The role of soluble checkpoint receptor and intestinal permeability in Hepatocellular Carcinoma (HCC)

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Introduction

Hepatocellular Carcinoma (HCC) is the fourth most common cause of death from cancer and the second most lethal cancer globally. Risk factors for HCC include chronic HBV or HCV infection, alcoholic liver disease and non-alcoholic fatty liver disease (NAFLD)[1].

Inhibitory checkpoint receptors, such as PD-1, have shown to be central to the impaired anti-tumour immunity observed in HCC patients[2,3,4].

Blocking antibodies targeting inhibitory checkpoint receptor pathways reinvigorate exhausted T-cells and are a promising therapeutic avenue [5].

Soluble forms of membrane-bound checkpoint molecules have shown to be functional [6] but their role in anti-HCC immunity is not well understood.

Some gut microbiome profiles have been associated with a favourable outcome to anti-PD-1 therapy but how they impart anti-tumour benefit is unknown. It has been suggested that bacterial translocation from the gut may be modulating immunity[7].

Aims

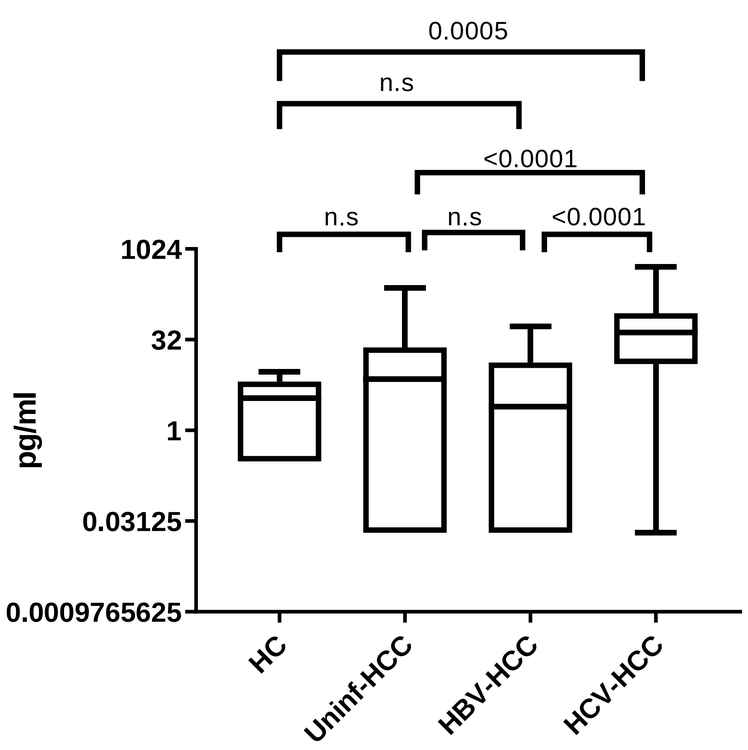
- To investigate whether soluble checkpoint receptors and/or markers of bacterial translocation are involved in HCC.

Methodology

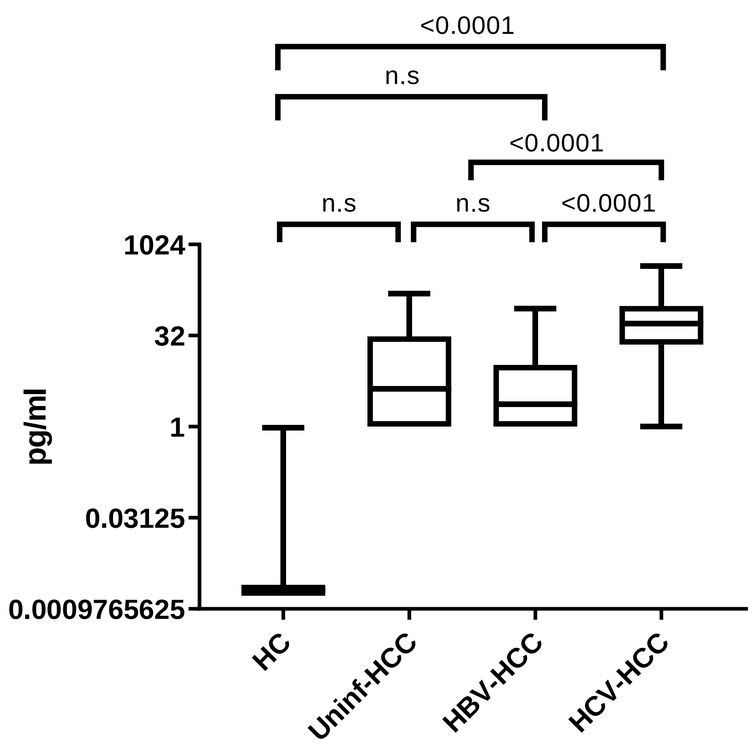
- Soluble (s)PD-1, sPD-L1, sPD-L2, sCTLA-4, sCD80 and D-Lactate (a marker of bacterial translocation) were quantified in baseline serum samples of 257 patients with advanced HCC who were subsequently treated with the anti-PD-1 antibody Nivolumab and 11 healthy controls using Multiplex ELISA and colorimetric assays.
- Soluble checkpoint receptor and D-Lactate levels were compared between 65 HBV infected HCC (HBV-HCC), 60 HCV infected HCC (HCV-HCC), 132 uninfected (Uninf)-HCC and 11 healthy controls (HC). Statistical analysis was performed using a Kruskal-Wallis test. A p-value≤0.05 was considered significant (Results 1).
- R were classed as those who had a complete response, partial response or stable disease and NR were classed as patients who had a progressive disease. Statistical analysis performed by two-tailed Mann-Whitney U test. p≤0.05 statistically significant (Results 2).

