Evaluation of Antibodies against Different Epstein-Barr Virus Nuclear Antigen 1 Peptides in Diagnosis of Nasopharyngeal Carcinoma

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Epstein-Barr virus nuclear antigen 1 (EBNA1) is a protein expressed consistently in nasopharyngeal carcinoma (NPC). Although antibody levels against three different EBNA1 peptides were all high in NPC patients, the correlation between any two biomarkers was low. Therefore, the selection of distinct EBNA1 peptides could render different results in serological detection for individuals with NPC.

Epstein-Barr virus (EBV) infection is closely associated with several malignant diseases, including nasopharyngeal carcinoma (NPC). NPC is highly prevalent in southern China, and NPC patients usually have higher EBV antibody levels than healthy controls. Examination of the serum immunoglobulin A (IgA) antibodies against EBV capsid antigen, diffuse early antigens (EA-D), and EBV nuclear antigen 1 (EBNA1) are widely used for NPC diagnosis and prognosis (3).

EBNA1 plays a role in the maintenance of latent EBV infection and is expressed in all EBV-associated malignant tissues (7), resulting in the hypothesis that EBNA1 is critical for initiating and developing these tumors (6). Encoded by the BamHI K fragment of the EBV genome, EBNA1 contains a Gly-Ala repeat domain flanked by unique regions (1). The repeat region, C terminus, and N terminus are antigenic (2, 8).

To evaluate the commercial EBNA1 proteins for EBV serological examination, two EBNA1 peptides, a recombinant full-length peptide (rEBNA1) (Biodigest, Saco, ME) and a recombinant fusion fragment containing amino acids 1 to 90 (sEBNA1, GSGPRHRDGVRRPQKRPS) by adding a biotinylated linker to the N terminus (Hanyu, Shenzhen, China).

To compare antibody responses in NPC patients and healthy controls, including 78 males and 10 females with an age range of 25 to 71 (mean, 46.6 ± 13.1) years. Written informed consent was obtained from all participants.

As shown in Table 1, the IgA values against the three peptides were significantly higher for samples from the NPC patients than from the healthy controls (P < 0.0001). The areas under the concentration-time curve for IgA xMAP assays were all above 0.8, and the sensitivities and specificities ranged from 80 to 88% for NPC diagnosis according to the optimal cutoff values. The IgG levels against the fusion fragment or synthesized peptide were higher in samples from the NPC patients. However, the IgG levels against full-length EBNA1 were higher in samples from the healthy controls. These results might be due to the nonspecific response to the Gly-Ala repeat region presented in the full-length peptide.

The correlation between any two biomarkers was low, as the correlation coefficient (r) ranged from 0.314 to 0.456. Moreover, the levels of IgG-rEBNA1 had no correlation with the levels of IgG-sEBNA1 (r = –0.066, P = 0.537) or IgG-IEBNA1.
(\(r = 0.072, P = 0.333\)), indicating that the serum samples recognized the EBNA1 peptides variously. This may be due to various individual immune responses to EBNA1 after EBV infection. Alternatively, the peptides might have different conformations, consequently altering the immunogenic regions and resulting in distinct affinities with the same serum.

Therefore, the selection of distinct EBNA1 peptides could render different results in serological detection for individuals with NPC, and it might be more efficient for NPC screening and diagnosis in regions where the disease is endemic if any combination of these peptides is analyzed. Indeed, when IgA-rEBNA1 and sEBNA1 were combined, only 7 of 95 NPC patients had IgA levels below both cutoff values, and the sensitivity of NPC diagnosis increased to 92.6\% (95\% confidence interval [CI], 85.4 to 97.0\%), providing a better strategy for NPC screening and diagnosis. As for other combinations, the sensitivities of IgA-rEBNA1 and sEBNA1, IgA-fEBNA1 and sEBNA1, and IgG-fEBNA1 and sEBNA1 reached 93.6\% (95\% CI, 82.5 to 98.7\%), 97.9\% (95\% CI 88.7 to 99.9\%), and 80.9\% (66.7 to 90.9\%), respectively, with better discriminatory values than the individual peptides. Since the EBV EBNA1 serology examination was performed with members of the Cantonese population, which has the highest NPC incidence, the diagnostic values of these methods await further confirmation in regions where NPC is not endemic.

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REFERENCES

<table>
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<th>Anti-EBNA1 peptide</th>
<th>No. of serum samples from:</th>
<th>FI* value (mean ± SEM) for:</th>
<th>Receiver operating characteristic analysis result for:</th>
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<tr>
<td></td>
<td>NPC patients</td>
<td>Healthy controls</td>
<td>NPC patients</td>
</tr>
<tr>
<td>IgA-rEBNA1</td>
<td>95</td>
<td>88</td>
<td>2,804.7 ± 296.4</td>
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<tr>
<td>IgA-fEBNA1</td>
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<td>IgA-sEBNA1</td>
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<td>IgG-sEBNA1</td>
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<td>44</td>
<td>11,202.5 ± 502.5</td>
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</tbody>
</table>

* FI, fluorescence intensity.
\(^{a}\) AUC, area under the concentration-time curve.