Phenotypic characterization of untreated chronic hepatitis B infected individuals in an Ethiopian cohort using fixed whole blood samples.

Julia MacLeod (1, 2), Asgeir Johannessen (1,3), Nega Berhe (3,4), Hailemichael Desalegn (3,5), Fikadu Gudissa (6), Asiya Jeylan (6), Lasse Rossvoll (1,3), Dag Henrik Reikvam (1, 2), Niklas Björkström (7), Annika Niehrs (7).

Institute of Clinical Medicine, University of Oslo, Oslo, Norway (1). Department of Infectious Diseases, Oslo University Hospital, Oslo Norway (2). Vestfold Hospital, Tønsberg, Norway (3). Addis Ababa University, Addis Ababa, Ethiopia (4). St. Paul's Hospital Millenium Medical College, Addis Ababa, Ethiopia (5). Adama Comprehensive Specialized Hospital Medical College, Adama, Ethiopia (6). Centre for Infectious Medicine, Karolinska Institutet, Stockholm, Sweden (7).

BACKGROUND

- Hepatitis B virus (HBV) infection affects 64.7 million people in Africa alone and the immunophenotype of African patients with untreated HBV infection is rarely described.
- In this study we used a novel cryopreservation method and flow cytometry to investigate the immune cell phenotype of untreated chronically HBV-infected African patients.

CONCLUSION

- Cytodelics® is a reliable cryopreservation method for identification of all major immune cell subtypes.
- Preliminary results suggest cirrhosis patients display a distinct immunophenotype in this untreated, Ethiopian population.

.

METHOD

Patients enrolled at Adama Hospital, Ethiopia:
- Untreated HBV infection
- HBV and HIV negative

→ Grouped based on:
- Clinical examination
- ALT, HBV DNA
- Fibroscan/ ultrasound
- APRI score
- Pregnancy

→ Following 187 patients included in herein dataset:
- Cirrhosis - 20
- Inflammation - 37
- Immune control - 65
- Greyzone - 25
- Pregnancy - 40

→ Cryoreservation using Cytodelics® whole blood stabiliser.

→ Samples thawed, stained with primary labeled antibodies and analysed using a 27- colour flow cytometry panel.

→ CD45 barcoding with three patients in each group, de-barcoded using FlowJo®

RESULTS

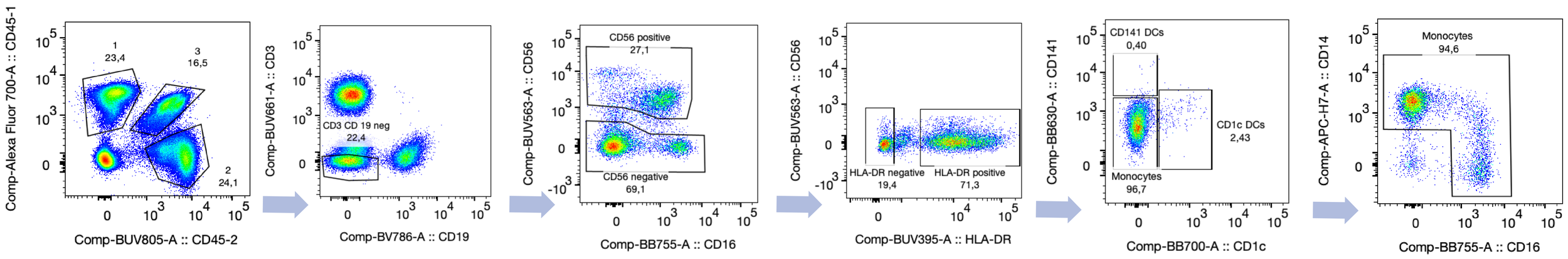

Figure 1: Gating strategy above showing debarcoding of CD45 positive cells then identification of Natural Killer cells (CD56 positive), CD141+ Dendritic cells (Dcs), CD1c+ Dendritic cells and monocytes.

