Article

Clinicopathological Significance of EBV-Infected Gastric Carcinomas: A Meta-Analysis

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Abstract: Background and objectives: The present study aims to elucidate the clinicopathologic significance of Epstein–Barr virus (EBV) infection in gastric carcinomas (GCs) through a meta-analysis. Materials and Methods: Sixty-one eligible studies were included in the present meta-analysis. The included patients, with and without EBV infection, were 2063 and 17,684, respectively. We investigated the clinicopathologic characteristics and various biomarkers, including programmed death-ligand 1 (PD-L1) expression and tumor-infiltrating lymphocytes (TILs). Results: The estimated EBV-infected rate of GCs was 0.113 (95% confidence interval (CI): 0.088–0.143). The EBV infection rates in GC cells were 0.138 (95% CI: 0.096–0.194), 0.103 (95% CI: 0.077–0.137), 0.080 (95% CI: 0.061–0.106), and 0.042 (95% CI: 0.016–0.106) in the population of Asia, America, Europe, and Africa, respectively. There was a significant difference between EBV-infected and noninfected GCs in the male: female ratio, but not other clinicopathological characteristics. EBV infection rates were higher in GC with lymphoid stroma (0.573, 95% CI: 0.428–0.706) than other histologic types of GCs. There were significant differences in high AT-rich interactive domain-containing protein 1A (ARID1A) and PD-L1 expressions, and high CD8+ TILs between EBV-infected and noninfected GCs. Conclusions: Our results showed that EBV infection of GCs was frequently found in male patients and GCs with lymphoid stroma. EBV infection was significantly correlated with ARID1A and PD-L1 expressions and CD8+ TILs in GCs.

Keywords: gastric carcinoma; Epstein–Barr virus; clinicopathological characteristics; histologic type; meta-analysis

1. Introduction

The Epstein–Barr virus (EBV) is a ubiquitous human herpesvirus associated with several lymphoid and epithelial malignancies, including Burkitt’s lymphoma, Hodgkin’s lymphoma, nasal NK/T cell lymphoma, and a subset of gastric carcinomas (GCs) [1–6]. In 1990, Burke et al. first detected the EBV genomes in a small group of GCs using a polymerase chain reaction [1]. Shibata et al. demonstrated that EBV genomes were uniformly present in GC cells, resembling lymphoepithelioma cells [4]. After that, EBV involvement was detected not only in lymphoepithelioma-like GCs but also in a subset of ordinary GCs [4,7].
EBV-associated gastric cancers (EBVaGCs) have a unique molecular signature, which has defined this group of tumors as a distinctive molecular subtype of gastric cancer that accounts for approximately 10% of all GCs [2–4]. Thus, EBVaGC is the most common cancer among EBV-related malignancies. However, the prevalence of EBV infection in GCs has differed by reports and histologic subtypes [7–67]. Furthermore, cumulative information cannot be obtained from individual studies. As part of The Cancer Genome Atlas (TCGA) project, EBVaGCs are associated with distinct molecular changes, as follows: DNA hypermethylation, high frequency of PIK3CA mutation, JAK2 gene amplification, programmed death-ligand 1/programmed cell death 1 ligand 2 (PD-L1/PD-L2) overexpression, and cyclin-dependent kinase inhibitor 2A (CDKN2A) silencing [2]. Recently, the loss of AT-rich interactive domain-containing protein 1A (ARID1A) was found in 20% of GCs and significantly correlated with EBVaGCs, PD-L1 status, as well as microsatellite instability (MSI) [64]. As the incidences and clinical features of GCs differ between regions, the clinicopathological characteristics of EBVaGCs may vary according to the various factors. In the present study, we investigate the clinicopathologic significance of EBVaGCs from eligible studies and perform the subgroup analysis to elucidate the EBV infection rate. We also evaluate the differences in the expression of various markers between EBVaGCs and non-EBVaGCs.

2. Materials and Methods

2.1. Published Study Search and Selection Criteria

Relevant articles were obtained by searching the PubMed database on 31 January 2020. For the search, the following keywords were used: “gastric carcinoma or gastric cancer or stomach cancer” and “Epstein–Barr virus or EBV”. The titles and abstracts of all searched articles were screened for the inclusion and exclusion of each article. Included articles contained information on the correlation between EBV positivity and clinicopathological characteristics in GCs. However, case reports, nonoriginal articles, or those not written in English were excluded from the present study. The PRISMA checklist is shown in Table S1.

2.2. Data Extraction

Data associated with clinicopathological characteristics based on EBV positivity in GCs were extracted from each of the eligible studies [7–67]. Two independent authors obtained all the data. The data extracted were the author’s information, study location, number of patients analyzed, EBV-positive rates, and clinicopathological characteristics by EBV infection. Additional information on immunohistochemical stains is shown in Table S2.

2.3. Statistical Analyses

The meta-analysis was performed using the Comprehensive Meta-Analysis software package 2.0 (Biostat, Englewood, NJ, USA). The EBV positivity rate was investigated in GCs. In addition, a subgroup analysis based on study location and histologic subtypes of GCs was performed. The correlations between EBV infection and clinicopathological characteristics were evaluated in GCs. In the present study, the following were included in the evaluated clinicopathological characteristics: age, sex, tumor size, tumor differentiation, histologic type, lymphatic, vascular, and perineural invasions, lymph node metastasis, and pTNM stages. Furthermore, the correlations between EBV positivity and p53, ARID1A, human epidermal growth factor receptor (HER2), and PD-L1 expressions, tumor-infiltrating lymphocytes (TILs), and microsatellite instability (MSI) in GCs were analyzed. We checked the heterogeneity between the studies by Q and \( I^2 \) statistics, expressed as \( p \)-values. Additionally, we conducted a sensitivity analysis to assess the heterogeneity of the eligible studies and the impact of each study on the combined effects. In the meta-analysis, as the eligible studies used various populations, a random-effect model (rather than a fixed-effect model) was determined to be more suitable. The statistical difference between subgroups was evaluated by a metaregression test. We used Begg’s funnel plot and Egger’s test to assess the publication bias; if significant publication bias was
3. Results

3.1. Selection and Characteristics of the Studies

In this study, 1301 relevant articles were found from the PubMed database and reviewed for a meta-analysis. Of these, 405 articles had no or a lack of sufficient information for the meta-analysis. A further 346 were excluded due to nonoriginal articles. Among the remaining articles, 489 reports were excluded for the following reasons: nonhuman studies (n = 238), articles on other diseases (n = 185), in a language other than English (n = 40), and duplication (n = 26); see Figure 1. Finally, 61 eligible articles were selected and included for the meta-analysis (Table 1). These studies included 19,747 GC patients with and without EBV infection (2,063 and 17,684, respectively).

