Title page

Title: Hepatitis B Virus Reactivation in Breast Cancer Patients Undergoing Chemotherapy: a review and meta-analysis of prophylaxis management

authors: Zihao Liu[1], Lei Jiang[3], Gehao Liang[1], Erwei Song[1], Wen G. Jiang[4], Yubao Zheng[2,5,*], Chang Gong[1, 4,*]

[1] Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetic and Gene Regulation, Breast Tumor Center, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, 510120, China

[2] Department of Infectious Diseases, Third Affiliated Hospital of Sun Yat-sen University, 600 Tianhe Road, Guangzhou 510630, China

[3] Department of oncology Surgery, The First Hospital of Lanzhou University, 1 Donggang West Road, Lanzhou, 730000, China

[4] Cardiff China Medical Research Collaborative, School of Medicine, Cardiff University, Cardiff, CF14 4XN, UK

[5] Division of Medicine, Liver Failure Group ILDH, UCL Medical School, London, UK

*Corresponding authors and these authors contributed equally to this study and share corresponding authorship.

*Correspondence:

*Chang Gong, Breast Tumor Center, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, 107 Yanjiang West Road, Guangzhou, 510120, P.R.China, Phone: +86-20-8133783, Fax:

+86-20-81332853, E-mail: changgong282@163.com or GongC1@cardiff.ac.uk

*Yubao Zheng, Department of Infectious Diseases, Third Affiliated Hospital of Sun Yat-sen University, 600 Tianhe Road, Guangzhou 510630, China, E-mail: zhybao@mail.sysu.edu.cn

*These authors contributed equally to this study and share corresponding authorship.

Abbreviations: the American Association for the Study of the Liver, AASLD; alanine aminotransfease , ALT; Asian–Pacific Association for the Study of the Liver, APASL; antibody to hepatitis B core antigen, anti-HBc; antibody to hepatitis B surface antigen, anti-HBs; covalently closed circular DNA, cccDNA; chronic HBV infection ,CHB; confidence interval, CI; Chinese society of hepatology, CMA; China National Knowledge Infrastructure, CNKI; CCAAT/enhancer-binding protein α, C/EBPα; European Association for the Study of the Liver, EASL; hepatitis A virus, HAV; hepatitis Be antigen, HBeAg; hepatitis B surface antigen, HBsAg; hepatitis B virus, HBV; hepatocellular carcinoma, HCC; hepatitis C virus, HCV; hepatitis D virus, HDV; the Korean Association for the Study of the Liver, KASL; mammalian target of rapamycin, mTOR; nucleos(t)ide analogues, NAs; odds ratio, OR; tyrosine-methionine-aspartate-aspartate, YMDD

Abstract:

Background & Aims: Hepatitis B virus (HBV) reactivation during or after chemotherapy in breast cancer patients has became a remarkable clinical problem. Prophylactic nucleos(t)ide analogues (NAs) are recommended for breast patients with hepatitis B surface antigen (HBsAg) positive before chemotherapy. We performed an up-to-date meta-analysis to compare the efficacy of prophylactic lamivudine use with non-prophylaxis in HBsAg positive breast cancer patients undergoing chemotherapy.

Methods: PubMed, the Cochrane Library and China National Knowledge Infrastructure (CNKI) databases were searched for literature until June 2016. Eligible articles that comparing the efficacy of prophylactic lamivudine use with non-prophylaxis in HBsAg positive breast cancer patients undergoing chemotherapy were identified.

Results: Eight studies enrolled 709 HBsAg positive breast cancer patients undergoing chemotherapy were analyzed. Lamivudine prophylaxis significantly reduces the rates of chemotherapy-associated hepatitis B flares in chronic hepatitis B in breast cancer compared with patients with non-prophylaxis (odds ratio [OR] = 0.15, 95% confidence interval [CI]: 0.07-0.35, P < 0.00001). Chemotherapy disruption rates attributed to HBV reactivation in the Prophylaxis groups were significant lower than Non-prophylaxis groups (OR = 0.17, 95% CI: 0.07-0.43, P = 0.0002). Patients with lamivudine prophylaxis have higher risk for tyrosine-methionine-aspartate-aspartate (YMDD) mutation than patients with non-prophylaxis (OR = 6.33, 95% CI: 1.01-39.60, P = 0.05).

Conclusions: Prophylactic antiviral therapy management is necessary for HBsAg positive breast cancer patients undergoing chemotherapy, for especially highly correlates with lamivudine-resistant HBV variants with YMDD motif-mutation.

Key words: breast cancer, hepatitis B, reactivation, lamivudine, prophylaxis antiviral therapy

Introduction:

HBV infection is a global public issue and it is estimated approximately 400 million patients suffered chronic HBV infection worldwide (1). China is one of the major epidemic countries of HBV in the world. The prevalence of chronic HBV infection in Chinese population aged 1-59 is as high as 7.2% when compared with 0.3-0.5% carrier rate within some western countries (2, 3).

HBV reactivation is documented in breast cancer patients receiving anti-cancer chemotherapy (4-6). As adjuvant chemotherapy is a basic part for breast cancer treatment, HBV reactivation has became a significant clinical problem for those breast cancer patients with chronic HBV infection, especially in China which enjoys increasing breast cancer incidence with twofold greater than the world average in recent years (7). HBV reactivation will result in interruption of chemotherapy schedule which predict uncertain prognosis for cancer management and the degree of liver dysfunction attributed to HBV reactivation can be asymptomatic, moderate, fulminant, even leaded to hepatic failure or mortality potentially (8). Several studies have shown NAs prophylaxis can decrease hepatitis B flare and the severity of hepatitis attributed to HBV reactivation (5, 9-11).

In breast oncology, researchers indicate risk factors for hepatitis B flare such as: HBV serologic status and chemotherapy regimen have a great influence on reactivation rates which varies from 41% to 56% (4, 5). Thus, identifying risk factors can predict different prognosis for HBV carrier and guides clinical strategies. However, there are very few review articles illustrating the risk for breast cancer. We comprehensively conclude not only viral status and traditional chemotherapy regimen, but also emerging targeting drugs “everolimus” associated HBV reactivation, which are newly applied in breast cancer chemotherapy.

In this study, we reviewed the basis for managing HBV reactivation including: risk factors, screening and prophylactic antiviral therapy. Moreover, we conducted an up-to-date meta-analysis to assess lamivudine prophylaxis efficacy as well as lamivudine prophylaxis related drug resistance on HBsAg positive breast cancer patients undergoing chemotherapy and propose a practical strategy for management of HBV reactivation.

Overview of HBV reactivation

The natural course of HBV reactivation depends on the interplay between virus replication and host immune control (12). Breast cancer patients with chronic HBV infection (CHB), which are refereed as HBsAg positive, antibody to hepatitis B core antigen (anti-HBc) positive and HBV DNA ranges from undetectable to over 9 $\log_{10}$ copies/ml, usually have hypoimmunity after receiving immunosuppressive chemotherapy. The impaired immunity will lead to breaking balance between virus replication and host immune control, active virus replication, hepatitis Be antigen (HBeAg) turning seropositive and widespread HBV virus infection of hepatocytes (13, 14). When chemotherapy completes, T cells destroy hepatic cells

with HBV infection via cytotoxic effects which are associated with immune reconstitution. Furthermore, many studies indicate individuals with cleared or occult HBV infection were still at risk for HBV reactivation (15). Patients with cleared HBV infection which is defined as HBsAg negative, anti-HBc (IgG) positive, antibody to hepatitis B surface antigen (anti-HBs) positive and HBV DNA negative; or with occult HBV infection which is defined as HBsAg negative, anti-HBc positive, anti-HBs negative and HBV DNA positive/negative, have residual HBV DNA in the hepatocytes (16, 17). In these patients, the immunosuppression chemotherapy will disable host immunity on the control of "dormant" HBV replication and results in HBV reactivation. HBV reactivation in patients with breast cancer occurs after the first 1-6 cycles of chemotherapy (median 3 cycles) and the peak alanine aminotransfease (ALT) level emerges following the peak HBV DNA with a time lag of 2-3 weeks (4, 18). Some cancer patients experience asymptomatic HBV reactivation accompanied by serum ALT increasing during chemotherapy; others may suffer severe fulminant hepatitis and hepatic failure which even lead to the death of cancer patients (13).

