RESEARCH ARTICLE

High Risk of Hepatitis B Reactivation among Patients with Acute Myeloid Leukemia

Chien-Yuan Chen¹, Sheng-Yi Huang¹, Aristine Cheng², Wen-Chien Chou¹,³, Ming Yao¹, Jih-Luh Tang¹,⁴, Woei Tsay¹, Wang-Huei Sheng²*, Hwei-Fang Tien¹

¹ Division of Hematology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, ² Division of Infectious disease, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, ³ Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan, ⁴ Tai-Cheng stem cell therapy center, National Taiwan University Hospital, Taipei, Taiwan

* whsheng@ntu.edu.tw

Abstract

Background
Hepatitis B virus (HBV) infections are common and associated with significant morbidity and mortality in cancer patients. However, the incidence and risk factors of HBV reactivation in patients with acute myeloid leukemia (AML) are rarely investigated.

Methods
AML patients followed-up at the National Taiwan University Hospital between 2006 and 2012 were analyzed. The clinical characteristics and laboratory data were retrospectively reviewed.

Results
Four hundred and ninety patients comprising 265 men and 225 women were studied. The median age was 52 years (range, 18 - 94). Chronic HBV carriage was documented at the time of leukemia diagnosis in 57 (11.6%) patients. Forty-six (80.7%) of the 57 HBV carriers received prophylaxis with anti-HBV agents. Sixteen HBV carriers (28.1%) developed hepatitis B reactivation during or after chemotherapy, including 7 patients who had discontinued antiviral therapy. The incidence of hepatitis B reactivation among AML patients with HBV carriage was 9.5 per 100 person-years. Prophylaxis with anti-HBV agents significantly decreased the risk of hepatitis B reactivation among HBV carriers (13% vs. 61%, p<0.001).

Four (2.8%) of 142 patients with initial positive anti-HBsAb and anti-HBcAb experienced hepatitis B reactivation and lost their protective anti-HBsAb. Multivariate analysis revealed that diabetes mellitus (p=0.008, odds ratio (OR) = 2.841, 95% confident interval (CI): 0.985-8.193) and carriage of HBsAg (p<0.001, OR=36.878, 95% CI: 11.770-115.547) were independent risk factors for hepatitis B reactivation in AML patients.
Conclusions
Hepatitis B reactivation is not uncommon in the HBsAg positive AML patients. Prophylaxis with anti-HBV agent significantly decreased the risk of hepatitis B reactivation.

Introduction
Acute myeloid leukemia (AML) is a heterogeneous disorder with regards to the morphology and chromosome aberrations detected in the leukemic cells [1]. In spite of this, most AML patients receive standard induction chemotherapy containing anthracycline and cytarabine, and consolidation with high dose cytarabine [1]. The chemotherapy agents used for AML are similar to those used for lymphoma [2–4]. High dose cytarabine infusion can cause skin rash and fever [5], and thus, steroid is usually added as prophylaxis. Abnormal liver function tests are frequently found among AML patients receiving chemotherapy, possibly related to the chemotherapy itself, the concomitant use of antifungal agents and total parenteral nutrition, or to sepsis related organ dysfunction, leukemic infiltration, or graft versus host disease. Reactivation of viral hepatitis, including hepatitis B, is also a potential cause for abnormal liver function tests in leukemic patients receiving chemotherapy [6, 7].

Hepatitis B virus (HBV) affects 350 to 400 million persons worldwide and constitutes a major global health burden [8]. HBV is a DNA virus transmitted parenterally, sexually, and perinatally [8]. HBV infection can cause acute and chronic liver disease including cirrhosis and hepatocellular carcinoma. Following immunosuppression, HBV replication along with signs of hepatocellular injury in a silent hepatitis B surface antigen (HBsAg) carrier may occur [9]. The clinical presentation of hepatitis B reactivation is variable, ranging from asymptomatic to fulminant hepatitis, liver failure, and death. Previous reports of HBV reactivation have been observed in patients with lymphoma [10, 11], treated with corticosteroids [12, 13] and rituximab [14, 15] as well as in patients undergoing stem cell and bone marrow transplantation [16, 17]. However, the epidemiology and clinical manifestations of hepatitis B reactivation among AML patients are rarely described [10]. Taiwan has long been an area endemic for HBV, and, previously, the seroprevalences of anti-HBc approached 80–90% and of HBsAg 15–20% prior to the nationwide hepatitis B vaccination program [18]. In this study, we retrospectively reviewed the epidemiology, clinical and laboratory data of hepatitis B in AML patients at a medical center to understand the epidemiology and clinical outcomes of HBV reactivation among AML patients.

Patients and Method
Ethics Statement
The Institutional Review Board of the National Taiwan University Hospital Research Ethics Committee waived the need for written informed consent from the participants in the retrospective review of medical record and approved this study. This research conformed to the Helsinki Declaration and local legislation, and was approved by the Institutional Review Board National Taiwan University Hospital Research Ethics Committee.

Hospital setting and patients
National Taiwan University Hospital (NTUH) is a 2900-bed teaching hospital in northern Taiwan providing both primary and tertiary care. In this study, the clinical and laboratory data,
hepatitis B serology, hepatitis B virus DNA and outcome of all adult AML patients during the period January 2006 to December 2012 at NTUH were analyzed retrospectively by chart review. Liver function tests, hepatitis B serology titers, and HBV DNA levels were performed as clinically indicated.

