academicJournals

Vol. 6(2), pp. 13-19, March 2014
DOI: 10.5897/JCREO2014- 0109
ISSN 2141-2243 ©2014

http://www.academicjournals.org/JCREO

Journal of Cancer Research and Experimental Oncology

Full Length Research Paper

The prognostic value of IgA/[EBNA1+VCA-p18] on survival of nasopharyngeal cancer patients

Kartika Widayati Taroeno-Hariadi[1], Djohan Kurnianda[1], Jajah Fachiroh[1], Bambang Hariwiyanto[2], Sagung Rai Indrasari[3], Camelia Herdini[3], Ibnu Purwanto[1], Salugu Maesadji[4], Retno Dwi Danarti[4], Mardiah Suci Hardianti[1], Harijadi[5] and Sofia Mubarika Haryana[2]

[1]Department of Internal Medicine, Division of Hematology and Medical Oncology, Faculty of Medicine Universitas Gadjah Mada – Dr. Sardjito Hospital, Jl Kesehatan No. 1 Yogyakarta, DIY, Indonesia.
[2]Department of Histology and Cell Biology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3]Department of Ear, Nose, Throat, Dr Sardjito Hospital-Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.
[4]Department of Radiotherapy, Dr Sardjito Hospital – Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.
[5]Department of Pathology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Received 24 January, 2014; Accepted 5 March, 2014

Undifferentiated (World Health Organization (WHO) 3) type of nasopharyngeal cancer (NPC) is strongly correlated with Epstein-barr (EBV) virus latent infection. Post-treatment viral reactivation is associated with relapsed or recurrence of NPC. Viral activation can be measured indirectly via plasma IgA responses towards EBV proteins such as EBNA1 and VCA-p18. This study aims at determining the prognostic value of IgA/[EBNA1+VCA-p18] on progression free survival and overall survival of NPC patients. NPC patients aged > 18 years, with locally advanced disease receiving concurrent chemoradiation, with weekly cisplatin 40 mg/m^2 samples for blood plasma before treatment, 3 months post-treatment, and at 12 months after treatment completion or at the time of disease progression, whichever came first. An established enzyme linked immunosorbent assay (ELISA) method was used for evaluation of IgA/[EBNA1+VCA-p18] level reported as optical density 450 nm (OD_{450}) values. Forty six NPC patients, with male predominance and mostly in productive age were included. Twenty seven patients had disease progression or died during study follow up. Mean of pre-treatment IgA OD_{450} was higher in patients with progression compared to those still in remission (2.33 ± 1.08 versus 1.66 ± 1.19, p < 0.05). The higher risk serology group (OD_{450} ≥ 1.4) had shorter time to progression (RR 6.06; p = 0.014; median time to progression is 13.47 month). Overall survival was not influenced by plasma IgA. Pretreatment IgA/[EBNA-1+VCA-p18] may predict early progression for NPC

Key words: Nasopharyngeal cancer, prognosis factor, immunological response, IgA/(EBNA1+ VCA-p18).

INTRODUCTION

Nasopharyngeal cancer (NPC) is a common cancer in Southern China, South East Asia and North Africa (Raab-

*Corresponding author. E-mail: taroeno_hariadi@yahoo.com.

Traub, 2000; Chang et al., 2006).. In Indonesia, NPC is the fourth commonest cancer in men and sixth among women (Adham, 2012). More than 90% of NPC in Indonesia comprised of histology type World Health Organization (WHO) III or undifferentiated carcinoma which is strongly correlated with Epstein-barr virus (EBV) infection (Soeripto, 1997; Adham et al., 2012).

Consistent expression of EBV gene products in nasopharyngeal cancer cells, specific immune response to EBV antigen in NPC patients, as well as detection of EBV in premalignant lesion support the pathogenic role of EBV in NPC (Henle et al.,1970; Wolf et al., 1973; Henle, 1976; Ho et al., 1976; Zeng et al 1982, 1983; Yeung et al., 1993; Sam et al., 1994; Pathmanathan et al., 1995; Gulley, 2001; Chien et al., 2001; Middeldorp et al., 2003). Although NPC is sensitive to radiotherapy and chemotherapy, recurrence rate of NPC during the first 2-year post treatment remains high (2-year progression free survival is less than 53% with median time to progression is 17.4 month) in our centre (Taroeno-Hariadi et al., 2005 unpublished observation).

Wildeman et al. (2013) reported that median overall survival of NPC patients in our centre is 21 months (95% CI = 18 to 35) from day of diagnosis. Treatment modality, tumor stage, patient performance status, viral load and viral reactivation may influence recurrence and progression (Farias et al., 2003; Twu et al., 2007; Sham and Choy, 2010; Wu et al., 2012).

