Prognostic value of tumour-infiltrating CD68⁺ macrophages is dependent upon location within cholangiocarcinoma tumours

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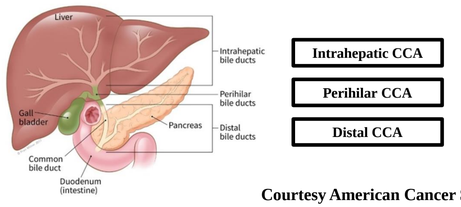
Pathological Society — Understanding Disease — Guiding Therapy

Introduction

- Cholangiocarcinoma (CCA) is the second commonest form of liver cancer worldwide and arises from a malignant transformation of cholangiocytes (epithelial lining of the bile ducts/ductules within the liver).
- Rising incidence and mortality rates, combined with a dismal prognosis (5-year survival < 10%) make this an important clinical problem to be addressed .
- Recognized risk factors for CCA, including chronic Hepatitis B or C infection, liver fluke infection and autoimmune-related diseases such as Primary Sclerosing Cholangitis, all point to an immune component in the pathogenesis of this disease. However, immune-related mechanisms of disease pathogenesis and progression in CCA remain largely understudied.
- A better understanding of the immune tumour microenvironment (TME) in this disease is necessary to enhance development of new treatment and prognostication strategies for patients with CCA.
- We therefore aimed to investigate the potential contribution of immune cells to CCA pathology.



Courtesy American Cancer Society

Figure 1: Anatomic classification of CCA

Methods

- Using immunohistochemistry, we first analyzed formalin-fixed, paraffin-embedded CCA tumours to investigate the presence of tumour-infiltrating immune cells in human CCAs (n=20).
- We next conducted a systematic review and meta-analysis of existing literature in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[1,2], to determine the correlation between immune cell presence (assessed histologically) and clinical outcomes in patients diagnosed with CCA. Also see Systematic Review and Meta-Analysis Protocol on next slide.
- Finally, using NanoString™ Digital Spatial Profiling (DSP), we preliminarily undertook spatially-resolved analysis of immune protein signatures within CCA tumours (n=2).

References

1.Liberati A, et al., The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. 2009;339:b2700.

2. Egbuniwe IU. et al. 2020. PROSPERO Int. Prospect. Regist. Syst. Rev

Results

Our results demonstrate the following:

- Preferential accumulation of CD68⁺ macrophages and CD3⁺ T lymphocyte subsets within CCA tumours compared with non-tumour bile duct tissue.
- Presence of intra-tumoural CD68⁺ macrophages and CD3⁺ CD8⁺ (Cytotoxic) T lymphocytes are most significantly associated with overall patient survival in patients diagnosed with CCA.
- Prognostic significance of intra-tumoural CD68⁺ macrophages appears dependent upon spatial location within CCA tumours.
- Corroboration of differential presence of CD68⁺ macrophages within distinct tumour regions by quantification analysis.
- Distinct clustering patterns between CD68⁺ macrophages and the immune checkpoint molecule B7-H3 using cluster analysis.

Macrophages preferentially accumulate within CCA tumour tissue compared to non-tumour bile duct tissue

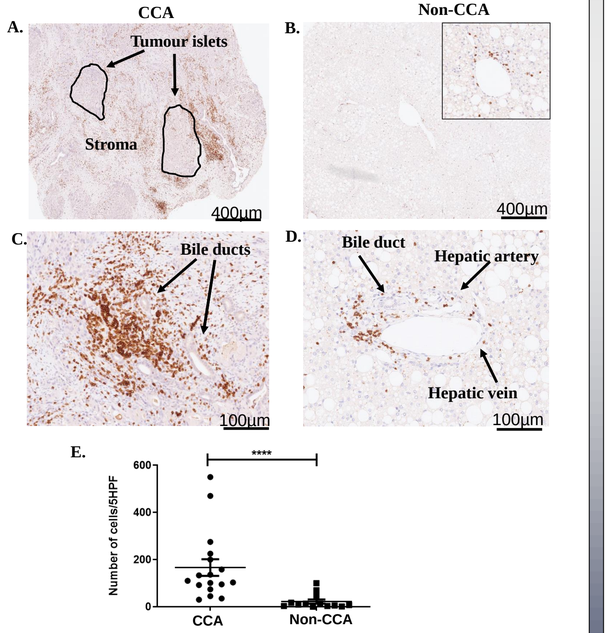


Figure 2: Immunohistochemical staining of CCA tumours (A and C), and matched non-tumour bile duct tissue (B and D) shows tumour-infiltrating CD68⁺ macrophages (brown dots) within tumour islets and stroma. Comparatively fewer CD68⁺ macrophages are present within non-tumour tissue obtained from the same patient sample. (E) Quantification of CD68⁺ macrophages within CCA tumour and non-tumour tissue. P<0.0001; Mann-Whitney U Test; Error bars = Mean ± SEM.