Definitions
The serum biochemistry (alanine transaminase, ALT) was measured by the Beckman Coulter AU5800 platform (Beckman Coulter Inc, Brea, CA, USA); HBsAg, anti-HBsAb, Anti-HBc, HBeAg, and anti-HCV were performed with Abbott ARCHITECT i2000SR (Abbott Laboratories, Abbott Park, North Chicago, IL, USA); HBV DNA was analyzed with COBAS AmpliPrep /COBAS TaqMan HBV Test, v2.0 (Roche, Basel, Switzerland) according to the manufacturer's instruction. The serological results were defined by the following cut-off values: positive hepatitis B surface antigen (HBsAg) if \( \geq 0.05 \) IU/mL; positive anti-hepatitis B surface antibody (anti-HBsAb) with a threshold of 10 mIU / mL \([19]\); positive anti-hepatitis B core antibody (anti-HBcAb) if \( \geq 1.0 \). Chronic hepatitis B carrier status was defined by the detection of positive hepatitis B surface antigen (HBsAg) for more than 6 months. Hepatitis B reactivation was defined as a greater than 10-fold increase, compared with previous nadir levels, of HBV DNA or by the re-appearance of hepatitis B "e" antigen in the serum for patients whose baseline HBeAg was negative. HBV-related hepatitis was defined as a greater than 3-fold increase of ALT level (the upper normal limit is 41 U/L at NTUH) accompanying or following HBV reactivation. In July 1984, the Taiwan government launched a nationwide universal HBV vaccination program \([20]\). The following catch-up program of the nationwide HBV vaccination had a coverage rate of 86.9 to 98.0% \([21]\).

The diagnosis of cirrhosis was based on clinical symptoms / signs and imaging study (ultrasound, computed tomography). The diagnostic criteria of hepatocellular carcinoma(HCC) in this study was based on histologic and/or clinical findings and on the presence of all of the following criteria: chronic viral hepatitis infection and liver cirrhosis, hepatic tumor with imaging (ultrasound, computed tomography) characteristics compatible with a diagnosis of HCC and without evidence of gastrointestinal or other primary tumor, and a persistent elevation of the serum level of alpha-fetoprotein (AFP) to 400 ng / mL or higher.

Chemotherapy of acute myeloid leukemia
Standard induction chemotherapy consisting of cytarabine and anthracycline were used for patients with AML at NTUH. Standard dose chemotherapy was defined as cytarabine 100mg / m\(^2\) body surface area administered as a continuous infusion for seven days. Low dose chemotherapy was defined as cytarabine 10 to 20 mg / m\(^2\) body surface area for 7 to 14 days. Consolidation chemotherapy consisted of high-dose cytarabine-based regimens. Steroid was used routinely for prevention of fever and skin rash in AML patients receiving high dose cytarabine chemotherapy. Methylprednisolone 40 mg twice daily by intravenous injection was administered before cytarabine for 6 to 8 doses. Patients with acute promyelocytic leukemia (APL) were treated with all trans retinoic acid (ATRA) in combination with anthracycline-based chemotherapy. Hypomethylating agents such as azacitidine were given at a dose of 75 mg / m\(^2\) body surface area for 7 days every 4 weeks.

Statistical analysis
Survival was estimated by the Kaplan-Meier analysis and compared using the log-rank test. Categorical variables were compared using the Chi-square test. Univariate and multivariate analyses of factors associated with time to HBV reactivation were performed using the Cox
proportional hazards model. Factors with p-value of ≤0.10 in the univariate analysis were considered in the multivariate model. All statistical analyses were performed using the statistical package SPSS for Windows v.18 (SPSS Inc., Chicago, IL). A p-value of ≤0.05 was considered significant and all statistical tests were two-tailed.

Results

Epidemiology

There were 593 AML patients diagnosed and regularly followed at NTUH during the study period. Four hundred and ninety patients with at least one hepatitis B serology test were enrolled in this study, of which 265 were men and 225 were women. The median age was 52 years and ranged from 18 to 94 years. The median observation period was 596 days (ranged from one to 3065 days). The cytogenetic of this population compromised 234 patients with normal karyotype, 27 patients with t(8;21), 35 patients with t(15;17), 21 patients with inv(16), 13 patients with chromosomal 11q23 changes, 15 patients with del(5q)/monosomy5 or del(7q)/monosomy7, 57 patients with complex (i.e. more than three) chromosomal changes, and 80 patients with other simple chromosomal changes. There were 6 patients whose cytogenetic study revealed no mitosis and 2 patients without cytogenetic analysis. There were 35 patients diagnosed as acute promyelocytic leukemia. The factors of cytogenetic change, leukemic subtype, preceding myelodysplastic syndrome and therapy-related were not significantly correlated with HBV reactivation and carrier status.

The clinical characteristics of these 490 AML patients are shown in Table 1. At the time of leukemia diagnosis, there were 57 patients with positive HBsAg giving an estimated prevalence of 11.6% and 433 patients with negative HBsAg (Fig 1). The anti-HBsAb serology was positive in 324 of 433 patients, negative in 50 patients, and not tested in 59 patients. Fifteen (3%) patients had chronic hepatitis C at diagnosis of leukemia and two patients had both hepatitis B and C. The incidence of hepatitis B reactivation and HBV-related hepatitis were 9.5 and 8.3 per 100 person-years among chronic HBV carriers of AML patients. Three of 32 HBV vaccinees who were born after July 1984 were HBV carriers.

