3% of those with EBV-positive *vs.* 15% with EBV-negative tumors (adjusted OR=0.16, p=0.021). Antibodies to p53 were not statistically significantly correlated with anti-EBV antibodies in the combined set (*Rho* coefficients ranged from 0.02 to −0.13).

Meta-analysis

The literature search identified a total of 111 reports mentioning anti-p53 antibodies in gastric cancer. After excluding 97 irrelevant publications (mainly reports of anti-p53 immunohistochemical staining), 14 full-text reports were retrieved for further evaluation; six additional publications regarding anti-p53 antibody prevalence across multiple cancer sites were identified from a previous meta-analysis (19) (Supplementary Table 1). Thus, there were a total of 20 reports (19 written in English and 1 in Polish) published between 1997 and 2017 regarding the seroprevalence of anti-p53 antibodies in patients with gastric cancer (Supplementary Figure). Fifteen studies were conducted in Asian countries and five in European countries. The total sample size ranged from 25 to 501 gastric cancer cases. Two reports each presented data on two independent populations. Eighteen (90%) studies assessed IgG anti-p53 antibodies by enzyme-linked immunosorbent assay (ELISA), 1 study used a Luminex-based multiplex assay, and 1 study used immunofluorescence.

None of the studies reported separately values for patients with EBV-positive gastric cancer. Across the 22 independent series represented in the 20 reports, population-specific seroprevalences ranged from 7 to 32%. The pooled seroprevalence of anti-p53 was 15% (95% CI, 13–18%), with high between-study heterogeneity (I^2=61%; Figure 2). Seroprevalence varied significantly by test method ($p<0.01$), with lower seroprevalence by Luminex-based multiplex and immunoblot as compared to ELISA.

DISCUSSION

This multi-country case-case comparison found higher antibody levels against EBV-specific proteins in patients with EBV-positive gastric cancer as compared to EBV-negative cases. These serological patterns are consistent with viral reactivation in the presence of EBV-positive tumors as three (anti-VCA, anti-EA-D and anti-ZEBRA) of the four studied antibodies target proteins expressed during lytic replication. This pattern of viral reactivation could represent lytic phase infection in either EBV-positive gastric cancer cells or in benign lymphocytes related to reduced immune function.

Our findings are in agreement with higher antibodies to VCA, EA and EBNA in EBV-positive gastric cancer as previously reported in two cross-sectional comparisons and a prospective (nested case-control) study (Supplementary Table 2) (20–22). A unique feature of our study was the evaluation of anti-ZEBRA. ZEBRA is encoded by the EBV immediate early gene *BZLF1*,and is a key mediator of reactivation from latency to the viral productive cycle. Elevated antibodies to ZEBRA have also been detected in patients with nasopharyngeal carcinoma and acquired immune deficiency syndrome (23–25).

Antibody reactivity to EBV has been variably linked to gastric cancer overall. In a case-control study from Spain increasing antibody reactivity against EBNA-1 and VCA-p18 was associated with gastric cancer (26). On the other hand, a nested case-control study from East Asia found no association (27). Notably, these studies may be relatively insensitive since EBV-positive cases comprise less than 10% of gastric cancer overall.

Gastric carcinogenesis is usually characterized by identifiable precursor lesions, and the presence of EBV prior to cancer is uncertain (28–31). In a longitudinal study of gastric atrophy by Schetter *et al.* (32), individuals with elevated baseline VCA IgG and EBNA IgG titers had a higher likelihood of progressing to more severe gastric lesions. Additional studies of preneoplastic stages are needed to fully elucidate the role of EBV in gastric cancer development.

EBV is strongly associated with nasopharyngeal carcinoma and several studies have indicated that EBV antibody testing has diagnostic utility (33–35). High antibody levels, particularly IgA antibodies directed against EBV structural proteins, precede the development of this neoplasia (36–38). For other EBV-associated malignancies, serological results are inconsistent (39). Given the rarity of EBV-positivity in gastric cancer, it may never be justifiable to use anti-EBV antibodies for general population cancer screening. Among patients diagnosed with gastric cancer, EBV-positive tumor status may be a useful predictor of response to immunotherapy (40). Anti-viral antibody tests thus have potential

value for both non-invasive clinical management as well as research applications in settings where gastric tissue is unavailable for EBV assessment. Additional serologic discrimination of tumor EBV status could be achieved by combination with blood tests for other viral (e.g., EBV microRNAs) or host response factors (e.g., PD-L1) (41).

