

# Characterization of Blood Immune Cells in patients with Decompensated Cirrhosis (SDC, UDC, and pre-ACLF): A pilot study

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## INTRODUCTION

Liver cirrhosis has been traditionally classified into 2 major states: **compensated cirrhosis** and **decompensated cirrhosis**. The last is characterized by the occurrence of complications (ascites, variceal bleeding, and hepatic encephalopathy), which are associated with poor survival and quality of life (1).

Recently, more in detail, 4 pathophysiological/prognostic groups in patients with decompensated cirrhosis have been proposed (2):

- Stable decompensated cirrhosis (SDC)
- Unstable decompensated cirrhosis (UDC)
- Pre-acute-on-chronic liver failure (pre-ACLF)
- ACLF



However, the biological mechanisms involved in decompensation of cirrhosis and its progression need to be well-defined.

## OBJECTIVE

Patients with cirrhosis present their immune system altered, indicated by an increase in circulating immune-deficient monocytes. The aim of this study is to estimate **changes in cell-type proportion** as well to perform **differential expression analysis** between different stages of decompensated cirrhosis at cell-type level.

## SAMPLE OF STUDY & DATA ANALYSIS

### Baseline Characteristics

	DECISION <i>de novo</i> recruitment				p value
	Healthy	SDC	UDC	Pre-ACLF	
<b>Patients</b>	3	6	6	7	-
<b>Characteristics</b>					
Age, median (range)	56 (56 - 60)	59.5 (54 - 81)	55.5 (43 - 57)	70 (49 - 79)	0.134
Female Sex, yes, n (%)	1 (33)	2 (33)	0	2 (29)	0.479
Diabetes mellitus, yes, n (%)	-	2 (33)	2 (33)	3 (43)	0.614
CKD, yes, n (%)	-	0	0	2 (29)	0.194
Cardiovascular disease, yes, n (%)	-	0	1 (17)	1 (14)	0.672
BMI >30, yes, n (%)	-	2 (33)	0	0	0.118
COPD, yes, n (%)	-	0	0	1 (14)	0.523
HCC, yes, n (%)	-	0	0	1 (14)	0.523
Alcohol Consumption, active, n (%)	-	1 (17)	2 (33)	1 (14)	0.643
<b>Etiology of cirrhosis, n (%)</b>					
Alcohol	-	1 (17)	3 (50)	2 (29)	0.452
Viral (HBV/HCV)	-	1 (17)	1 (17)	0	0.259
NAFLD	-	2 (33)	1 (17)	2 (29)	0.828
Alcohol and viral	-	0	0	2 (29)	0.147
Alcohol and NAFLD	-	1 (17)	1 (17)	0	0.521
other	-	1 (17)	0	1 (14)	0.591

### Data Generation

Preliminary analysis is based on a pilot study. It consists on single-cell RNAseq Gene Expression analysis (GEX) of peripheral Blood Mononuclear Cells (PBMCs) from 3 patients (1 SDC, 1 UDC and 1 pre-ACLF) and one healthy donor.



### Data Processing

Cell Ranger Software 5.0.1. (3) was used to perform simple demultiplexing, barcode processing and single-cell 3' gene counting using standards default parameters and human build hg38.

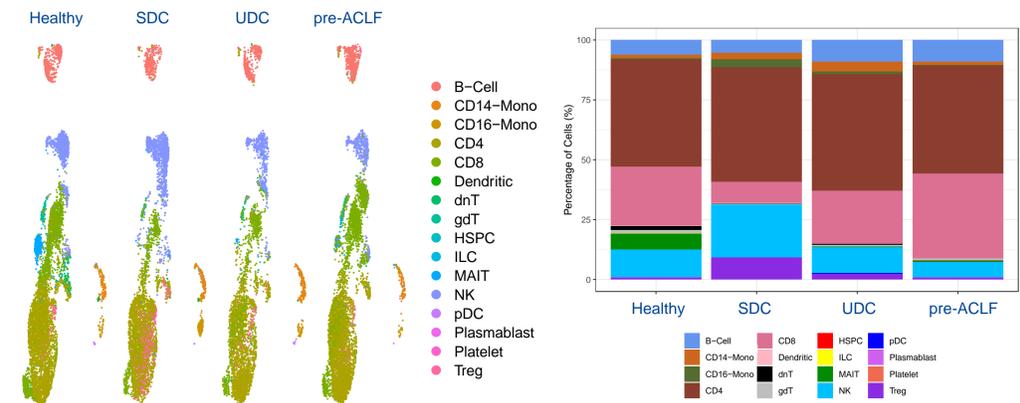
### Sequencing Control

	Estimated Number of Cells	Mean Reads per Cell	Median Genes per Cell	Number of Reads	Reads Mapped Confidently to Genome/ Transcriptome
Healthy	8,327	17,015	1,531	141,682,646	84.50% - 72.50%
SDC	6,597	21,445	1,930	141,474,048	86.40% - 73.60%
UDC	5,237	25,257	1,759	132,272,104	83.40% - 71.70%
pre-ACLF	8,218	13,252	1,398	108,902,141	82.80% - 71%