Clinical characteristics and outcome

Comparing HBs carriers to non-HBs patients, the median HBsAg was ≥ 250 IU/mL (n = 57, range 0.72 to ≥250 IU/mL) v.s 0 IU/mL (n = 433, range 0 to 0.04 IU/mL); median anti-HBsAb was 0.08 mIU/mL (n = 40, range 0 to 16.37 mIU/mL) v.s 90.21 mIU/mL (n = 374, range 0 to ≥1000 mIU/mL); median Anti-HBcAb was 12.75 (n = 17, range 10.58 to 18.12) v.s 3.97 (n = 204, range 0.17 to 20.74); median HBeAg was 0.32 (n = 33, range 0.16 to 1568.99) v.s 0.27 (n = 27, range 0.0 to 0.601); and median anti-HBeAb was 0.02 (n = 25, range 0.0 to 59.53) v.s 1.14 (n = 15, range 0.08 to 2.22), at initial diagnosis of leukemia, respectively.

Twenty AML patients suffered from hepatitis B reactivation, including 16 (28%) of 57 patients with positive HBsAg and 4 (2.8%) of 142 patients with positive anti-HBsAb and anti-HBcAb. The median time to hepatitis B reactivation from time of leukemia diagnosis was 276 days (range from 32 to 718 days). Hepatitis B reactivation is significantly higher in AML patients with positive HBsAg than the patients with positive anti-HBsAb and anti-HBcAb (16/57 (28%), vs 4/142 (2.8%), p<0.001). The median HBV DNA viral load at the time of HBV reactivation was 1.88X10^6 IU/mL. The baseline serum HBV DNA and HBsAg levels were not associated with HBV reactivation in HBsAg-positive patients. The median peak ALT level was 361 U/L (range from 51 to 2182) in the patients with hepatitis B reactivation. There were 18 patients with documented HBV-related hepatitis, including 14 patients who were chronic
Table 1. Clinical characteristics of 490 acute myeloid leukemia patients with positive and negative hepatitis B surface antigen (HBsAg).

|                          | Positive HBsAg (n = 57) | Negative HBsAg (n = 433) | p value |
|--------------------------|-------------------------|--------------------------|---------|
| **Age**                  |                         |                          | 0.104   |
| ≥65 years                | 9                       | 115                      |         |
| <65 years                | 48                      | 318                      |         |
| **Gender**               |                         |                          | 0.482   |
| Men                      | 30                      | 235                      |         |
| Women                    | 27                      | 198                      |         |
| **Hypertension**         |                         |                          | 0.593   |
| Yes                      | 9                       | 86                       |         |
| No                       | 48                      | 347                      |         |
| **Diabetes mellitus**    |                         |                          | 0.492   |
| Yes                      | 4                       | 46                       |         |
| No                       | 53                      | 387                      |         |
| **HSCT**                 |                         |                          | 0.205   |
| Yes                      | 20                      | 114                      |         |
| No                       | 37                      | 319                      |         |
| **Liver Cirrhosis**      |                         |                          | 0.013   |
| Yes                      | 2                       | 0                        |         |
| No                       | 55                      | 433                      |         |
| **Hepatocellular carcinoma** |                    |                          | 0.013   |
| Yes                      | 2                       | 0                        |         |
| No                       | 55                      | 433                      |         |
| **HBV Vaccination**      |                         |                          | 1.000   |
| Yes                      | 3                       | 29                       |         |
| No                       | 54                      | 404                      |         |
| **HBV reactivation**     |                         |                          | <0.001  |
| Yes                      | 16                      | 4                        |         |
| No                       | 41                      | 429                      |         |
| **Hepatitis C**          |                         |                          | 0.690   |
| Yes                      | 2                       | 13                       |         |
| No                       | 55                      | 420                      |         |
| **Preceding MDS**        |                         |                          | 1.000   |
| Yes                      | 2                       | 21                       |         |
| No                       | 55                      | 412                      |         |
| **Acute promyelocytic leukemia** |          |                          | 1.000   |
| Yes                      | 4                       | 31                       |         |
| No                       | 53                      | 402                      |         |
| **Cytogenetics***        |                         |                          | 0.210   |
| Good                     | 11                      | 72                       |         |
| Intermediate             | 30                      | 284                      |         |
| Poor                     | 15                      | 70                       |         |
| unknown                  | 1                       | 7                        |         |
| **Initial Treatment**    |                         |                          | 0.417   |
| Supportive Care          | 2                       | 24                       |         |
| Standard dose chemotherapy | 1                     | 21                       |         |
| Low dose chemotherapy    | 1                       | 9                        |         |
| Oral chemotherapy        | 48                      | 313                      |         |
| Hypomethylating agent    | 5                       | 66                       |         |

(Continued)
hepatitis B carriers and four patients with positive anti-HBsAb and anti-HBcAb. One HBsAg positive patients died of fulminant hepatitis B.