Results 1

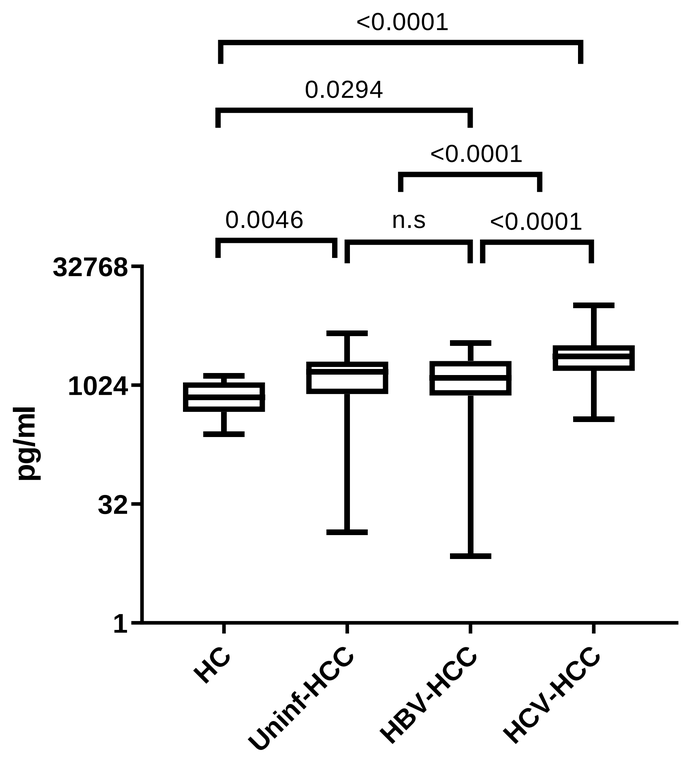
1A Soluble PD-1 expression in HC, Uninf-HCC, HBV-HCC and HCV-HCC



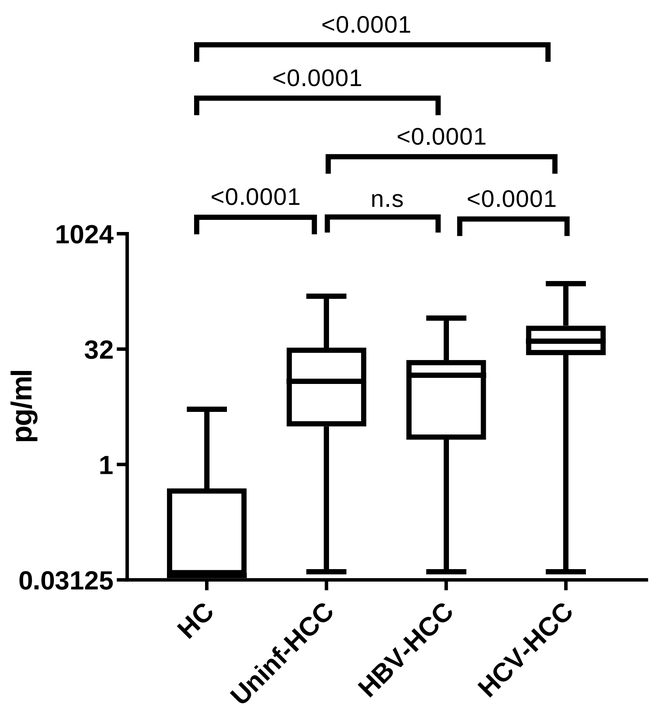
1B Soluble PD-L1 expression in HC, Uninf-HCC, HBV-HCC and HCV-HCC



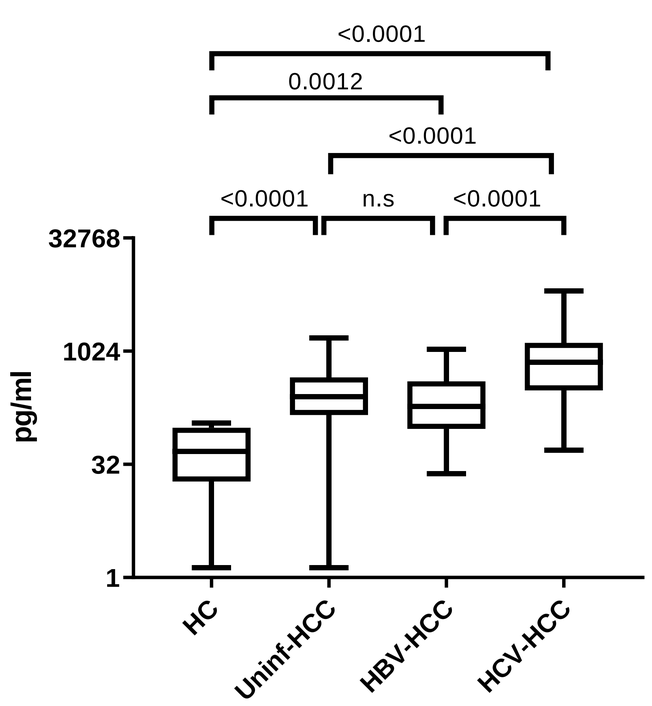
1C Soluble PD-L2 expression in HC, Uninf-HCC, HBV-HCC and HCV-HCC



