Editorial

Screening of nasopharyngeal carcinoma using plasma Epstein-Barr virus DNA for at-risk population

Hsin-Pai Li[1,2,3], Cheng-Lung Hsu[3], Yu-Sun Chang[1,2,4]

[1]Department of Microbiology and Immunology, [2]Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan; [3]Department of Internal Medicine, Division of Hematology-Oncology, Chang Gung Memorial Hospital, Chang Gung University, Lin-Kou, Taiwan; [4]Department of Otolaryngology-Head and Neck Surgery, Chang Gung Memorial Hospital, Lin-Kou, Taiwan

Correspondence to: Yu-Sun Chang. Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan. Email: ysc@mail.cgu.edu.tw.

Comment on: Chan KCA, Woo JKS, King A, *et al.* Analysis of Plasma Epstein-Barr Virus DNA to Screen for Nasopharyngeal Cancer. N Engl J Med 2017;377:513-22.

Received: 27 February 2018; Accepted: 25 March 2018; Published: 28 March 2018.

doi: 10.21037/anpc.2018.03.01

View this article at: http://dx.doi.org/10.21037/anpc.2018.03.01

Nasopharyngeal carcinoma (NPC) is a prominent cancer in Southeast Asia. Decades of studies have shown that NPC is closely associated with infection of the human herpesvirus, Epstein-Barr virus (EBV). Quantitative real-time PCR-based technique was developed to detect circulating cell-free EBV DNA (cf-EBV DNA) as a biomarker for NPC in 1999 by a team led by Dr. Lo in Hong Kong (1). The cf-EBV DNA in plasma has proven useful for early detection, prognosis, treatment response and disease recurrence monitoring of NPC (2). A recent large prospective cf-EBV DNA screening study was published in *New England Journal of Medicine* (3); it was carried out by the same Hong Kong team, collecting blood between July 2013 and February 2016 from more than 20,000 volunteers (ethnically at-risk group, Chinese males aged 40–62) who had participated in the 147 health education sessions. This screening study demonstrated several key benefits related to using a rather conserved W repeat sequence of cf-EBV DNA as a marker for NPC. First, circulating cf-EBV DNA in plasma samples showed a very high sensitivity (97.1%) and specificity (98.6%) for detecting NPC in asymptomatic ethnically at-risk males. Second, a significantly higher proportion of participants diagnosed with NPC using cf-EBV DNA had an early stage (stage I or II) disease (Figure 2 in Chan *et al.*, NEJM 2017) compared with that of previous cohort in Hong Kong (4). Third, the quantitative cf-EBV DNA test is a well-established method with a fairly low estimated cost (approximately US$30). Given that early detection can reduce mortality, morbidity, and treatment costs, the use of cf-EBV DNA test to screen the at-risk population for NPC appears to be a technically feasible and cost-effective practice for regions with a high incidence of this disease.

In contrast to the technical challenges associated with screening for cancers that are not associated with viral infection, viral nucleic acid markers can be good biomarkers for detecting virus-associated cancers. In addition to the use of EBV DNA screening in NPC, HPV and HBV DNA are screened in Pap smear samples for the detection of cervical carcinoma and in blood samples for detection of hepatocellular carcinoma, respectively. However, HPV has more than 100 genotypes, and at least 13–14 genotypes are high risk based on oncogenic potential and geographic differences, indicating the need for a multiplexed-DNA screening tool (5). Similarly, specific HBV genotypes and mutants are recommended for the risk of hepatocellular carcinoma (6).

The collection of liquid biopsies or body fluids and the analysis of circulating DNA derived from cancer cells are becoming increasingly important for oncology researches and patient care. The clinical analysis of circulating tumor DNA in patient plasma samples has a high sensitivity for detecting cancer. To date, this strategy has mainly been used to guide treatment selection and monitor treatment response by detecting residual disease or recurrence. EBV contains a double-stranded DNA genome of approximately 170 kb and persistently infects almost all tumor cells in EBV-associated NPC. EBV sequences in cell-free plasma

can thus serve as a surrogate for tumor DNA. In addition, the unique structural features of EBV DNA including internal repetitive *Bam*HI W sequences (mostly 10 or more repeats in each EBV genome), high copy numbers of EBV episome presence in infected NPC cells (~50–30,000 EBV genome equivalents) (7,8) and high level of EBV DNA in the patient plasma sample offer researchers an excellent opportunity to detect cf-EBV DNA by sensitive PCR-based method and to allow large-scale screening of samples (1). Thus, the EBV repetitive sequence of the *Bam*H1 W fragment turned out to be the most sensitive EBV-PCR target. The current method has a detection limit of 20 EBV copies or 200 copies of the *Bam*H1 W target sequences per milliliter of plasma sample for standard quantitative PCR analysis. Even a small tumor releases enough EBV DNA into the circulation to allow this sensitive method to detect cancer-associated changes. Compared with the analysis of mutated DNA sequences or DNA copy number variations in tumor cells, the detection of EBV sequences in NPC is not affected by the presence of wild-type cellular genomic DNA and tumor cell heterogeneity.

