

Human cytomegalovirus and Epstein-Barr virus infections increase the risk of death in patients with head and neck cancers receiving radiotherapy or radiochemotherapy

Dorota Kiprian, MD, PhD^a, Bożena Czarkowska-Paczek, MD, PhD^{b,*}, Aleksandra Wyczalkowska-Tomasik, PhD^c, Leszek Paczek, MD, PhD, Prof^{c,d}

Abstract

Viral infections, including cytomegalovirus (CMV) and Epstein-Barr virus (EBV), play an important role in carcinogenesis and can influence patients' prognosis and condition during cancer treatment.

The goal of this study was to investigate CMV and EBV infections in patients receiving radiotherapy or radiochemotherapy due to head and neck cancers to determine the influence of these infections on the risk of death. The observation period was 2 years.

Of 41 patients enrolled, 11 received radiotherapy (simultaneous-integrated boost intensity-modulated radiation therapy [SIB-IMRT], 2.25 Gy/fraction, 30 fractions, [n=7] or IMRT, 2 Gy per fraction, 35 fractions, [n=4]) and 30 received radiochemotherapy (cisplatin 100 mg/m² and SIB-IMRT [n=13] or IMRT [n=17]). Plasma CMV and EBV DNA levels were assessed using real-time PCR before or during treatment or 4 weeks posttreatment.

The risk of death in the group positive for plasma CMV or EBV deoxyribonucleic acid (DNA) was significantly higher compared to the group without detectable plasma CMV (odd ratio [OR]: 7.5, 95% confidence interval [CI]: 1.11–50.67) or EBV DNA (OR: 10.91, 95% CI: 1.135–104.8). Results were confirmed using the Bayesian method. Plasma positivity for CMV or EBV DNA was associated with a higher risk of death (both $P=.04$).

Viral infections negatively affect the survival of patients with head and neck cancers. Diagnosing and treating these viral infections in patients with positive results should be considered.

Abbreviations: CI = confidence intervals, CMV = cytomegalovirus, COX-2 = cyclooxygenase-2, DNA = deoxyribonucleic acid, EBV = Epstein-Barr virus, FPI = focus primarius ignotus, HIV = human immunodeficiency virus, IL-6 = interleukin 6, IMRT = intensity-modulated radiation therapy, LMP-1 = latent membrane protein-1, OR = odds ratio, SCT = stem cell transplant, SIB-IMRT = simultaneous-integrated boost intensity-modulated radiation therapy, TNM = tumor-node-metastasis classification of the International Union Against Cancer, VEGF = vascular endothelial growth factor.

Keywords: CMV, EBV, head and neck carcinomas, incidence of death

1. Introduction

Viral infections including Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are considered to play an important role in

carcinogenesis. Viral infections could also influence patients' prognosis and condition during cancer treatment.

The CMV is a herpes virus carried by 70 to 100% of the world's population; however, in most cases it is asymptomatic. The CMV can be detected in solid malignant tumours; for example, glioblastoma multiforme, colon cancer, breast cancer, and others. The CMV is not believed to be oncogenic, rather it contributes to cancer development.^[1] The EBV also belongs to the herpes family and infection with EBV is ubiquitous in most humans, with no obvious symptoms. The EBV is an etiological agent of infectious mononucleosis, and is also associated with Burkitt's lymphoma, oral cancers, and nasopharyngeal cancer.^[2,3] In addition to the suspected role of these viruses in the development of particular cancers, latent infections could become reactivated during radiochemotherapy applied during cancer treatment. In patients treated with radiochemotherapy due to brain gliomas, 48% developed CMV viremia during or up to 28 days after the cessation of treatment, and 87% of them required specific anti-viral treatment for CMV-associated encephalitis. The reactivation of CMV could also explain some deaths.^[4,5] Data in the literature regarding EBV infection mostly refers to patients with nasopharyngeal cancers. Patients with advanced nasopharyngeal cancer have high plasma EBV deoxyribonucleic acid (DNA) levels at diagnosis, and monitoring EBV DNA is a sensitive and highly specific marker for detecting disease

Editor: Martin S. Staeger.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The authors have no conflicts of interest to disclose.

