Hepatocellular carcinomas (HCC) develop on a background of chronic liver inflammation. Neutrophils are key mediators of the tumour immune microenvironment. Elevated circulating neutrophils in HCC patients is independently associated with poorer survival, while neutrophil depletion in a murine HCC model had profound anti-tumour effects. Pan-neutrophil depletion rendering patients susceptible to infection is not a viable therapeutic option, particularly as some neutrophils may also have anti-tumour functions. Here we have interrogated tissue neutrophils in HCC patients, aiming to understand associations with circulating cells, clinical features and prognosis.

Biopsy tissue from HCC patients (n=61) presenting to a Newcastle centre from 2016 to 2019 were studied for the presence and number of neutrophils. Immunohistochemistry for the neutrophil marker CD66b was performed on an automated system, and biopsy slides were analysed using the Aperio Leica system paired with ImageScope software. Data were acquired using an algorithm and presented as number of neutrophils per unit of area (mm^2). Associations between number of neutrophils within peritumour and intratumour areas and clinical features of the patients were assessed using SPSS statistical software package, with statistical significance considered as p<0.05.

Numbers of circulating neutrophils correlated with both peritumour and intratumour neutrophils (Spearman r=0.357, p=0.03, and r=0.328, p=0.025, respectively). Focused on intratumour tissue neutrophils, higher numbers (>52 cells/mm^2 median) were associated with smaller tumours <5 cm and earlier TNM stage (p=0.037 and p=0.025, respectively, Chi-squared test). Elevated intratumour neutrophil number was also significantly associated with better responses in treated patients at 1 year (p=0.017, Chi-squared test), as well as in a smaller more uniform sub-cohort (n=12) treated with transarterial therapy (p=0.031). Higher versus lower intratumour neutrophils was also associated with improved progression-free survival (median 20.93 versus 6.9 months; p=0.009, Kaplan-Meier; HR 4.085, CI 1.317–12.672, p=0.015, univariate cox regression). These positive associations were restricted to intratumour neutrophils only.

In combination, these data suggest that while elevated circulating neutrophils are associated with a poor outcome in patients with HCC, correlating with neutrophil infiltration in the tumour tissues, the location of the neutrophils in the tissues may be key. In cases where neutrophils enter the tumour, tumour progression may be delayed and responses to treatment enhanced. Understanding the cues (neutrophil phenotype or the tumour microenvironment) governing location and interactions within tumours may aid the development of therapeutic strategies that will benefit more patients.

**REFERENCES**

1. Margetts J, et al., 2017  
2. Wilson CL, et al., 2015