Autoantibodies as additive biomarkers to AFP for the detection of HCC

Christopher Welberry* 1, William Irving 2, Andrea Murray 3, Caroline Chapman 4

1 Division of Medical Sciences and Graduate Entry Medicine University of Nottingham

2 Division of Microbiology and Infectious Diseases, University of Nottingham,

3 Oncimmune Ltd

4 Bowel Cancer Screening Programme, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom

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HCC Screening

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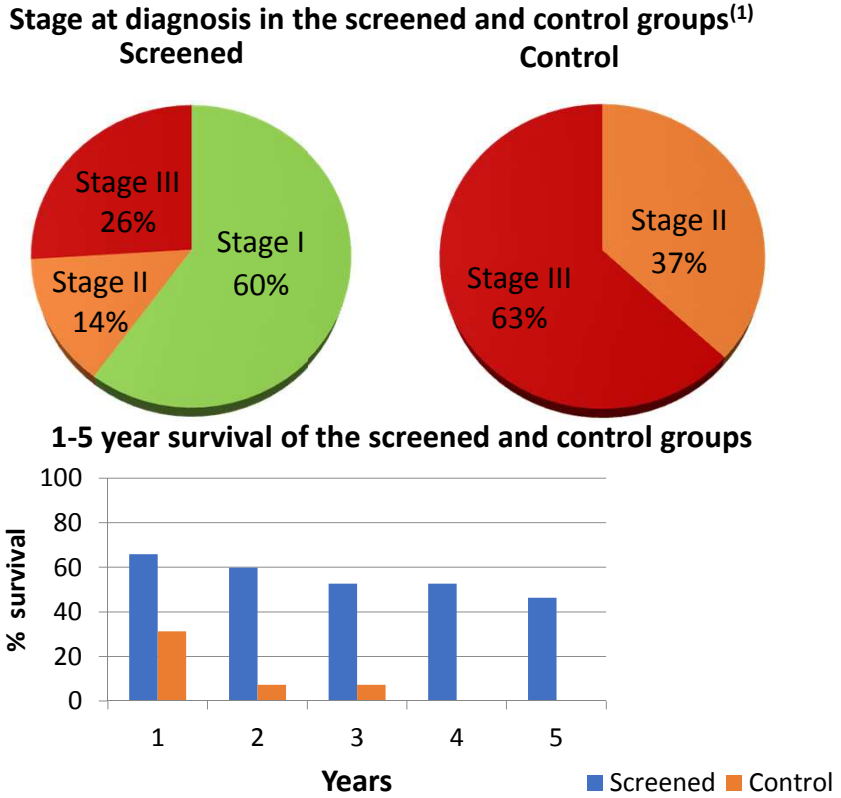
- Detection of Hepatocellular carcinoma (HCC) at an early stage is crucial for a positive prognostic outcome.
- Assessment of Alpha-fetoprotein (AFP) as a screening/diagnostic tool for HCC is still under scrutiny as many patients with HCC do not show elevated levels while raised AFP can be seen in some non-malignant liver diseases.
- Autoantibodies have been shown to be present in numerous solid malignancies including HCC and in some cases have been reported to be detectable before clinical diagnosis.
- A randomised controlled trial of HCC screening in Chinese HBV patients with AFP + Ultrasound, demonstrated HCC survival benefits and detection at an earlier stage(1)
- To increase viability and health economic benefit of screening either test performance must increase or costs must decrease

Outcomes of Zhang et al. Screening trial(1)



