



Key Publications

For **EarlyCDT[®]—Liver** for lesions

Development Studies

Serum Autoantibody Measurement for the Detection of Hepatocellular Carcinoma.

Middleton CH, Irving W, Robertson JFR, et al. *PLoS ONE*. 2014; 9(8), e103867.

Autoantibodies as Additive Biomarkers to AFP for the Detection of HCC.

Welberry C, Irving W, Murray A, et al. Poster P-083 presented at: 11th International Liver Cancer Association Annual Conference. September 16th, 2017, Seoul.

Technical & Clinical Validation Studies

Development and Validation of an ELISA Test Detecting a Panel of Autoantibodies in Combination with AFP for Early Detection of Hepatocellular Carcinoma.

Macdonald I, Welberry C, Murray A, et al. Poster P-149 presented at: 12th International Liver Cancer Association Annual Conference, September 15th, 2018, London.

Risk Assessment of Liver Nodules

Improvement in HCC Risk Stratification Following Positive Imaging for Liver Nodules By Application of a Blood Biomarker Test.

Murray A, Macdonald I, Welberry C, et al. (2018), Posters (Abstract 886).

Hepatology. 68: 184–1353.



Publications Spotlight

For **EarlyCDT—Liver** for lesions

Development and Validation of an ELISA Test Detecting a Panel of Autoantibodies in Combination with AFP for Early Detection of Hepatocellular Carcinoma

Macdonald I, Welberry C, Murray A, et al. Poster P-149 presented at: 12th International Liver Cancer Association Annual Conference, September 15th, 2018, London.

Introduction

Assessment of Alpha-fetoprotein (AFP) as a clinical tool for Hepatocellular Carcinoma (HCC) is still under scrutiny. Tumour associated autoantibodies (AABs) have been detected in a variety of solid malignancies, including HCC. The aim of this study was to identify a panel of AABs that could discriminate patients with HCC from those with chronic non-malignant liver disease. The resultant AAB panel's performance for detecting HCC was validated on two independent cohorts (training and validation) in combination with AFP antigen.

Methods

IgG AABs against recombinant Tumour associated-antigens (TAAs) were measured via enzyme-linked immunosorbent assay (ELISA). Cut-Offs for 7 TAAs were determined, using Monte Carlo direct search, in a training cohort of 200 HCC patients matched by age and sex to a control cohort of 200 patients with chronic non-malignant liver disease. Circulating AFP antigen levels measured using an ELISA kit (Monobind Inc) and a cut-off of 200ng/ml applied to determine positivity. These cut-offs were validated in an independent validation cohort of 100 HCC patients and 100 controls, and the sensitivities and specificities determined.



Results

Cut-offs were set for an optimal panel of EpCAM, RalA, NY-ESO-1, MAGE A4, CAGE, MMP9 and AFP AAbs using the training cohort, detecting HCC with a sensitivity of 44.1%, increasing to 55.9% in combination with AFP antigen. Specificity was 91.3%, reducing to 91.1% when combined with AFP. When the same cut-offs were applied to the validation cohort, the sensitivity and specificity of the AAb panel were 30.6% and 87.6% respectively, increasing to 51.0% and 88.2% with the addition of AFP. There was no significant difference in performance of the combined AAb and AFP panel between training and validation ($p=0.30$). Sensitivity for early stage disease (BCLC 0-B) was 54.8% and 55.2% for the training and validation cohorts, respectively. Sensitivity of the AAb panel was independent of disease stage ($p=0.13$). Addition of AFP caused a significant difference between early and late stage ($p=0.007$), however its use still significantly increased sensitivity for early ($p<0.0001$) and late ($p=0.0003$) stage disease detection.

Conclusion

We have successfully developed and validated a minimally invasive blood test based on measurement of a panel of 7 AAbs and AFP antigen to distinguish patients with HCC from those with chronic non-malignant liver disease. This test can detect those patients with early stage disease at a sensitivity of $>50\%$ whilst maintaining a high specificity in high risk patients.

Improvement in HCC Risk Stratification Following Positive Imaging for Liver Nodules By Application of a Blood Biomarker Test

Murray A, Macdonald I, Welberry C, et al. (2018), Posters (Abstract 886).
Hepatology. 68: 184–1353.

Background

Ultrasound screening for hepatocellular carcinoma (HCC) in risk populations, followed by cross sectional imaging for suspicious lesions, still leaves 18-43% of lesions indeterminate. Previous studies have shown tumor associated autoantibody (AAb) levels to be independent of tumor size and disease stage,



with raised levels prior to clinical diagnosis in some instances. Measuring AAb may well be additive to monitoring AFP levels in early detection of HCC or risk stratification of indeterminate lesions. The aim of this study was to develop a high specificity blood biomarker test based on AAb to aid in the risk stratification of patients with liver lesions.

Methods

14 tumor associated antigens were produced recombinantly in *E. Coli* and used to measure IgG AAb via ELISA. Circulating AFP antigen levels were assessed using a commercially available ELISA kit. A panel of AAb plus AFP which could discriminate both early and late stage HCC from chronic non-malignant liver disease using two cut-offs “moderate” (specificity $\geq 90\%$) and “high” (specificity $\geq 98\%$) was identified using a training set (188 HCC/196 chronic liver disease). These cut-offs were validated using an independent cohort of 98 HCC cases and 100 chronic liver disease controls.

Results

A panel of; EpCAM, RalA, NY-ESO-1, MAGE A4, CAGE, MMP9 and AFP AAb plus circulating AFP (EarlyCDT-Liver), was identified using the training set. Performance between training and validation was not significantly different using either the moderate ($p=0.304$) or high cut-offs ($p=0.095$). Sensitivities of the combined training and validation sets were 54% and 41% at specificities of 90% and 97% for moderate and high cut-offs respectively. Sensitivity for early stage disease (BCLC 0-B) was 54.9% and 40.5% using the moderate and high cut-off respectively. A HCC risk model for lesions detected by imaging before and after **EarlyCDT—Liver** was developed. A positive test following Ultrasound+CT or MRI would increase risk of HCC to a minimum of 96% and up to 99% following a high result after Ultrasound+CT. A high result following ultrasound alone increases risk 3.5-fold (23% to 80% increase).

Conclusions

We have developed and validated a minimally non-invasive blood test based on measurement of a panel of 7 AAb and AFP antigen to distinguish patients with HCC, even at early stage, from those with chronic non-malignant liver disease. This test may have clinical utility as a “rule-in” for lesions especially following indeterminate or non-concurrent imaging results.



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