Figure 1. Flow chart of the searching strategy.
Table 1. Main characteristics of the eligible studies.

| Study     | Location      | Number of Patients | EBV   | Study     | Location | Number of Patients | EBV   |
|-----------|---------------|--------------------|-------|-----------|----------|--------------------|-------|
|           |               |                    | Positive | Negative | Study     | Location | Positive | Negative |
| Ahn 2017  | Korea         | 349                | 26     | 323       | Ma 2017  | China       | 571    | 31       | 540       |
| Castaneda 2019 | Peru   | 375                | 72     | 303       | Martinez-Ciarpaglini 2019 | Spain | 209 | 13 | 196       |
| Birkman 2018 | Finland | 238                | 17     | 221       | Min 2016 | Korea       | 145    | 124      | 21        |
| Böger 2017 | Germany      | 484                | 22     | 462       | Nogueira 2017 | Portugal | 82    | 9        | 73        |
| Bösch 2019 | Germany      | 189                | 11     | 178       | Noh 2018 | Korea       | 449    | 36       | 413       |
| Baek 2018  | Korea         | 276                | 59     | 217       | Osumi 2017 | Japan  | 898    | 71       | 827       |
| Chapel 2000 | France  | 56                 | 7      | 49        | Pereira 2018 | Brazil  | 286    | 30       | 256       |
| Cho 2004   | Korea         | 24                 | 19     | 5         | Ramos 2019 | Brazil  | 178    | 18       | 160       |
| de Lima 2012 | Brazil  | 160                | 11     | 149       | Ribeiro 2017 | Portugal | 179   | 15       | 164       |
| De Rosa 2018 | Italy   | 169                | 33     | 136       | Roh 2019 | Korea       | 582    | 41       | 541       |
| de Souza 2014 | Brazil  | 125                | 12     | 113       | Saito 2017 | Japan  | 232    | 96       | 136       |
| de Souza 2018 | Brazil  | 302                | 62     | 240       | Setia 2019 | USA/Korea | 486   | 33       | 453       |
| Dong 2016  | China         | 855                | 59     | 796       | Shen 2017 | China       | 202    | 42       | 160       |
| Gasenko 2019 | Latvia  | 302                | 26     | 276       | Shibata 1993 | USA   | 187    | 19       | 168       |
| Grogg 2003 | USA           | 110                | 7      | 103       | Shinozaki 2009 | Japan | 111    | 43       | 68        |
| Guo 2019   | China         | 270                | 18     | 252       | Sun 2019  | China       | 165    | 2        | 163       |
| Han 2016   | Korea         | 410                | 30     | 380       | Trimeche 2009 | Tunisia | 96    | 4        | 92        |
| Huang 2014 | Taiwan        | 1020               | 52     | 968       | Truong 2009 | USA   | 235    | 12       | 223       |
| Huang 2019 | Taiwan        | 1248               | 65     | 1183      | Valentini 2019 | Italy  | 70     | 2        | 68        |
| Irkkan 2017 | Turkey       | 105                | 8      | 97        | van Beek 2004 | Netherlands | 566   | 41       | 525       |
| Kawazoe 2017 | Japan    | 487                | 25     | 462       | Vo 2002 | USA       | 108    | 11       | 97        |
| Kawazoe 2019 | Japan    | 225                | 14     | 211       | Wang 2005 | China       | 58     | 13       | 45        |
| Kijima 2003 | Japan        | 420                | 28     | 392       | Wu 2017  | China       | 340    | 17       | 323       |
| Kim 2019 (a) | Korea    | 273                | 25     | 248       | Xing 2017 | China       | 967    | 34       | 933       |
| Kim 2019 (b) | USA        | 43                 | 6      | 37        | Yanagi 2019 | Japan  | 1067   | 69       | 988       |
| Koriyama 2007 | Japan    | 149                | 49     | 100       | Zhang 2017 | China  | 218    | 64       | 154       |
| Kwon 2017  | Korea         | 394                | 26     | 368       | Yoon 2019 | USA       | 107    | 3        | 104       |
| Leung 1999 | China (Hong Kong) | 79            | 18     | 61        | Yen 2014 | Brunei Darussalam | 81  | 25       | 56        |
| Li 2016    | China         | 137                | 30     | 107       | Zhang 2019 | China  | 1013   | 58       | 955       |
| Lim 2017   | Korea         | 241                | 215    | 26        | Zhou 2019 | China       | 300    | 28       | 272       |
| Ma 2016    | USA           | 44                 | 7      | 37        |                     |          |        |          |           |

EBV, Epstein-Barr virus.
3.2. Epstein–Barr virus (EBV) Infected Rates of Gastric Carcinomas (GCs)

First, we investigated and analyzed the EBV-positive rates of GCs. The estimated EBV-positive rate was 0.113 (95% CI: 0.088–0.143) in overall GC cases. In the subgroup analysis based on study location, the EBV infected rate was the highest in Asia, compared to that in other regions. The EBV infected rate in the Asia region was 0.138 (95% CI: 0.096–0.194). In other areas, the EBV infected rates were 0.103, 0.080, and 0.042 in America, Europe, and Africa, respectively (Table 2).

Table 2. The estimated rates of Epstein–Barr virus positivity in gastric carcinoma.

| Number of Subsets | Fixed Effect (95% CI) | Heterogeneity Test (p-value) | Random Effect (95% CI) | Egger’s Test (p-value) |
|-------------------|-----------------------|-----------------------------|------------------------|----------------------|
| EBV positive rate | 61                    | 0.116 (0.111, 0.121)        | <0.001                 | 0.113 (0.088, 0.143)  | 0.912                |
| Asia              | 34                    | 0.121 (0.115, 0.128)        | <0.001                 | 0.138 (0.096, 0.194)  | 0.238                |
| America           | 13                    | 0.132 (0.118, 0.148)        | <0.001                 | 0.103 (0.077, 0.137)  | 0.002                |
| Europe            | 12                    | 0.083 (0.073, 0.095)        | <0.001                 | 0.080 (0.061, 0.106)  | 0.558                |
| Africa            | 1                     | 0.042 (0.016, 0.106)        | 1.000                  | 0.042 (0.016, 0.106)  | -                    |

CI, confidence interval; EBV, Epstein–Barr virus.

3.3. Correlations Between Epstein–Barr virus (EBV) Infection and Clinicopathological Characteristics in Gastric Carcinomas (GCs)

The clinicopathological characteristics, according to EBV positivity, were investigated in GCs. The male patients showed a significantly higher estimation rate in the EBV-positive group than in the EBV-negative group (0.824 vs. 0.639; \( p < 0.001 \) in a metaregression test). Other clinicopathological characteristics, including age, tumor size, tumor differentiation, lymphatic, vascular, and perineural invasions, pT stage, lymph node metastasis, and pTNM stage, had no significant differences between EBV-infected and noninfected GCs (Table 3). Next, the EBV-positive rates by histologic type of GC were investigated (Table 4). The EBV-positive rate of GC with lymphoid stroma was 0.573 (95% CI: 0.428–0.706). This GC with lymphoid stroma showed higher EBV-positive rates compared to other tumor subtypes such as tubular adenocarcinoma (0.174), poorly cohesive carcinoma (0.078), papillary carcinoma (0.022), mucinous carcinoma (0.053), and undifferentiated carcinoma (0.111).