Currently, the criteria of HBV reactivation is defined as (1) HBV-DNA level rising (≥10-fold increase greater than baseline level or the absolute value of HBV DNA copy exceeds 1×10^9 copies/ml); (2) HBV DNA turns from seronegativity to seropositivity while excluding of co-infection with hepatitis A, C and D virus (HAV, HCV, HDV). (3) HBsAg seroreversion with HBV DNA *de novo* detection in HBsAg negative/ anti-HBc positive patients. HBV-related hepatitis flare is defined as serum ALT is 3-fold increase greater than upper limit of normal or ALT absolute value is beyond 100 U/L (ALT ≥ 3-fold of upper limit of normal or ALT ≥100 U/L) with the coexisting HBV reactivation (19-21).

Risk factors for HBV reactivation in breast cancer patients

It is significantly important to identify risk factors for HBV reactivation in breast cancer patients clinically for adopting effective prevention strategies via assessing these factors. We have summarized risk factors for HBV reactivation as following.

Virus status

Serum HBV viral load is the most fundamental risk factor of HBV reactivation and it is, therefore, thought to be the earliest indicator for HBV reactivation. In a study of 39 HBeAg negative breast cancer patients who underwent cytotoxic chemotherapy, HBV reactivation preferred in patients serum HBV DNA load is over 3×10^5 copies/ml ($\geq 3\times10^5$ copies/ml) (22, 23). HBsAg positivity and HBeAg positivity further increase the risk for HBV reactivation. HBsAg positivity confers approximate 8-fold greater risk for reactivation than HBsAg negative patients (24, 25). However, HBeAg positivity is not applied as a risk factor in the case universally due to other viral status, for example: HBsAg positivity and detectable HBV DNA (20, 26). It should be noted HBeAg low-level and HBeAg seroconversion are highly susceptible to have HBV precore/core promoter mutation which is a particular risk underestimated (27, 28). Precore/core mutation commonly trigger spontaneous reactivation in chronic HBV infection patients which often result in severe liver dysfunction; when meet tumors, patients with precore/core mutation tend to occur reactivation with a rate approaching 60% (29-31). Research shows that intrahepatic covalently closed circular DNA (cccDNA) which is replicative intermediate of pregenomic RNA, highly correlates with HBV reactivation with a specificity of 100% and an accuracy of 88.9% (32-34). Since persistent

cccDNA is detected in the hepatocyte nucleus, intrahepatic cccDNA level can be used as a risk factor for HBV reactivation in patients with cleared HBV infection. In addition, serum cccDNA\serum HBV DNA ratio validates as a severity index of HBV reactivation, but further studies are required to elucidate different grades of this ratio (35).

Cytotoxic anthracycline and steroid

It becomes evident that cytotoxic anthracycline and steroid, acting as an integral part of monitoring breast cancer, make a major contribution to HBV reactivation. Cancer patients who receive steroid-free chemotherapy have a significant lower HBV reactivation rate. Besides their effect on T-cell normal function, researchers indicate a glucocorticoid-responsive element residing in HBV DNA (36). This element activated by glucocorticoid will simulate HBV-DNA enhancer activity, which in turn promotes HBV replication as well as transcription. We do know reactivation is steroid dose- and time-dependent without knowing the threshold dose, as steroid is used in combination with other chemotherapy regimen frequently (26, 37).

Breast cancer patients receiving anthracycline-based chemotherapy have higher reactivation rates than those receiving anthracycline-free treatment even though two groups accept similar dose of steroid (38). Immunosuppression conducted by anthracycline was considered as the main cause of HBV reactivation. In addition to immunosuppresion, anthracycline results in HBV reactivation via other mechanism. Doxorubicin and epirubicin can mediate HBV DNA replication, HBV DNA secretion and HBeAg secretion in HBV-producing cell line (39, 40). Furthermore, the enhancement of cell regulator p21 upon doxorubicin treatment increases CCAAT/enhancer-binding protein α (C/EBPα) expression and promotes C/EBPα binding with

HBV promoters which results in HBV replication (41).

Molecular targeted drug everolimus

Everolimus is one of the mammalian target of rapamycin (mTOR) inhibitors, which is currently approved in breast cancer. Three cases, one of breast cancer and two of renal cell carcinoma, were reported to have HBV reactivation related to everolimus (42-44). These three cases developed into fatal fulminant hepatitis resulted from HBV reactivation. The author had also observed one breast cancer case displayed severe hepatitis attributed to everolimus-associated HBV reactivation in clinical practice. Strikingly, two cases had received "reactivation risk" chemotherapy (sorafenib for renal cell cancer patient and adriamycin for breast cancer patient), but neither had HBV reactivation during their "reactivation risk" chemotherapy schedule. In HBsAg positive hepatocellular carcinoma (HCC) patients, everolimus treatment can increase serum HBV DNA levels which results in HBV-related hepatitis flare in 19%-53.8% patients (45, 46). An EVOLVE-1 study of everolimus for advanced HCC reported 37% patients had HBV reactivation despite of prophylactic antiviral management (47). Everolimus contributes to HBsAg synthesis and induces HBV replication in cells (48, 49). However, the hepatitis B exacerbation should be attributed to strong immunosuppressive effects of everolimus, as it is commonly used as immunity inhibitor after organ transplantation (50, 51).

Interestingly, the HBV serologic status of the two cases from the foregoing was characterized as IgM anti-HBc negative, which indicates they potentially occurred cleared/occult HBV infection (52, 53). On the background of cleared/occult HBV infection, the HBV reactivation rates occur overtly lower than HBsAg positive patients, even reaching 1% in some clinical

trials (54). Yet, these patients tend to develop *de novo* HBV-related hepatitis which results in higher fulminant hepatic failure rates and mortality. Upon case series, nearly 20%-37.5% of patients occurred fatal fulminant hepatitis (55, 56). This scenario of low HBV reactivation rates but high fatal fulminant hepatitis risk raises concern and more systematic data is needed to illustrate the mechanism of the severity of de novo HBV-related hepatitis.

Prevention and prophylaxis management

Screening

Intervention is consequentially inferior to prevention before reactivation aggravates. Thus the key in prevention is the screening for patients at risk of reactivation by taking their viral status and chemotherapy regimen into consideration. Since a vast portion of cancer patients are not aware of their chronic HBV infection and they might not cognize they are at high risk of fulminant hepatitis, it is great importance to screen their serologic HBV status given the potential poor outcome of reactivation. Although targeted screening can be cost-effective, it is not advocated. Thus such universal HBV routine tests are recommended for all candidates prior to initiation of chemotherapy by European Association for the Study of the Liver (EASL) (57), Asian–Pacific Association for the Study of the Liver (APASL) (58), the Korean Association for the Study of the Liver (KASL) (59) and Chinese society of hepatology (CMA) (60). The American Association for the Study of the Liver (AASLD) recommends targeted screening, that is, patients at high risk for HBV infection should be tested with HBsAg and anti-HBc before initiation of chemotherapy (61). Table 1 shows current guidelines for HBV reactivation.