Results

T lymphocyte subsets are also enriched within CCA tumour tissue compared to non- tumour bile duct tissue

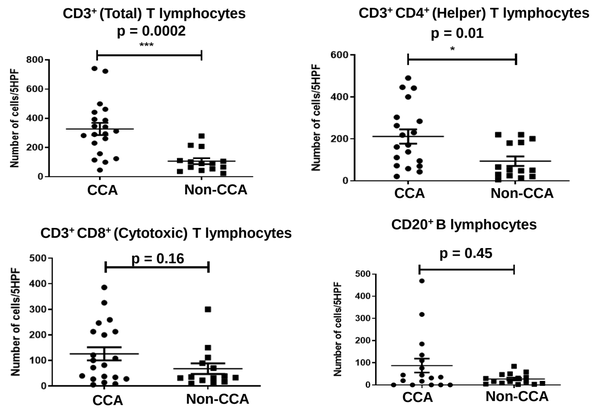


Figure 3: Increased numbers of total (CD3⁺), helper (CD3⁺CD4⁺) and cytotoxic (CD3⁺CD8⁺) T lymphocytes are present within CCA tumours compared to non-tumour bile duct tissue.

Prognostic significance of intra-tumoural macrophages is dependent upon location within CCA tumours

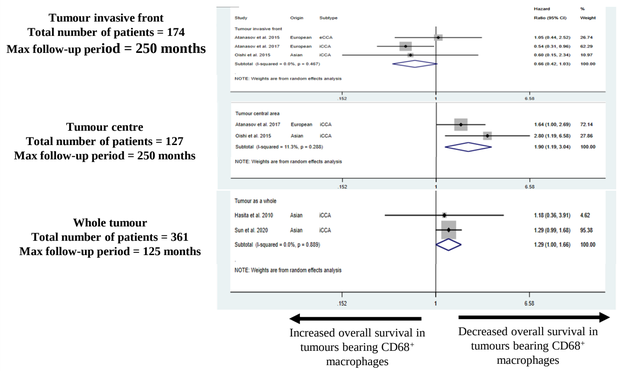


Figure 4: Forest plots show correlation between intra-tumoural location of CD68⁺ macrophages and overall survival in patients diagnosed with CCA. Measure of Effect = Pooled Hazard Ratio; Measure of Statistical Significance = 95% Confidence Interval (CI).

Results

Quantification and Cluster analyses corroborate differential presence of CD68⁺ macrophages within distinct tumour regions

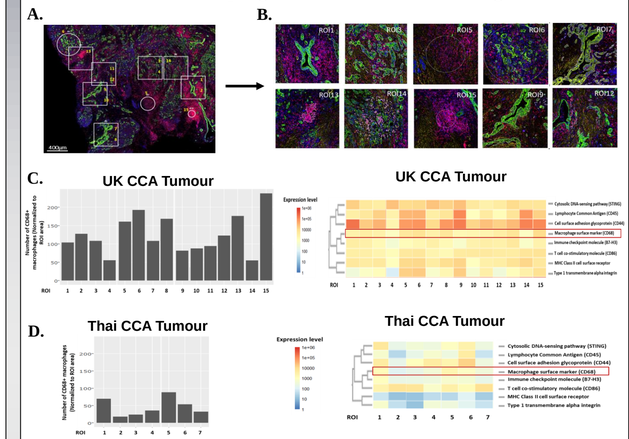


Figure 5: Patient CCA tumours were stained to identify tumour cells (green = pan-cytokeratin),T cells (red = CD3), stromal cells (yellow = smooth muscle actin) and cell nuclei (blue = DAPI stain). A and B = images of tumour obtained from a UK patient. Regions of interest (ROIs - white boxes/circles) representing tumour-rich, immune-rich or stroma-rich areas were further probed to generate quantification graphs and heatmaps (C and D; left and right panels respectively) of stromal and immune signatures. Differential presence of CD68⁺ macrophages is seen within different regions in CCA tumours from one UK (C) and one Thai (D) patient.
Heatmaps also show clustering of CD68⁺ macrophages with immune checkpoint molecule (B7-H3) within both tumours.

Conclusions

- CD68⁺ macrophages and CD3⁺ (Total) T lymphocytes are enriched within CCA tumours compared to non-tumour tissue.
- CD68⁺ tumour-infiltrating macrophages correlate with overall survival in patients with CCA.
- Prognostic significance of intra-tumoural CD68⁺ macrophages appears dependent upon location within CCA tumours – likely related to underlying intra and inter-tumoural heterogeneity
- Findings highlight the need to consider spatial context in the evaluation of tumour specimens in research and diagnostic/ prognostic endeavours.