PD-L1 expressions in tumor and immune cells were significantly higher in EBVaGCs than in non-EBVaGCs (Table 5). In detail, PD-L1 expression rates of tumor cells were 0.573 (95% CI: 0.449–0.688) and 0.183 (95% CI: 0.118–0.272) in EBVaGCs and non-EBVaGCs, respectively. In addition, the PD-L1 expression rates of immune cells were 0.832 (95% CI: 0.630–0.935) and 0.487 (95% CI: 0.357–0.619) in EBVaGCs and non-EBVaGCs, respectively. ARID1A was highly expressed in EBVaGCs compared to non-EBVaGCs (0.29 vs. 0.170; \( p = 0.021 \) in a metaregression test). HER2 expression was higher in non-EBVaGCs than in EBVaGCs (0.104 vs. 0.048), but with no significant difference in a metaregression test (\( p = 0.051 \)). There was no significant difference in MSI between EBVaGCs and non-EBVaGCs. CD8+ TILs were significantly higher in EBVaGCs than in non-EBVaGCs. In addition, there was no significant correlation between EBV positivity and loss of E-cadherin (Table S3).
### Table 3. Clinicopathological significance of Epstein–Barr virus positivity in gastric carcinomas.

|                | Number of Subsets | Fixed Effect (95% CI) | Heterogeneity Test (p-Value) | Random Effect (95% CI) | Egger’s Test (p-Value) | MRT (p-Value) |
|----------------|-------------------|-----------------------|-----------------------------|------------------------|------------------------|---------------|
| Age            |                   |                       |                             |                        |                        |               |
| EBV-positive   | 20                | 61.848 (61.115, 62.581)| <0.001                      | 62.161 (60.126, 64.197)| 0.693                  | 0.568         |
| EBV-negative   | 16                | 63.532 (63.219, 63.846)| <0.001                      | 63.519 (60.349, 66.690)| 0.788                  |               |
| Male ratio     |                   |                       |                             |                        |                        |               |
| EBV-positive   | 44                | 0.823 (0.802, 0.843)   | 0.063                       | 0.824 (0.796, 0.849)   | 0.189                  | <0.001        |
| EBV-negative   | 40                | 0.638 (0.629, 0.647)   | <0.001                      | 0.639 (0.620, 0.658)   | 0.945                  |               |
| Size (cm)      |                   |                       |                             |                        |                        |               |
| EBV-positive   | 12                | 3.840 (3.666, 4.015)   | <0.001                      | 4.890 (4.223, 5.556)   | <0.001                 | 0.918         |
| EBV-negative   | 7                 | 4.595 (4.507, 4.683)   | <0.001                      | 4.588 (4.354, 4.823)   | 0.957                  |               |
| Tumor differentiation, poorly |   |                       |                             |                        |                        |               |
| EBV-positive   | 20                | 0.674 (0.630, 0.716)   | 0.004                       | 0.682 (0.611, 0.745)   | 0.514                  | 0.112         |
| EBV-negative   | 20                | 0.608 (0.595, 0.622)   | <0.001                      | 0.597 (0.525, 0.665)   | 0.761                  |               |
| Lymphatic invasion |         |                       |                             |                        |                        |               |
| EBV-positive   | 7                 | 0.487 (0.429, 0.546)   | <0.001                      | 0.476 (0.299, 0.659)   | 0.843                  | 0.523         |
| EBV-negative   | 7                 | 0.496 (0.483, 0.513)   | <0.001                      | 0.522 (0.454, 0.588)   | 0.583                  |               |
| Vascular invasion |           |                       |                             |                        |                        |               |
| EBV-positive   | 7                 | 0.297 (0.249, 0.350)   | <0.001                      | 0.286 (0.189, 0.408)   | 0.636                  | 0.890         |
| EBV-negative   | 7                 | 0.276 (0.263, 0.290)   | <0.001                      | 0.297 (0.202, 0.413)   | 0.873                  |               |
| Perineural invasion |       |                       |                             |                        |                        |               |
| EBV-positive   | 8                 | 0.415 (0.350, 0.482)   | <0.001                      | 0.399 (0.213, 0.619)   | 0.807                  | 0.094         |
| EBV-negative   | 8                 | 0.517 (0.498, 0.535)   | <0.001                      | 0.521 (0.458, 0.584)   | 0.875                  |               |
| Low pT stage (pT1/pT2) |     |                       |                             |                        |                        |               |
| EBV-positive   | 33                | 0.435 (0.401, 0.471)   | <0.001                      | 0.366 (0.274, 0.469)   | 0.066                  | 0.670         |
| EBV-negative   | 31                | 0.413 (0.402, 0.424)   | <0.001                      | 0.350 (0.283, 0.422)   | 0.141                  |               |
| Lymph node metastasis |     |                       |                             |                        |                        |               |
| EBV-positive   | 40                | 0.493 (0.461, 0.526)   | <0.001                      | 0.595 (0.496, 0.686)   | 0.014                  | 0.127         |
| EBV-negative   | 37                | 0.593 (0.583, 0.604)   | <0.001                      | 0.655 (0.595, 0.711)   | 0.064                  |               |
| pTNM stage | |                       |                             |                        |                        |               |
| EBV-positive   | 25                | 0.507 (0.469, 0.544)   | <0.001                      | 0.500 (0.419, 0.580)   | 0.738                  | 0.236         |
| EBV-negative   | 25                | 0.451 (0.439, 0.463)   | <0.001                      | 0.460 (0.425, 0.496)   | 0.411                  |               |

CI, confidence interval; MRT, metaregression test; EBV, Epstein–Barr virus.

### Table 4. The estimated rates of Epstein–Barr virus positivity in gastric carcinomas according to the histologic types.

| Histologic Type          | Number of Subsets | Fixed Effect (95% CI) | Heterogeneity Test (p-Value) | Random Effect (95% CI) | Egger’s Test (p-Value) |
|--------------------------|-------------------|-----------------------|-----------------------------|------------------------|------------------------|
| Tubular adenocarcinoma   | 6                 | 0.152 (0.132, 0.174)  | <0.001                      | 0.174 (0.086, 0.320)   | 0.531                  |
| Poorly cohesive carcinoma| 8                 | 0.102 (0.083, 0.160)  | 0.038                       | 0.078 (0.053, 0.173)   | 0.263                  |
| Mixed carcinoma          | 4                 | 0.043 (0.016, 0.109)  | 0.306                       | 0.039 (0.013, 0.113)   | 0.084                  |
| Papillary carcinoma      | 2                 | 0.022 (0.004, 0.101)  | 0.530                       | 0.022 (0.004, 0.101)   | -                      |
| Mucinous carcinoma       | 4                 | 0.053 (0.013, 0.190)  | 0.688                       | 0.053 (0.013, 0.190)   | 0.042                  |
| GCLS                     | 5                 | 0.576 (0.468, 0.676)  | 0.203                       | 0.573 (0.428, 0.706)   | 0.748                  |
| Solid carcinoma          | 2                 | 0.130 (0.046, 0.316)  | 0.828                       | 0.130 (0.046, 0.316)   | -                      |
| Undifferentiated carcinoma| 1                 | 0.111 (0.015, 0.500)  | 1.000                       | 0.111 (0.015, 0.500)   | -                      |

CI, confidence interval; GCLS, gastric carcinoma with lymphoid stroma.
Table 5. The estimated rates of various markers in gastric carcinomas according to the Epstein–Barr virus positivity.

