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NPC-REAAD™ EBV IgA-EA ELISA

A Reliable and Accurate Nasopharyngeal Carcinoma (NPC) Detection

Report Summary

Nasopharyngeal Carcinoma (NPC) is a commonly diagnosed head and neck malignancy in Southeast Asia, and is associated with a number of potential causative factors. The EBV seromarkers have been demonstrated to be associated with the presence of the malignancy. NPC-REAAD™ is a sensitive and specific ELISA assay that uses proprietary proteins developed by Restalyst to detect the presence of a segment of the IgA antibody to EA seromarker in a patient's serum. Its use can aid in detection of the condition early on and improve prognosis.

Nasopharyngeal Cancer (NPC) is difficult to detect in its early stages and challenging to distinguish from other ENT related diseases which present NPC-like symptoms, even for experienced clinicians. NPC-REAAD™ utilises a cocktail of 4 NPC-specific antigens for early NPC detection. Clinical studies have shown that NPC-REAAD™ is also able to distinguish NPC from non-NPC cases. The performance of NPC-REAAD™ shows a sensitivity of 96% and specificity of 94% at cut-off of 115RU.

Literature Review

Introduction

Epstein–Barr virus specific serology is useful in the diagnosis of NPC. A number of antibodies have been proposed as suitable markers of the disease, the most common being the IgA antibodies to the Viral Capsid Antigen (VCA), Early Antigen (EA) and Nuclear Antigen (EBNA). The IgA antibody to VCA can be a sensitive diagnostic test for NPC although it has a much lower specificity compared to other markers such as EA. Data in the literature demonstrates that approximately 75%–100% of the patients with NPC had elevated IgA VCA levels (Tam, 1999). On the other hand, the IgA antibody to the early EA has a high specificity and a raised titre almost certainly indicates the presence of NPC. An assay based on EA detection is therefore particularly useful to physicians managing NPC in endemic area because raised titres of the antibodies will raise the suspicion of the presence of the disease.

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Case-Control Studies on NPC and Anti-EBV Seromarkers

The association between EBV seromarkers and NPC has been reported in several case-control studies since 1997. In a cross-sectional case-control study of 124 NPC patients (93 pre-treatment, 13 relapsed, and 18 in remission) and 40 controls in Hong Kong (Fan, et al., 2004), the seroprevalence of anti-EBV EA IgA was 73% for untreated NPC patients, 88% for relapsed NPC patients, 44% for remission patients, and 0% for health controls.

NPC-REAAD™ Performance Data: ROC, Sensitivity and Specificity Characteristics

Background

Published studies have demonstrated the benefit of EBV EA as a suitable marker for NPC screening in populations (Henle & Henle, 1976) . NPC-REAAD™ uses proprietary proteins to detect segments of this marker. Data was generated to assess sensitivity and specificity characteristics for V420 using known positive and negative WHO clinical samples (n=143). Testing was carried out by an independent laboratory (customer site). Samples were assayed and data generated used to construct a representative ROC curve (a plot of the true positive rate against the false positive rate for the different possible cut off points of the assay).

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Materials and Methods

Evidences indicate using a combination of the Ribonucleotide Reductase Small subunit (RR-S) and the DNA Polymerase Accessory subunit (EA-D) recombinant antigens in differentiating clinically confirmed NPC patients from non-NPC patients in a clinical setting. The ELISA under consideration – the NPC-REAAD™ (REAAD™: acronym for Recombinant Antigen-Antibody Detection) uses a combination of four recombinant antigens: Ribonucleotide Reductase Large subunit, Ribonucleotide Reductase Small subunit, the DNA Polymerase Accessory subunit and a novel proprietary protein discovered by Restalyst during the development of NPC-REAAD™ to enhance both the specificity and sensitivity of serological detection of NPC in the attempt to achieve the goal of early detection, single-modality treatment and good control of NPC.

The measurement of serum IgA antibodies to EBV Early Antigens and Ribonucleotide Reductases was performed according to the manufacturer's ELISA procedure. In summary, recombinant NPC-specific antigens dissolved in coating buffer solution were coated onto each well of a 96-well micro-titre plate. The sera were diluted 20 folds with NPC-REAAD™ sample conjugate buffer. Antibodies specific to these antigens if present in diluted human sera will bind to the coated antigens in microplate thus forming antigen-antibody complexes. This reaction was incubated at 37°C for 30 minutes. Plates were washed with wash buffer 3 times to remove any excess antibody before 100 µL of diluted horseradish peroxidase-conjugate Goat-Anti-Human IgA was added to each well. This reaction was incubated a second time at 37°C for 30 minutes. After which, excess conjugate was removed by washing with wash buffer 3 times. Finally, the colorimetric reaction was facilitated by adding Tetramethylbenzidine (TMB) to each well with 15minutes incubation at 37°C. TMB forms a blue colour in the presence of bound conjugates. This enzymatic reaction was then halted by the addition of 1N H_2SO_4, which turns blue coloration to yellow. The wells were read on a spectrophotometer or a micro-well ELISA plate reader at 450 nm against a 620-630 nm reference filter to eliminate any possible causes of interferences. Each sample was done in duplicate.