IgG (and specifically IgA) response to EBV antigens is the hallmark of NPC (Henle et al., 1970; Henle and Henle, 1976; Ho et al., 1976). With the advent of polymerase chain reaction (PCR) technology, nowadays viral reactivation can be measured more directly by detecting EBV-DNA. EBV-DNA quantification has been reported as sensitive and specific method for NPC diagnosis, treatment monitoring and prognosis (Lo et al., 2000). However, this method is quite expensive to be applied in low income countries such as Indonesia. Fachiroh et al. (2006) have developed serodiagnostic tools based on enzyme-linked immunosorbent assay (ELISA) to measure IgA antibody response to combination of EBV immunodominant epitopes [EBNA1+VCA-p18] in one assay to diagnose NPC.

This method had a reported sensititivity of 85.4% and specificity of 90.1%. Sensitivity, a specificity of IgA/[EBNA1+VCA-p18] is better than either IgA EBNA-1 or IgA VCAp-18 alone (Fachiroh et al., 2006). The application of this serodiagnostic tool to predict survival or recurrence in NPC requires further clinical evidence. This is the first study reporting the potential role of IgA/[EBNA1+VCA-p18] as predictor of progression or survival.

MATERIALS AND METHODS

This preliminary prospective study was held in Dr Sardjito Hospital Yogyakarta Indonesia from January, 2007 to October, 2010 and included all newly diagnosed, locally advanced NPC. Diagnosis of NPC was confirmed by histology examination and clinical staging was performed by a Multi Slice Computed Tomography scan of head and neck region for primary tumor, by abdominal ultrasonography, chest x-ray and skeletal survey for detecting metastases. Patients with stage III, IVA and IVB as designated by American Joint Commitee of Cancer (AJCC), 7th edition, aged above 18 year-old and have performance status of WHO 0, 1, 2, 3, were included in this study. Patients had normal complete blood count and blood chemistry results as requirements to receive chemoradiation with weekly low dose cisplatin (40 mg/m^2) for 8 cycles concurrently with radiotherapy for 70 Gy in 35 fractions. Hemoglobin was > 10 g/dl, white blood cell (WBC) count > 4,000/L or absolute neutrophil count > 1,500/L, platelet > 100,000/mmk and creatinin clearance ≥50 ml/mnt. Alanine aminotransferase (ALAT) or aspartate aminotransferase (ASAT) ≤ 2 × upper limit of normal and bilirubin ≤ 2 × upper limit of normal.

Patients who received less than 80% of planned treatment, patients with severe infection or co-morbid ilnesses were excluded from the study. Plasma samples for IgA/[EBNA1+VCA-p18] ELISA were taken at pre-treatment, at the time of tumor assessment (12-weeks post-treatment) and at 12 months after treatment completion, or at the time of disease progression, whichever came first. Treatment responses were assessed at 12-weeks after treatment completion. Responses were catagorized as: complete response (CR) = disappearance of all target lesions (any pathological lymph nodes must have reduction in short axis to < 10 mm), partial response (PR) = at least a 30% decrease in the sum of diameters of target lesions, stable disease (SD) = neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD and progression disease (PD) = at least 20% increase in the sums of diameters of the target lesions, according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1.

IgA/[EBNA1+VCA-p18] ELISA

The method was described in previous publication (Fachiroh et al., 2006) by the use of EBNA1 and VCA-p18 peptides (Cyto-Barr, Zuidhorn, The Netherlands, kindly provided through KWF funding) in one ELISA well. All OD_{450} values were normalized by subtracting the value for 1:100-diluted EBV-negative sera used in duplicate in each ELISA run. The receiver operating characteristic analysis was done to predict the cut-off value of IgA, giving best sensitivity and specificity to predict progression (Fachiroh et al., 2006).

Statistical analysis

IgA/[EBNA1+VCA-p18]-level at pre-treatment, at 12-weeks post-treatment, and at 12-months post-treatment or at the time of disease progression were calculated as mean of OD_{450} ± standard deviation and grouped according to the treatment responses. The difference of mean IgA according to treatment response were analyzed with student t-test. Association of IgA reactivity and treatment response were analyzed with χ^2 test. Kaplan-Meir plots of overall survival and event-free survival were established for patients group of different serological groups. Log rank tests were performed to assess survival probabilities between patients subsets (high risk serological group versus low risk).

Ethics

The study protocol was reviewed and approved by the institutional review board of the Faculty of Medicine, Universitas Gadjah Mada, and all patients were required to fill in written informed consent before participation.

Table 1. Characteristic of subjects.