| Markers                      | Number of Subsets | Fixed Effect (95% CI) | Heterogeneity Test (p-Value) | Random Effect (95% CI) | Egger's Test (p-Value) | MRT (p-Value) |
|------------------------------|-------------------|-----------------------|-----------------------------|------------------------|------------------------|---------------|
| **PD-L1 in tumor cells**     |                   |                       |                             |                        |                        |               |
| EBV-positive                 | 14                | 0.500 (0.447, 0.554)  | <0.001                      | 0.573 (0.449, 0.688)   | 0.047                  | <0.001        |
| EBV-negative                 | 14                | 0.337 (0.323, 0.352)  | <0.001                      | 0.183 (0.118, 0.272)   | 0.008                  |               |
| **PD-L1 in immune cells**    |                   |                       |                             |                        |                        |               |
| EBV-positive                 | 8                 | 0.610 (0.531, 0.683)  | <0.001                      | 0.832 (0.630, 0.935)   | 0.007                  | 0.002         |
| EBV-negative                 | 8                 | 0.572 (0.552, 0.592)  | <0.001                      | 0.487 (0.357, 0.619)   | 0.081                  |               |
| **p53 overexpression**       |                   |                       |                             |                        |                        |               |
| EBV-positive                 | 5                 | 0.359 (0.256, 0.477)  | 0.223                       | 0.194 (0.067, 0.446)   | 0.023                  | 0.090         |
| EBV-negative                 | 4                 | 0.464 (0.418, 0.511)  | <0.001                      | 0.439 (0.314, 0.572)   | 0.502                  |               |
| **ARID1A**                   |                   |                       |                             |                        |                        |               |
| EBV-positive                 | 4                 | 0.295 (0.206, 0.403)  | 0.309                       | 0.295 (0.196, 0.418)   | 0.519                  | 0.021         |
| EBV-negative                 | 4                 | 0.176 (0.153, 0.201)  | 0.055                       | 0.170 (0.134, 0.214)   | 0.530                  |               |
| **HER2**                     |                   |                       |                             |                        |                        |               |
| EBV-positive                 | 8                 | 0.048 (0.024, 0.093)  | 0.723                       | 0.048 (0.024, 0.093)   | 0.167                  | 0.051         |
| EBV-negative                 | 8                 | 0.101 (0.088, 0.115)  | <0.001                      | 0.104 (0.070, 0.152)   | 0.739                  |               |
| **Microsatellite instability**|                   |                       |                             |                        |                        |               |
| EBV-positive                 | 5                 | 0.087 (0.040, 0.179)  | 0.240                       | 0.077 (0.028, 0.190)   | 0.230                  | 0.536         |
| EBV-negative                 | 5                 | 0.104 (0.089, 0.121)  | <0.001                      | 0.108 (0.069, 0.166)   | 0.637                  |               |
| **CD8+ TILs**                |                   |                       |                             |                        |                        |               |
| EBV-positive                 | 4                 | 0.705 (0.584, 0.802)  | 0.100                       | 0.761 (0.547, 0.894)   | 0.163                  | 0.001         |
| EBV-negative                 | 4                 | 0.307 (0.275, 0.341)  | <0.001                      | 0.269 (0.141, 0.450)   | 0.851                  |               |

CI, confidence interval; MRT, metaregression test; PD-L1, programmed death-ligand 1; EBV, Epstein–Barr virus; ARID1A, AT-rich interactive domain-containing protein 1A; HER2, human epidermal growth factor receptor 2; TIL, tumor-infiltrating lymphocyte.

4. Discussion

In other epithelial malignancies, the prevalence of EBV positivity was found to be 26.37%, 33.44%, and 45.37% in breast, cervical, and oral squamous cell carcinomas, respectively [68–70]. The range of EBV positivity reported was variable in GC tissues [7–67]. However, Chen et al. reported that non-neoplastic gastric tissue did not detect EBV positivity [71]. A TCGA study stated that the incidence of EBVaGCs was 9% [2]. Previous meta-analyses have reported the range as 2–20% and 6–33% [72,73]. In addition, the clinicopathological features of EBV positivity in GCs were variable, according to reports [72,73]. Therefore, the impact of variable EBV positivity on the controversy of clinicopathological implications of EBV in GCs needs to be elucidated. The present study includes a detailed meta-analysis of the clinicopathological implications of EBV positivity in GCs.

In the present study, the estimated EBV positive rate was 11.3%. EBV positive rates ranged from 1.2% to 89.2% in the individual eligible studies [7–67]. In previous meta-analyses, EBV positive rates have been reported as 7.5% and 12.6% in 2010 and 2019, respectively [74,75]. Various factors, including the eligible studies, may have affected the differences of EBV positivity between meta-analyses. In Murphy’s report, a subgroup analysis based on study location was performed, and the estimated EBV positive rates in America, Asia, and Europe were 9.88%, 8.28%, and 8.70%, respectively [72]. In the current study, the positive rate was highest in Asia at 13.8%. However, there were no significant differences between study locations in the metaregression test. Lee et al. reported that locations with a high prevalence of GCs had low EBV positivity [76]. They showed only odds ratios according to study locations, but not the estimated rates. As the criteria of the odds ratio were not described, interpretation of the odds ratio was not possible. They described that the EBV-positive rate of Asians was 8.4% through simple estimation using the raw data of each study. A meta-analysis did not obtain
this result. Moreover, the estimated EBV-positive rates of Caucasian and Hispanic patients did not differ from Asians. In another meta-analysis, there was no significant difference in EBV-positive rates between study locations [75].

In addition, EBV positivity rates can differ according to the histologic type of GC. The highest EBV-positive rate was found in GC with lymphoid stroma at 57.3%. The implications of study location and ethnicity on EBV positivity may be less important when compared to the subtype of GC. Furthermore, the impact of studied years can contribute to varying EBV-positive rates. Additionally, we investigated EBV positivity in tubular adenocarcinoma according to study years. Based on 2017 data, EBV-positive rates were 0.113 (95% CI: 0.063–0.195) and 0.375 (95% CI: 0.132–0.703) after 2017 and before 2017, respectively, with a significant difference between subgroups (p = 0.012 in a metaregression test; data not shown). The possible causes are different methodologies and different histologic subtypes of the included cases. The cellular component can affect EBV positivity. In GCs, TILs can show EBV positivity [71]. Of course, the use of a PCR method with microdissection is possible for a more detailed examination; however, this limitation cannot be solved by microdissection due to intratumoral and peritumoral lymphocytes. Although PCR methods are more sensitive than in situ hybridization (ISH) methods, EBV positivity should be elucidated by evaluating cellular fractions, such as in ISH [71]. However, a definitive cause for the difference of EBV positivity by study years could not be found.

In previous studies, EBV positivity has been significantly correlated with some clinicopathological characteristics, sex, and tumor location [22,26,53]. In the present study, there was a significant correlation between EBV positivity and the patient’s sex; however, EBV positivity was not correlated with lymphovascular invasion or pTNM stage. The clinicopathological significance of EBV infection is different by reports [24,25,74–76]. Huang et al. reported that EBV infection in GCs was correlated with high pTNM stages and lymphatic tumor invasion, as opposed to our results [24,25]. Lee et al. reported that EBV positivity was higher in younger patients than in older patients [76]. Li et al. reported a correlation between EBV positivity and lymph node metastasis [74]. However, other meta-analyses showed no correlation between EBV positivity and lymph node metastasis, in agreement with our result [75,76]. For the evaluation of correlation with lymph node metastasis, Li’s meta-analysis and our meta-analysis included 5 and 40 datasets, respectively. Moreover, they analyzed their data using odds ratios, unlike our analysis. These discrepancies could be involved in the difference of results between the meta-analyses.

Although the molecular characteristics of GCs have been studied [2], previous meta-analyses have not dealt with their correlation with various molecular markers [75]. In our results, CD8+ TILs and PD-L1 expressions of the tumor and immune cells were more frequently found in EBVaGCs than in non-EBVaGCs. Abundant TILs are one of the histologic features in GCs with EBV infection [77–79]. In the TCGA report, PD-L1 gene amplification was elevated in EBVaGCs [2]. Furthermore, PD-L1 immunohistochemical expression in tumor cells was more frequently found in EBVaGCs than in non-EBVaGCs [28]. However, the impact of TILs in GCs is not yet fully understood. In addition, further evaluation of the tumor-infiltrating and peritumoral lymphocytes will be needed in GC with lymphoid stroma, which was significantly associated with high EBV positivity. In GCLS, EBV-positive tumors had more PI3K/AKT pathway mutations than EBV-negative tumors [80]. In addition, because EBVaGCs are significantly correlated with high TILs, new immunotherapeutic strategies associated with T-cells are challenging for the treatment of advanced EBVaGCs [81,82]. ARID1A expression was higher in EBVaGCs than in non-EBVaGCs. In the previous meta-analysis, correlations between EBV positivity and molecular markers, such as p53 and CpG island methylator phenotype, were found [76].

