Development of a Serum Autoantibody Assay to Help with the Detection of Hepatocellular Carcinoma

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BACKGROUND

Hepatocellular carcinoma (HCC) is the sixth most common cancer and third most common cause of cancer related death worldwide. A lack of efficient screening, leading to detection at late stage, is the main cause for the poor prognosis and the high mortality rates of this disease. Patients with liver disease (Hepatitis B and C, alcoholic liver disease) have an increased risk of developing HCC. Early detection could significantly improve the outcome for patients by identifying the disease when tumours are still operable and curative treatment available.
*Early*CDT®-Lung is a commercial autoantibody assay designed to aid the early detection of lung cancer. This fully validated ELISA test utilises a panel of tumour associated antigens (TAA) to detect serum autoantibodies (AAb) and monitor the humoral immune response. The initial development of a similar test for HCC is described here and work is still ongoing to further improve the results presented. .

METHODS

Protein purification:
A thorough literature search identified 40 HCC and cancer associated antigens (Table 1). Their human cDNA sequences were sub-cloned, with addition of a small tag, into an E.coli expression vector. Competent cells, transformed with the resultant plasmids were expressed to produce the antigens of interest. Purification of the expressed antigens were performed by immobilised metal affinity chromatography (IMAC)[1].

Table 1: Antigen identification by type and specific relation to cancer

HCC AAb evidence	Cancer AAb evidence	No Cancer AAb evidence
Over expressed TAA in HCC (n=20)		
n=9 *(Calrectulin, Ck8, Cyclin B1, FASN, RalA, GRP78…)*	n=5 *(DKK1, LMYC2, Wilms tumour…)*	n=6 *(β-HCG, Gankyrin, GPC-3, HDGF…)*
Aberrantly Expressed TAA in HCC (n=11)		
n=8 *(NY-ESO-1…)*	n=2	n=1 *(AFP)*
Mutated TAA in HCC (n=7)		
n=2 *(p53…)*	n=2	n=3 *(β-catenin, HCC1 & HRAS)*
Un-assigned TAA (n=2)		
n=0	n=1	n=1

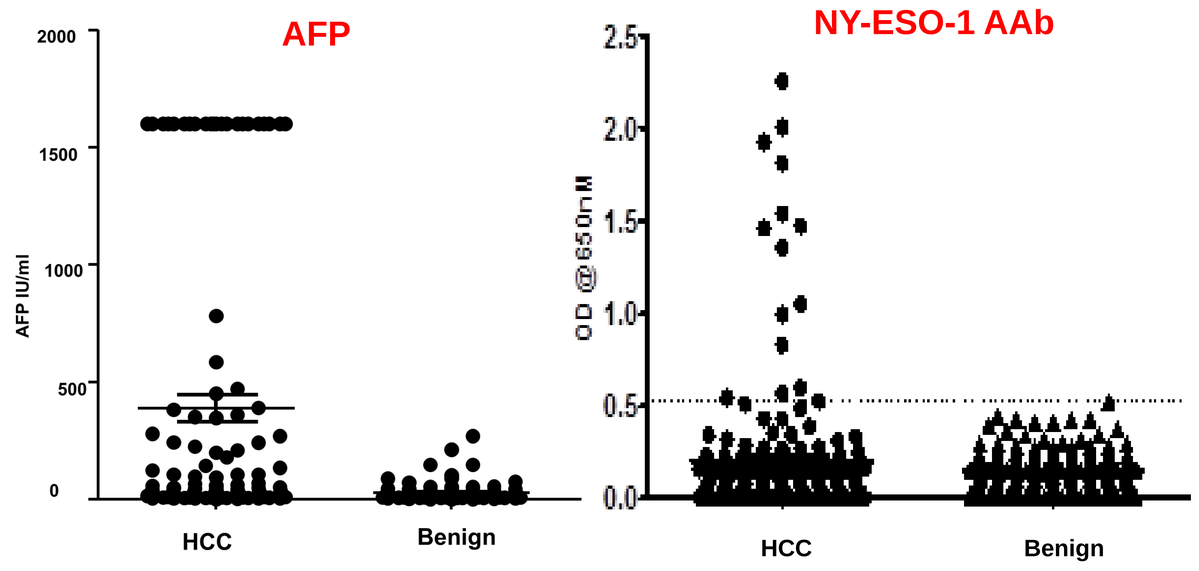
Immuno-assay (pilot study):
Serum from patients with HCC (n=96), as well as matched healthy control sera (n=96) and controls with benign liver disease (n=169), were investigated, by high-throughput ELISA, for the presence of AAb to the 40 antigens tested. The presence of AAb was evaluated using a 2 point semi-automated ELISA[1].