^a Department of Head and Neck Cancer, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, ^b Department of Clinical Nursing, Medical University of Warsaw, ^c Department of Immunology, Transplantology, and Internal Diseases, Medical University of Warsaw, ^d Department of Bioinformatics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.

* Correspondence: Bożena Czarkowska-Paczek, Department of Clinical Nursing, Medical University of Warsaw, E. Ciołka str. 27, 01-445 Warsaw, Poland (e-mail: bożena.czarkowska-paczek@wum.edu.pl).

Copyright © 2018 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Medicine (2018) 97:51(e13777)

Received: 15 February 2018 / Accepted: 28 November 2018

<http://dx.doi.org/10.1097/MD.0000000000013777>

recurrence and metastasis. Patients with nasopharyngeal carcinoma who have persistently detectable plasma EBV DNA after radiotherapy have a higher rate of treatment failure and poorer survival.^{16,71}

Head and neck carcinomas arise from the epithelium of the aero-digestive tract. Despite the advances in current treatments, the overall 5-year survival rate remains at 50%.¹⁸¹

The goal of the present study was to investigate the plasma levels of CMV DNA and EBV DNA in patients receiving radiotherapy or radiochemotherapy due to head and neck cancers and determine the influence of positivity for these viruses on the risk of death over a 2-year observation period.

2. Materials and methods

2.1. Patients

The bioethics Committee of the Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw approved the study (No. 44/2012). Every person enrolled in the study provided written informed consent to participate in the study.

The group investigated comprised 41 patients with newly diagnosed and histologically proven head and neck cancer who had been admitted to the Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology during about 6 months between 2013 and 2014. The mean age of the patients was 53 (range, 23–73) years. All patients underwent a complete medical examination including hematological and biochemical tests and radiological examinations, which included magnetic resonance imaging, chest x-ray, and computed tomography (CT) scans. Clinical and pathological staging was done according to the tumor-node-metastasis (TNM) classification of the International Union Against Cancer.¹⁹¹ Twenty three patients were diagnosed with oral cavity cancer (14 palatine tonsils cancers, 5 base of the tongue cancers, 1 soft palate cancer, 1 oral cavity cancer, and 2 molar triangle cancers). Nasopharyngeal cancers were diagnosed in 10 patients, hypopharynx cancer in 4 patients, laryngeal cancer in 2 patients, and maxillary sinus cancer in 1 patient. In 1 patient, the primary localization of the cancer was unknown (FPI, focus primarius ignotus). The cancers were advanced, categorized as clinical stage III or IVa. The TNM stage at pre-treatment evaluations were categorized as follows: clinical T1 (n=5), T2 (n=19), T3 (n=9), T4 (n=7), and Tx (n=1) by tumor depth; clinical N0 (11), N1 (n=7), N2 (n=22), and Nx (n=1) by lymph node metastasis; and clinical M1 (n=1) by distant metastasis.

All patients underwent radical radiotherapy (n=11) or radiochemotherapy (n=30). Patients referred to radiotherapy alone were treated with accelerated simultaneous-integrated boost intensity-modulated radiation therapy (SIB-IMRT; n=7), with 2.25 Gy per fraction and a total dose of 67.5 Gy in 30 fractions, or with IMRT (intensity-modulated radiation therapy, n=4), with 2 Gy per fraction and a total dose of 70 Gy in 35 fractions (standard fractionation). Patients treated with radiochemotherapy received 2 cycles (patients with nasopharyngeal cancer received 3 cycles) of cisplatin-based chemotherapy at a dose of 100 mg/m². Seventeen patients from this group were irradiated using the IMRT technique, and the remaining 13 patients received radiotherapy delivered using the SIB-IMRT technique as described above. Blood samples were collected before treatment, over 6–7 weeks during the treatment, and 1 month after the cessation of treatment. Patients were observed

for 2 years after the last patient/participant finished the therapy. During this period 6 patients died due to cancer progression.