This study has some limitations. First, a subgroup analysis based on EBV detection methods could not be performed due to the methods used in the eligible studies. Second, the impact of study years on EBV positivity could not be fully investigated based on subtypes of GCs. We evaluated only tubular adenocarcinomas among the various GC subtypes. Third, the eligible studies used different antibodies and evaluation criteria for immunohistochemistry. However, subgroup analysis based on antibody and evaluation criteria could not be performed due to insufficient information.
5. Conclusions

Taken together, our results show that the EBV positivity of GCs is frequently found in male patients and GC with lymphoid stroma. Although EBV positivity was highest in Asians, there was no significant difference between study locations. EBV positivity is significantly correlated with ARID1A and PD-L1 expressions, as well as CD8+ TILs in GCs.

Supplementary Materials: The following are available online at http://www.mdpi.com/1010-660X/56/7/345/s1, Table S1 PRISMA Checklist, Table S2 Antibody information and evaluation criteria of immunohistochemical stains in eligible studies, and Table S3 The estimated rates of various markers in gastric carcinoma according to the Epstein-Barr virus positivity.

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References

1. Burke, A.P.; Yen, T.S.; Shekitka, K.M.; Sobin, L.H. Lymphoepithelial carcinoma of the stomach with Epstein-Barr virus demonstrated by polymerase chain reaction. Mod. Pathol. Off. J. United States Can. Acad. Pathol. Inc. 1990, 3, 377–380.

2. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014, 513, 202–209. [CrossRef] [PubMed]

3. Chen, J.N.; Ding, Y.G.; Feng, Z.Y.; Li, H.G.; He, D.; Du, H.; Wu, B.; Shao, C.K. Association of distinctive Epstein-Barr virus variants with gastric carcinoma in Guangzhou, southern China. J. Med. Virol. 2010, 82, 658–667. [CrossRef] [PubMed]

4. Shihata, D.; Weiss, L.M. Epstein-Barr virus-associated gastric adenocarcinoma. Am. J. Pathol. 1992, 140, 769–774.

5. Shannon-Lowe, C.; Rickinson, A.B.; Bell, A.I. Epstein-Barr virus-associated lymphomas. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 2017, 372. [CrossRef]

6. Guidry, J.T.; Birdwell, C.E.; Scott, R.S. Epstein-Barr virus in the pathogenesis of oral cancers. Oral Dis. 2018, 24, 497–508. [CrossRef]

7. Cho, M.Y.; Kim, T.H.; Yi, S.Y.; Jung, W.H.; Park, K.H. Relationship between Epstein-Barr virus-encoded RNA expression, apoptosis and lymphocytic infiltration in gastric carcinoma with lymphoid-rich stroma. Med. Princ. Pract. Int. J. Kuwait Univ. Health Sci. Cent. 2004, 13, 353–360. [CrossRef]

8. Ahn, S.; Lee, S.J.; Kim, Y.; Kim, A.; Shin, N.; Choi, K.U.; Lee, C.H.; Huh, G.Y.; Kim, K.M.; Setia, N.; et al. High-throughput Protein and mRNA Expression-based Classification of Gastric Cancers Can Identify Clinically Distinct Subtypes, Concordant With Recent Molecular Classifications. Am. J. Surg. Pathol. 2017, 41, 106–115. [CrossRef]

9. Baek, D.W.; Kang, B.W.; Kim, J.G. The Predictive Value of Epstein-Barr Virus-Positivity in Patients Undergoing Gastrectomy Followed by Adjuvant Chemotherapy. Chonnam Med. J. 2018, 54, 173–177. [CrossRef]

10. Birkman, E.M.; Mansuri, N.; Kurki, S.; Ålgars, A.; Lintunen, M.; Ristamäki, R.; Sundström, J.; Carpén, O. Gastric cancer: Immunohistochemical classification of molecular subtypes and their association with clinicopathological characteristics. Virchows Arch. Int. J. Pathol. 2018, 472, 369–382. [CrossRef]

11. Böger, C.; Krüger, S.; Behrens, H.M.; Bock, S.; Haag, J.; Kalthoff, H.; Röcken, C. Epstein-Barr virus-associated gastric cancer reveals intratumoral heterogeneity of PIK3CA mutations. Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 2017, 28, 1005–1014. [CrossRef]

12. Bösch, F.; Todorova, R.; Link, H.; Westphalen, C.B.; Boeck, S.; Heinemann, V.; Werner, J.; Kirchner, T.; Angele, M.K.; Neumann, J. Molecular subtyping of gastric cancer with respect to the growth pattern of lymph-node metastases. J. Cancer Res. Clin. Oncol. 2019, 145, 2689–2697. [CrossRef]

13. Castaneda, C.A.; Castillo, M.; Chavez, I.; Barreda, F.; Suarez, N.; Nieves, J.; Bernabe, L.A.; Valdivia, D.; Ruiz, E.; Dias-Neto, E.; et al. Prevalence of Helicobacter pylori Infection, Its Virulent Genotypes, and Epstein-Barr Virus in Peruvian Patients With Chronic Gastritis and Gastric Cancer. J. Glob. Oncol. 2019, 5, 1–9. [CrossRef] [PubMed]
14. Chapel, F.; Fabiani, B.; Davi, F.; Raphael, M.; Tepper, M.; Champaulet, G.; Guettier, C. Epstein-Barr virus and gastric carcinoma in Western patients: Comparison of pathological parameters and p53 expression in EBV-positive and negative tumours. Histopathology 2000, 36, 252–261. [CrossRef]

15. de Lima, M.A.; Ferreira, M.V.; Barros, M.A.; Pardini, M.I.; Ferrasi, A.C.; Rabenhorst, S.H. Epstein-Barr virus-associated gastric carcinoma in Brazil: Comparison between in situ hybridization and polymerase chain reaction detection. Braz. J. Microbiol. [Publ. Braz. Soc. Microbiol.] 2012, 43, 393–404. [CrossRef] [PubMed]

16. De Rosa, S.; Sahnane, N.; Tibiletti, M.G.; Magnoli, F.; Vanoli, A.; Sesso, F.; Chiaravalli, A.M. EBV+ and MSI Gastric Cancers Harbor High PD-L1/PD-1 Expression and High CD8+ Intratumoral Lymphocytes. Cancers 2018, 10, 102. [CrossRef] [PubMed]

17. de Souza, C.R.T.; de Oliveira, K.S.; Ferraz, J.J.; Leal, M.F.; Calcagno, D.Q.; Seabra, A.S.; Khayat, A.S.; Montenegro, R.C.; Alves, A.P.; Assumpção, P.P.; et al. Occurrence of Helicobacter pylori and Epstein-Barr virus infection in endoscopic and gastric cancer patients from Northern Brazil. BMC Gastroenterol. 2014, 14, 179. [CrossRef] [PubMed]

18. de Souza, C.R.T.; Almeida, M.C.A.; Khayat, A.S.; da Silva, E.L.; Soares, P.C.; Chaves, L.C.; Burbano, R.M.R. Association between Helicobacter pylori, Epstein-Barr virus, human papillomavirus and gastric adenocarcinomas. World J. Gastroenterol. 2018, 24, 4928–4938. [CrossRef] [PubMed]

19. Dong, M.; Wang, H.Y.; Zhao, X.X.; Chen, J.N.; Zhang, Y.W.; Huang, Y.; Xue, L.; Li, H.G.; Du, H.; Wu, X.Y.; et al. Expression and prognostic roles of PIK3CA, JAK2, PD-L1, and PD-L2 in Epstein-Barr virus-associated gastric carcinoma. Hum. Pathol. 2016, 53, 25–34. [CrossRef]