Prophylaxis of Hepatitis B reactivation in the AML patients

Currently, there is no consensus for antiviral prophylaxis for AML patients with HBV carrier status in Taiwan. In clinical practice, antiviral prophylaxis generally starts at diagnosis of leuke-

mia, and continues for at least one year after chemotherapy according to the clinical condition. Accordingly, forty-six of 57 positive HBsAg patients received HBV prophylaxis with antiviral agents after diagnosis of leukemia; 28 patients received lamivudine, 13 patients received enteca-

vir, two patients received adefovir, two patients received telbivudine, and one patient received
tenofovir. Ten patients were not prescribed any prophylaxis after diagnosis of leukemia. Over-

all, sixteen of 57 positive HBsAg patients suffered from recrudescence of hepatitis B virus dur-

ing or after chemotherapy, including four patients who were antiviral-naive, seven patients

who had discontinued initial HBV prophylaxis after a median of 118 days (range, 30 to 447
days), and five patients who were taking antiviral prophylaxis but still had hepatitis B reactiva-

tion. The latter five were under antiviral (lamivudine n = 2, adefovir n = 1, telbivudine n = 1,
entecavir n = 1,) prophylaxis at the time of HBV reactivation, documented by a 10-fold or
more elevation of hepatitis B viral load and accompanied by a median rise of ALT to 116 U/L,
range 84–378 U/L). These five patients had good antiviral compliance. The durations from antiviral therapy to hepatitis B reactivation were 4, 12, 7, 14, and 3 months, respectively. Case 2 and case 4 had received hematopoietic stem cell transplantation (HSCT) and hepatitis B reactiva-

tion developed after 2 and 7 months after HSCT. The two patients under lamivudine pro-

phylaxis with HBV reactivation were subsequently shown to harbor HBV virus with the
YMDD mutation. Nevertheless, AML patients with positive HBsAg under antiviral prophylaxis
had significantly decreased risks of hepatitis B reactivation than AML patients with positive
HBsAg without prophylaxis (5 of 39 (13%), vs. 11 of 18 (61%), p <0.001).

Risk Factors For hepatitis B reactivation

We compared the 20 patients who had hepatitis B reactivation and the other 419 patients who
did not have hepatitis B reactivation to evaluate the risk factors for hepatitis B reactivation
among patients previously exposed to HBV (Table 2). Fifty-one patients with negative anti-
HBcAb were excluded. By univariate analysis, diabetes mellitus (p = 0.006), hematopoietic
stem cell transplant (p = 0.014), liver cirrhosis (p = 0.002), positive HBsAg (p<0.001) and age
less than 65 years (p = 0.020) appeared to be associated with HBV reactivation. By multivariate
analysis, diabetes mellitus (p = 0.008, odds ratio (OR) = 2.841, 95% confident interval (CI):
0.985–8.193), and positive HBsAg (p<0.001, OR = 36.878, 95% CI: 11.770–115.547) were independent predictors of hepatitis B reactivation among AML patients.

The subgroup analysis for HBV reactivation in 57 HBsAg-positive patients is shown in Table 3. Univariate analysis showed diabetes mellitus was associated with higher risk of HBV reactivation (p = 0.005) and antiviral therapy with less risk of hepatitis B reactivation (p<0.001). Multivariate analysis showed that HBsAg positive patients who had received antiviral therapy had significantly lower risk of hepatitis B reactivation (p = 0.033, odds ratio (OR) = 0.094, 95% confident interval (CI): 0.025–0.355).

Four of 142 negative HBsAg AML patients had hepatitis B reactivation. Their unusual clinical courses are shown in Table 4. All four patients had seroconverted with positive anti-HBsAb

---

**Fig 1. Study flow and hepatitis B serological data of 490 AML patients at Leukemia Diagnosis.**

doi:10.1371/journal.pone.0126037.g001
Table 2. Overall risk factors for hepatitis B reactivation in 439 acute myeloid leukemia patients*.

|                          | No Reactivation (n = 419) | Reactivation (n = 20) | Univariate P value | Multivariate P value | Odds ratio (95% CI) |
|--------------------------|--------------------------|-----------------------|--------------------|----------------------|-------------------|
| Age                      |                          |                       | 0.020              | 0.996                | NA                |
| <65 years                | 299                      | 19                    |                    |                      |                   |
| ≥65 years                | 120                      | 1                     |                    |                      |                   |
| Gender                   |                          |                       | 0.255              |                      |                   |
| Women                    | 192                      | 12                    |                    |                      |                   |
| Men                      | 227                      | 8                     |                    |                      |                   |
| Hypertension             |                          |                       | 0.778              |                      |                   |
| No                       | 331                      | 17                    |                    |                      |                   |
| Yes                      | 88                       | 3                     |                    |                      |                   |
| Diabetes                 |                          |                       | 0.060              | 0.008                | 2.841 (0.985–8.193) |
| No                       | 375                      | 15                    |                    |                      |                   |
| Yes                      | 44                       | 5                     |                    |                      |                   |
| HSCT*                    |                          |                       | 0.014              | 0.178                | NA                |
| No                       | 321                      | 10                    |                    |                      |                   |
| Yes                      | 88                       | 10                    |                    |                      |                   |
| Liver cirrhosis          |                          |                       | 0.002              | >0.999               | NA                |
| No                       | 419                      | 18                    |                    |                      |                   |
| Yes                      | 0                        | 2                     |                    |                      |                   |
| Hepatocellular carcinoma|                          |                       | 0.089              | >0.999               | NA                |
| No                       | 418                      | 19                    |                    |                      |                   |
| Yes                      | 1                        | 1                     |                    |                      |                   |
| HBV Vaccination          |                          |                       | >0.999             |                      |                   |
| No                       | 398                      | 19                    |                    |                      |                   |
| Yes                      | 21                       | 1                     |                    |                      |                   |
| Hepatitis B carrier      |                          |                       | <0.001             | <0.001               | 36.878 (11.770–115.547) |
| No                       | 378                      | 4                     |                    |                      |                   |
| Yes                      | 41                       | 16                    |                    |                      |                   |
| Hepatitis C              |                          |                       | 0.509              |                      |                   |
| No                       | 405                      | 19                    |                    |                      |                   |
| Yes                      | 14                       | 1                     |                    |                      |                   |
| Preceding MDS²           |                          |                       | 0.614              |                      |                   |
| No                       | 398                      | 20                    |                    |                      |                   |
| Yes                      | 21                       | 0                     |                    |                      |                   |
| APL³                     |                          |                       | >0.999             |                      |                   |
| No                       | 387                      | 19                    |                    |                      |                   |
| Yes                      | 32                       | 1                     |                    |                      |                   |
| Cytogenetic⁴             |                          |                       | 0.235              |                      |                   |
| Good                     | 66                       | 6                     |                    |                      |                   |
| Intermediate             | 270                      | 10                    |                    |                      |                   |
| Poor                     | 76                       | 13                    |                    |                      |                   |
| Unknown                  | 7                        | 1                     |                    |                      |                   |
| Treatment                |                          |                       | 0.200              |                      |                   |
| Supportive               | 26                       | 0                     |                    |                      |                   |
| Standard chemotherapy    | 293                      | 19                    |                    |                      |                   |
| Low dose chemotherapy    | 68                       | 1                     |                    |                      |                   |