Character	N (%)
Sex	
Male	32 (69.6)
female	14 (30.4)
Mean of age: 45.2 year-old ± 12.3	
< 45 year-old	19 (41.3)
≥ 45 year-old	27 (58.7)
Clinical Performance	
0, 1	17 (37)
2	24 (52.2)
3	5 (10.9)
Histology type	
WHO 2	3 (6.52)
WHO 3	43 (93.5)
Tumor size	
T1	5 (10.9)
T2	13 (28.2)
T3	15 (32.6)
T4	13 (28.2)
Lymphnodes metastasis	
N0	10 (21.7)
N1	10 (21.7)
N2	11 (23.9)
N3	15 (32.6)

RESULTS

Characteristic of subjects

Forty six patients were eligible for this study as shown as Table 1. Most of them were men at productive age, with moderate performance status. Patients were characterized by larger tumor size (60.8%) and extensive neck lymphnodes involvement (56.5%). Ninety five percent subjects completed their treatment according to protocol. Pre-treatment serology data were available from all subjects, while in post treatment, serology data was missing for 13.3% patients due to early progression before the scheduled sample collection. Treatment response could be assessed in 95.6% patients. Twenty one patients achieved complete response (47.7%), 19 patients were in partial response (43.2%), 2 patients were stable (4.5%), and 2 patients (4.5%) were in disease progression. A follow-up was done during 36 months. Median time to progression was 10.81 ± 11.8 months, with 27 events of death or progression during follow up.

Serology test IgA/[EBNA1+VCA-p18] reactivity and treatment response

Dynamic fluctuation of plasma IgA/[EBNA1+VCA-p18] levels was observed during follow up. Patients with complete response had lower pre-treatment OD_{450} IgA/[EBNA1+VCA-p18] than those with partial response or stable disease; but did not reach statistical significance (1.85 ± 1.18 versus 2.40 ± 0.98, p = 0.113; 95% CI: 1.22 to 0.135). Patients with complete response had more often declined post-treatment plasma IgA/[EBNA1+VCA-p18] levels than other groups (χ^2 = 12.25; p = 0.016). Pre-treatment plasma IgA/[EBNA1+VCA-p18] were higher in those who had disease progression or who died compared to those who achieved good clinical response (OD_{450} = 2.33 ± 0.2 versus 1.66 ± 0.27; p < 0.05) as shown in Figure 1.

Pre-treatment IgA/[EBNA1+VCA-p18] plasma as prognostic marker of disease progression

To determine the OD_{450} level that can define progression risk, a calculation based on receiver operating characteristic (ROC) was done and cut-off value for IgA/[EBNA1+VCA-p18] was determined at OD_{450} 1.44 with 81.3% of sensitivity and 58.9% of specificity. Kaplan-Meir analysis was performed to estimate survival difference based on high risk serology (IgA/[EBNA1+VCA-p18] OD_{450} ≥1.4) and low risk serology (OD_{450} < 1.4). Twenty seven of 46 NPC patients (58.1%) had disease progression or died during follow-up. The estimation of survival difference by serology yielded 30 patients at high risk and 16 at low risk. Disease progression or death were more frequent in high-risk serology group (22 of 30 subjects or 73%), with median time to progression of 13.5 month. In low risk serology group (n = 16), 5 patients had progression (31.3%) while median time of progression was not reached. There were significant differences in progression free-survival (PFS) according to serology risk (p = 0.014) as shown in Figure 2A. In high risk serology group, median overal survival (OS) was 17.3 month, whereas in low risk serology group, median OS was not reached (p = 0.114) as shown in Figure 2B.

Serology IgA/[EBNA1+VCA-p18] changes and disease relapse

The changes of serology level in this study failed to indicate a difference between those who had poor outcome (disease progression or relapse) and good outcome (remission and stable disease, without progression). During follow-up, patients with disease progression showed elevated serology, with mean elevation of OD_{450} 0.73 ± 1.00; whereas those without progression had mean