20. Gasenko, E.; Isajevs, S.; Camargo, M.C.; Offerhaus, G.J.A.; Polaka, I.; Gulley, M.L.; Skapars, R.; Sivirs, A.; Kojalo, I.; Kirsners, A.; et al. Clinicopathological characteristics of Epstein-Barr virus-positive gastric cancer in Latvia. Eur. J. Gastroenterol. Hepatol. 2019, 31, 1328–1333. [CrossRef]

21. Grogg, K.L.; Lobse, C.M.; Pankratz, V.S.; Halling, K.C.; Smyrk, T.C. Lymphocyte-rich gastric cancer: Associations with Epstein-Barr virus, microsatellite instability, histology, and survival. Mod. Pathol. Off. J. United States Can. Acad. Pathol. Inc. 2003, 16, 641–651. [CrossRef] [PubMed]

22. Guo, C.; Wei, J.; Scott, R.S.; Chen, Y.; Chen, Z.; Zhao, W.; Zhang, C.; Wang, B.; Chai, C.; Dai, G.; et al. Prevalence and characteristics of Epstein-Barr virus associated gastric carcinoma in Gansu Province, Northwest China with mRNA expression of glycoprotein BMRF2. J. Med. Virol. 2020, 92, 356–363. [CrossRef] [PubMed]

23. Han, N.; Kim, M.A.; Lee, H.S.; Kim, W.H. Loss of ARID1A Expression is Related to Gastric Cancer Progression, Epstein-Barr Virus Infection, and Mismatch Repair Deficiency. Appl. Immunohistochem. Mol. Morphol. 2016, 24, 320–325. [CrossRef] [PubMed]

24. Huang, S.C.; Ng, K.F.; Chen, K.H.; Hsu, J.T.; Liu, K.H.; Yeh, T.S.; Chen, T.C. Prognostic factors in Epstein-Barr virus-associated stage I-III gastric carcinoma: Implications for a unique type of carcinogenesis. Oncol. Rep. 2014, 32, 530–538. [CrossRef]

25. Huang, S.C.; Ng, K.F.; Yeh, T.S.; Cheng, C.T.; Lin, J.S.; Liu, Y.J.; Chuang, H.C.; Chen, T.C. Subtraction of Epstein-Barr virus and microsatellite instability genotypes from the Lauren histotypes: Combined molecular and histologic subtyping with clinicopathological and prognostic significance validated in a cohort of 1,248 cases. Int. J. Cancer 2019, 145, 3218–3230. [CrossRef]

26. Irkkan, C.; Balei, S.; Güler Tezel, G.; Akinci, B.; Yalçın, B.; Güler, G. Comparison of Clinicopathologic Parameters and Survivals Between Epstein-Barr Virus-positive and Her2-positive Gastric Cancers. Appl. Immunohistochem. Mol. Morphol. 2017, 25, 609–614. [CrossRef]

27. Kawazoe, A.; Kuwata, T.; Kuboki, Y.; Shirata, K.; Nagatsuma, A.K.; Aizawa, M.; Yoshino, T.; Doi, T.; Ohtsu, A.; Ochiai, A. Clinicopathological features of programmed death ligand 1 expression with tumor-infiltrating lymphocyte, mismatch repair, and Epstein-Barr virus status in a large cohort of gastric cancer patients. Gastric Cancer Off. J. Int. Gastric Cancer Assoc. Jpn. Gastric Cancer Assoc. 2017, 20, 407–415. [CrossRef]

28. Kawazoe, A.; Shirata, K.; Kuboki, Y.; Bando, H.; Kojima, T.; Yoshino, T.; Ohtsu, A.; Ochiai, A.; Togashi, Y.; Nishikawa, H.; et al. Clinicopathological features of 22C3 PD-L1 expression with mismatch repair, Epstein-Barr virus status, and cancer genome alterations in metastatic gastric cancer. Gastric Cancer Off. J. Int. Gastric Cancer Assoc. Jpn. Gastric Cancer Assoc. 2019, 22, 69–76. [CrossRef]