(Continued)
and anti-HBcAb at the time of diagnosis of leukemia. Three of the four patients had relative lower anti-HBsAb serology titer before hepatitis B reactivation (24.95 IU/mL, 18.68 mIU /mL and 28.19 mIU /mL, respectively). Two patients (case no. 2 and no. 3) had received hematopoietic stem cell transplantation with long-term immunosuppressant medication and hepatitis B reactivation after 12 and 10 months of transplantation. Of the other two patients (case no. 1 and no. 4) who did not received transplantation, reactivation of hepatitis B occurred 7 and 11 months after chemotherapy. All four cases had hepatitis (the liver transaminases were greater than 100 U/L), and three died of leukemia within 4 months.

Discussion

There is still controversy regarding the role of screening for hepatitis B reactivation in cancer patients. The American Society of Clinical Oncology Provisional Clinical Opinion reported that the evidence is insufficient to determine the net benefits and harms of routine screening for chronic HBV infection in individuals with cancer who are about to receive cytotoxic or immunosuppressive therapy or who are already receiving such therapy [22]. To the best of our knowledge, this is the first large cohort study to investigate hepatitis B reactivation in AML patients. In this study that was conducted in a country of HBV hyperendemicity, we showed that AML patients had a similar risk for HBsAg sero-positivity (11.6%), because most exposure to HBV that results in either development of anti-HBs antibody or chronic carriers of HBV in Taiwan before the implementation of the nationwide HBV vaccination program, occurred during the perinatal period or in early childhood. Our results showed that AML patients, especially those with diabetes and with HBsAg carriage, are also at high risk for hepatitis B reactivation. Prophylaxis with antiviral therapy significantly decreased the risk of hepatitis B reactivation. However, development of lamivudine resistance during antiviral therapy should be suspected if one observes a rebound of HBV plasma viral loads.

The incidence of hepatitis B reactivation and HBV-related hepatitis in our study were 9.5 and 8.3 per 100 person-years in AML patients who are also chronic hepatitis B carriers, which is similar to the incidence of hepatitis B reactivation in lymphoma patients (10.4 per 100 person-years) [11, 23]. While Nakamura et al. [24] reported that 10 of 13 (76.9%) AML patients had severe hepatitis due to HBV in 85 patients with hematological malignancy; Yeo et al. [10]
found that patients with either leukemia or myeloma were at comparatively less risk of hepatitis B reactivation than patients with lymphoma. Since fulminant hepatitis B is a catastrophic event for HBsAg positive AML patients [6, 25, 26], periodic assessment of liver function and follow up of HBV serological status is important during chemotherapy. Further prospective cohort studies of patients with AML especially in the endemic hepatitis B area should be undertaken to capture the true incidence of HBV flare-ups.

In this study, AML patients with positive HBsAg under antiviral agents were significantly protected from hepatitis B reactivation. We therefore recommend that all HBsAg positive AML patients should be initiated on antiviral prophylaxis prior to chemotherapy. Although, there is no current consensus for antiviral prophylaxis for AML patients with HBV carrier status in Taiwan, the National Comprehensive Cancer Network (NCCN) guidelines indeed have some recommendation with regards to patients with high risk for HBV [27]. Moreover, the major HBV treatment guidelines proposals originating from America, Europe, and the Asia-Pacific region, recommended that all cancer patients should check HBV markers, including