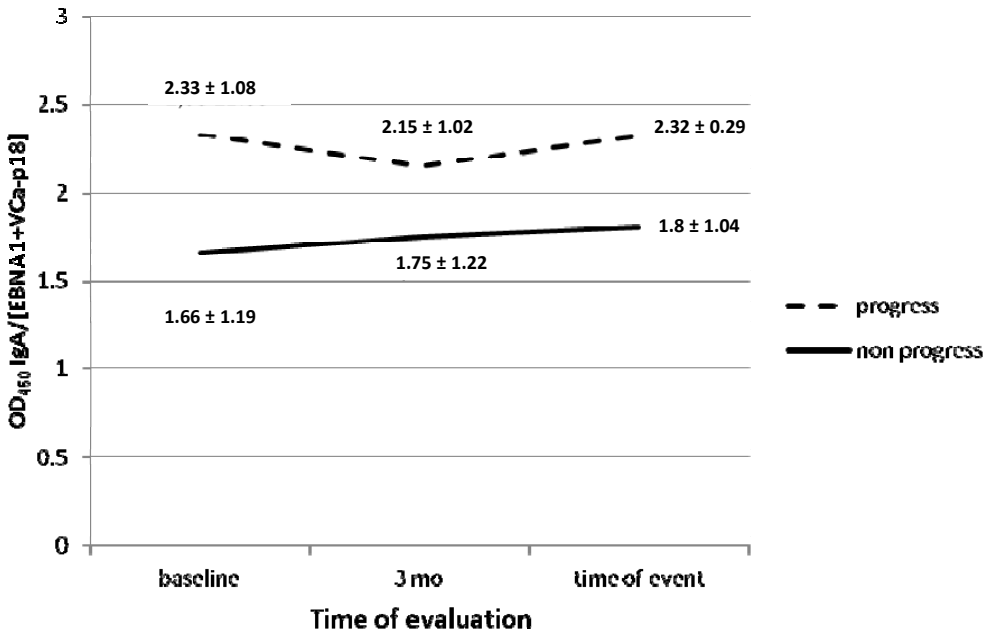


Figure 1. Dynamic changes of OD $_{450}$ plasma IgA/[EBNA1+VCA-p18] based on time of evaluation between NPC groups that progressed (n=27) and non progressed (n=19). Evaluation was performed at baseline (pre-treatment), 3-month or 12-week post-treatment, and at 1-year post-treatment or at the time of disease progression ($p<0.05$).

mean elevation OD_{450} 0.50 ± 0.89 ($p = 0.49$; 95% CI: 0.45 to 0.90). Eighteen out of 28 patients (64.3%) with stable or elevated IgA within 1 year had disease progression; whereas 2 out of 7 patients (28.6%) with decreased IgA during the same period had progression ($X^2 = 3.85$; $p = 0.14$).