The EBV genome encodes 85 genes (42). Previous serologic studies of EBV-positive gastric cancer have focused on a small number of EBV proteins, limiting our understanding of the role of the humoral immune response in cancer development. Taking advantage of emerging technologies (43), future research efforts should investigate wider ranges of viral epitopes, antibody functional types and immunoglobulin classes and subclasses. Longitudinal studies should also evaluate whether changes in EBV serology patterns over time are predictive of EBV-positive gastric cancer risk. Elevation of specific antibodies years before cancer onset may support a viral role in carcinogenesis, while altered antibody patterns close to the time of diagnosis could reflect impaired immunity in individuals with EBV-positive gastric cancer (i.e., reverse causality).

Although rare in EBV-positive gastric cancer, mutation of the p53 tumor suppressor gene is found in about half of gastric carcinomas overall (6, 44). The protein product of a mutated p53 gene generally has a longer half-life than wild-type p53 protein (45), leading to accumulation of mutant protein and production of anti-p53 antibodies. The lower prevalence of anti-p53 in our patients with EBV-positive gastric cancer is consistent with the expected rarity of the corresponding mutation (6). Since p53 inhibition is a central carcinogenic pathway, EBV-positive gastric cancer may have an alternative mechanism to abrogate p53 activity. One possible mediator is EBV-miR-BART5–3p, which facilitates degradation of p53 proteins and also targets the 3’-UTR of TP53 to consequently down-regulate CDKN1A, BAX and FAS expression. The clinical implication of anti-p53 antibodies in gastric cancer patients is largely unknown, although seropositivity has been associated with poor survival in some studies. Hot spot mutations in *TP53* have been associated with worse survival (46) and tumor EBV-positivity with better survival (5). These findings are similar to the case of human papillomavirus-related head and neck carcinoma, in which viral presence is associated with both low prevalence of *TP53* mutation as well as better survival (47).

In conclusion, patients with EBV-positive gastric tumors have elevated antibodies to EBV. Our results further implicate EBV in gastric carcinogenesis, potentially as an alternative pathway to p53 inhibition, and may provide a useful diagnostic marker for clinical and research applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans: Schistosomes, liver flukes and Helicobacter pylori. First ed. Lyon, France: IARC Press; 1994.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018.
3. Murphy G, Pfeiffer R, Camargo MC, Rabkin CS. Meta-analysis shows that prevalence of Epstein-Barr virus-positive gastric cancer differs based on sex and anatomic location. Gastroenterology. 2009;137(3):824–33. [PubMed: 19445939]
4. Camargo MC, Koriyama C, Matsuo K, Kim WH, Herrera-Goepfert R, Liao LM, et al. Case-case comparison of smoking and alcohol risk associations with Epstein-Barr virus-positive gastric cancer. International journal of cancer Journal international du cancer. 2014;134(4):948–53. [PubMed: 23904115]
5. Camargo MC, Kim WH, Chiaravalli AM, Kim KM, Corvalan AH, Matsuo K, et al. Improved survival of gastric cancer with tumour Epstein-Barr virus positivity: an international pooled analysis. Gut. 2014;63(2):236–43. [PubMed: 23580779]
6. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014;513(7517):202–9. [PubMed: 25079317]
7. Ribeiro J, Oliveira C, Malta M, Sousa H. Epstein-Barr virus gene expression and latency pattern in gastric carcinomas: a systematic review. Future Oncol. 2017;13(6):567–79. [PubMed: 28118740]
8. Young LS, Yap LF, Murray PG. Epstein-Barr virus: more than 50 years old and still providing surprises. Nature Reviews Cancer. 2016;16(12):789–802. [PubMed: 27687982]
9. Horwitz CA, Henle W, Henle G, Rudnick H, Latts E. Long-term serological follow-up of patients for Epstein-Barr virus after recovery from infectious mononucleosis. J Infect Dis. 1985;151(6):1150–3. [PubMed: 2987370]
10. Schaeffner M, Mrozek-Gorska P, Buschle A, Woellmer A, Tagawa T, Cernilogar FM, et al. BZLF1 interacts with chromatin remodelers promoting escape from latent infections with EBV. Life Sci Alliance. 2019;2(2).
11. Murata T Regulation of Epstein-Barr virus reactivation from latency. Microbiology and immunology. 2014;58(6):307–17. [PubMed: 24786491]
12. Ryan JL, Morgan DR, Dominguez RL, Thorne LB, Elmore SH, Mino-Kenudson M, et al. High levels of Epstein-Barr virus DNA in latently infected gastric adenocarcinoma. Laboratory investigation; a journal of technical methods and pathology. 2009;89(1):80–90. [PubMed: 19002111]
13. Song HJ, Srivastava A, Lee J, Kim YS, Kim KM, Kang WK, et al. Host Inflammatory Response Predicts Survival of Patients With Epstein-Barr Virus-Associated Gastric Carcinoma. Gastroenterology. 2010;139(1):84–U123. [PubMed: 20398662]
14. Brenner N, Mentzer AJ, Butt J, Michel A, Prager K, Brozy J, et al. Validation of Multiplex Serology detecting human herpesviruses 1–5. PloS one. 2018;13(12):e0209379.
15. Teras LR, Gapstur SM, Maliniak ML, Jacobs EJ, Gansler T, Michel A, et al. Prediagnostic Antibodies to Serum p53 and Subsequent Colorectal Cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2018;27(2):219–23.
16. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986;7(3):177–88. [PubMed: 3802833]
17. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557–60. [PubMed: 12958120]
18. Nyaga V, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. Archives of Public Health. 2014;72(39).
19. Werner S, Chen H, Tao S, Brenner H. Systematic review: serum autoantibodies in the early detection of gastric cancer. International journal of cancer Journal international du cancer. 2015;136(10):2243–52. [PubMed: 24615018]