2.2. Analytical methods

The EDTA-treated plasma samples were centrifuged at 1000 × g for 15 minutes then stored at -80°C until further processing.

Isolation of nucleic acid from plasma samples was performed using BioRobot EZ1 (Qiagen, Germany) and the EZ1 Virus Mini Kit v.2.0 (Qiagen, Germany) according to the manufacturer's instructions. After the quality and quantity control using a ND-1000 camera (NanoDrop Technologies, Wilmington, Delaware), DNA was stored at -80°C.

Real-time polymerase chain reaction was performed using ABI 7500 (Applied Biosystems, Waltham, Massachusetts). Reaction mixture was successively added to each well of a MicroAmp Optical 96-well reaction plate containing a barcode (Applied Biosystems, Waltham, Massachusetts). The reaction mixture included the following: TaqMan Universal PCR Master Mix (Applied Biosystems, Waltham, Massachusetts) and appropriately selected primers for the TaqMan Gene Expression Assay (Thermo Fisher Scientific, Waltham, Massachusetts) to test for CMV and EBV as well as for the World Health Organization (WHO) standard control curve.

2.3. Statistical analysis

We assessed the presence of plasma CMV or EBV DNA at any time point and evaluated its influence on the risk of death. Due to having only a few samples, we categorized data as follows: the presence of viral DNA ([CMV DNA (+) or EBV DNA (+)] – 1) or the absence of viral DNA ([CMV DNA (-) or EBV DNA (-)] – 0); survival (– S), and death (– D).

To make the results more reliable in the case of small groups, we applied 4 different statistical methods to investigate the relationship between the presence of plasma viral DNA and the occurrence of death. First, Fisher's exact test was performed then we estimated the odds ratio (OR) with 95% confidence intervals (CI) for risk of death in patients without or with plasma virus DNA at any time point. Binary dependent logistic regression models were built, with death as a dependent variable and plasma viral DNA as an independent variable. Finally, Bayesian analysis with Markov-Chain Monte Carlo algorithm was also applied. Each logistic regression model was evaluated using mildly informed prior distributions and 3 independent chains with 5000 steps of warm-up, followed by 5000 steps of sampling. Results for each model were analyzed using Gelman-Rubin convergence diagnostic and number of effective steps, and in each case a satisfactory quality of chains were observed.

All statistical analyses were performed in R statistical package, version 3.2. Markov chains were run with RStan, version 2.14.

3. Results

3.1. CMV

The number and percentage of patients with plasma positive for CMV DNA in the group of those who survived (n=34) and those who died (n=6) are shown in Table 1. The total number of patients was n=40.

There was no statistically significant relationship between the plasma CMV DNA (+) and the incidence of death; however, the *P* value only slightly exceeded the established level of

Table 1

The number and percentage of patients positive (+) or negative (-) for plasma CMV DNA in the group of patients who survived (S, n=34 and those who died (D, n=6).

Number of patients (n=40)	CMV DNA (-)	CMV DNA (+)
S (n=34)	30 (88.24%)	4 (11.76%)
D (n=6)	3 (50%)	3 (50%)

CMV = cytomegalovirus, DNA = deoxyribonucleic acid.

significance ($P = .055$). The risk of death in the group of patients who were positive for plasma CMV DNA was almost 7.5 times higher compared to the group of patients who were negative for plasma CMV DNA (OR: 7.5, 95% CI: 1.11–50.67).

According to the results of the logistic regression analysis, positivity for plasma CMV DNA was associated with a significantly higher risk of death ($P = .03$).

The OR for the death in the group who were positive for CMV DNA was estimated using Bayesian methods, and resulted in a value of at least 1.15, with a probability of 97.5%; the most likely OR was 8.34 (mean value of a posteriori distribution).

3.2. EBV

The number and percentage of patients with positive and negative results for plasma EBV DNA in the group of those who survived (n=35) and those who died (n=6) are shown in Table 2. The total number of patients was 41.