| Table 3. Risk factors for hepatitis B reactivation in the subgroup of AML patients with positive HBsAg (n = 57). |
|---------------------------------------------------------------|
| **Reactivation (n = 16)** | **No reactivation (n = 41)** | **Univariate P value** | **Multivariate P value** | **Odds ratio (95% CI)** |
| Age | | | | |
| ≥65 years | 1 | 8 | | |
| <65 years | 15 | 33 | | |
| Gender | | | | |
| Men | 5 | 25 | 0.075 | 0.106 | NA |
| Women | 11 | 16 | | |
| Hypertension | | | | |
| Yes | 2 | 7 | 1.000 | | |
| No | 14 | 34 | | |
| Diabetes mellitus | | | | |
| Yes | 4 | 0 | 0.005 | 0.999 | NA |
| No | 12 | 41 | | |
| HSCT | | | | |
| Yes | 8 | 12 | | |
| No | 8 | 29 | | |
| Liver cirrhosis | | | | |
| Yes | 2 | 0 | 0.075 | 0.999 | NA |
| No | 14 | 41 | | |
| Hepatocellular carcinoma | | | | |
| Yes | 1 | 1 | | |
| No | 15 | 40 | | |
| Antiviral prophylaxis | | | | |
| Yes | 5 | 34 | <0.001 | 0.033 | 0.094(0.025–0.355) |
| No | 11 | 7 | | |
| Hepatitis C | | | | |
| Yes | 1 | 1 | | |
| No | 15 | 40 | | |
| Survival | | | | |
| Death | 9 | 22 | 1.000 | | |
| Alive | 7 | 31 | | |

HSCT: Hematopoietic stem cell transplantation, NA: not available

doi:10.1371/journal.pone.0126037.t003
HBsAg and anti-HBc, prior to initiation of chemotherapy, and to use routine prophylactic antiviral therapy for individuals who are positive for HBsAg before the start of cancer chemotherapy [28–30]. The optimal timing, duration and choice of anti-HBV agent for prophylaxis remain unknown [30–32]. In our study cohort, despite antiviral prophylaxis, seven AML patients experienced hepatitis B reactivation after discontinuation of prophylaxis. Hence, close monitoring of the liver functional assays as well as hepatitis B plasma viral loads may be indicated in the first year after discontinuation of antiviral prophylaxis in patients with ongoing immune suppression. Of the patients with reactivation under ongoing anti-HBV prophylaxis, two of the four patients underwent genotypic resistance testing. Both patients had proven YMDD (rtM204I/V) mutation under lamivudine prophylaxis. Hence, the risk for emerging resistance should be considered in the event of virologic breakthrough during lamivudine therapy. Resistance to other anti-HBV viral agent in the first year is less frequently encountered.

In this study, four patients with positive anti-HBsAb lost seroprotection with reappearance of HBsAg. Of cancer patients receiving chemotherapy or transplantation, anti-HBsAb levels might decline below the threshold for protection against new HBV infection or reactivation [33–36]. Although HBV vaccination is highly immunogenic and efficacious in inducing protective antibody against HBV [37], a gradual decline in titers decades after vaccination have been reported [38]. Moreover, serologic evidence of recovery from hepatitis B infection does not preclude its reactivation after immunosuppression. Thus, screening for serologic evidence of hepatitis B and booster HBV vaccination for those without protective anti-HBsAb should be considered in individuals in whom immunosuppressive therapy is planned.

The finding that diabetes mellitus is associated with HBV reactivation is biologically plausible since the hyperglycemic environment increases the virulence of viral pathogens. The underlying mechanisms may involve reduced production of interleukins in response to infection.
reduced chemotaxis and phagocytic activity, and reduced humoral immunity [39]. Wang et al. reported diabetes was associated with serum alanine aminotransferase activity elevation in patients with hepatitis B infection [40]. Diabetes mellitus has also been documented as an independent risk factor for HBV related hepatocellular carcinoma [41] and occult HBV infection [42]. In general, diabetic patients are considered to be relatively immunocompromised. However, the exact role of hyperglycemia in hepatitis B reactivation should be further clarified.

This study suffered from several limitations. Since the study is a retrospective review, the clinical data was derived from medical records in accordance to clinical practice. Therefore, data were limited or unavailable for several variables. Hence, subclinical hepatitis or asymptomatic reactivations, newly acquired HBV infection or seroconversion of HBsAg may be potentially missed. In addition, patients with early mortality during AML treatment may have contributed to underestimated risks of HBV reactivation.

In conclusion, hepatitis B virus on reactivation is not uncommon in HBsAg positive AML patients receiving or undergoing chemotherapy. Prophylaxis or early preemption with an anti-HBV agent significantly reduced the risk of hepatitis B reactivation and HBV related hepatitis. Diabetes mellitus and chronic hepatitis B carrier status were independent risk factors of hepatitis B reactivation in AML patients.

Acknowledgments
We thank the staff of the Eighth Core Lab., Department of Medical Research, National Taiwan University Hospital, for their technical support during the study.

Author Contributions
Conceived and designed the experiments: CYC AC SYH WHS. Performed the experiments: CYC AC SYH WHS. Analyzed the data: CYC AC SYH WHS. Contributed reagents/materials/analysis tools: WCC WT MY JLT HFT. Wrote the paper: CYC AC SYH WHS.