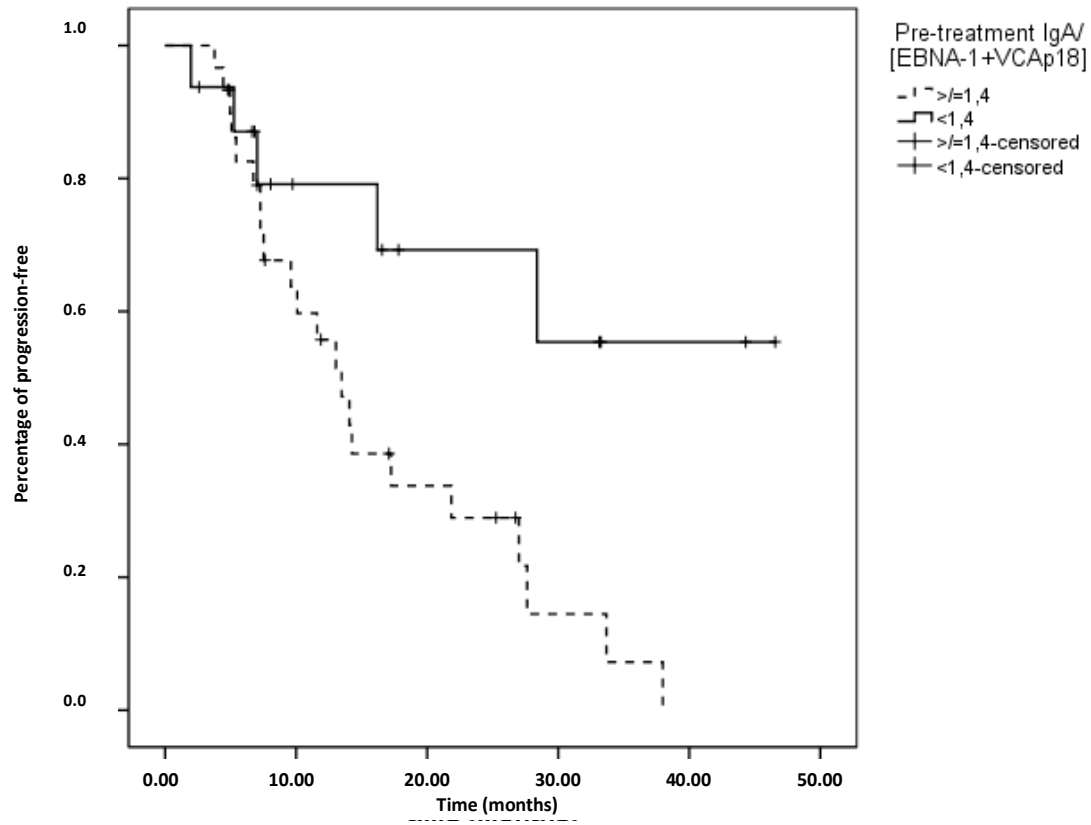
DISCUSSION

People infected with EBV will develop specific antibody response including IgM against viral capsid antigen (VCA) during acute primary infection, followed by IgG against VCA and EBV nuclear antigen 1 (EBNA1) that persist for life (Tsuchiya, 2002; Hess, 2004). Aberrant level of antibody response against EBV has been evident in various EBV-related malignancies (Tao et al., 2006). Nasopharyngeal cancer patients often shows increase in antibody response of IgA and IgG against VCA, EA, EBNA1 and transcription activator Zta and Rta, as well as other EBV lytic cycle protein (Henle and Henle, 1976; Fachiroh et al., 2004). Elevation of antibody responses to EBV may precede onset of clinical manifestation of NPC by 1 to 5 year (Yip et al., 1994; Ji MF et al., 2007). Combined EBV serological biomarkers could improve diagnostic value of NPC (Neel and Taylor, 1990; Fachiroh et al., 2006; Liu et al., 2012; Ai et al., 2013; Chang et al., 2013).

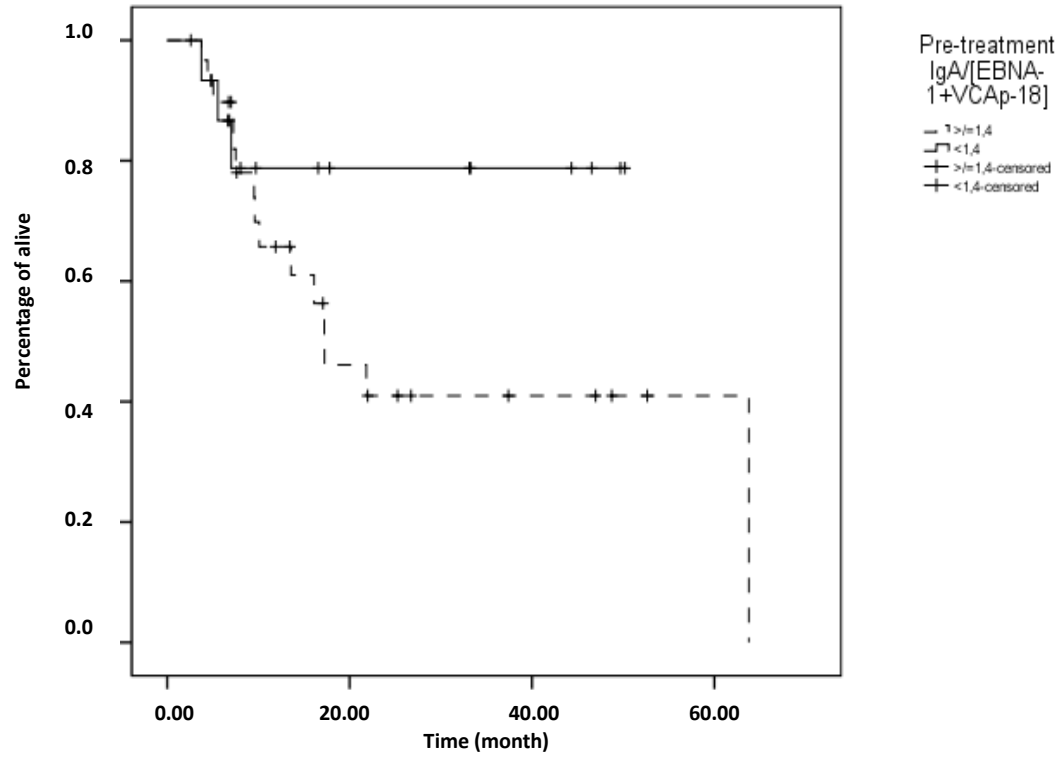
Furthermore, dynamic fluctuation of antibody level after treatment of NPC raised the possibilities of humoral response to be used as prognostic marker (Yip et al., 1994). Specific antibody responses to EBV proteins have become a powerful tool to detect reactivation of this virus in human body. Previous studies reported various serological biomarkers as prognostic factors with inconsistent results (Karray et al., 2005; Liu et el., 2004; Neel and Taylor, 1990; Yip et al., 1994; de Vathaire et al., 1988). Fan et al. (2004) reported the use of IgA early antigen (EA) serology to predict post treatment outcome. IgA/EA was still detected in 44% of patients and IgG/EA was detected in 94% NPC patients in remission; whilst EBV DNA became undetectable during remission.

This led to conclusion that the role of EA serology was less important than viral EBV DNA load (Liu et al., 2004; Twu et al., 2007). Similar findings was reported by Adham et al. (2013) among NPC patients in Indonesia. Adham et al. (2013) reported that there was no significant reduction at 2-months post-treatment of IgA EBV either IgA EBNA1 or IgA VCA-p18.