There was a statistically significant relationship between positivity for plasma EBV DNA and the risk of death ($P = .03$). The risk of death in the group of patients positive for plasma EBV DNA was almost 11 times higher in comparison to the group that was negative for plasma EBV DNA (OR: 10.91, 95% CI: 1.135–104.8).

According to the results of the logistic regression analysis, plasma EBV DNA positivity was associated with a significantly higher risk of death ($P = .04$).

The Bayesian estimation of the OR for death in the group that was positive for EBV DNA gives a 97.5% of probability to a value of at least 1.58, while the most probable OR was 16.22.

4. Discussion

The highlight of this study is the finding that CMV or EBV viremia detected before, during, or immediately after radiotherapy or radiochemotherapy, confirmed by the presence of virus DNA in the plasma, increases the risk of death in patients with head and neck cancers. The CMV or EBV viremia therefore negatively influences the overall survival rate. In the present study, no attempts were made to distinguish CMV or EBV antigenemia from CMV or EBV disease.

Table 2

The number and percentage of patients who were positive (+) or negative (-) for plasma EBV DNA in the group of patients who survived (S, n=35) and who died (D, n=6). There was a statistically significant relationship between plasma EBV DNA positivity and the incidence of death ($P = .03$).

Number of patients, n=41	EBV DNA (-)	EBV DNA (+)
S (n=35)	24 (68.57%)	11 (31.43%)
D (n=6)	1 (16.67%)	5 (83.33%)

DNA = deoxyribonucleic acid, EBV = Epstein-Barr virus.

The CMV and EBV infections are very common, but usually remain silent; however, once infected, an individual remains a carrier for life.^[1,2] These infections, including reactivation, occur mainly in immunocompromised patients; for instance, in solid organ transplant recipients or patients with HIV (human immunodeficiency virus).^[10,11] Cancer patients collectively belong to a group with nonspecific immunosuppression and therefore seem to be susceptible to viral infections. Radiation therapy could also activate viruses in vitro and in vivo.^[15,12]

The incidence of CMV infection among patients with carcinomas depends on the underlying disease and applied therapy. Han et al analyzed 4382 cancer patients, revealing that plasma CMV DNA was present in about 9 to 40% of patients depending on whether they had received a stem cell transplant (SCT). The lowest rates were observed in patients without SCT, and the highest rates were observed in patients who had received allogeneic SCTs. Patients who had not received a SCT and had solid tumors had CMV antigenemia rates of 8.5%; however, this rate could be underestimated because most patients with solid tumors did not have severe complications or immune suppression that warrants testing for CMV.^[13] In our group comprising only patients with head and neck carcinomas, the incidence of CMV antigenemia was at 17.5%, and the incidence of EBV antigenemia was at 39%. Evidence has been provided that demonstrates that CMV is present in some high-grade gliomas and brain metastases. Patients whose gliomas were positive for low-grade CMV infections lived 2.5 times longer than those with high grade infections.^[11] Data regarding the presence of CMV DNA in peripheral blood are controversial; however, Mitchel showed that 80% of patients with newly diagnosed glioblastoma multiforme had CMV DNA in their peripheral blood.^[14] The CMV could be transmitted to tumor cells, and recent evidence suggests that CMV might play a role in modulating the tumor microenvironment as well as in the initiation and promotion of the tumor cells themselves. The US28, encoded by the human CMV, activates nuclear factor-kappa B, and in this way induces COX-2 (*cyclooxygenase-2*) expression and the production of vascular endothelial growth factor (VEGF) and interleukin 6 (IL-6) resulting in inflammation, cellular migration, angiogenesis, and tumor invasiveness. Other CMV proteins are also involved in telomerase activation, which is associated with cellular immortalization and transformation. Cells infected with CMV are characterized by protooncogene activation.^[1,15,16] Neurological decline and encephalopathy, which occur over the course of radiotherapy for brain tumors as well as for other indications, could also be attributed to CMV infection.^[14,5,10]