References
1. Estey E, Döhner H. (2006) Acute myeloid leukemia. Lancet. 368: 1894–1907. PMID: 17126723
2. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. (2002) CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 346: 235–242. PMID: 11807147
3. Velasquez WS, McLaughlin P, Tucker S, Hagemeister FB, Swan F, Rodriguez MA, et al. (1994) ESHAP—an effective chemotherapy regimen in refractory and relapsing lymphoma: a 4-year follow-up study. J Clin Oncol. 12: 1169–1176. PMID: 8201379
4. Khouri IF, Romaguera J, Kantarjian H, Palmer JL, Pugh WC, Korbling M, et al. (1998) Hyper-CVAD and high-dose methotrexate/cytarabine followed by stem-cell transplantation: an active regimen for aggressive mantle-cell lymphoma. J Clin Oncol. 16: 3803–3809. PMID: 9850025
5. Čelkovská P, Pizinger K, Čelkovský P. (2002) High-dose cytosine arabinoside-induced cutaneous reactions. J Eur Acad Dermatol Venereol. 16: 481–485. PMID: 12428842
6. Ishiga K, Kawatani T, Suou T, Tajima F, Omura H, Idobe Y, et al. (2001) Fulminant hepatitis type B after chemotherapy in a serologically negative hepatitis B virus carrier with acute myelogenous leukemia. Int J Hematol. 73: 115–118. PMID: 11372746
7. Wong GC, Tan P, Goh YT, Ng HS, Chong R, Lee LH. (1996) Exacerbation of hepatitis in hepatitis B carriers following chemotherapy for haematological malignancies. Ann Acad Med Singapore. 25: 500–503. PMID: 8993918
8. Dienstag JL. (2008) Hepatitis B virus infection. N Engl J Med. 359: 1486–1500. doi: 10.1056/NEJMra0801644 PMID: 18832247
9. Shouval D, Shibolet O. (2013) Immunosuppression and HBV reactivation. Semin Liver Dis. 33: 167–177. doi: 10.1055/s-0033-1345722 PMID: 23749673
10. Yeo W, Chan PK, Zhong S, Ho WM, Steinberg JL, Tam JS, et al. (2000) Frequency of hepatitis B virus reactivation in cancer patients undergoing cytotoxic chemotherapy: a prospective study of 626 patients with identification of risk factors. J Med Virol. 62: 299–307. PMID:11055239
11. Hsu C, Tsou HH, Lin SJ, Wang MC, Yao M, Hwang WL, et al. (2014) Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: A prospective study. Hepatology. 59: 2092–2100. doi:10.1002/hep.26718 PMID:24002804
12. Cheng AL, Hsiung CA, Su J, Chen PJ, Chang MC, Tsao CJ, et al. (2003) Steroid-free chemotherapy decreases risk of hepatitis B virus (HBV) reactivation in HBV-carriers with lymphoma. Hepatology. 37: 1320–1328. PMID:12774010
13. Yeo W, Zee B, Zhong S, Chan PK, Wong WL, Ho WM, et al. (2004) Comprehensive analysis of risk factors associating with Hepatitis B virus (HBV) reactivation in cancer patients undergoing cytotoxic chemotherapy. Br J Cancer. 90: 1306–1311. PMID:15054446
14. Kusumoto S, Tanaka Y, Ueda R, Mizokami M. (2011) Reactivation of hepatitis B virus following rituximab-plus-steroid combination chemotherapy. J Gastroenterol. 46: 9–16. doi:10.1007/s00535-010-0331-4 PMID:20924616
15. Tsutsumi Y, Yamamoto Y, Shimojo N, Ohhigashi H, Teshima T. (2013) Hepatitis B virus reactivation with rituximab-containing regimen. World J Hepatol. 5: 612–620. doi:10.4254/wjh.v5.i11.612 PMID:24303089
16. Martin BA, Rowe JM, Koides WA, DiPersio JF. (1995) Hepatitis B reactivation following allogeneic bone marrow transplantation: case report and review of the literature. Bone Marrow Transplant. 15: 145–148. PMID:7742749
17. Liang R, Lau GK, Kwong YL. (1999) Chemotherapy and bone marrow transplantation for cancer patients who are also chronic hepatitis B carriers: a review of the problem. J Clin Oncol. 17: 394–398. PMID:10458258
18. Sung J, Chen DS, Lai MY, Yu JY, Wang TH, Wang CY, et al. (1984) Epidemiological study on hepatitis B virus infection in Taiwan. Chin. J. Gastroenterol. 1: 1–9
19. ul-Haq N, Hasnain SS, Umar M, Abbas Z, Valenzuela-Silva C, Lopez-Saura P. (2003) Immunogenicity of 10 and 20 microgram hepatitis B vaccine in a two-dose schedule. Vaccine. 21: 3179–3185. PMID:12804846
20. Chang MH, Chen CJ, Lai MS, Hsu HM, Wu TC, Kong MS, et al. (1997) Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med. 336: 1855–1859. PMID:9197213
21. Ni YH, Huang LM, Chang MH, Yen CJ, Lu CY, You SL, et al. (2007) Two decades of universal hepatitis B vaccination in Taiwan: impact and implication for future strategies. Gastroenterology 132: 1287–1293. PMID:17433322
22. Arzt AS, Somerfield MR, Feld JJ, Giusti AF, Kramer BS, Sabichi AL, et al. (2010) American Society of Clinical Oncology provisional clinical opinion: chronic hepatitis B virus infection screening in patients receiving cytotoxic chemotherapy for treatment of malignant diseases. J Clin Oncol. 28: 3199–3202. doi:10.1200/JCO.2010.30.0673 PMID:20516452
23. Manzano-Alonso ML, Castellano-Tortajada G. (2011) Reactivation of hepatitis B virus infection after cytotoxic chemotherapy or immunosuppressive therapy. World J Gastroenterol. 17: 1531–1537. doi: 10.3748/wjg.v17.i12 PMID:21427116
24. Nakamura Y, Motokura T, Fujita A, Yamashita T, Ogata E. (1996) Severe hepatitis related to chemotherapy in hepatitis B virus carriers with hematologic malignancies. Survey in Japan, 1987–1991. Cancer. 76: 2210–2215. PMID:8918416
25. Kojima H, Abei M, Takei N, Mukai Y, Hasegawa Y, Iijima T, et al. (2002) Fatal reactivation of hepatitis B virus following cytotoxic chemotherapy for acute myelogenous leukemia: fibrosing cholestatic hepatitis. Eur J Haematol. 69: 101–104. PMID:12366713
26. Yujiri T, Tanaka M, Taguchi A, Tanaka Y, Nakamura Y, Tanizawa Y. (2014) Reactivation of hepatitis B virus in a hepatitis B surface antigen-negative patient with acute promyelocytic leukemia treated with arsenic trioxide. Ann Hematol. 93: 351–352. doi: 10.1007/s00277-013-1804-3 PMID:23728611
27. Baden LR, Bensinger W, Angarone M, Casper C, Dubberke ER, Freifeld AG, et al. (2012) Prevention and treatment of cancer-related infections. J Natl Compr Canc Netw. 10: 1412–1445. PMID:23138169
28. Lok AS, McMahon BJ. (2009) Chronic hepatitis B: update 2009. Hepatology. 50: 661–662. doi: 10.1002/hep.23190 PMID:19714720
29. European Association For The Study Of The Liver. (2012) EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. J Hepatol. 57: 167–185. doi:10.1016/j.jhep.2012.02.010 PMID:22436845
30. Liaw YF, Kao JH, Piratvisuth T, Chan LY, Chien RN, Liu CJ, et al. (2012) Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int 6: 531–561.