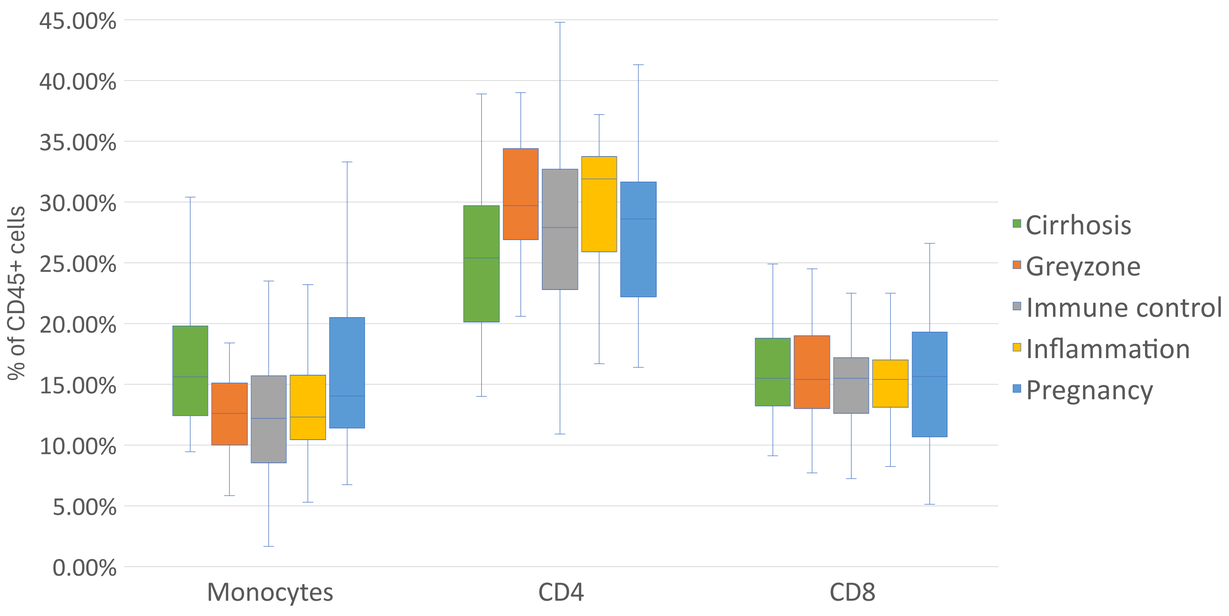

Figure 2: Horizontal line represents the median, box represents interquartile range, and whiskers represent the maximum and minimum. Patients with cirrhosis have a higher median percentage of monocytes (median percentage = 15.60%) in the CD45+ cell population compared with the pregnancy, inflammation, immune control and greyzone groups. The median percentage of CD8 positive T cells appears similar across all groups, however, there appears to be a lower median percentage of CD4 positive T cells (median percentage = 25,40%) compared with the other groups which could explain the lower percentage of regulatory T cells observed in Figure 4.

Figure 3: Example plots above showing to the left IgD negative, IgD positive/CD27 positive and naive B cells in the CD19 positive B cell population. On the right regulatory T cells in the CD4 positive population.

