IL-33/ST2 axis during chronic rejection after liver transplantation

Marco Santillán[1], Paula Constanza Arriola Benitez[2], Luis Pérez[3], Jeremías Moreira[1], Claudio Tiribelli[4], Valeria Descalzi[2], Martin Rumbo[5], Anastasios Giannou[6], María Virginia Gentilini[1], Gabriel Gondolesi[1,2]

[1]Instituto de Medicina Traslacional, Trasplante y Bioingeniería, IMETTyB, Universidad Favaloro, Buenos Aires, Argentina; [2]Instituto de Investigaciones en Medicina Traslacional, IIMT, Universidad Austral, Pilar, Argentina; [3]Cirugía General, Hepatología y Trasplante Hepático, Hospital Universitario Fundación Favaloro, Buenos Aires, Argentina; [4]Fondazione Italiana Fegato - ONLUS, Italian Liver Foundation, Trieste, Italy; [5]Instituto de Estudios Inmunológicos y Fisiopatológicos, IIFP, Universidad Nacional de La Plata, La Plata, Argentina; [6]University Medical Center Hamburg, Eppendorf, Hamburgo, Germany

BACKGROUND & AIM

Liver fibrosis is the hallmark characteristic of the progression to liver chronic rejection (CR), an irreversible, progressive disease that leads to graft loss, requiring re-transplantation. Emerging evidence from our laboratory and other groups has suggested that IL-33 and its primary functional receptor suppression of tumorigenicity 2 (ST2) are involved in the pathogenesis of hepatic fibrosis. Considering that, **our aim was to study the role of IL-33/ST2 axis in the fibrogenic mechanism involved in the CR after liver transplant (LT) in humans**.

MATERIAL & METHODS

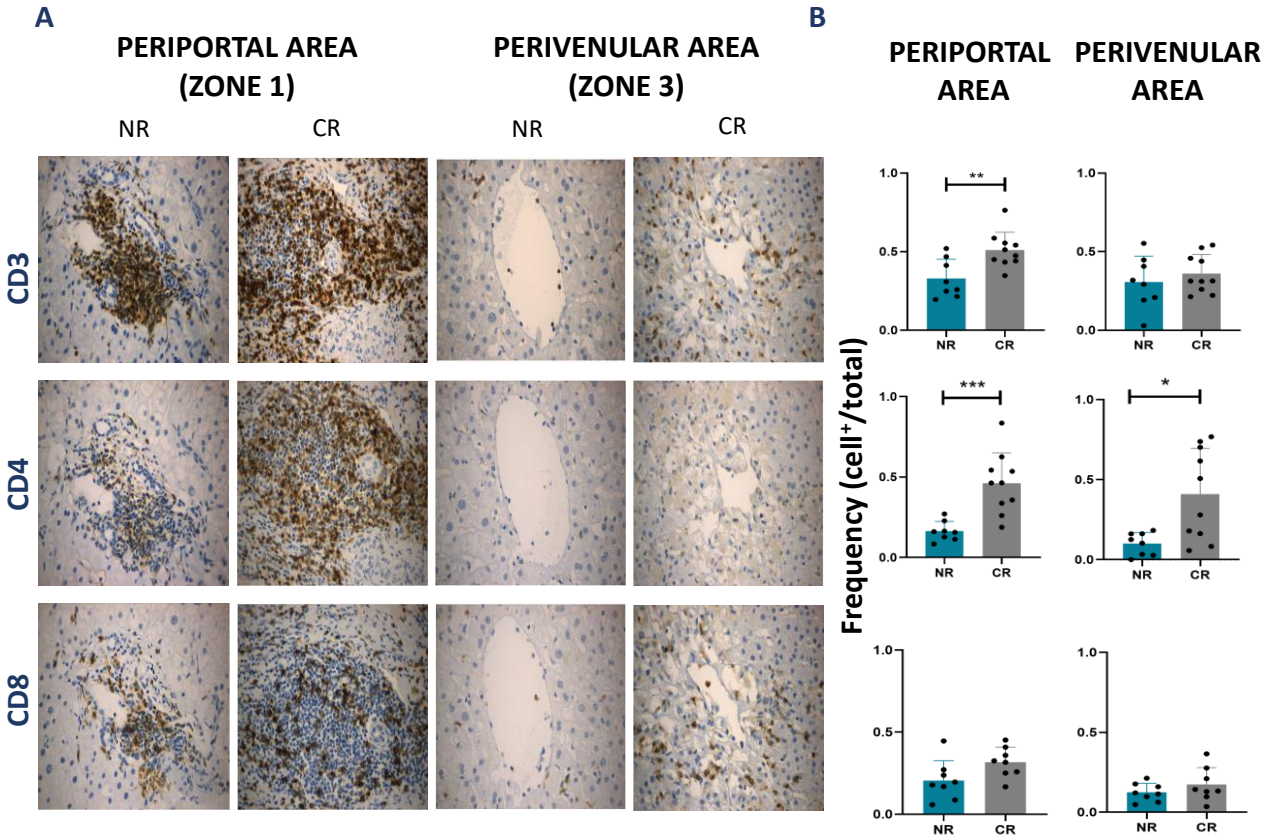
The study included liver biopsies and tissue samples from LT patients [Non-rejection [NR=12], patients with chronic rejection [CR=6] and control samples from donor allograft [Control=9]. The frequency and distribution of immune cell populations were evaluated by immunohistochemistry. Liver fibrosis stage was determined by Masson's trichrome staining. Histological analyses of collagen deposition were performed by Sirius Red staining. Gene expressions were evaluated by quantitative real-time PCR. Analysis of immunological parameters, clinical laboratory and METAVIR score were made using the Kruskal–Wallis with Dunn's post-test. Correlations were evaluated with the Spearman rank correlation test, $p<0.05$ was considered statistically significant. The protocol was approved by the Institutional Review Board of HUFF (DDI [1490] 2419).