20. Levine PH, Stemmermann G, Lennette ET, Hildesheim A, Shibata D, Nomura A. Elevated antibody titers to Epstein-Barr virus prior to the diagnosis of Epstein-Barr-virus-associated gastric adenocarcinoma. International journal of cancer Journal international du cancer. 1995;60(5):642–4. [PubMed: 7860138]
21. Shinkura R, Yamamoto N, Koriyama C, Shinmura Y, Eizuru Y, Tokunaga M. Epstein-Barr virus-specific antibodies in Epstein-Barr virus-positive and -negative gastric carcinoma cases in Japan. Journal of medical virology. 2000;60(4):411–6. [PubMed: 10686024]
22. Imai S, Koizumi S, Sugiura M, Tokunaga M, Uemura Y, Yamamoto N, et al. Gastric carcinoma: monoclonal epithelial malignant cells expressing Epstein-Barr virus latent infection protein. Proceedings of the National Academy of Sciences of the United States of America. 1994;91(19):9131–5. [PubMed: 8090780]
23. Joab I, Nicolas JC, Schwaab G, de-The G, Clausse B, Perricaudet M, et al. Detection of anti-Epstein-Barr-virus transactivator (ZEBRA) antibodies in sera from patients with nasopharyngeal carcinoma. International journal of cancer Journal international du cancer. 1991;48(5):647–9. [PubMed: 1649137]
24. Dardari R, Khyatti M, Benider A, Jouhadi H, Kahlain A, Cochet C, et al. Antibodies to the Epstein-Barr virus transactivator protein (ZEBRA) as a valuable biomarker in young patients with nasopharyngeal carcinoma. International Journal of Cancer. 2000;86(1):71–5. [PubMed: 10728597]
25. Tedeschi R, Dillner J, DePaoli P. Antibody responses to defined epitopes in the Epstein-Barr virus BZLF1-encoded transactivator protein among human immunodeficiency virus-infected patients. Clin Diagn Lab Immun. 1996;3(3):363–4.
26. Aragones N, Fernandez de Larrea N, Pastor-Barriuso R, Michel A, Romero B, Pawlita M, et al. Epstein Barr virus antibody reactivity and gastric cancer: A population-based case-control study. Cancer epidemiology. 2019;61:79–88. [PubMed: 31154081]
27. Varga MG, Cai H, Waterboer T, Murphy G, Shimazu T, Taylor PR, et al. Epstein-Barr Virus Antibody Titers Are Not Associated with Gastric Cancer Risk in East Asia. Digestive diseases and sciences. 2018;63(10):2765–72. [PubMed: 29948559]
28. Shibata D, Weiss LM. Epstein-Barr virus-associated gastric adenocarcinoma. The American journal of pathology. 1992;140(4):769–74. [PubMed: 1314023]
29. Zur Hausen A, van Rees BP, van Beek J, Craanen ME, Bloemena E, Offerhaus GJ, et al. Epstein-Barr virus in gastric carcinomas and gastric stump carcinomas: a late event in gastric carcinogenesis. Journal of clinical pathology. 2004;57(5):487–91. [PubMed: 15113855]
30. Ryan JL, Shen YJ, Morgan DR, Thorne LB, Kenney SC, Dominguez RL, et al. Epstein-Barr Virus Infection Is Common in Inflamed Gastrointestinal Mucosa. Digestive diseases and sciences. 2012;57(7):1887–98. [PubMed: 22410851]
31. Wanvimonsuk S, Thitiwanichpiwong P, Keelawat S, Mutirangura A, Kitkumthorn N. Distribution of the Epstein-Barr virus in the normal stomach and gastric lesions in Thai population. Journal of medical virology. 2019;91(3):444–9. [PubMed: 30229949]
32. Schetter AJ, You WC, Lennette ET, Gail MT, Rabkin CS. Association of Epstein-Barr virus antibody levels with precancerous gastric lesions in a high-risk cohort. Cancer science. 2008;99(2):350–4. [PubMed: 18201267]
33. Liu Z, Ji MF, Huang QH, Fang F, Liu Q, Jia WH, et al. Two Epstein-Barr virus-related serologic antibody tests in nasopharyngeal carcinoma screening: results from the initial phase of a cluster randomized controlled trial in Southern China. American journal of epidemiology. 2013;177(3):242–50. [PubMed: 23255783]
34. Li T, Guo X, Ji M, Li F, Wang H, Cheng W, et al. Establishment and validation of a two-step screening scheme for improved performance of serological screening of nasopharyngeal carcinoma. Cancer Med. 2018;7(4):1458–67. [PubMed: 29479857]
35. Coghill AE, Pfeiffer RM, Proietti C, Hsu WL, Chien YC, Lekieffre L, et al. Identification of a Novel, EBV-Based Antibody Risk Stratification Signature for Early Detection of Nasopharyngeal Carcinoma in Taiwan. Clin Cancer Res. 2018;24(6):1305–14. [PubMed: 29301829]