The results of the ELISA are given as follows: **REAAD™-Units (RU)** = 100 x [(OD_{Sample} – OD_{Blank}) / ($OD_{Reference}$ – OD_{Blank})]. A REAAD™-Unit (RU) reading higher than or equal to 115 (the cut-off) is determined to be positive result or a high risk of contracting NPC where as a RU reading lower than the cut-off of 115 is a negative result or a low risk of contracting NPC.

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Performance Data

Below is the ROC curve generated from V420 assay data with associated sensitivity and specificity characteristics. The performance of NPC-REAAD™ ELISA was determined at a REAAD™-Unit (RU) cut-off of 115RU with reference to Immunofluorescence Assay (IFA). The IFA anti-VCA IgA at the cut-off of 1:10 (Tam, 1999) and IFA anti-EA IgA at the cut-off of 1:5 are used by clinical laboratories to assist in the screening of NPC (Table 1).

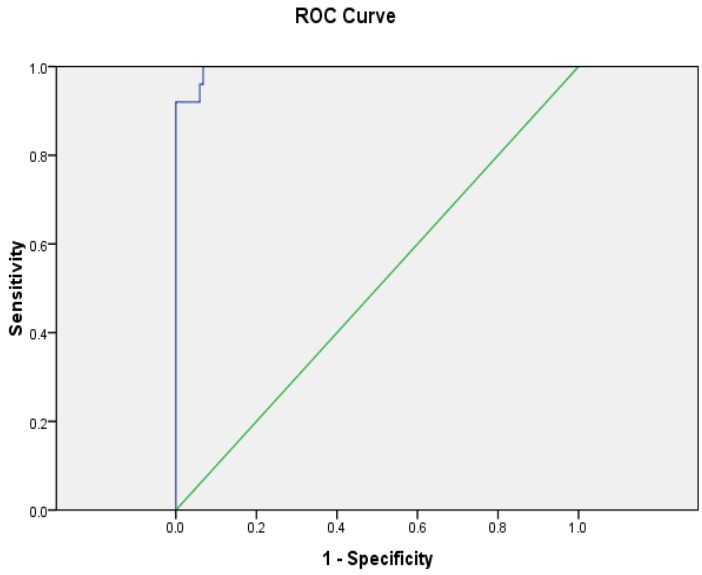

Figure 1: ROC Graph with respect to NPC-REAAD

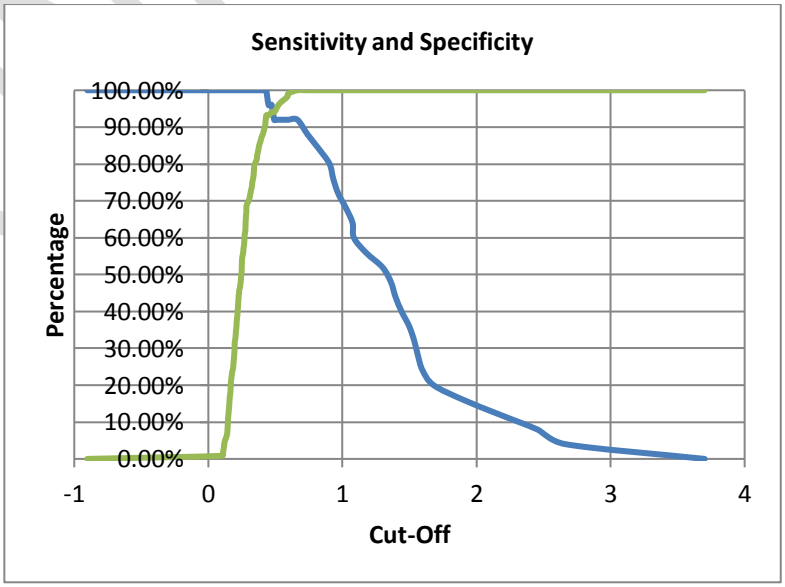

Figure 2: Sensitivity and Specificity of NPC-REAAD

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Table 1: Sensitivities, Specificities and Accuracies of NPC-REAAD™

Test	Positive Criteria	Sensitivity (%)	Specificity (%)
NPC-REAAD™	≥ 115RU	96.0%	94.0%
IFA anti-VCA IgA	≥ 1:10	85.1%	82.3%
IFA anti-EA IgA	≥ 1:5	78.7%	97.3%

Discussion and Conclusion

The ROC curve generated demonstrates the high accuracy of NPC REAAD ™ V420 (the curve follows the left-hand border and then the top border of the ROC space with significant area underneath). Sensitivity and specificity generated from SPSS details performance >90% for both at the defined cut-off point for the assay. It had better sensitivity and specificity to the IFA anti-EBV VCA IgA at cut-off 1:10 and the IFA anti-EBV EA IgA at cut-off 1:5. Results obtained for V420 demonstrate excellent performance characteristics of the assay. In conclusion, an ELISA using a combination of four recombinant antigens specifically detecting IgA antibodies against EBV Early Antigens and Ribonucleotide Reductases can be useful in the serological detection of NPC in an attempt to achieve the ultimate goal of early detection, treatment and control of NPC.

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