On the contrary, Ling et al. (2009) reported that pretreatment IgA/VCA serology test had prognostic value. Patients with higher IgA/VCA level had shorter survival. In this study, pre-treatment IgA/[EBNA1+VCA-p18] serology level could discriminate risk for progression. To our knowledge, there had not been a report of IgA/[EBNA1+VCA-p18] as a prognostic marker of NPC. This preliminary finding may help us to adjust treatment plan for high risk group for recurrence or progression. Higher dose of chemoradiation may be recommended to NPC patients with higher level of pre-treatment



(A)



(B)

Figure 2. Survival function based on pre-treatment OD 450 IgA/[EBNA1+VCA-p18] groups (low risk OD450 <1.4 vs. high risk OD450 ≥ 1.4). (A) is progression free-survival and (B) is overall survival.

IgA/[EBNA1+VCA-p18]. This study measured combined EBV serological biomarkers for prognostic use, added to its diagnostic capacity (Fachiroh et al., 2006; Ai-di et al., 2009). Only pre-treatment serology IgA/[EBNA1+VCA-p18] showed prognostic role for progression. Elevation of IgA/EBV titer within 1-year post-treatment tend to be followed by disease progression, however, this did not reach the statistical significance. Some patients had disease progression or died before the serology test (missing post-treatment serology). Hence it may contribute to this result. Another possible explanation for incapability of post-treatment IgA/EBNA as prognostic factor was that EBV might be harbored not only in nasopharingeal tumor cells, but also in activated infiltrating T-cell, B lymphocytes and epithelial cells which are able to produce EBV-related antigen. This may result in elevated IgA/EBV even after remission of disease.

Our unpublished data showed that IgA/[EBNA1+VCA-p18] level was not an independent prognostic factors for survival because of its dependency on patient's clinical performance. The prognostic role of serology IgA/[EBNA1+VCA-p18] to predict progression was strong enough (HR 3.19; 95% CI: 1.09 to 9.37; p = 0.03) without adjusting to clinical performance. Clinical performance of the patients declined continously even after treatment cessation due to difficulty in swallowing. Poor nutrition status affects the overall health status and furthermore may impair immune response. Antibody response to EBV antigen may decline after treatment and raise at the time of progression (Ai-di et al., 2009) but unfortunately this dynamic fluctuation is obscured by the declining of clinical performance, immunity and nutritional status.

Immunocompromised host responded inadequately to antigens so the reactivation of EBV might not be detected by measuring antibody response in this situation (Verschuuren et al., 2003). This may explain that only pre-treatment IgA/[EBNA1+VCA-p18] has a role to determine risk for progression. Post-treatment IgA-EBV serology failed to determine prognostic difference among NPC patients. This is consistent with the previous studies done by Adham et al. (2013) and Ai et al. (2013). Direct quantification of viral EBV DNA, therefore, might lend a hand to support the notion that viral reactivation play important role in disease progression and survival (Hassen et al., 2011; Wang et al., 2013; Yip et al., 2014). EBV DNA load from nasopharyngeal brushings and whole blood showed significant reduction at 2-month after treatment, which was not reflected by EBV-IgA serology (Adham et al., 2013).

Conclusion

Pre-treatment serology IgA/[EBNA1+VCA-p18] can predict progression of NPC. High risk serology group (OD_{450} IgA > 1.4) progresses earlier. The role of IgA/[EBNA1+VCA-p18] to replace EBV DNA load for disease monitoring is less convincing.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

This study was funded byRISBIN IPTEKDOK 2009-2010 No HK.06.01/1/1304/2010 and Netherland Cancer Society grant KWF-IN 2004-17 (PI: Prof dr JM Middeldorp and Prof dr SM Haryana).

REFERRENCES

Adham M,Greijer AE, Verkuijlen SAWM, Juwana H, Fleig S, Rahmadi L (2013). Epstein-Barr Virus DNA Load in nasopharyngeal brushings and whole blood in nasopharyngeal cancer patients before and after treatment. Clin. Cancer Res. 19(8):2175-2186. doi: 10.1158/1078-0432.CCR-12-2897.

Adham M, Kurniawan AN, Muhtadi AI, Roezin A, Hermani B, Gondhowiardjo S, Bing Tan I, Middeldorp JM (2012). Nasopharyngeal cancer in Indonesia: epidemiology, incidence, signs, and symptoms at presentation. Chin. J. Cancer. 31(4):185-196.

Ai P, Wang T, Zhang H, Wang Y, Song C, Zhang L, Li Z, Hu H (2013). Determination of antibodies directed at EBV proteins expressed in both latent and lytic cycles in nasopharyngeal carcinoma. Oral Oncol. 49(4):326-331.