If screen is undertaken by clinicians, serologic test including HBsAg and anti-HBc should be present. Breast cancer patients, who are HBsAg negative and anti-HBc positive, should be tested for HBV DNA in order to distinguish occult hepatitis B infection or cleared hepatitis B infection. As for HBsAg positive patients, their further serologic status including: HBV DNA level, HBeAg, precore/core mutation and serum cccDNA should be required to determine prevention strategies.

Prophylaxis use of antiviral drugs

Although antivirus drugs, NAs, have therapeutic effects on HBV reactivation, the liver function has significantly deteriorated when we notice severe clinical manifestation as the HBV replicates since the very beginning. Such condition potentially missed optimal time point for antiviral therapy. Therefore, especially for breast cancer specialists must find the optimal time for intervention. According to the recent studies, the intervention strategies can be divided into as: (1) prophylactic therapy (early pre-emptive therapy): use antivirus medication prior to the start of chemotherapy; (2) pre-emptive therapy (deferred pre-emptive therapy): tests HBV DNA regularly and use antivirus medication immediately when HBV DNA reaches the “positive” baseline and ALT level is within normal limits; (3) therapeutic intervention: use antivirus medication when ALT level rises (62, 63). Recent meta-analyses have addressed the efficacy of pre-emptive therapy for HBV reactivation in breast cancer patients (9, 64, 65). We also perform a meta-analysis to assess the benefits of prophylactic lamivudine for reactivation by adding more data. Based on the HBV reactivation mechanism as well as vast clinical data, the early pre-emptive therapy and the deferred pre-emptive therapy are better approaches for managing than the delayed intervention therapy. However, it

is remarkable fact that, in deferred pre-emptive therapy strategy, lamivudine was treated at the time when HBV DNA "significant rise" defined as: HBV DNA levels 10-fold increase if baseline HBV DNA levels 10^5 copies/mL; HBV DNA levels 100-fold increase or HBV DNA levels 10^5 copies if baseline level $<10^5$ copies/mL (66). HBV DNA elevation is the first stage of reactivation. Considering that such high HBV DNA levels have met HBV reactivation criteria partially and investigators decide lamivudine use by such high HBV DNA levels, we insist that deferred pre-emptive therapy is an alternative therapeutic intervention. In fact, two randomized controlled studies have proven prophylactic therapy is far more effective than pre-emptive therapy in lymphoma patients and the reactivation rate in pre-emptive therapy group is close to the rates in therapeutic group (63, 67).

Prophylaxis management is not requisite for all breast cancer patients undergoing chemotherapy. Several guidelines recommend that HBsAg positive or HBV DNA detected cancer patients should be tested for baseline HBV DNA levels and use NAs before the initiation of chemotherapy regardless of HBV DNA levels (58-61). As for cleared/occult HBV infection patients, they have lower risks for reactivation than HBsAg-positive patients when expose to chemotherapy. They are recommended to monitor ALT level, HBV DNA and HBsAg and be treated with NAs upon confirmation of HBV reactivation (57-59). Considering high risks for cleared/occult HBV infection patients such as: everolimus, intense anthracycline and steroid, we suggest that these breast cancer patients should receive prophylaxis antiviral therapy before the initiation of chemotherapy (68). In addition, prophylaxis therapy is expensive and it will, however, lead to the possibility of overtreatment for nearly half of the cancer patients without HBV reactivation. We propose that clinicians can

use of risk calculators to decide whether breast cancer patients require prophylaxis antiviral therapy or not by estimating HBV DNA level, HBeAg, precore/core mutation, serum cccDNA and types of chemotherapy. For instance, researchers establish a predictive model that consists of clinical and virological factors to estimate the probability of HBV reactivation in lymphoma and breast cancer patients (69). (Figure 1)

Lamivudine is extensively used as prophylaxis antiviral agent in most studies as it was first generation of NAs approved for hepatitis B treatment and its preventative efficacy in managing HBV reactivation has been proven in breast cancer patients. However, failure of preventative lamivudine was observed during clinical practice. In this study, we have shown that lamivudine prophylaxis patients have higher risk to develop tyrosine-methionine-aspartate-aspartate (YMDD) mutation than non-prophylaxis. Lamivudine has a low barrier to develop drug resistance. Variants in the YMDD motif tend to increasingly develop which can lead to its resistance over prolonged course of lamivudine (70). Emergence of YMDD mutation abrogates the benefits obtained with prophylaxis lamivudine treatment and may cause fatal hepatic failure (61, 71). Clinicians should be aware of YMDD mutation rate in prophylactic lamivudine treatment. Furthermore, delayed HBV reactivation after lamivudine withdrawal was reported in cancer patients who accepted preventative lamivudine until 4 weeks to 3.4 months after cessation of chemotherapy (72, 73). High baseline HBV DNA (> 2000 IU/mL) is a predictor of delayed HBV reactivation. Hence, for breast cancer patients whose anticipated antiviral duration is long (> 12 months) or pre-chemotherapy HBV DNA > 2000 IU/mL, entecavir or tenofovir is recommended and NAs treatment was recommended to continue at least 6 months after the cessation of chemotherapy for HBsAg

positive cancer patients undergoing conventional chemotherapy (57-59, 61).

Materials and methods

Data sources and study selection

Two independent authors (ZH.L. and L.J.) searched and reviewed citations from the Cochrane Library, PubMed and China National Knowledge Infrastructure (CNKI) by use the keywords "chemotherapy", "breast", "cancer", "carcinoma", "HBV", "reactivation", "prophylaxis", "preemptive", "lamivudine". The search was performed in June 2016 without limitation of language and time. This study is restricted to human studies and includes all clinical trials regardless of randomized, controlled, prospective and retrospective studies. This study excludes: (1) meeting abstract, reviews and case reports; (2) studies do not contain prophylaxis and non-prophylaxis groups; (3) studies involved HCV, HDV and human immunodeficiency virus co-infection; (4) the studies included patients receiving no immunosuppressive chemotherapy.

Definition

The criteria of HBV reactivation is defined as (1) HBV-DNA level rising (≥10-fold increase greater than baseline level or the absolute value of HBV DNA copy exceeds 1×10^9 copies/ml); (2) HBV DNA turns from seronegativity to seropositivity while excluding of co-infection with HAV, HCV and HDV. (3) HBsAg seroreversion with HBV DNA *de novo* detection in HBsAg negative/anti-HBc positive patients. HBV-related hepatitis flare is defined as ALT is 3-fold increase greater than upper limit of normal or ALT absolute value is beyond 100 U/L (ALT ≥ 3-fold of upper limit of normal or ALT ≥100 U/L) with the coexisting HBV

reactivation. Disruption of chemotherapy was defined as premature termination or a delay of chemotherapy over than 8 days between cycles (19-21).

Data extraction and quality assessment

By reading the included literature, one researcher (ZH.L.) extracted all data and another independent researcher (L.J.) verify the data and the data included details of the studies, number of enrolled patients, chemotherapy regimen and outcomes of patients. The study quality was independently estimated by two investigators (ZH.L. and L.J.) using the Newcastle-Ottawa Scale (NOS).

Statistical analysis

We perform meta-analysis by using Review Manager Software version 5.0 for window (Cochrane Collaboration, Oxford, UK). Statistical heterogeneity was assessed by the chi-squared and I-squared test. In these tests, $P < 0.10$ was regarded as significant heterogeneity. The outcome was measured as odds ratio (OR) with 95% confidence intervals (CI). A random effect model was applied for analysis in the cases where significant heterogeneity exists. A fixed effect model was applied in other cases. $P < 0.05$ was used to indicate statistical significance.

