Editorial

EBV-related serological biomarkers for nasopharyngeal cancer remain a hot topic

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Comment on: 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.

Received: 03 April 2018; Accepted: 16 April 2018; Published: 20 April 2018.

doi: 10.21037/anpc.2018.04.03

View this article at: http://dx.doi.org/10.21037/anpc.2018.04.03

The pathogenic role of Epstein-Barr virus (EBV) for the initiation and progression of nasopharyngeal carcinoma (NPC) has been highly investigated since EBV-DNA was identified in an NPC tumor specimen (1). In addition to elevated antibody titers against EBV antigens, especially viral capsid antigen (VCA) and early antigen (EA), specific elevation of the IgA antibody in NPC but not Burkitt lymphoma promoted the study of the diagnostic value of EBV serology. Therefore, identification of an EBV-associated antibody as a more accurate tumor biomarker of NPC is desired (2).

The incidence of NPC is high in Southeast Asia (more than 20 to 40 cases per 100,000 person-years) and low in Western countries (less than one case per 10,000 persons per year). Most NPC patients have advanced disease and did not exhibit any clinical symptoms in the early stages. NPC is directly diagnosed by examining biopsy samples from the primary nasopharyngeal site, and occasionally, metastatic neck disease. Improvements in treatment modalities and chemotherapy have saved many advanced NPC patients. However, comorbidity is still high. Thus, identification of patients at early stages and individuals at risk of developing NPC is needed to improve the prognosis. The clinical background of NPC facilitates EBV serology, especially anti-EBV IgA titers, for screening of NPC in endemic areas (3). The general Taiwanese population with elevated IgA antibodies against the lytic VCA protein showed an increased risk of developing NPC compared to those with normal VCA IgA titers, which persisted even more than 5 years after assessment of EBV antibody (HR, 13.9; 95% CI, 3.1–61.7) (4).

The discovery that serum or plasma of patients with infectious mononucleosis agglutinated horse or sheep erythrocytes lead to the original heterophile test. The test was then improved to serum-mediated agglutination of latex beads coated by bovine heterophile antigens, becoming the main serological diagnostic tool for EBV-associated diseases. Although indirect fluorescent antibody tests were more useful, they were hard work and subjective. Later, recombinant EBV proteins using enzyme-linked immunosorbent assays were introduced as a more economical and objective serological test for antibodies against EBV (2).

Coghill *et al.* have been developing suitable serological biomarkers for prediction and early detection of NPC. They demonstrated the superior predictive value of ELISA-based IgA antibodies against the defined VCAp18 region and the EBV nuclear antigen 1 (EBNA1) for NPC.

For VCAp18 IgA, keeping a threshold value of more than 80% sensitivity resulted in a specificity of only 20%, which is less than half of the specificity for EBNA1 IgA. For the combination of VCAp18 + EBNA1 IgA titer, the specificity with more than 80% sensitivity was 54%. Another important aspect in the evaluation of potential screening tools is that selection of the target population changes the number of screened individuals that is required per cancer case to be detected. The sensitivity of the EBNA1 IgA titer was lower when it was analyzed years before NPC diagnosis

(incident NPC) in high-risk multiplex family members compared with measurement at the time of NPC diagnosis (prevalent NPC) in a general population setting (4).

Although the association of anti-EBNA1 and VCA IgA antibody with the risk of NPC development has been well established, whether pattern of antibody titers can predict individuals who will develop NPC in the future still remains unclear. In the prospective study of anti-EBV antibodies and risk of NPC development among high-risk family members in Taiwan using research-based assays for EBNA1 and VCA IgA, they were confirmed as sensitive markers for detecting incident NPC, but the specificity did not reach 50% (4).

A recent report published in *Clin Cancer Res* is the first comprehensive evaluation of the EBV antibody repertoire for detection and prediction of NPC, in which IgA and IgG antibody responses against 199 EBV gene products were assessed. Using this high-dimensional microarray dataset, elevation of 133 antibodies in Stage I/IIa NPC patients was observed and a 14-antibody subset was selected. The selected 14 EBV gene products are composed of all EBV life-cycle proteins, and this subset predicted 5-year NPC risk with 89% sensitivity in high-risk family members and 93% sensitivity in the general population Taiwanese cohorts. This system was significantly improved (P<0·01) compared with VCAp18/EBNA1 IgA alone (AUC=82.3%; 95% CI 74.9–89.7%). Specificity ranging from 61–83%, compared with 60–67% for VCAp18/EBNA1 IgA biomarkers alone, was achieved for this sensitivity range. The reliability of the data obtained by multiplex technology for EBV was confirmed by comparing the present IgA data with the previous IgA data generated by ELISAs. There was a close correlation between the microarray IgA data and the previous ELISA data for IgA antibodies against VCAp18 and EBNA1 (Spearman =0.76 and 0.79, respectively, P<0.01).(5) However, as discussed by the article authors, the limitation of this microarray assay is that EBV proteins printed onto the microarray were produced via cell-free translated sequences, and antibody responses specific to conformational structure or post-translational processing, such as glycosylation, may have been missed. Moreover, the data from the protein microarray does not directly reflect the amount of antibody in the blood. From this viewpoint, it is not recommended to use the risk score or associated cutoff values for clinical application. However, this array technique introduced a new perspective for the value of EBV serology as an NPC biomarker (5).

Quantification of circulating EBV-DNA has become prevalent as a more sensitive tumor biomarker than anti-EBV IgA for NPC detection in endemic and non-endemic areas of NPC. Furthermore, the predictive value for prognosis, especially after initial treatment, of the cell-free EBV-DNA amount in NPC patients is widely accepted, whereas EBV serology markers failed to demonstrate such prognostic value (6-8). In addition, screening of circulating EBV-DNA for asymptomatic NPC was recently reported (9).

There are several reasons for why EBV serology remains a hot research area. The detection rate of circulating EBV-DNA is inconsistent among reports, attributable to the variation in assay methods. Many factors, such as the time point of EBV-DNA quantification during the initial treatment, the target EBV sequence for PCR amplification, the source and sample volume of eluted EBV-DNA, and cut-off levels for PCR products, vary among studies. When an FDA-approved assay for measurement of the circulating EBV-DNA load becomes available, the process described above will become one of verification rather than validation (10). Both EBV serology and EBV-DNA load have been clarified to reflect some aspect of NPC due to advances in laboratory technology and clinical trial design. Therefore, direct comparison of these two methods in well-organized prospective studies is desired in the future.

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: The author has completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/anpc.2018.04.03). The author has no other conflicts of interest to declare.

Ethical Statement: The author is 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.

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doi: 10.21037/anpc.2018.04.03
Cite this article as: Yoshizaki T. EBV-related serological biomarkers for nasopharyngeal cancer remain a hot topic. Ann Nasopharynx Cancer 2018;2:5.