Immuno-assay (development study):
27 antigens identified from the pilot study were tested on an expanded patient sera cohort (HCC n=193, including 25 from the previous cohort, controls with benign liver disease n=192, including 66 from the previous cohort; a sub-cohort of 106 HCC and 106 control sera were fully age matched). The results of this study and levels of AAb were analysed on a 5 point assay[2,3] with an additional NRI (Net Reclassification Improvement)[4] analysis in order to rank the tested antigens by contribution to a potential panel.
In addition to this study, AFP levels were measured with the Aviva AFP-ELISA kit (Insight Biotechnology) on a subset from the cohort described above (HCC n=112, benign liver disease n=82, healthy normal n=44).

RESULTS

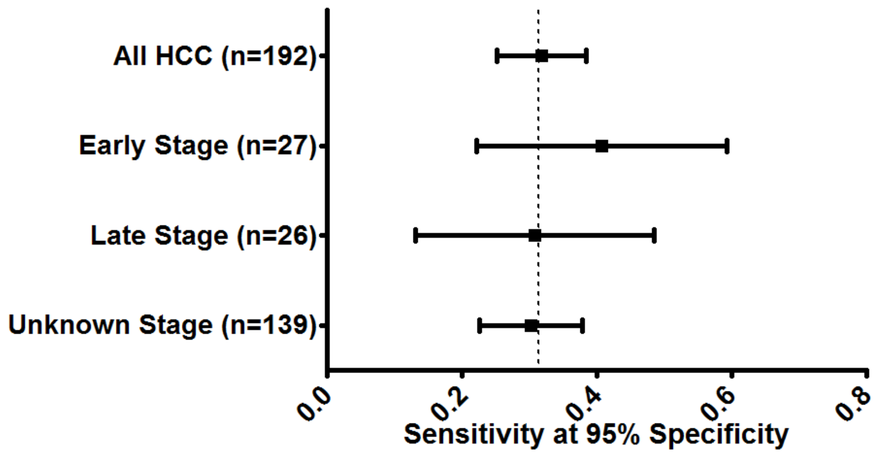
- 27 leads were identified out of the 40 antigens tested from the pilot study and 17 antigens out of these 27 antigens which showed clear cancer normal differentiation were then included in the final panel.
- Measurement of AFP gave a specificity of 98% and sensitivity of 33%.

Figure 1: Example of dot plots showing measurement of AFP and AAbs against NY-ESO-1



- NRI analysis identified a slightly different panel of 17 antigens (including both HCC associated TAAs and more generic cancer associated ones). When combined with AFP results, a specificity of 92.7% and a sensitivity of 53.6% against patients with benign liver disease was achieved.
- All stages of HCC could be detected equally well using this panel (Figure 2).

Figure 2: Sensitivity of Panel of AAbs by Stage of HCC



CONCLUSION

The studies described here have resulted in the identification of a panel of AAb assays that can detect all stages of HCC. When AFP measurements are performed alongside, it shows both AAb and AFP tests are additive and so in combination their individual performance are much improved with a specificity of 92.7% in high risk patients and sensitivity increased to 53.6%. This panel for detection of HCC can be run on the same platform as the *Early*CDT-Lung test which is already available from Oncimmune's CLIA accredited laboratory. When used in combination with AFP, the *Early*CDT-Liver test could prove invaluable in helping clinicians worldwide detect HCC at an earlier stage in high risk populations, thus improving the prognosis and decreasing the number of deaths due to this disease. Research is ongoing to finalise an AAb panel to maximise performance with the aim to release this test commercially in 2016.

REFERENCES

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