Result

Search results and description of studies

In all, 142 literatures were obtained and screened for retrieval via using the searching strategy mentioned above. After screening for title and abstract, 125 studies were excluded and 17 were retrieved for further assessment. Ultimately, 9 studies were removed by the exclusion

criteria and 8 studies were included by using strict inclusion criteria (5, 66, 74-79). Details on study selection are listed in Supplementary Figure 1.

One study was a randomized controlled study (74); two were retrospective cohort studies (75, 76) and five were prospective cohort studies (5, 66, 76, 77, 79) of the eight studies. Seven studies have compared prophylaxis lamivudine administration with non-prophylaxis management (5, 74-79) and one evaluated the efficacy-effectiveness distinction between prophylaxis lamivudine management and deferred pre-emptive lamivudine management (66). We combine with the concept of therapeutic strategy and deferred pre-emptive strategy as we have discussed deferred pre-emptive strategy is another kind of therapeutic strategy above. The populations of the eight studies were HBsAg positive and were all Asian, with five from China and three from Korea.

Comparing occurrence rates of hepatitis attributed to HBV reactivation between the prophylaxis and non-prophylaxis groups

The six studies which included 232 patients in the prophylaxis group and 387 patients in the non-prophylaxis group, reported hepatitis attributed to HBV reactivation. Meta-analysis reveals that there is a significant difference in hepatitis attributed to HBV reactivation between lamivudine prophylaxis and non-prophylaxis group (OR = 0.15, 95% CI: 0.07-0.35, $P < 0.00001$) (figure 2). There is no significant heterogeneity among the six studies (chi-squared = 2.47, $I^2 = 0\%$, P = 0.65).

Comparing chemotherapy disruption rates attributed to HBV reactivation between the prophylaxis and non-prophylaxis groups

The six studies which included 228 patients in the prophylaxis group and 292 patients in the

non-prophylaxis group reported chemotherapy disruption attributed to HBV reactivation. Patients with lamivudine prophylaxis have a significant lower disruption rate when compared patients with non-prophylaxis (OR = 0.17, 95% CI: 0.07-0.43, P = 0.0002) (figure 3). No significant heterogeneity was observed (chi-squared = 2.36, I^2 = 0%, P = 0.67).

Comparing YMDD mutation rates related to lamivudine prophylaxis between the prophylaxis and non-prophylaxis groups

Only four patients had YMDD mutation in the six studies which include 226 patients in the prophylaxis group and 346 patients in the non-prophylaxis group. Patients with lamivudine prophylaxis have higher risk for YMDD mutation statistically (OR = 6.33, 95% CI: 1.01-39.60, P = 0.05) (figure 4). No significant heterogeneity was noted in these data (chi-squared = 0.16, I^2 = 0%, P = 0.92).

Discussion

HBV reactivation upon immunosuppressive chemotherapy is strongly associated with hepatic impairment as well as chemotherapy disruption, which predicts poor prognosis for breast cancer patients and potentially indicates increasing mortality. Thus antiviral strategies are addressed to manage HBV reactivation in several studies. This meta-analysis indicates that breast cancer patients with prophylaxis management have better outcomes, including lower occurrence rates of hepatitis B flares attributed to HBV reactivation and lower chemotherapy disruption rates attributed to HBV reactivation, than patients with non-prophylaxis. Thus, prophylactic antiviral management is very necessary for HBsAg positive breast cancer patients.

In the past, we have focused on patients with lymphoma or undergoing hematopoietic stem cell transplantation and efficacy of prophylactic lamivudine use in these patients (80, 81). Little attention about prophylactic antiviral management was drawn in breast cancer. In this meta-analysis, we have discussed the necessity of antiviral therapy prophylaxis for HBsAg positive breast cancer patients. However, there are several obstacles to be addressed. According to another meta-analysis, approximately 85% breast cancer patients are over-treated with lamivudine for only 15% patients had developed HBV reactivation in the non-prophylaxis group (64). Therefore, most HBsAg positive breast cancer patients will benefit from a reactivation risk predictive model. Further researches are required to illustrate the risk predictive model in breast cancer.

In addition, we notice patients with lamivudine prophylaxis have higher risk for YMDD mutation statistically in our study, while the YMDD mutation rate is no significant difference between prophylaxis and non-prophylaxis because of 95% CI: 0.60-48.95 and P = 0.13 in another meta-analysis (64). It should be noted three of the four patients had received long lamivudine treatment course with duration time over 18 months (75, 76). Prolonged lamivudine use can result in increasing likelihood of YMDD mutants. Studies reported that YMDD mutation emerged in 24% of chronic HBV infected patients in the first year of lamivudine treatment, 38% in the second year, 50% in the third year and 67% in the fourth year (70, 82, 83). We suggest that lamivudine should no longer be regarded as first-line medicine for prophylactic management, even though we have some available remedial measures when HBV YMDD mutants arising. Those NAs such as entecavir and tenofovir which have higher barrier to resistance are proved to be more efficient in preventing HBV

reactivation, reducing HBV related mortality and lowering chemotherapy disruption rates than lamivudine (84, 85). No matter what, prophylactic antiviral therapy plays a prerequisite "escort" role during chemotherapy for those breast cancer patients with CHB (as showed in Figure 5).

Undoubtedly, our study has several limitations to be considered before the practice of our findings. First, we conducted this meta-analysis based on eight studies but not on patients' data. Confounding variables such as different chemotherapy schemes, cancer metastasis and patients co-morbidities were not taken into account. Second, some studies included had small sample sizes. We need more data to evaluate the relationship between lamivudine prophylaxis and YMDD mutation rate because of $P = 0.05$.

In conclusion, our study showed lamivudine prophylaxis is effective in reducing chemotherapy disruption rates attributed to HBV reactivation as well as occurrence rates of hepatitis B flares attributed to HBV reactivation for HBsAg positive/or HBsAg negative anti-HBc positive breast cancer patients undergoing chemotherapy. So, breast cancer specialists should monitor HBV infection situation and NAs such as entecavir and tenofovir are suggested as first-line prophylactic medicine for HBV reactivation management.

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Disclosure

The authors have nothing to disclose.

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Figure 1: (supplemental file)

Figure 1 proposed algorithm for the management of HBV reactivation in breast cancer patients undergoing chemotherapy. Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBc, hepatitis B core antibody; HBeAg, hepatitis Be antigen; anti-HBs, hepatitis B surface antibody; cccDNA, covalently closed circular DNA; LFT, liver function test.

Figure 2: (supplemental file)

Figure 2 Comparison of occurrence rates of hepatitis attributed to HBV reactivation.

Figure 3: (supplemental file)

Figure 3 Comparison of chemotherapy disruption rates of hepatitis attributed to HBV reactivation.

Figure 4: (supplemental file)

Figure 4 Comparison of YMDD mutation rates related to lamivudine.

Figure 5: (supplemental file)

Figure 5 A cartoon depicting the dynamic situation of HBV virus replication and host immune control during chemotherapy with antiviral therapy.