CHARACTERISTICS OF THE PATIENTS INCLUDED IN THE STUDY

CHARACTERISTIC	WHOLE BIOPSY GENE EXPRESSION		WHOLE BIOPSY IMMUNOHISTOCHEMISTRY	
GROUP	Non-Rejection (NR = 12)	Chronic Rejection (CR = 6)	Non-Rejection (NR = 8)	Chronic Rejection (CR = 7)
a) Age of recipient at time of Tx (years)	47,6 ± 4	17,5 ± 6,6	51,6 ± 5,3	31,8 ± 4,6
b) Gender				
Women	3	0	1	3
Men	9	6	7	4
c) Etiology/pathology				
Autoimmune	6	4	2	2
Metabolic	2	0	2	2
Alcoholic cirrhosis	1	0	2	1
Other	3	2	2	2
d) Age of recipient at time of sampling (years)	49,7 ± 3,6	24 ± 5,8	52,8 ± 5,3	24,4 ± 7,6
e) Time Post - Tx (days)	790,8 ± 440,8	3109,8 ± 1212,6	423,8 ± 47,8	2677,4 ± 1276,8
f) Immunosuppression				
Steroids	1	1	-	1
Tacrolimus	-	2	-	2
Tacrolimus- Steroids	3	2	2	2
Tacrolimus- Steroids- MMF	8	1	6	2