29. Kijima, Y.; Ishigami, S.; Hokita, S.; Koriyama, C.; Akiba, S.; Eizuru, Y.; Aikou, T. The comparison of the prognosis between Epstein-Barr virus (EBV)-positive gastric carcinomas and EBV-negative ones. Cancer Lett. 2003, 200, 33–40. [CrossRef]
30. Kim, Y.B.; Ahn, J.M.; Bae, W.J.; Sung, C.O.; Lee, D. Functional loss of ARID1A is tightly associated with high PD-L1 expression in gastric cancer. *Int. J. Cancer* **2019**, *145*, 916–926. [CrossRef]
31. Kim, T.S.; da Silva, E.; Coit, D.G.; Tang, L.H. Intratumoral Immune Response to Gastric Cancer Varies by Molecular and Histologic Subtype. *Am. J. Surg. Pathol.* **2019**, *43*, 851–860. [CrossRef] [PubMed]
32. Koriyama, C.; Akiba, S.; Itoh, T.; Sueyoshi, K.; Minakami, Y.; Corvalan, A.; Yonezawa, S.; Eizuru, Y. E-cadherin and beta-catenin expression in Epstein-Barr virus-associated gastric carcinoma and their prognostic significance. *World J. Gastroenterol.* **2007**, *13*, 3925–3931. [CrossRef]
33. Kwon, M.J.; Kim, K.C.; Nam, E.S.; Cho, S.J.; Park, H.R.; Min, S.K.; Seo, J.; Cho, J.Y.; Lee, H.K.; Kang, H.S.; et al. Programmed death ligand-1 and MET co-expression is a poor prognostic factor in gastric cancers after resection. *Oncotarget* **2017**, *8*, 82399–82414. [CrossRef] [PubMed]
34. Leung, S.Y.; Yuen, S.T.; Chung, L.P.; Chu, K.M.; Wong, M.P.; Branicki, F.J.; Ho, J.C. Microsatellite instability, Epstein-Barr virus, mutation of type II transforming growth factor beta receptor and BAX in gastric carcinomas in Hong Kong Chinese. *Br. J. Cancer* **1999**, *79*, 582–588. [CrossRef] [PubMed]
35. Li, Z.; Lai, Y.; Sun, L.; Zhang, X.; Liu, R.; Feng, G.; Zhou, L.; Jia, L.; Huang, X.; Kang, Q.; et al. PD-L1 expression is associated with massive lymphocyte infiltration and histology in gastric cancer. *Hum. Pathol.* **2016**, *55*, 182–189. [CrossRef] [PubMed]
36. Lim, H.; Lee, I.S.; Lee, J.H.; Park, Y.S.; Kang, H.J.; Na, H.K.; Ahn, J.Y.; Kim, D.H.; Choi, K.D.; Song, H.J.; et al. Clinical application of early gastric cancer with lymphoid stroma based on lymph node metastasis status. *Gastric Cancer Off. J. Int. Gastric Cancer Assoc. Jpn. Gastric Cancer Assoc.* **2017**, *20*, 793–801. [CrossRef]
37. Ma, C.; Patel, K.; Singh, A.D.; Ren, B.; Zhu, B.; Shaikh, F.; Sun, W. Programmed Death-Ligand 1 Expression Is Common in Gastric Cancer Associated with Epstein-Barr Virus or Microsatellite Instability. *Am. J. Surg. Pathol.* **2016**, *40*, 1496–1506. [PubMed]
38. Ma, J.; Li, J.; Hao, Y.; Nie, Y.; Li, Z.; Qian, M.; Liang, Q.; Yu, J.; Zeng, M.; Wu, K. Differentiated tumor immune microenvironment of Epstein-Barr virus-associated and negative gastric cancer: Implication in prognosis and immunotherapy. *Oncotarget* **2017**, *8*, 67094–67103. [CrossRef]
39. Martinez-Ciarpaglini, C.; Fleitas-Kanonnikoff, T.; Gambardella, V.; Llorca, M.; Mongort, C.; Mengual, R.; Nieto, G.; Navarro, L.; Huerta, M.; Rosello, S.; et al. Assessing molecular subtypes of gastric cancer: Microsatellite unstable and Epstein-Barr virus subtype. Methods for detection and clinical and pathological implications. *ESMO Open* **2019**, *4*, e000470. [CrossRef]
40. Min, B.H.; Tae, C.H.; Ahn, S.M.; Kang, S.Y.; Woo, S.Y.; Kim, S.; Kim, K.M. Epstein-Barr virus infection serves as an independent predictor of survival in patients with lymphoepithelioma-like gastric carcinoma. *Gastric Cancer Off. J. Int. Gastric Cancer Assoc. Jpn. Gastric Cancer Assoc.* **2016**, *19*, 852–859. [CrossRef]
41. Nogueira, C.; Mota, M.; Gradiz, R.; Cipriano, M.A.; Caramelo, F.; Cruz, H.; Alarc, A.; FC, E.S.; Oliveira, F.; Martinho, F.; et al. Prevalence and characteristics of Epstein-Barr virus-associated gastric carcinomas in Portugal. *Infect. Agents Cancer* **2017**, *12*, 41. [CrossRef] [PubMed]
42. Noh, B.J.; Kim, J.H.; Eom, D.W. Prognostic Significance of Categorizing Gastric Carcinoma by PD-L1 Expression and Tumor Infiltrating Lymphocytes. *Ann. Clin. Lab. Sci.* **2018**, *48*, 695–706. [PubMed]
43. Osumi, H.; Kawachi, H.; Yoshio, T.; Ida, S.; Yamamoto, N.; Horiuchi, Y.; Ishiyama, A.; Hirasawa, T.; Tsuchida, T.; Hiki, N.; et al. Epstein-Barr virus status is a promising biomarker for endoscopic resection in early gastric cancer: Proposal of a novel therapeutic strategy. *J. Gastroenterol.* **2019**, *54*, 774–783. [CrossRef]
44. Pereira, M.A.; Ramos, M.; Faraj, S.F.; Dias, A.R.; Yagi, O.K.; Zilberstein, B.; Cecconello, I.; Alves, V.A.F.; de Mello, E.S.; Ribeiro, U., Jr. Clinicopathological and prognostic features of Epstein-Barr virus infection, microsatellite instability, and PD-L1 expression in gastric cancer. *J. Surg. Oncol.* **2018**, *117*, 829–839. [CrossRef]
45. Ramos, M.; Pereira, M.A.; Amorim, L.C.; de Mello, E.S.; Faraj, S.F.; Ribeiro, U.; Hoff, P.M.G.; Cecconello, I.; de Castris, T.B. EBV gastric cancer molecular classification and adjuvant therapy: Is there a different benefit according to the subtype? *J. Surg. Oncol.* **2020**, *121*, 804–813. [CrossRef] [PubMed]
46. Ribeiro, J.; Oliveira, A.; Malta, M.; Oliveira, C.; Silva, F.; Galagher, A.; Afonso, L.P.; Neves, M.C.; Medeiros, R.; Pimentel-Nunes, P.; et al. Clinical and pathological characterization of Epstein-Barr virus-associated gastric carcinomas in Portugal. *World J. Gastroenterol.* **2017**, *23*, 7292–7302. [CrossRef]
47. Roh, C.K.; Choi, Y.Y.; Choi, S.; Seo, W.J.; Cho, M.; Jang, E.; Son, T.; Kim, H.I.; Kim, H.; Hyung, W.J.; et al. Single Patient Classifier Assay, Microsatellite Instability, and Epstein-Barr Virus Status Predict Clinical Outcomes in Stage II/III Gastric Cancer: Results from CLASSIC Trial. *Yonsei Med. J.* **2019**, *60*, 132–139. [CrossRef]
48. Saito, R.; Abe, H.; Kunita, A.; Yamashita, H.; Seto, Y.; Fukayama, M. Overexpression and gene amplification of PD-L1 in cancer cells and PD-L1(+)-immune cells in Epstein-Barr virus-associated gastric cancer: The prognostic implications. Mod. Pathol. Off. J. United States Can. Acad. Pathol. Inc 2017, 30, 427–439. [CrossRef]

49. Setia, N.; Ahn, S.; Han, H.S.; Park, D.Y.; Lauwers, G.Y. Predictive value of WHO classification for PD-L1 and Her2/Neu expression and distinct associations with protein expression based classification in gastric carcinoma. Hum. Pathol. 2019, 94, 64–70. [CrossRef]

50. Shen, H.; Zhong, M.; Wang, W.; Liao, P.; Yin, X.; Rotroff, D.; Kneppe, T.C.; McLeod, H.L.; Zhou, C.; Xie, S.; et al. EBV infection and MSI status significantly influence the clinical outcomes of gastric cancer patients. Clin. Chim. Acta; Int. J. Clin. Chem. 2017, 471, 216–221. [CrossRef]

51. Shibata, D.; Hawes, D.; Stemmermann, G.N.; Weiss, L.M. Epstein-Barr virus-associated gastric adenocarcinoma among Japanese Americans in Hawaii. Cancer Epidemiol. Biomark. Prev. A Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol. 1993, 2, 213–217.

52. Shinozaki, A.; Ushiku, T.; Morikawa, T.; Hino, R.; Sakatani, T.; Uozaki, H.; Fukayama, M. Epstein-Barr virus-associated gastric carcinoma: A distinct carcinoma of gastric phenotype by claudin expression profiling. J. Histochem. Cytochem. Off. J. Histochem. Soc. 2009, 57, 775–785. [CrossRef] [PubMed]

53. Sun, Y.; Yu, W.; Guan, W.; Cai, L.; Qiao, M.; Zheng, L.; Jiang, R.; Wang, R.; Wang, L. Integrated assessment of PD-L1 expression and molecular classification facilitates therapy selection and prognosis prediction in gastric cancer. Cancer Manag. Res. 2019, 11, 6397–6410. [CrossRef]

54. Valentini, A.M.; Di Pinto, F.; Coletta, S.; Guerra, V.; Armentano, R.; Caruso, M.L. Tumor microenvironment immune types in gastric cancer are associated with mismatch repair however, not HER2 status. Oncol. Lett. 2019, 18, 1775–1785. [CrossRef] [PubMed]

55. van Beek, J.; zur Hausen, A.; Klein Kranenbarg, E.; van de Velde, C.J.; Middeldorp, J.M.; van den Brule, A.J.; Meijer, C.J.; Bloemena, E. EBV-positive gastric adenocarcinomas: A distinct clinicopathologic entity with a low frequency of lymph node involvement. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 2004, 22, 664–670. [CrossRef]

56. Vo, Q.N.; Geradts, J.; Gulley, M.L.; Boudreau, D.A.; Bravo, J.C.; Schneider, B.G. Epstein-Barr virus in gastric adenocarcinomas: Association with ethnicity and CDKN2A promoter methylation. J. Clin. Pathol. 2002, 55, 669–675. [CrossRef]