36. Chien YC, Chen JY, Liu MY, Yang HI, Hsu MM, Chen CJ, et al. Serologic markers of Epstein-Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. The New England journal of medicine. 2001;345(26):1877–82. [PubMed: 11756578]
37. Cao SM, Liu Z, Jia WH, Huang QH, Liu Q, Guo X, et al. Fluctuations of epstein-barr virus serological antibodies and risk for nasopharyngeal carcinoma: a prospective screening study with a 20-year follow-up. PloS one. 2011;6(4):e19100.
38. Ji MF, Wang DK, Yu YL, Guo YQ, Liang JS, Cheng WM, et al. Sustained elevation of Epstein-Barr virus antibody levels preceding clinical onset of nasopharyngeal carcinoma. British journal of cancer. 2007;96(4):623–30. [PubMed: 17285127]
39. Coghill AE, Hildesheim A. Epstein-Barr virus antibodies and the risk of associated malignancies: review of the literature. American journal of epidemiology. 2014;180(7):687–95. [PubMed: 25167864]
40. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nat Med. 2018;24(9):1449–58. [PubMed: 30013197]
41. Camargo MC, Sivins A, Isajevs S, Folkmanis V, Rudzite D, Gulley ML, et al. Associations of Epstein-Barr Virus-Positive Gastric Adenocarcinoma with Circulating Mediators of Inflammation and Immune Response. Cancers (Basel). 2018;10(9).
42. Santpere G, Darre F, Blanco S, Alcami A, Villoslada P, Mar Alba M, et al. Genome-wide analysis of wild-type Epstein-Barr virus genomes derived from healthy individuals of the 1,000 Genomes Project. Genome Biol Evol. 2014;6(4):846–60. [PubMed: 24682154]
43. Zheng DS, Wan J, Cho YG, Wang LY, Chiou CJ, Pai S, et al. Comparison of Humoral Immune Responses to Epstein-Barr Virus and Kaposi's Sarcoma-Associated Herpesvirus Using a Viral Proteome Microarray. Journal of Infectious Diseases. 2011;204(11):1683–91.
44. Fenoglio-Preiser CM, Wang J, Stemmermann GN, Noffsinger A. TP53 and gastric carcinoma: a review. Hum Mutat. 2003;21(3):258–70. [PubMed: 12619111]
45. Davidoff AM, Iglehart JD, Marks JR. Immune-Response to P53 Is Dependent Upon P53/Hsp70 Complexes in Breast Cancers. Proceedings of the National Academy of Sciences of the United States of America. 1992;89(8):3439–42. [PubMed: 1373500]
46. Tahara T, Shibata T, Okamoto Y, Yamazaki J, Kawamura T, Horiguchi N, et al. Mutation spectrum of TP53 gene predicts clinicopathological features and survival of gastric cancer. Oncotarget. 2016;7(27):42252–60. [PubMed: 27323394]
47. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. The lancet oncology. 2010;11(8):781–9. [PubMed: 20451455]