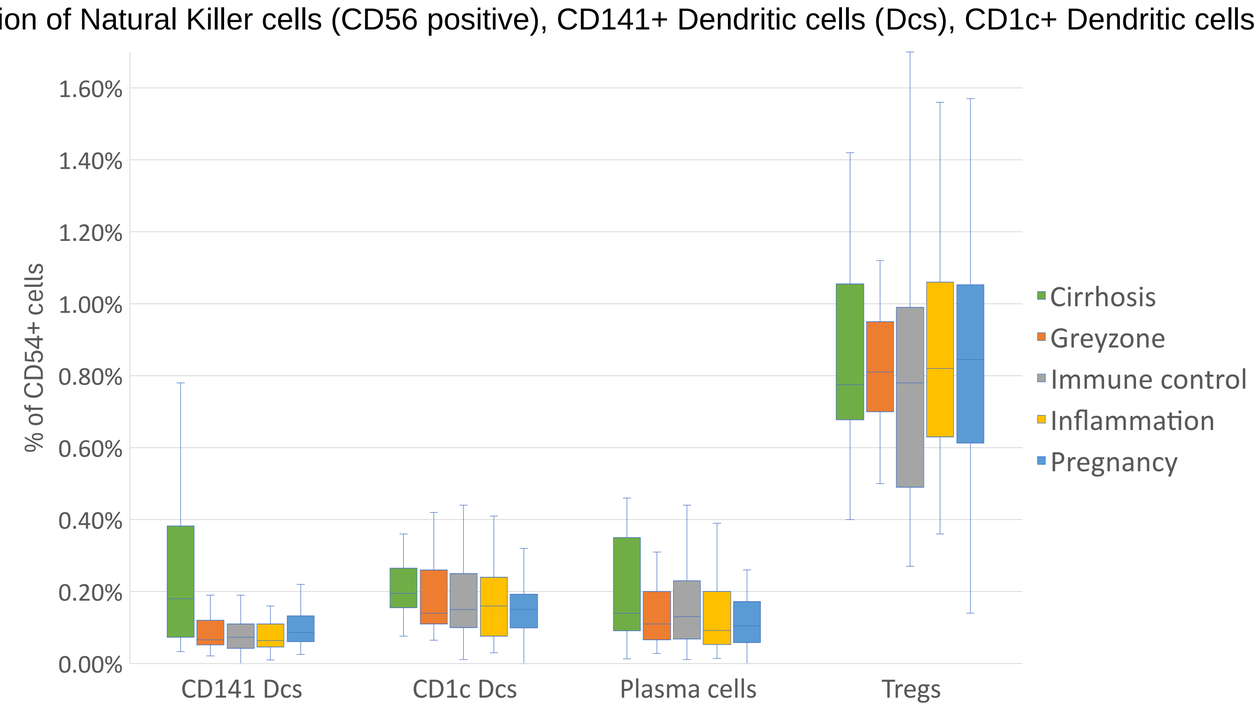

Figure 4: Patients with cirrhosis display a higher percent of both CD141Dcs (median percentage = 0.18%), CD1c Dcs (median percentage 0.20%) and plasma cells (median percentage 0.14%) compared to the other groups. There is observed a slight reduction in median percent positive regulatory T cells (Tregs) (median percentage = 0.78%).

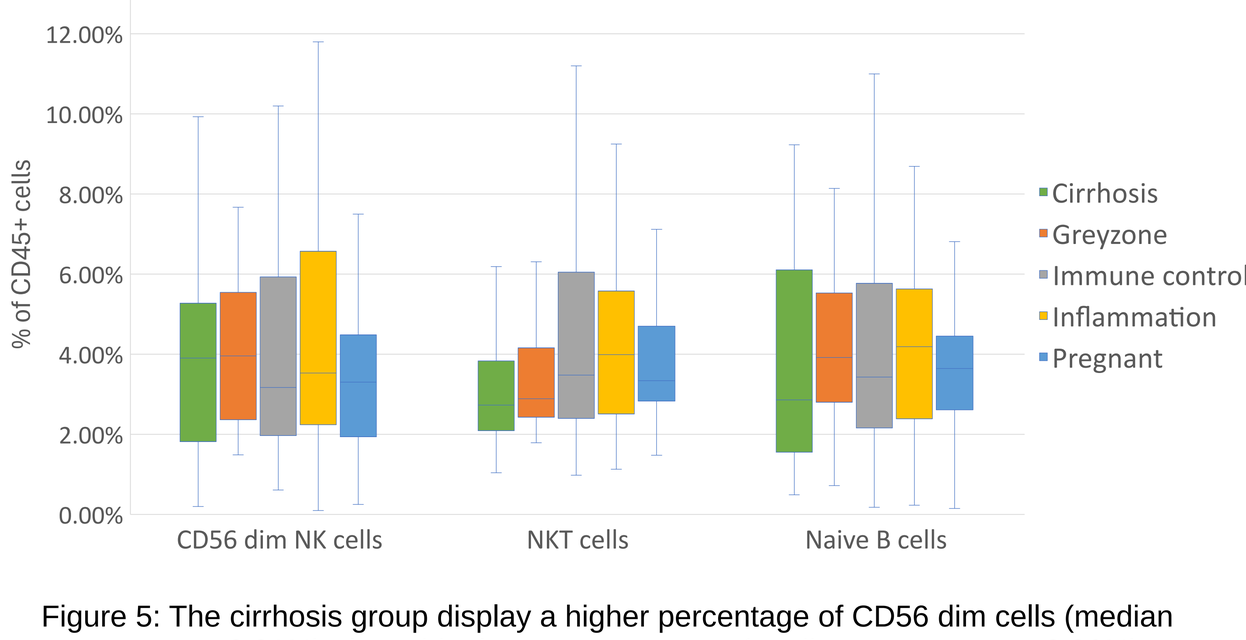

Figure 5: The cirrhosis group display a higher percentage of CD56 dim cells (median percentage = 3,91%) alongside the greyzone group (median percentage = 3.96%) compared with the other groups. The cirrhosis group also have a lower percentage of NKT cells (median percentage = 2.73%) and naive B cells (median percentage 2.86%) compared with all the other groups.

Oslo University Hospital · UiO : University of Oslo · Funded by The Research Council of Norway · Karolinska Institutet

Correspondence to: Julia MacLeod
MBChB, PhD student
juliamac@uio.no