Early detection of cancer including NPC can offer the greatest chance for cure. In the recent study by Chan *et al.*, the cf-EBV DNA test for NPC screening identified 16 patients with stage I disease, eight with stage II disease, eight with stage III disease, and two with stage IV disease. In contrast, more than 80% of NPC cases were identified at a late stage of disease in an historical cohort, the 2013 Hong Kong Cancer Registry (3). Thus, the recent prospective clinical trial study demonstrated the clinical utility for the diagnostic cf-EBV DNA test.

In the context of NPC population screening, the EBV DNA test may outperform the current strategies that use antibodies against EBV proteins or immune response signatures. Antibodies to the viral capsid antigen (VCA), the EBV nuclear antigen 1 (EBNA 1), and early antigen (EA), individually or in combination, have been examined in serum samples from at-risk populations in southeast China, Hong Kong, and Taiwan. The anti-VCA and anti-EBNA1 antibodies, which are used clinically as serological biomarkers for NPC diagnosis, recently underwent a cluster randomized trial in Southern China (9) and Taiwan (10) to assess their ability to identify disease-free individuals at high risk for developing NPC. The most recent study comprehensively measured EBV IgG and IgA antibody responses using a custom protein microarray targeting 199 sequences from 86 EBV proteins. The levels of 60 IgA and 73 IgG antibodies were found to be elevated in stage I/II NPC patients compared to controls, and the use of a 14-antibody signature that included anti-VCA and anti-EBNA1 was found to improve the NPC screening results. However, the output of antibody level was not found to directly reflect the amount of antibody in the blood (11).

To summarize, Chan *et al.* demonstrated that the cf-EBV DNA test can be a feasible and sensitive means for population screening of NPC. From a practical standpoint, it is likely that this screening test can identify at-risk individuals who initially show a negative screening result in anti-EBV antibody test and/or lacking noticeable symptom but may develop NPC within 2 years. Finally, public health campaigns and the large-scale sensitive quantitative cf-EBV DNA screening test effectively improve the awareness as well as the early NPC detection for populations at-risk. Both the campaigns and EBV screening test should be encouraged, supported and conducted routinely to fight against EBV-associated NPC.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Annals of Nasopharynx Cancer*. The article did not undergo external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/anpc.2018.03.01). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Lo YM, Chan LY, Lo KW, et al. Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. Cancer Res 1999;59:1188-91.
2. Kim KY, Le QT, Yom SS, et al. Clinical Utility of Epstein-Barr Virus DNA Testing in the Treatment of Nasopharyngeal Carcinoma Patients. Int J Radiat Oncol Biol Phys 2017;98:996-1001.
3. Chan KCA, Woo JKS, King A, et al. Analysis of Plasma Epstein-Barr Virus DNA to Screen for Nasopharyngeal Cancer. N Engl J Med 2017;377:513-22.
4. Lee AW, Sze WM, Au JS, et al. Treatment results for nasopharyngeal carcinoma in the modern era: the Hong Kong experience. Int J Radiat Oncol Biol Phys 2005;61:1107-16.
5. Shah SS, Senapati S, Klacsmann F, et al. Current Technologies and Recent Developments for Screening of HPV-Associated Cervical and Oropharyngeal Cancers. Cancers (Basel) 2016;8.
6. Yang HI, Yeh SH, Chen PJ, et al. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. J Natl Cancer Inst 2008;100:1134-43.
7. Andersson-Anvret M, Forsby N, Klein G, et al. Relationship between the Epstein-Barr virus and undifferentiated nasopharyngeal carcinoma: correlated nucleic acid hybridization and histopathological examination. Int J Cancer 1977;20:486-94.
8. Chang YS, Tyan YS, Liu ST, et al. Detection of Epstein-Barr virus DNA sequences in nasopharyngeal carcinoma cells by enzymatic DNA amplification. J Clin Microbiol 1990;28:2398-402.
9. Liu Z, Ji MF, Huang QH, et al. Two Epstein-Barr virus-related serologic antibody tests in nasopharyngeal carcinoma screening: results from the initial phase of a cluster randomized controlled trial in Southern China. Am J Epidemiol 2013;177:242-50.
10. Coghill AE, Hsu WL, Pfeiffer RM, et al. Epstein-Barr virus serology as a potential screening marker for nasopharyngeal carcinoma among high-risk individuals from multiplex families in Taiwan. Cancer Epidemiol Biomarkers Prev 2014;23:1213-9.
11. Coghill AE, Pfeiffer RM, Proietti C, et al. Identification of a Novel, EBV-Based Antibody Risk Stratification Signature for Early Detection of Nasopharyngeal Carcinoma in Taiwan. Clin Cancer Res 2018;24:1305-14.

doi: 10.21037/anpc.2018.03.01
Cite this article as: Li HP, Hsu CL, Chang YS. Screening of nasopharyngeal carcinoma using plasma Epstein-Barr virus DNA for at-risk population. Ann Nasopharynx Cancer 2018;2:3.