57. Wang, Y.; Luo, B.; Yan, L.P.; Huang, B.H.; Zhao, P. Relationship between Epstein-Barr virus-encoded proteins with cell proliferation, apoptosis, and apoptosis-related proteins in gastric carcinoma. World J. Gastroenterol. 2005, 11, 3234–3239. [CrossRef]

58. Wu, Y.; Cao, D.; Qu, L.; Cao, X.; Jia, Z.; Zhao, T.; Wang, Q.; Jiang, J. PD-1 and PD-L1 co-expression predicts favorable prognosis in gastric cancer. Oncotarget 2017, 8, 64066–64082. [CrossRef]

59. Xing, X.; Guo, J.; Ding, G.; Li, B.; Dong, B.; Feng, Q.; Li, S.; Zhang, J.; Ying, X.; Cheng, X.; et al. Analysis of PD1, PDL1, PDL2 expression and T cells infiltration in 1014 gastric cancer patients. Oncoimmunology 2018, 7, e1356144. [CrossRef] [PubMed]

60. Yoon, J.Y.; Sy, K.; Brezden-Masley, C.; Streutker, C.J. Histo- and immunohistochemistry-based estimation of the TCGA and ACRG molecular subtypes for gastric carcinoma and their prognostic significance: A single-institution study. PLoS ONE 2019, 14, e0224812. [CrossRef] [PubMed]

61. Zhang, Y.; Chen, J.N.; Dong, M.; Zhang, Z.G.; Zhang, Y.W.; Wu, J.Y.; Du, H.; Li, H.G.; Huang, Y.; Shao, C.K. Clinical significance of spasmolytic polypeptide-expressing metaplasia and intestinal metaplasia in Epstein-Barr virus-associated and Epstein-Barr virus-negative gastric cancer. Hum. Pathol. 2017, 63, 128–138. [CrossRef]
66. Zhang, Y.W.; He, D.; Tan, C.; Dong, M.; Zhou, L.; Shao, C.K. Differential expression of HER2 and downstream proteins in prediction of advanced tumor phenotypes and overall survival of patients with Epstein-Barr virus-positive vs. negative gastric cancers. *Pathol. Res. Pract.* 2019, 215, 152675. [CrossRef]

67. Zhou, H.; Tan, S.; Li, H.; Lin, X. Expression and significance of EBV, ARID1A and PIK3CA in gastric carcinoma. *Mol. Med. Rep.* 2019, 19, 2125–2136. [CrossRef]

68. Farahmand, M.; Monavari, S.H.; Shoja, Z.; Ghaffari, H.; Tavakoli, M.; Tavakoli, A. Epstein-Barr virus and risk of breast cancer: A systematic review and meta-analysis. *Future Oncol.* 2019, 15, 2873–2885. [CrossRef]

69. de Lima, M.A.P.; Neto, P.J.N.; Lima, L.P.M.; Gonçalves Júnior, J.; Teixeira Júnior, A.G.; Teodoro, I.P.P.; Facundo, H.T.; da Silva, C.G.L.; Lima, M.V.A. Association between Epstein-Barr virus (EBV) and cervical carcinoma: A meta-analysis. *Gynecol. Oncol.* 2018, 148, 317–328. [CrossRef]

70. de Lima, M.A.P.; Teodoro, I.P.P.; Galiza, L.E.; Filho, P.; Marques, F.M.; Pinheiro Junior, R.F.F.; Macedo, G.E.C.; Facundo, H.T.; da Silva, C.G.L.; Lima, M.V.A. Association between Epstein-Barr Virus and Oral Carcinoma: A Systematic Review with Meta-Analysis. *Crit. Rev. Oncog.* 2019, 24, 349–368. [CrossRef]

71. Chen, X.Z.; Chen, H.; Castro, F.A.; Hu, J.K.; Brenner, H. Epstein-Barr virus infection and gastric cancer: A systematic review. *Medicine* 2015, 94, e792. [CrossRef] [PubMed]

72. Murphy, G.; Pfeiffer, R.; Camargo, M.C.; Rabkin, C.S. Meta-analysis shows that prevalence of Epstein-Barr virus-positive gastric cancer differs based on sex and anatomic location. *Gastroenterology* 2009, 137, 824–833. [CrossRef] [PubMed]

73. Polom, K.; Marano, L.; Marrelli, D.; De Luca, R.; Roviello, G.; Savelli, V.; Tan, P.; Roviello, F. Meta-analysis of microsatellite instability in relation to clinicopathological characteristics and overall survival in gastric cancer. *Br. J. Surg.* 2018, 105, 159–167. [CrossRef]

74. Li, S.; Du, H.; Wang, Z.; Zhou, L.; Zhao, X.; Zeng, Y. Meta-analysis of the relationship between Epstein-Barr virus infection and clinicopathological features of patients with gastric carcinoma. *Sci. China. Life Sci.* 2010, 53, 524–530. [CrossRef] [PubMed]

75. Qiao, Y.W.; Zhao, X.Q.; Liu, J.; Yang, W.J. Clinicopathological features of Epstein-Barr virus-associated gastric carcinoma: A systematic review and meta-analysis. *J. B.U.ON. Oncol.* 2019, 143, 1517. [CrossRef]

76. Lee, J.H.; Kim, S.H.; Han, S.H.; An, J.S.; Lee, E.S.; Kim, Y.S. Clinicopathological and molecular characteristics of Epstein-Barr virus-associated gastric carcinoma: A meta-analysis. *J. Gastroenterol. Hepatol.* 2009, 24, 354–365. [CrossRef]

77. Chiaravalli, A.M.; Cornaggia, M.; Furlan, D.; Capella, C.; Fiocca, R.; Tagliabue, G.; Klersy, C.; Solcia, E. The role of histological investigation in prognostic evaluation of advanced gastric cancer. *Virchows Arch. Int. J. Pathol.* 2001, 439, 158–169. [CrossRef]

78. Elsaleh, H.; Powell, B.; Soontarapornchai, P.; Joseph, D.; Goria, F.; Spry, N.; Jacobetta, B. p53 gene mutation, microsatellite instability and adjuvant chemotherapy: Impact on survival of 388 patients with Dukes’ C colon carcinoma. *Onecology* 2000, 58, 52–59. [CrossRef] [PubMed]

79. Tokunaga, M.; Land, C.E.; Uemura, Y.; Tokudome, T.; Tanaka, S.; Sato, E. Epstein-Barr virus in gastric carcinoma. *Am. J. Pathol.* 1993, 143, 1250–1254. [CrossRef]

80. Fang, W.L.; Chen, M.H.; Huang, K.H.; Lin, C.H.; Chao, Y.; Lo, S.S.; Li, A.F.; Wu, C.W.; Shyr, Y.M. The Clinicopathological Features and Genetic Alterations in Epstein-Barr Virus-Associated Gastric Cancer Patients after Curative Surgery. *Cancers* 2020, 12, 1517. [CrossRef]

81. Dasari, V.; Sinha, D.; Neller, M.A.; Smith, C.; Khanna, R. Prophylactic and therapeutic strategies for Epstein-Barr virus-associated diseases: Emerging strategies for clinical development. *Expert Rev. Vaccines* 2019, 18, 457–474. [CrossRef] [PubMed]

82. Rodriguenez, M.G.; Roviello, G.; D’Angelo, A.; Lavacchi, D.; Roviello, F.; Polom, K. MSI and EBV Positive Gastric Cancer’s Subgroups and Their Link With Novel Immunotherapy. *J. Clin. Med.* 2020, 9, 1427. [CrossRef] [PubMed]