Table 1 Summary of current guidelines for hepatitis B virus reactivation management

(supplemental file)

	United states AASLD 2009 (61)	**Europe** EASL 2012 (57)	**Asian-pacific** APASL 2015 (58)	**Korea** KASL 2016 (59)	**China** CMA 2015 (60)
Screening indication	Patients who are at high risk for HBV infection	All candidates for chemotherapy	All candidates for chemotherapy	All candidates for chemotherapy	All candidates for chemotherapy
Screening parameters	HBsAg and anti-HBc	HBsAg and anti-HBc	HBsAg and anti-HBc	HBsAg, anti-HBc and Serum HBV DNA	HBsAg, anti-HBc and Serum HBV DNA
Screening time	Before chemotherapy	Before chemotherapy	Before chemotherapy	Before chemotherapy	Before chemotherapy
Indication of prophylaxis	HBV carriers	HBsAg (+)	HBsAg (+)	HBsAg (+) or HBV DNA (+)	HBsAg (+)
Time of starting antiviral therapy	With start of chemotherapy	During chemotherapy	Before chemotherapy	With start of chemotherapy	Before chemotherapy
Antiviral agents	Lamivudine or telbivudine, if anticipated duration is short (<12 mo) Tenofovir or entecavir if anticipated duration if long (> 12 mo)	Lamivudine for HBsAg (+) and HBV DNA < 2000 IU/mL Entecavir or tenofovir for HBsAg (+), HBV DNA > 2000 IU/mL and (or) lengthy or repeated cycles of chemotherapy	NAs (entecavir and tenofovir should be considered if prolonged antiviral therapy is indicated)	NAs (Entecavir or tenofovir for HBV DNA > 2000 IU/mL or lengthy or repeated cycles of chemotherapy)	NAs
Duration endpoint	6 mo after cessation of chemotherapy for baseline HBV DNA < 2000 IU/mL Until reaching treatment endpoints of immunocompetent for cases of baseline HBV DNA > 2000 IU/mL	12 mo after cessation of chemotherapy	12 mo after cessation of chemotherapy	6 mo after cessation of chemotherapy	6 mo after cessation of chemotherapy
HBsAg (-) anti-HBc (+) management	Not specific	Test with ALT and HBV DNA, Treat with NA upon confirmation of reactivation regardless of anti-HBs	Test with ALT and HBV DNA, Treat with NA upon confirmation of reactivation regardless of anti-HBs	Test with serum HBV and HBsAg Treat with NA upon serum HBV and HBsAg reappears	Not specific

Abbreviations: the American Association for the Study of the Liver, AASLD; European Association for the Study of the Liver, EASL; Asian–Pacific Association for the Study of the Liver, APASL; the Korean Association for the Study of the Liver, KASL; Chinese society of hepatology, CMA; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBc, hepatitis B core antibody; alanine aminotransfease ,ALT; nucleos(t)ide analogues, NAs

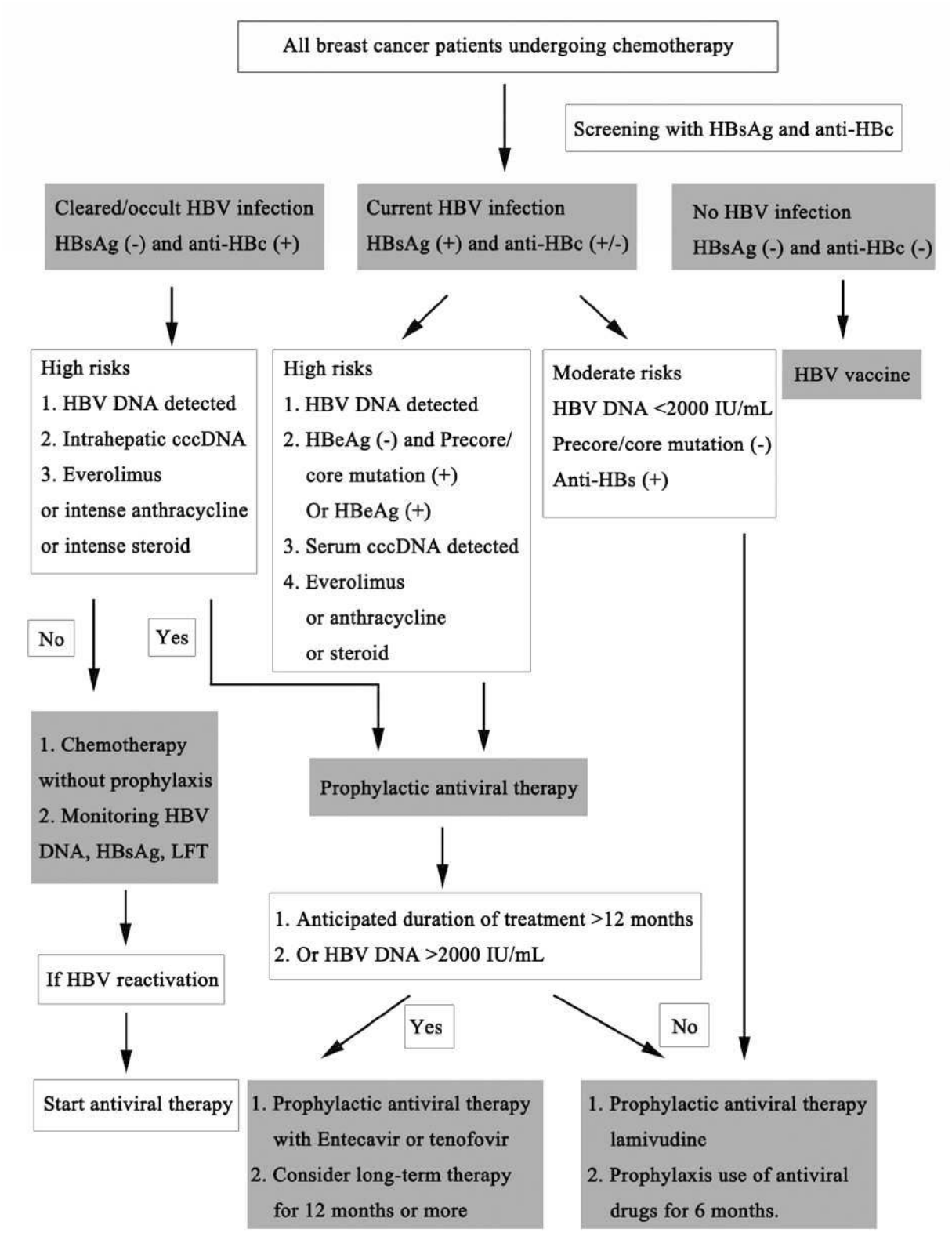

All breast cancer patients undergoing chemotherapy
Screening with HBsAg and anti-HBc
Cleared/occult HBV infection
HBsAg (-) and anti-HBc (+)
Current HBV infection
HBsAg (+) and anti-HBc (+/-)
No HBV infection
HBsAg (-) and anti-HBc (-)
High risks
1. HBV DNA detected
2. Intrahepatic cccDNA
3. Everolimus
or intense anthracycline
or intense steroid
High risks
1. HBV DNA detected
2. HBeAg (-) and Precore/
core mutation (+)
Or HBeAg (+)
3. Serum cccDNA detected
4. Everolimus
or anthracycline
or steroid
Moderate risks
HBV DNA <2000 IU/mL
Precore/core mutation (-)
Anti-HBs (+)
HBV vaccine
No
Yes
1. Chemotherapy
without prophylaxis
2. Monitoring HBV
DNA, HBsAg, LFT
Prophylactic antiviral therapy
1. Anticipated duration of treatment >12 months
2. Or HBV DNA >2000 IU/mL
If HBV reactivation
Yes
No
Start antiviral therapy
1. Prophylactic antiviral therapy
with Entecavir or tenofovir
2. Consider long-term therapy
for 12 months or more
1. Prophylactic antiviral therapy
lamivudine
2. Prophylaxis use of antiviral
drugs for 6 months.