1D Soluble CTLA-4 expression in HC, Uninf-HCC, HBV-HCC and HCV-HCC



1E Soluble CD80 expression in HC, Uninf-HCC, HBV-HCC and HCV-HCC



1F D-Lactate expression in HC, Uninf-HCC, HBV-HCC and HCV-HCC

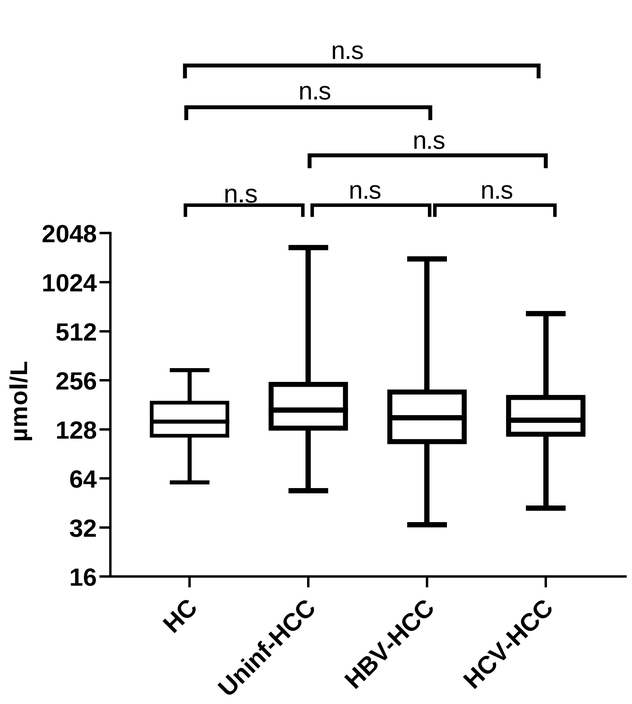


Figure 1: Differences in expression of baseline sPD-1 **(1A)**, sPD-L1 **(1B)**, sPD-L2 **(1C)**, sCTLA-4 **(1D)**, sCD80 **(1E)** and D-Lactate **(1F)** between uninfected-HCC (Uninf-HCC), HBV infected HCC (HBV-HCC) and HCV infected HCC (HCV-HCC) patients given Nivolumab treatment in the serum and between healthy controls (HC). **(1A-1F)** Concentrations of markers were quantified respectively in the serum of patients treated with Nivolumab at baseline using Luminex technology and colorimetric assays. Uninf-HCC n= 132; HBV-HCC n= 65; HCV-HCC n= 60; HC n= 11.

Results 2

2A D-Lactate expression R vs NR in HCV-HCC

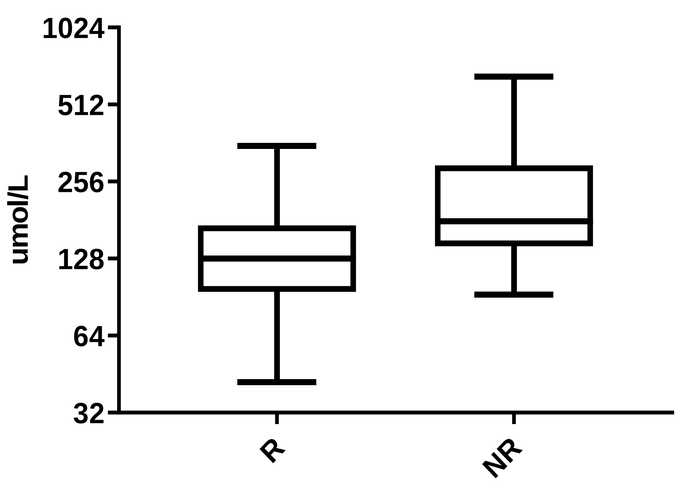


Figure 2. Difference in baseline serum levels of D-lactate between Nivolumab responders and non-responders who were HCV-HCC infected only **(2A)**. D-Lactate was quantified using a colorimetric assay and compared between responders (R) n=34 and non-responders (NR) n=26 with HCV-HCC only.

Conclusions

- Our results found that HCV-HCC patients express higher levels of soluble checkpoint receptors at baseline than HBV-HCC and uninfected-HCC patients, suggesting an important role of soluble checkpoint receptors in HCV-HCC immunity.
- Our results also indicate that non-responder HCV-HCC may have bacterial translocation although how this affects the efficacy of checkpoint receptor inhibition treatment is yet to be determined.

References
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