1. Adapted from Zhang et al. (2004). Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol*, *130*(7), 417–22.

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Study Aims and Rationale

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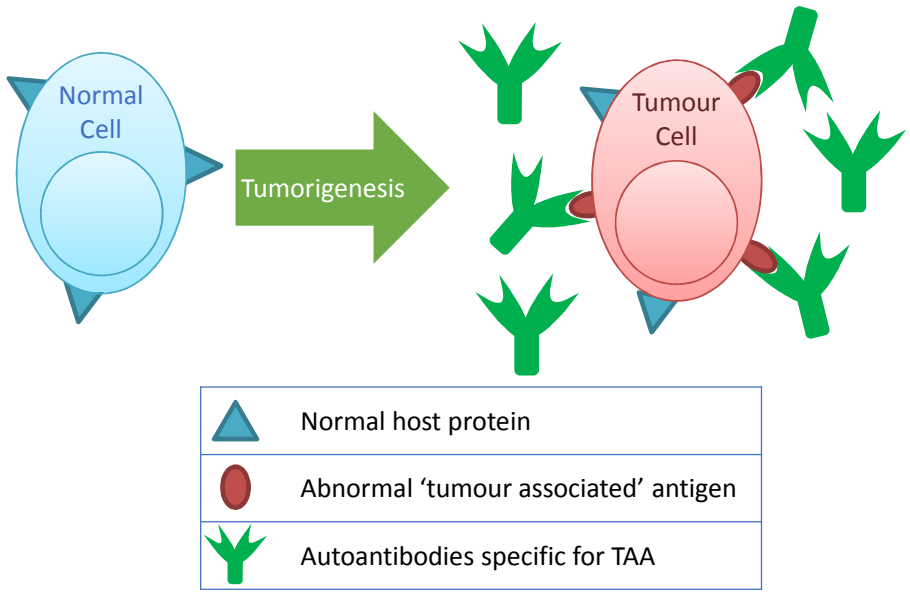
Aims

- Assess autoantibody responses against 21 HCC related antigens in a group of HCC patients and high risk control patients
- Compare the performance of an autoantibody test with that of a commercially available AFP test
- Combine autoantibody data and AFP data to form an improved panel

Rationale

- Early cancer detection based on measurement of autoantibodies
- Produced early in tumour genesis
- Absent or low concentrations in non-malignant patients
- One abnormal (cancer) antigen will lead to many 1,000's of autoantibodies = early measurable amplified signal



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Methods

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Method

Autoantibody ELISA

- Recombinant HCC related capture antigens produced in *E.coli* and purified via affinity chromatography
- Sample set of 98 cancer and 99 high risk controls screened against 21 antigens by ELISA
- Individual antigen cut-offs assigned as the value which gave the max Youden's value with a specificity of >=95%
- Autoantibody panel was formed using net reclassification improvement

AFP ELISA

- Commercially available kit from Aviva Systems Biology
- Cut-off of 200ng/ml used to determine positivity

Sample set

Group	Number	Mean Age (years)	Males/Females
HCC	98	62.6	68/30
TNM Stage 1	*35*		
TNM Stage 2	*20*		
TNM Stage 3	*21*		
TNM Stage 4	*2*		
Stage N/A	*20*		
High risk control	99	50.7	69/30
HCV	*58*		
HBV	*31*		
ALD	*6*		
AIH	*2*		
Haemochromatosis	*1*		
PBC	*1*		

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Results

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- A panel of 10 autoantibodies (NY-ESO-1, DDX3X, HSPA4, Transferrin, MAGE-A4, MMP9, AIF-1, CAGE, RalA and EPCAM) could detect HCC with a sensitivity and specificity of 37% and 90% respectively
- Panel positivity was not significantly associated with stage (p=0.263) or tumour size (p=0.3)
- AFP had a sensitivity and specificity of 32% and 100% respectively
- AFP positivity was not significantly associated with stage (p=0.174), however size was significantly greater in those with a positive AFP test (p=0.007).
- There was no significant difference in ROC AUC between the autoantibody panel and AFP (p=0.489)
- The 10 autoantibody panel was significantly additive to AFP alone, increasing the AUC from 0.66 to 0.7215 (p=0.011) with a resulting sensitivity and specificity of 55% and 90% respectively.

Panel	Sensitivity (%)	Specificity (%)
AFP	32	100
AAb	37	90
AFP + AAb	55	90

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Conclusions

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- Autoantibody measurement has the potential to differentiate HCC patients from those at high risk due to underlying liver disease
- Performance of the autoantibody panel is similar to the performance of AFP
- Autoantibody measurement combined with AFP measurement performed significantly better than either one alone
- The increased performance of the two tests combined could increase the viability of use in screening or surveillance programs