31. Koh C, Zhao X, Samala N, Sakiani S, Liang TJ, Talwalkar JA. (2013) AASLD clinical practice guidelines: a critical review of scientific evidence and evolving recommendations. Hepatology. 58:2142–52. doi: 10.1002/hep.26578 PMID: 23775835

32. Bihl F, Alaei M, Negro F. (2010) The new EASL guidelines for the management of chronic hepatitis B infection adapted for Swiss physicians. Swiss Med Wkly. 140:154–9. doi: smw-12969 PMID: 20104374

33. Wu C, Shi H, Lu M, Xu Y, Chen X. (2013) A case of hepatitis B reactivation in an anti-HBs positive, anti-HBc positive non-Hodgkin’s lymphoma patient. Virol Sin. 28: 49–52. doi: 10.1007/s12250-013-3285-x PMID: 23385354

34. Koo YX, Tay M, Teh YE, Teng D, Tan DS, Tan IB, et al. (2011) Risk of hepatitis B virus (HBV) reactivation in hepatitis B surface antigen negative/hepatitis B core antibody positive patients receiving rituximab-containing combination chemotherapy without routine antiviral prophylaxis. Ann Hematol. 90:1219–1223. doi: 10.1007/s00277-011-1241-0 PMID: 21520001

35. Sarrecchia C, Cappelli A, Aiello P. (2005) HBV reactivation with fatal fulminating hepatitis during rituximab treatment in a subject negative for HBsAg and positive for HBsAb and HBcAb. J Infect Chemother. 11: 189–191. PMID: 16133710

36. Palmore TN, Shah NL, Loomba R, Borg BB, Lopatin U, Feld JJ, et al. (2009) Reactivation of Hepatitis B with Reappearance of Hepatitis B Surface Antigen After Chemotherapy and Immune Suppression. Clin Gastroenterol Hepatol. 7: 1130–1137. doi: 10.1016/j.cgh.2009.06.027 PMID: 19577007

37. Ni YH, Chang MH, Huang LM, Chen HL, Hsu HY, Chiu TY, et al. (2001) Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. Ann Intern Med. 135: 796–800. PMID: 11694104

38. Lu CY, Chiang BL, Chi WK, Chang MH, Ni YH, Hsu HM, et al. (2004) Waning immunity to plasma-derived hepatitis B vaccine and the need for boosters 15 years after neonatal vaccination. Hepatology. 40: 1415–1420. PMID: 15565627

39. Casqueiro Juliana, Casqueiro Janine, Alves Cresio. (2012) Infections in patients with diabetes mellitus: A review of pathogenesis. Indian J Endocrinol Metab. 16(Suppl1): S27–S36.

40. Wang YY, Lin SY, Sheu WH, Liu PH, Tung KC. (2010) Obesity and diabetic hyperglycemia were associated with serum alanine aminotransferase activity in patients with hepatitis B infection. Metabolism. 2010; 59: 486–491. doi: 10.1016/j.metabol.2009.07.038 PMID: 19846182

41. Gao C, Zhao HC, Li JT, Yao SK. (2010) Diabetes mellitus and hepatocellular carcinoma: Comparison of Chinese patients with and without HBV-related cirrhosis. World J Gastroenterol. 16: 4467–4475. PMID: 20845516

42. Demir M, Serin E, Göktürk S, Oztürk NA, Kulaksizoglu S, Yılmaz U. (2008) The prevalence of occult hepatitis B virus infection in type 2 diabetes mellitus patients. Eu J Gastroenterol Hepatol. 20: 668–673.