The EBV has been implicated in several human carcinomas including nasopharyngeal carcinoma. Especially, EBV-encoded latent membrane protein-1 (LMP-1) has been known to have oncogenic properties. All signaling cascades triggered by LMP-1 lead to the disruption of the cell cycle and cell immortalization.^[17] The prognostic role of plasma EBV-DNA is well known in patients with nasopharyngeal carcinomas. The presence of pretreatment plasma EBV DNA correlates with the initial TNM stage, probability of relapse, and distant metastasis.^[6,18] It is also an independent factor associated with progression-free survival, distant metastasis-free survival, and overall survival in patients with nonmetastatic local and regionally advanced nasopharyngeal carcinomas treated with IMRT and cisplatin-based concurrent chemotherapy.^[19] Persistent plasma EBV DNA at the midpoint of radiotherapy or postradiation is associated with worse clinical outcomes.^[7,20] There are no established recommendations regarding prevention of EBV infection in

patients with nasopharyngeal carcinomas; however, further research has focused on development of anti EBV strategies, like vaccination or luteolin or other therapies directed towards EBV.^[2,21] The results of these previous studies are generally in line with the results of our study; however, we took into consideration all types of head and neck carcinomas and not only nasopharyngeal carcinoma, and considered plasma EBV DNA at any time point from diagnosis up to 1 month post radiotherapy or radiochemotherapy.

In summary, CMV and EBV infections in patients with solid tumors are considered to be rare and prophylactic treatment is not recommended. Even screening for latent viral infection is not routine in clinical practice. But, since confirmed viral infections negatively affect the survival rate of patients with head and neck cancers, diagnosis of viral infections and treatment of patients with positive results should be considered.

The weak point of our study is the low number of patients in the investigated groups. Due to this fact additional statistical analysis, for instance the survival curves of patients with or without viral infection were not performed. And more study is required to investigate the correlations between CMV or EBV viremia and the infection status in relation to the implementation of the treatment and other variables, for instance, particular type of carcinoma, TMN stage or the type of treatment. Also further interventional studies are required to investigate the possible improvement resulting from the implementation of anti-viral treatment in patients with confirmed viremia.”

Acknowledgments

Statistical analysis was performed by Biostat.

English language was edited by American Manuscript Editors.

Author contributions

Conceptualization: Dorota Kiprian, Bozena Czarkowska-Paczek, Leszek Paczek.

Data curation: Dorota Kiprian, Bozena Czarkowska-Paczek, Aleksandra Wyczalkowska-Tomasik, Leszek Paczek.

Formal analysis: Dorota Kiprian, Bozena Czarkowska-Paczek, Leszek Paczek.

Methodology: Aleksandra Wyczalkowska-Tomasik.

Writing - original draft: Bozena Czarkowska-Paczek.

Writing - review & editing: Dorota Kiprian, Aleksandra Wyczalkowska-Tomasik, Leszek Paczek.

Bozena Czarkowska-Paczek orcid: 0000-0002-1023-3057.