Figure 1

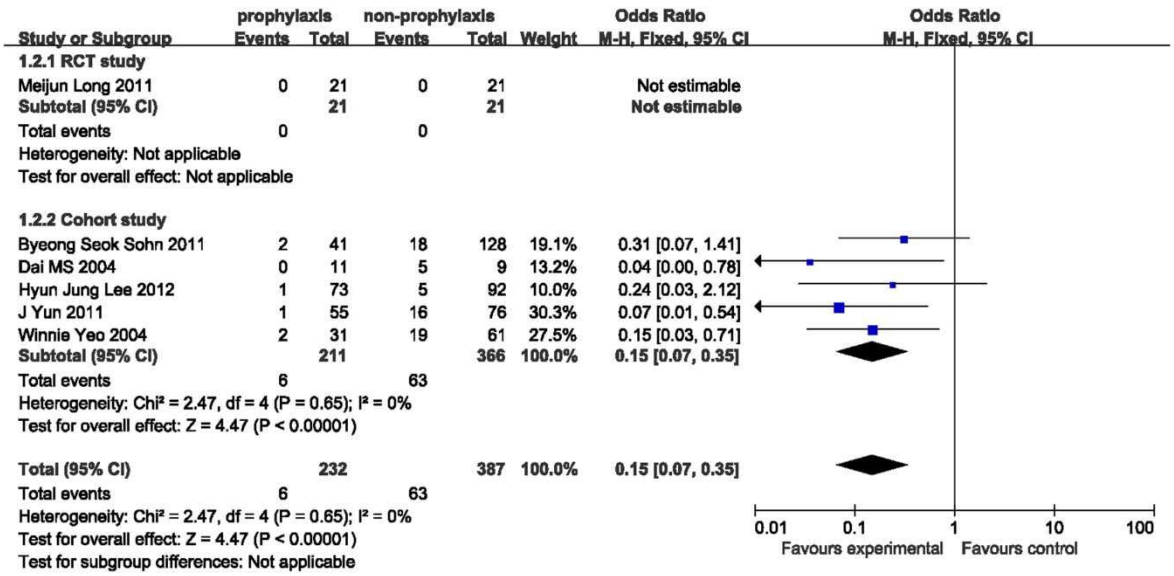

prophylaxis
non-prophylaxis
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
1.2.1 RCT study
Meijun Long 2011 0 21 0 21 Not estimable
Subtotal (95% CI) 21 21 Not estimable
Total events 0 0
Heterogeneity: Not applicable
Test for overall effect: Not applicable
1.2.2 Cohort study
Byeong Seok Sohn 2011 2 41 18 128 19.1% 0.31 [0.07, 1.41]
Dai MS 2004 0 11 5 9 13.2% 0.04 [0.00, 0.78]
Hyun Jung Lee 2012 1 73 5 92 10.0% 0.24 [0.03, 2.12]
J Yun 2011 1 55 16 76 30.3% 0.07 [0.01, 0.54]
Winnie Yeo 2004 2 31 19 61 27.5% 0.15 [0.03, 0.71]
Subtotal (95% CI) 211 366 100.0% 0.15 [0.07, 0.35]
Total events 6 63
Heterogeneity: Chi² = 2.47, df = 4 (P = 0.65); I² = 0%
Test for overall effect: Z = 4.47 (P < 0.00001)
Total (95% CI) 232 387 100.0% 0.15 [0.07, 0.35]
Total events 6 63
Heterogeneity: Chi² = 2.47, df = 4 (P = 0.65); I² = 0%
Test for overall effect: Z = 4.47 (P < 0.00001)
Test for subgroup differences: Not applicable
0.01
0.1
1
10
100
Favours experimental
Favours control

Figure 2

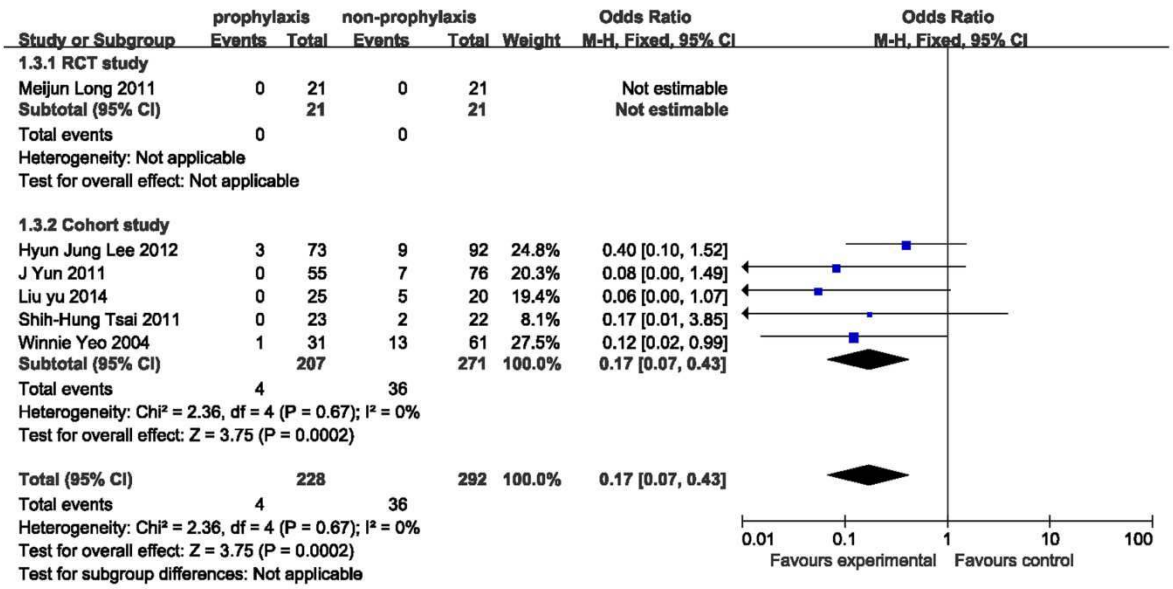

prophylaxis
non-prophylaxis
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
1.3.1 RCT study
Meijun Long 2011 0 21 0 21 Not estimable
Subtotal (95% CI) 21 21 Not estimable
Total events 0 0
Heterogeneity: Not applicable
Test for overall effect: Not applicable
1.3.2 Cohort study
Hyun Jung Lee 2012 3 73 9 92 24.8% 0.40 [0.10, 1.52]
J Yun 2011 0 55 7 76 20.3% 0.08 [0.00, 1.49]
Liu yu 2014 0 25 5 20 19.4% 0.06 [0.00, 1.07]
Shih-Hung Tsai 2011 0 23 2 22 8.1% 0.17 [0.01, 3.85]
Winnie Yeo 2004 1 31 13 61 27.5% 0.12 [0.02, 0.99]
Subtotal (95% CI) 207 271 100.0% 0.17 [0.07, 0.43]
Total events 4 36
Heterogeneity: Chi² = 2.36, df = 4 (P = 0.67); I² = 0%
Test for overall effect: Z = 3.75 (P = 0.0002)
Total (95% CI) 228 292 100.0% 0.17 [0.07, 0.43]
Total events 4 36
Heterogeneity: Chi² = 2.36, df = 4 (P = 0.67); I² = 0%
Test for overall effect: Z = 3.75 (P = 0.0002)
Test for subgroup differences: Not applicable
0.01
0.1
1
10
100
Favours experimental
Favours control