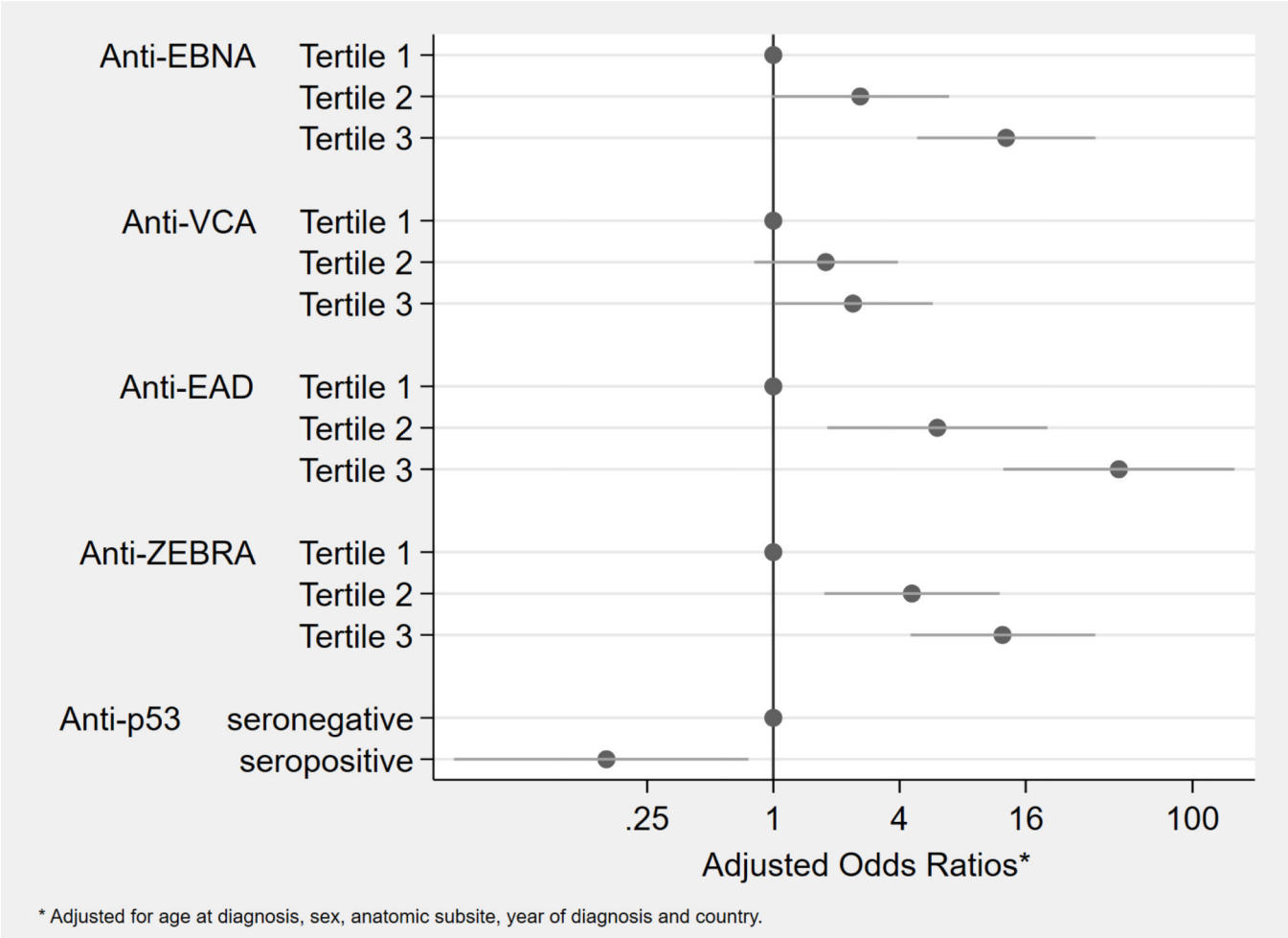

Figure 1.
Adjusted associations between EBV-positive gastric cancer and antibodies to EBV and p53 proteins.