Chang C, Middeldorp JM, Yu KJ, Juwana H, Hsu WL, Lou PJ (2013). Characterization of ELISA detection of broad-spectrum anti-Epstein-Barr antibodies associated with nasopharyngeal carcinoma. J. Med. Virol. 85(3):524-529.

Chang ET, Adami HO (2006). The Enigmatic Epidemiology of Nasopharyngeal Cancer. Cancer Epidemiol. Biomarkers Prev. 15:1755-1777.

Chien YC, Chen JY, Liu MY (2001). Serologic markers of Epstein-Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. N. Engl. J. Med. 345:1877-1882.

Fachiroh J, Schouten T, Hariwiyanto B (2004). Molecular diversity of Epstein-Barr molecular IgG and IgA responses in nasopharyngeal carcinoma: comparison of Chinese, Indonesian, and European subjects. J. Infect. Dis. 190:53-62.

Fachiroh J, Paramita DK, Hariwiyanto B (2006). Single-Assay Combination of Epstein-Barr Virus (EBV) EBNA1- and Viral Capsid Antigen-p18-Derived Synthetic Peptides for Measuring Anti-EBV Immunoglobulin G (IgG) and IgA Antibody Levels in Sera from Nasopharyngeal Carcinoma Patients: Options for Field Screening. J. Clin. Microbiol. 44:1459-1467.

Fan H, Nichols J, Chua D (2004). Laboratory Markers of Tumor Burden in Nasopharyngeal Carcinoma: A comparison of Viral Load and Serologic test for Epstein-Barr Virus. Int. J. Cancer 112(6):1036-1041.

Farias TP, Dias FL, Lima RA (2003). Prognostic factors and outcome for nasopharyngeal carcinoma. Arch. Otolaryngol. Head Neck Surg.129:794-799.

Gu AD, Lu XL, Xie YB, Chen LZ, Feng QS, Kang T, Jia WH, Zeng YX (2009). Clinical values of multiple Epstein-Barr virus (EBV) serological biomarkers detected by xMAP technology. J. Transl. Med. 7:73.

Gulley M (2001) Review: molecular diagnosis of Epstein-Barr virus-related diseases. J. Mol. Diagn. 1:1-10.

Hassen E, Farhat K, Gabbouj S, Bouaouina N, Abdelaziz H, Chouchane L (2011). Epstein-Barr virus DNA quantification and follow up in Tunisian nasopharyngeal cancer patients. Biomarker. 16(3):274-280.

Henle W, Henle G, Ho HC (1970). Antibodies to Epstein-Barr virus in nasopharyngeal carcinoma, other head and neck neoplasms and control groups. J. Natl. Cancer Inst. 44:225-231.

Henle G, Henle W (1976). Epstein-Barr virus-specific IgA serum antibodies as an outstanding feature of Nasopharyngeal carcinoma. Int. J. Cancer 17:1-7.

Hess RD (2004). Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. J. Clin. Microbiol. 4(8):3381-3387.

Ho HC, Ng MH, Kwan HC (1976). Epstein-Barr virus-specific IgA and IgG serum antibodies nasopharyngeal carcinoma. Br. J. Cancer. 34:655-660.

Ji MF, Wang DK, Yu YL (2007). Sustained elevation of Epstein-Barr virus antibody levels preceding clinical onset of nasopharyngeal carcinoma. Br. J. Cancer 96:623-630.

Karray H, Ayadi W, Fki L, Hamami A (2005). Comparison of three different serological techniques for primary diagnosis and monitoring of nasopharyngeal carcinoma in two age groups from Tunisia. J. Med. Virol. 75:6-29.

Ling W, Cao SM, Huang QH, Li YH, Deng MQ (2009). Prognostic implication of pretreatment titer of serum immunoglobulin A against Epstein-Barr virus capsid antigen in nasopharyngeal carcinoma patients in Sihui, Guangdong. Ai Zheng 28:57-59.

Liu MY, Huang YT, Sheen TS, Chen JY, Tsai CH (2004). Immune responses to Epstein–Barr virus lytic proteins in patients with nasopharyngeal carcinoma. J. Med. Virol. 73:575-582.

Liu Y, Huang Q, Liu W,Liu Q, Jia W,Chang E (2012). Establishment of VCA and EBNA 1 IgA-based combination by enzyme-linked immunosorbent assay as preferred screening method for naopharyngeal carcinoma: a two stage design with a preliminary performance study and a mass screening in southern China. Int. J. Cancer 131:406-416.

Lo YM, Chan AT, Chan LY (2000). Molecular prognostication of nasopharyngeal carcinoma by quantitative analysis of circulating Epstein-Barr virus DNA. Cancer Res. 60:6878-6881.