References

- [1] Rahbar A, Orrego A, Peredo I, et al. Human cytomegalovirus infection levels in glioblastoma multiforme are of prognostic value. *J Clin Virol* 2013;57:36–42.
- [2] Wu CC, Fang CY, Hsu HY, et al. EBV reactivation as a target of luteolin to repress NPC tumorigenesis. *Oncotarget* 2016;7:18999–9017.
- [3] Lucchese A. Viruses and oral cancers: crossreactivity as a potential link. *Anticancer Agents Med Chem* 2015;15:1224–9.
- [4] Goerig NL, Frey B, Korn K, et al. Frequent occurrence of therapeutically reversible CMV-associated encephalopathy during radiotherapy of the brain. *Neuro Oncol* 2016;18:1664–72.
- [5] Goerig N, Semrau S, Frey B, et al. Clinically significant CMV (re) activation during or after radiotherapy/chemotherapy of the brain: correlation with neurological deterioration and improvement upon antiviral treatment. *Strahlenther Onkol* 2016;192:489–97.
- [6] Ferrari D, Codeca C, Bertuzzi C, et al. Role of plasma EBV DNA levels in predicting recurrence of nasopharyngeal carcinoma in western population. *BMC Cancer* 2012;12:208.
- [7] Wang W-Y, Lin T-Y, Twu C-W, et al. Long-term clinical outcome in nasopharyngeal carcinoma patients with post-radiation persistently detectable plasma EBV DNA. *Oncotarget* 2016;7:42608–16.
- [8] Poth KJ, Guminski AD, Thomas GP, et al. Cisplatin treatment induces a transient increase in tumorigenic potential associated with high interleukine-6 expression in head and neck squamous cell carcinoma. *Mol Cancer Ther* 2010;9:2430–9.
- [9] Sobin LH, Wittekind CH. UICC TNM classification of malignant tumours. 5-th edition 1997; Wiley-Liss, Inc, New York:66-69.
- [10] De Jesus A, Grossman SA, Paun O. Cytomegalovirus associated colonic pseudotumor: a consequence of iatrogenic immunosuppression in a patient with primary brain tumor receiving radiation and temozolomide. *J Neurooncol* 2009;94:445–8.
- [11] Manuel O, Kralidis G, Mueller NJ, et al. Impact of antiviral preventive strategies on the incidence and outcomes of cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant* 2013;13:2402–10. doi: 10.1111/ajt.12388.
- [12] Westphal EM, Blackstock W, Feng W, et al. Activation of lytic Epstein-Barr virus (EBV) infection by radiation and sodium butyrate in vitro and in vivo: a potential method for treating EBV-positive malignancies. *Cancer Res* 2000;60:5781–8.
- [13] Han XY. Epidemiologic analysis of reactivated cytomegalovirus antigenemia in patients with cancer. *J Clin Microbiol* 2007;45:1126–32.
- [14] Mitchell DA, Xie W, Schmittling R, et al. Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma. *Neuro Oncol* 2008;10:10–8.
- [15] Slinger E, Maussang D, Schreiber A, et al. HCMV-encoded chemokine receptor US28 mediates proliferative signaling through the IL-6-STAT3 axis. *Sci Signal* 2010;3:ra58doi: 10.1126/scisignal.2001180.
- [16] Soroceanu L, Cobbs CS. Is HCMV a tumor promoter? *Virus Res* 2011;157:193–203. doi: 10.1016/j.virusres.2010.10.026.
- [17] Tao Y, Shi Y, Jia J, et al. Novel roles and therapeutic targets of Epstein-Barr virus-encoded latent membrane protein 1-induced oncogenesis in nasopharyngeal carcinoma. *Expert Rev Mol Med* 2015;17:e15doi: 10.1017/erm.2015.13.
- [18] Du XJ, Tang LL, Mao YP, et al. Circulating EBV DNA, globulin and nodal size predict distant metastasis after intensity-modulated radiotherapy in stage II nasopharyngeal carcinoma. *J Cancer* 2016;7:664–70. doi: 10.7150/jca.14183.
- [19] Chen WH, Tang LQ, Guo SS, et al. Prognostic value of plasma Epstein-Barr virus DNA for local and regionally advanced nasopharyngeal carcinoma treated with Cisplatin-based concurrent chemoradiotherapy in intensity-modulated radiotherapy era. *Medicine (Baltimore)* 2016;95:e2642doi: 10.1097/MD.0000000000002642.
- [20] Leung SF, Chan KC, Ma BB, et al. Plasma Epstein-Barr viral DNA load at midpoint of radiotherapy course predicts outcome in advanced-stage nasopharyngeal carcinoma. *Ann Oncol* 2014;25:1204–8. doi: 10.1093/annonc/mdu117.
- [21] Tan WL, Tan EH, Lim DW, et al. Advances in systemic treatment for nasopharyngeal carcinoma. *Chin Clin Oncol* 2016;5:21doi: 10.21037/cco.2016.03.03.