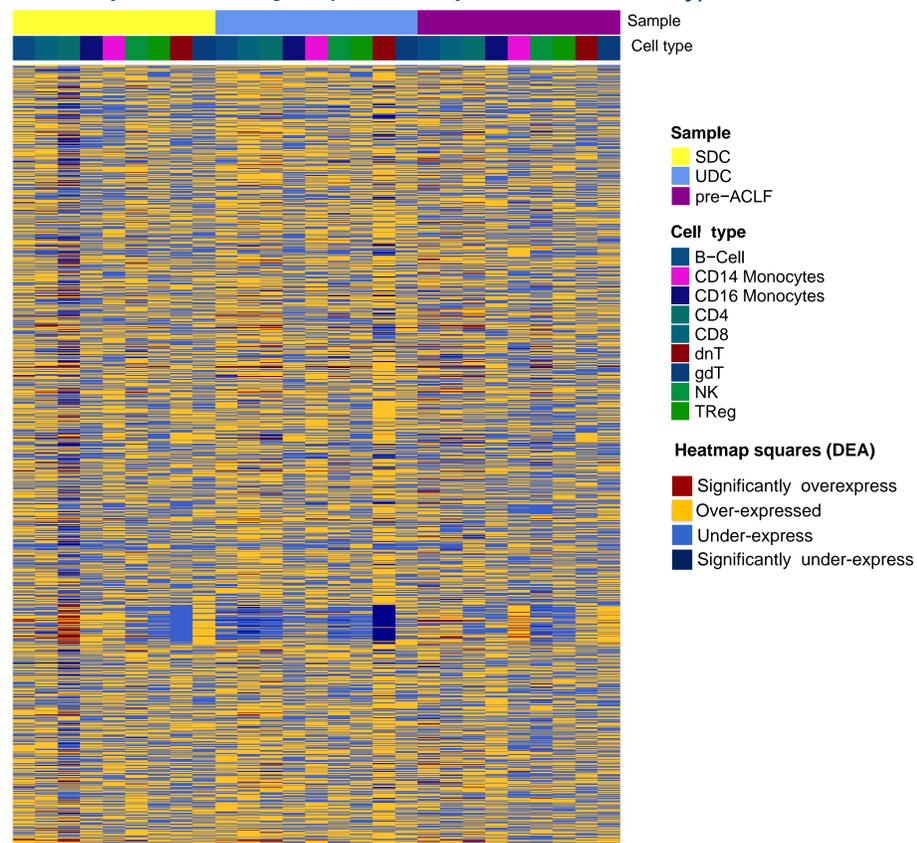
### Data Analysis

Seurat 4.0.1 was used to perform clustering analysis of single-cell data (4). Azimuth "reference-based mapping" pipeline was used in order to validate cell annotation (5).

### Cell Proportion across Samples



### Differential Expression Analysis (SDC/UDC/pre-ACLF vs Healthy)



## NEXT STEPS

- 1) Complete studio with all samples
- 2) Multiomic solution to study Immunology: GEX + Surface proteins + TCR + BCR
- 3) Differ and study the stages of decompensation of cirrhosis at cell level
- 4) Integration of scRNAseq data with genome-wide association studies

## REFERENCES

- (1) Gambino, C. and Piano, S. (2021), Identifying the four shades of acute decompensation of cirrhosis. United European Gastroenterol J, 9: 421-422. doi: 10.1002/ueg2.12074
- (2) Trebicka J, Fernandez J, [...] PREDICT STUDY group of the EASL-CLIF Consortium. The PREDICT study uncovers three clinical courses of acutely decompensated cirrhosis that have distinct pathophysiology. J Hepatol. 2020 Oct;73(4):842-854. doi: 10.1016/j.jhep.2020.06.013.
- (3) Zheng, G., Terry, J., Belgrader, P. et al. Massively parallel digital transcriptional profiling of single cells. *Nat Commun* 8, 14049 (2017). . doi:10.1038/ncomms14049
- (4) Satija R, Farrell JA, Gennert D, Schier AF, Regev A (2015). Spatial reconstruction of single-cell gene expression data. *Nature Biotechnology*, 33, 495-502. doi: 10.1038/nbt.3192
- (5) Lafzi, A., Moutinho, C., Picelli, S. et al. Tutorial: guidelines for the experimental design of single-cell RNA sequencing studies. *Nat Protoc* 13, 2742-2757 (2018). doi: 10.1038/s41596-018-0073-y



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