Figure 3

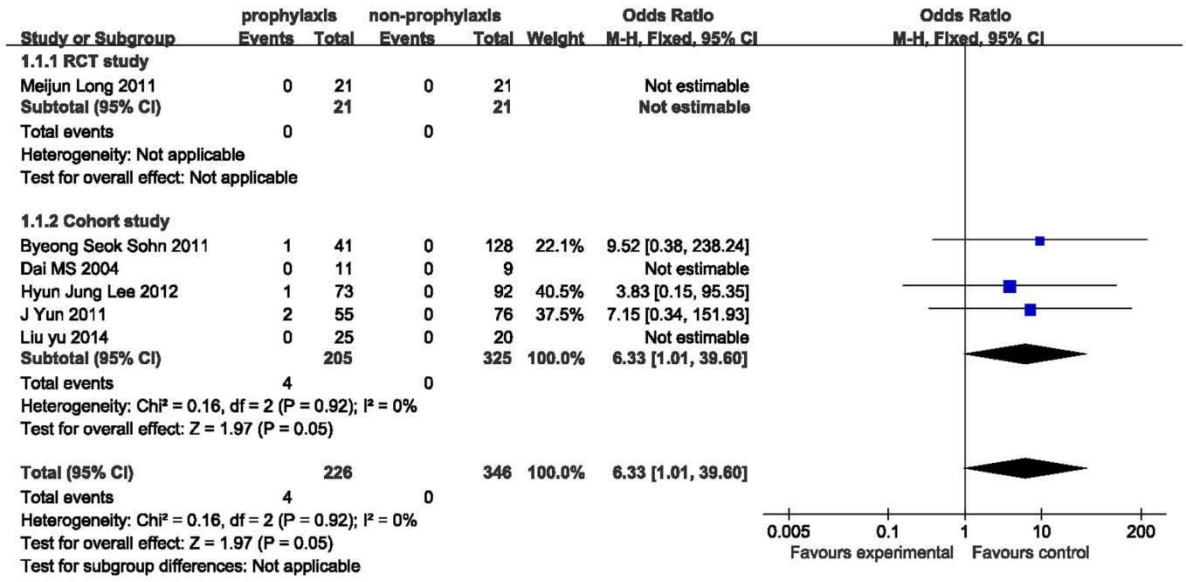

prophylaxis
non-prophylaxis
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
1.1.1 RCT study
Meijun Long 2011 0 21 0 21 Not estimable
Subtotal (95% CI) 21 21 Not estimable
Total events 0 0
Heterogeneity: Not applicable
Test for overall effect: Not applicable
1.1.2 Cohort study
Byeong Seok Sohn 2011 1 41 0 128 22.1% 9.52 [0.38, 238.24]
Dai MS 2004 0 11 0 9 Not estimable
Hyun Jung Lee 2012 1 73 0 92 40.5% 3.83 [0.15, 95.35]
J Yun 2011 2 55 0 76 37.5% 7.15 [0.34, 151.93]
Liu yu 2014 0 25 0 20 Not estimable
Subtotal (95% CI) 205 325 100.0% 6.33 [1.01, 39.60]
Total events 4 0
Heterogeneity: Chi² = 0.16, df = 2 (P = 0.92); I² = 0%
Test for overall effect: Z = 1.97 (P = 0.05)
Total (95% CI) 226 346 100.0% 6.33 [1.01, 39.60]
Total events 4 0
Heterogeneity: Chi² = 0.16, df = 2 (P = 0.92); I² = 0%
Test for overall effect: Z = 1.97 (P = 0.05)
Test for subgroup differences: Not applicable
0.005
0.1
1
10
200
Favours experimental
Favours control

Figure 4

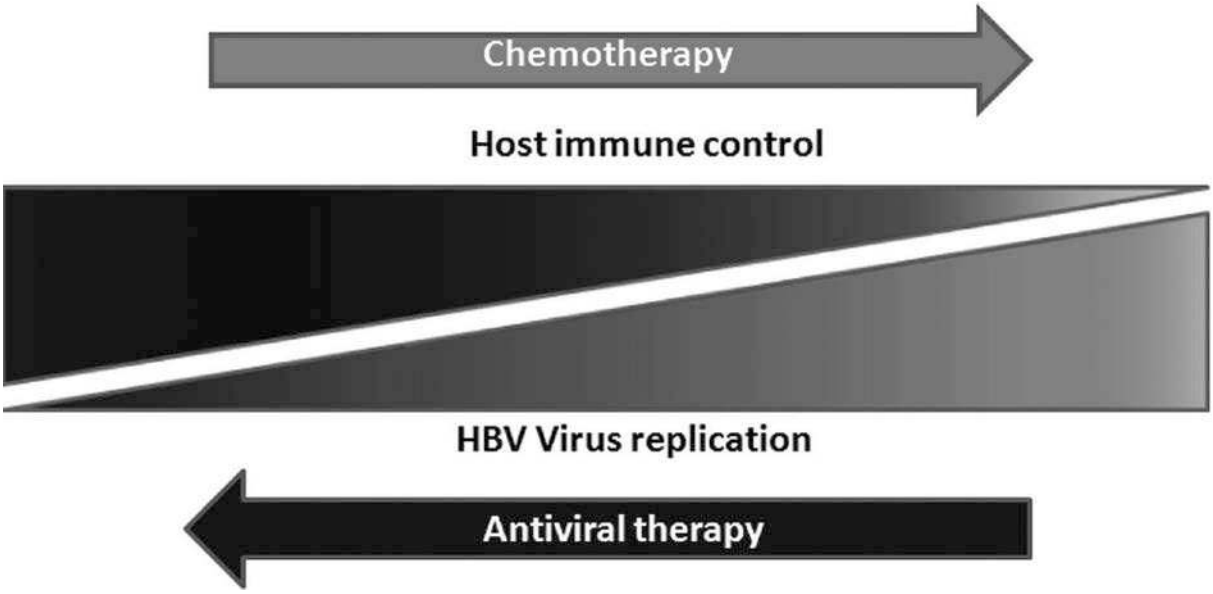
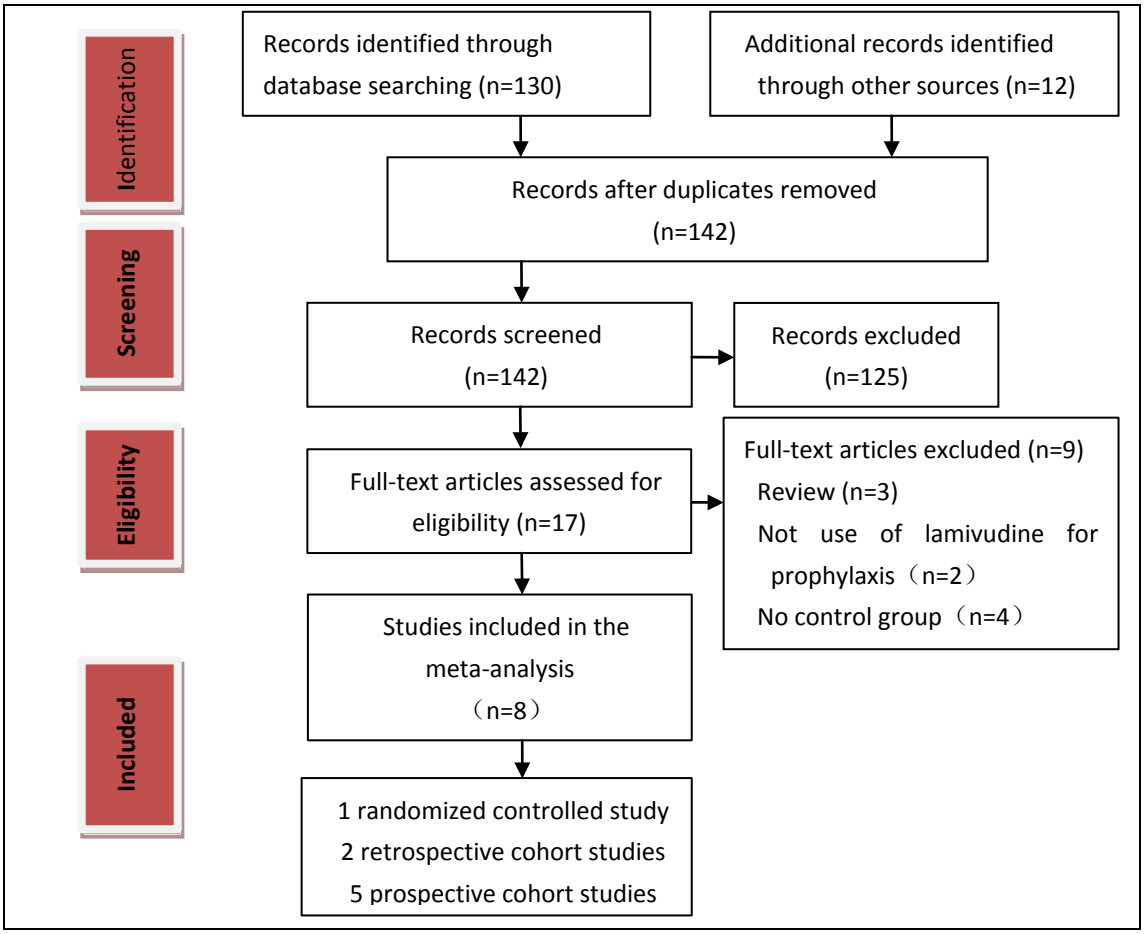