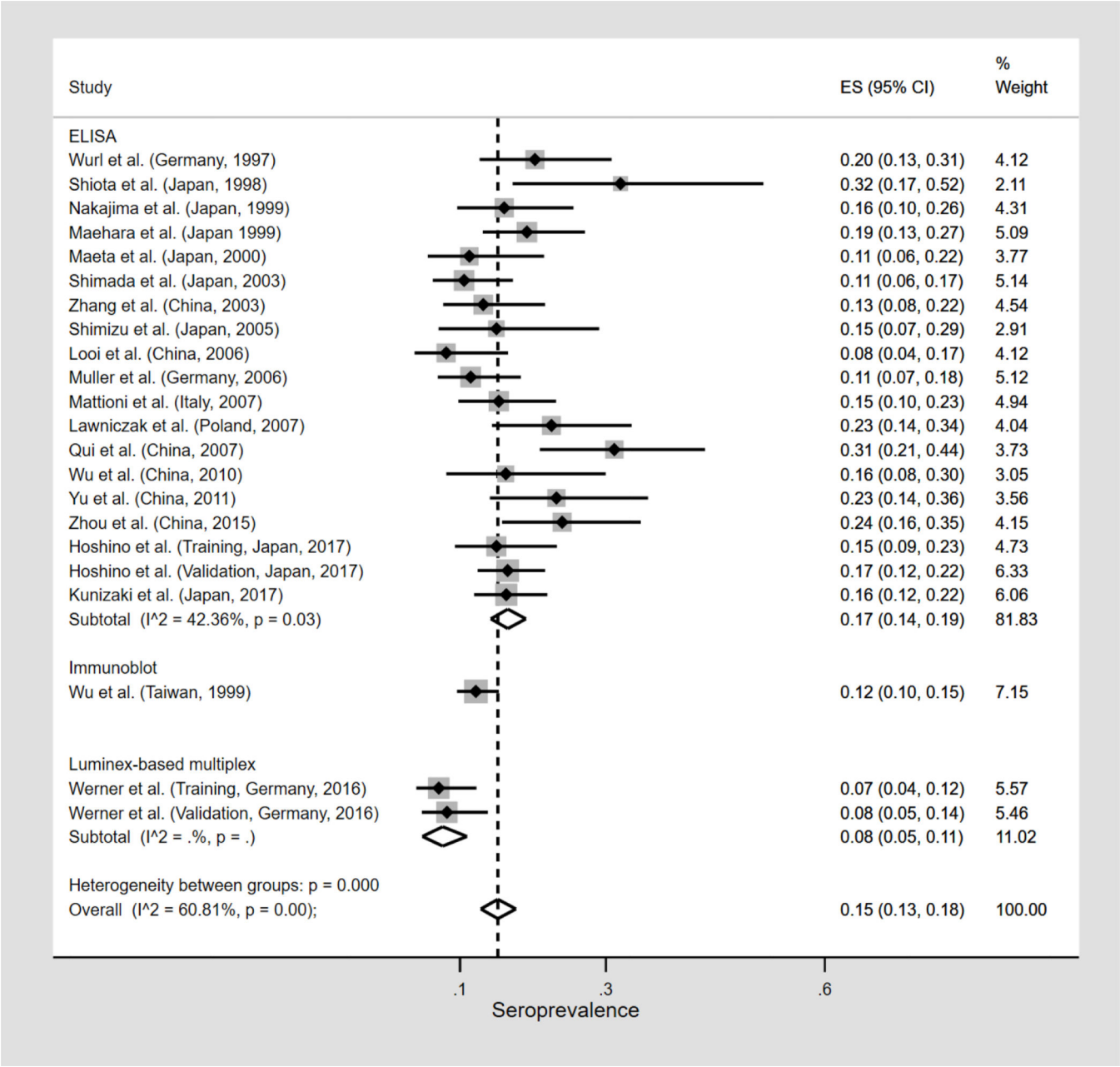

Figure 2.

Estimated prevalences and 95% CIs of anti-p53 seropositivity among patients with gastric cancer. Study-specific prevalences are shown as squares, with the size of the symbol inversely proportional to the study-specific variance. Random-effects pooled prevalences are shown as diamonds, with the middle corresponding to the point estimate and the width representing the 95% CI.