Middeldorp JM, Brink A, van den Brule A, Meijer C (2003). Pathogenesis roles of Epstein-Barr virus (EBV) gene products in EBV-associated proliferative disorders. Crit. Rev. Oncol. Hematol. 45:1-36.

Neel HB, Taylor WF (1990). Epstein-Barr virus-related antibody. Change in titers after therapy for nasopharyngeal carcinoma. Arch. Otolaryngol. Head Neck Surg.116:1287-1290.

Pathmanathan R, Prasad U, Sadler R (1995). Clonal proliferations of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. N. Engl. J. Med. 333:693-698.

Raab-Traub N (2000). Epstein-Barr virus and nasopharyngeal carcinoma, In J. A. Goedert (ed.), Infectious causes of cancer: targets for intervention. Humana Press,Totowa. N. J. 93-111.

Sam CK, Abu-Samah AJ, Prasad U (1994). IgA/VCA as follow-up marker in the monitoring of nasopharyngeal carcinoma. Eur. J. Surg. Oncol. 120:561-564.

Sham ST, Choy D (1990). Prognostic factors of nasopharyngeal carcinoma: a review of 759 patients. Br. J. Radiol. 63:51-58.

Soeripto K (1997). Epidemiology of nasopharyngeal carcinoma. Berita Kedokteran Masyarakat 13:207-211.

Tao Q, Young LS, Woodman CB, Murray PG (2006). Epstein-Barr virus (EBV) and its associated human cancers-genetics, epigenetics, pathobiology and novel therapeutics. Front Biosci. 11:2672-2713.

Tsuchiya S (2002). Diagnosis of Epstein-Barr virus-associated diseases. Crit. Rev. Oncol. Hematol. 44:227-238.

Twu CW, Wang WY, Liang WM (2007). Comparison of the prognostic impact of serum anti-EBV antibody and plasma EBV DNA assays in nasopharyngeal carcinoma. Int. J. Radiat. Oncol. Biol. Phys. 67:130-137.

de Vathaire F, Sancho-Garnier H, de-The H (1988). Prognostic value of EBV markers in the clinical management nasopharyngeal carcinoma (NPC): A multicenter follow-up study. Int. J. Cancer 42:176-181.

Verschuuren E, van der Bij W, de Boer W, Timens W, Middeldorp J, The TH (2003).Quantitative Epstein-Barr Virus (EBV) serology in lung transplant recipients with primary EBV infections and/or post-transplant lympho proliferative disease. J. Med. Virol. 69(2):258-266.

Wang WY, Twu CW, Chen HH, Jiang RS, Wu CT, Liang KL (2013). Longterm survival analysis of nasopharyngeal carcinoma by plasma Epstein-Barr virus DNA level. Cancer 119(5):963-970.

Wildeman MA, Fles R, Herdini C, Indrasari RS, Vincent AD (2013). Primary Treatment Results of Nasopharyngeal Carcinoma (NPC) in Yogyakarta, Indonesia. Plos one 8(5):e63706. doi:10.1371/journal.pone. 00637 06.

Wolf H, zur Hausen H, Becker Y (1973). Epstein-Barr viral genomes in epithelial nasopharyngeal carcinoma cells. Nature. 244:245-257.

Wu Z, Zeng RF, Su Y, Gu MF, Huang SM (2013). Prognostic significance of tumor volume in patients with nasopharyngeal carcinoma undergoing intensity-modulated radiation therapy. Head Neck 35(5):689-694.

Yeung WM, Zong YS, Chiu CT (1993). Epstein-Barr virus carriage by nasopharyngeal carcinoma in situ. Int. J. Cancer 53:746-750.

Yip TTC, Ngan RKC, Lau WH, Poon YF, Joab I, Cochet C (1994). A possible prognostic role of immunoglobulin-G antibody against recombinant Epstein-Barr virus BZLF-1 transactivator protein ZEBRA in patients with nasopharyngeal carcinoma. Cancer 74:2414-2424.

Yip TTC, Ngan RKC, Fong AHW, Law SCK (2014). Application of circulating plasma/serum EBV DNA in clinical management of nasopahryngeal carcinoma. Oral Oncol. http://dx.doi.org/10.1016/j.oraloncology.2013.12.011.

Zeng Y, Zhang LG, Li H, Jan MG, Zhang Q, Wu YC, Wang YS, Su GR (1982) Serological mass survey for early detection of nasopharyngeal carcinoma in Wuzhou city, China. Int. J. Cancer 29:139-141.

Zeng Y, Zhong JM, Li LY (1983). Follow-up studies on Epstein-Barr virus IgA/VCA antibody-positive persons in Zangwu County, China. Intervirology 20:190-194.