Chemotherapy
Host immune control
HBV Virus replication
Antiviral therapy

Figure 5



Supplemental Figure 1 Details on study selection of studies included in the meta-analysis.

MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies*

Title: Hepatitis B Virus Reactivation in Breast Cancer Patients Undergoing Chemotherapy: a review and meta-analysis of prophylaxis management

authors: Zihao Liu[1], Lei Jiang[3], Gehao Liang[1], Erwei Song[1], Wen G. Jiang[4], Yubao Zheng[2,5,*], Chang Gong[1, 4,*]

[1] Guangdong Provincial Key Laboratory of Malignant Tumor Epigenetic and Gene Regulation, Breast Tumor Center, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, 510120, China

[2] Department of Infectious Diseases, Third Affiliated Hospital of Sun Yat-sen University, 600 Tianhe Road, Guangzhou 510630, China

[3] Department of oncology Surgery, The First Hospital of Lanzhou University, 1 Donggang West Road, Lanzhou, 730000, China

[4] Cardiff China Medical Research Collaborative, School of Medicine, Cardiff University, Cardiff, CF14 4XN, UK

[5] Division of Medicine, Liver Failure Group ILDH, UCL Medical School, London, UK

*Corresponding authors and these authors contributed equally to this study and share corresponding authorship.

Criteria		**Brief description of how the criteria were handled in the meta-analysis**
Reporting of background should include		
√	The clinical problem	Hepatitis B virus reactivation during chemotherapy in breast cancer patients has became a remarkable clinic problem. Prophylactic nucleos(t)ide analogues are recommended for the prevention of HBV reactivation before chemotherapy. The efficacy of prophylactic lamivudine use in HBsAg positive breast cancer patients undergoing chemotherapy remains to be summarized.
√	The hypothesis	Prophylactic antiviral therapy management is efficient in reducing hepatitis attributed to HBV reactivation and chemotherapy disruption attributed to HBV reactivation, but increases the risk of tyrosine-methionine-aspartate-aspartate mutation.
√	Description of study outcomes	Observed objects developed HBV reactivation
√	Type of exposure or intervention used	lamivudine
√	Type of study designs used	Retrospective cohort studies, prospective cohort studies and a randomized controlled study in unselected population
√	Study population	No restriction.
Reporting of search strategy should include		
√	Qualifications of searchers	Patients and methods/Data extraction and quality assessment**.**

		Researchers experienced in systematic reviews, breast cancer and infectious diseases.
√	Search strategy, including time period included in the synthesis and keywords	Patients and methods/ Data sources and study selection. PubMed, the Cochrane Library and China National Knowledge Infrastructure (CNKI) up to June 2016. Key word: chemotherapy, breast, cancer, carcinoma, HBV, reactivation, prophylaxis, lamivudine.
√	Databases and registries searched	PubMed, the Cochrane Library and China National Knowledge Infrastructure (CNKI)
√	Search software used, name and version, including special features	We did not employ a search software. EndNote X7 was used to merge retrieved citations and eliminate duplications
√	Use of hand searching	We hand-searched bibliographies of retrieved papers for additional references,
√	List of citations located and those excluded, including justifications	Details of the literature search are presented in the Result/ Search results and description of studies. The citation list is available upon request
√	Method of addressing articles published in languages other than English	We placed no restrictions on language; local scientists fluent in the original language of the article were contacted for translation
√	Method of handling abstracts and unpublished studies	We did not include studies only published as abstracts
√	Description of any contact with authors	We contacted experts to identify potentially eligible trials, published and unpublished.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria were described in the Patients and methods section.
√	Rationale for the selection and coding of data	Data extracted from each of the studies were relevant to the population characteristics, study design, exposure, outcome, and possible effect modifiers of the association.
√	Documentation of how data were classified and coded	We expressed results for dichotomous outcomes as relative risk (RR) with 95% confidence intervals (CIs)
√	Assessment of confounding	Not stated
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Not stated
√	Assessment of heterogeneity	Statistical heterogeneity was assessed by the chi-squared and I-squared test. In these tests, $P < 0.10$ was regarded as significant heterogeneity
√	Statistical methods (eg, complete description of fixed or random	Not state

	effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	
√	Provision of appropriate tables and graphics	In the manuscript
Reporting of results should include		
√	A graph summarizing individual study estimates and overall estimate	See Fig 1. PRISMA statement of search results
√	A table giving descriptive information for each study included	No
√	Results of sensitivity testing	No
√	Indication of statistical uncertainty of findings	The outcome was measured as odds ratio with 95% confidence intervals, statistical heterogeneity was assessed by the chi-squared and I-squared test. In these tests, $P < 0.10$ was regarded as significant heterogeneity.
Reporting of discussion should include		
√	Quantitative assessment of bias	Not stated
√	Justification for exclusion	Described in Patients and methods / Data sources and study selection
√	Assessment of quality of included studies	We thoroughly observed every study and discussed the quality of each included study. Only included the well-designed cohort studies and randomized controlled studies that meet our criterias.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	In the Discussion paragraph 3 we consider the patients had received long lamivudine treatment course before prophylaxis lamivudine use. Limitations of the meta-analysis should be considered. These confounders may alter the result.
√	Generalization of the conclusions	Our meta-analysis, though with limitations, concludes that prophylactic antiviral therapy management is efficient in reducing hepatitis attributed to HBV reactivation and chemotherapy disruption attributed to HBV reactivation, but increases the risk of tyrosine-methionine-aspartate-aspartate mutation. We noted the lack of clinical data from Africa, America and Europe.
√	Guidelines for future research	We recommend future studies on the efficacy of prophylactic use of nucleos(t)ide analogues such as entecavir and tenofovir, as entecavir and tenofovir is widely used in clinical practice and associates with less emergence of drug resistance.
√	Disclosure of funding source	No separate funding was necessary for the undertaking of this systematic review.