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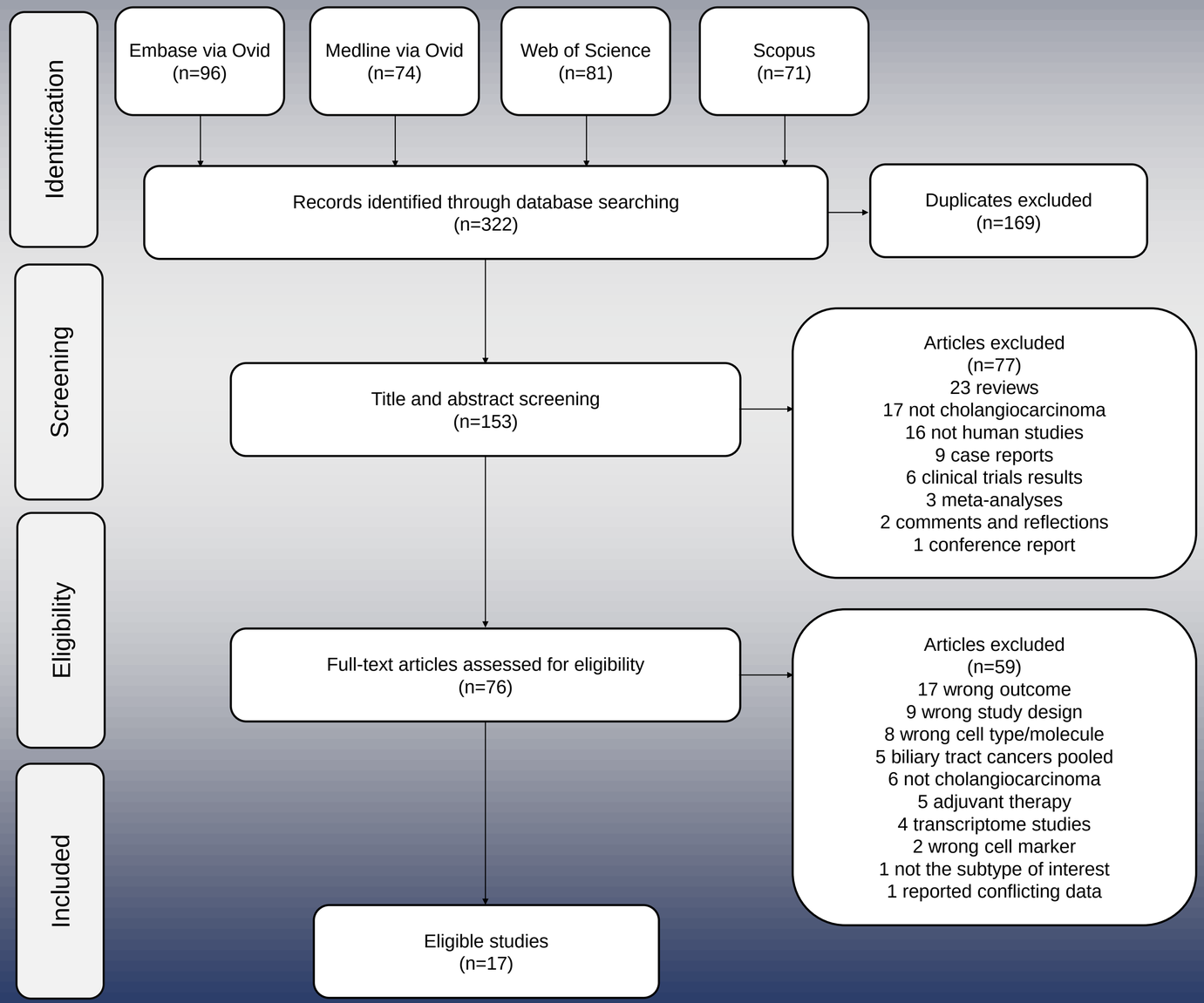
Systematic Review and Meta-analysis Protocol

- Study conducted following PRISMA guidelines[1]
- Protocol registered on PROSPERO database[2]
- Study screened using Rayaan QCRI web tool (https://rayyan.qcri.org/welcome)
- Data extraction/Inclusion criteria:
  - Excel spreadsheet
  - Histopathological assessment
  - Meta-analysis of pooled hazard ratios for overall survival (OS)
- Risk of Bias Assessment conducted using QUIPS tool[3]



References

1. Liberati A. et al. 2009. *BMJ. 339*
2. Egbuniwe IU. et al. 2020. *PROSPERO Int. Prospect. Regist. Syst. Rev*
3. Hayden JA. et al. 2013. *Ann. Intern. Med. 158:280-286*