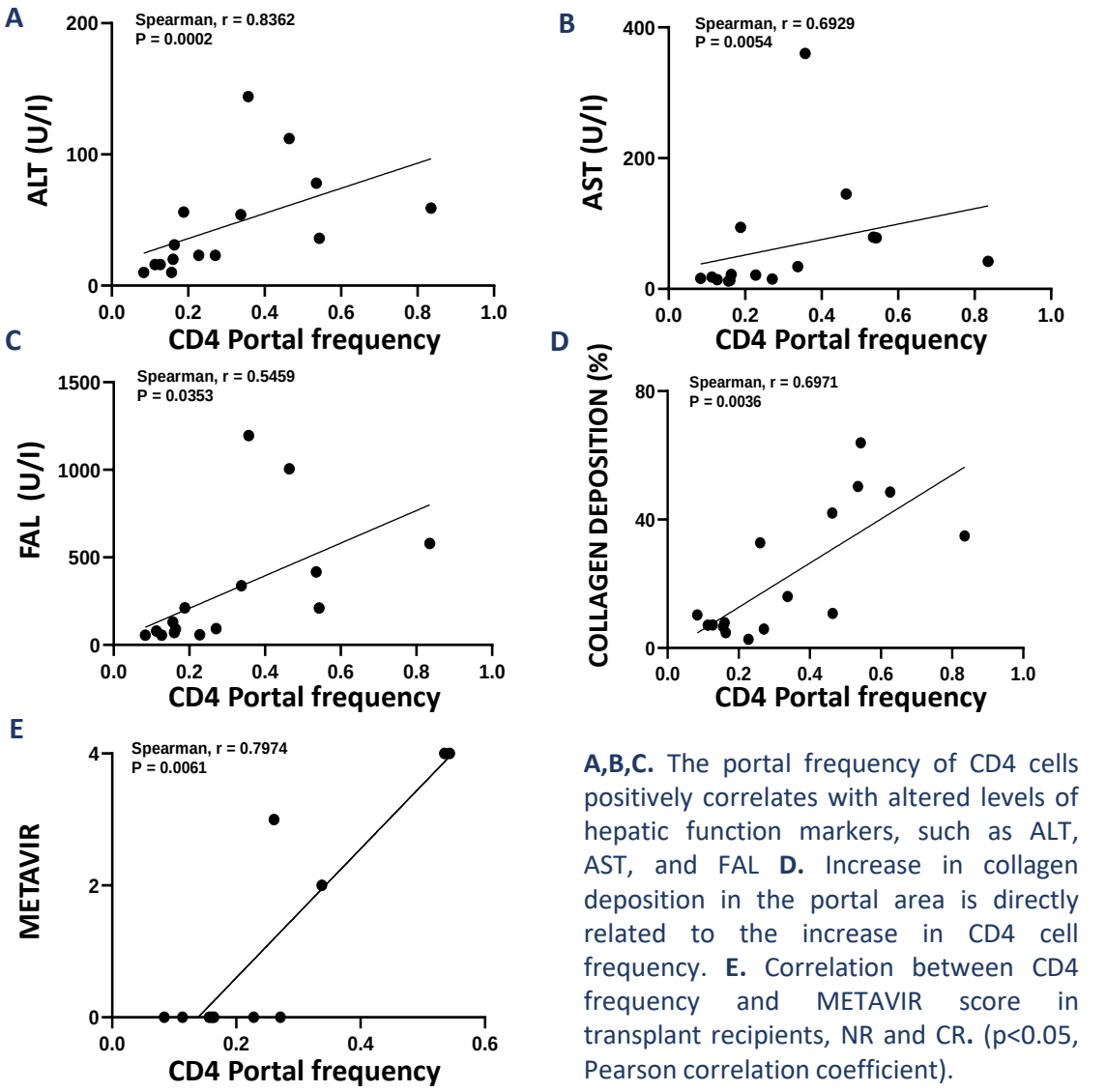
MMF: Mycophenolate Mofetil

CD4 T CELLS ARE PREDOMINANT IN IMMUNE INFLAMATION DURING CHRONIC LIVER REJECTION

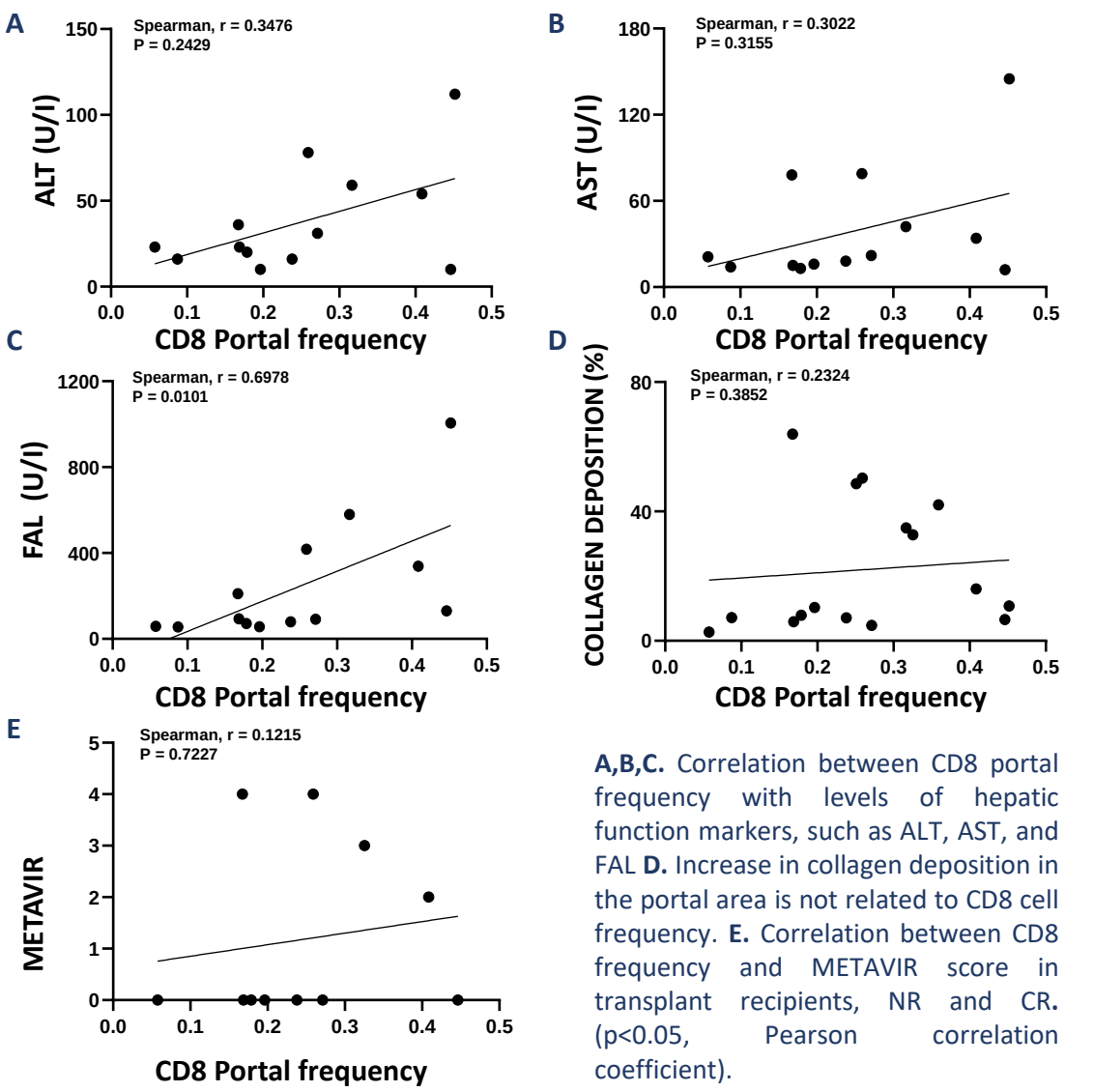


Significant increase of CD3 and CD4 positive cells in the periportal area. A. Representative photos of CD3, CD4, and CD8 detection in liver biopsies and explants from patients with chronic rejection (CR) and without rejection (NR) using immunohistochemistry. **B.** Frequency of CD3, CD4, and CD8 cells in the periportal and lobular zones. The cell frequency was calculated by counting positive cells over the total number of cells in both areas (×400). $p<0.05$, Mann-Whitney test.

INCREASED CD4 FREQUENCY CORRELATES WITH ELEVATED HEPATIC FUNCTIONAL MARKERS

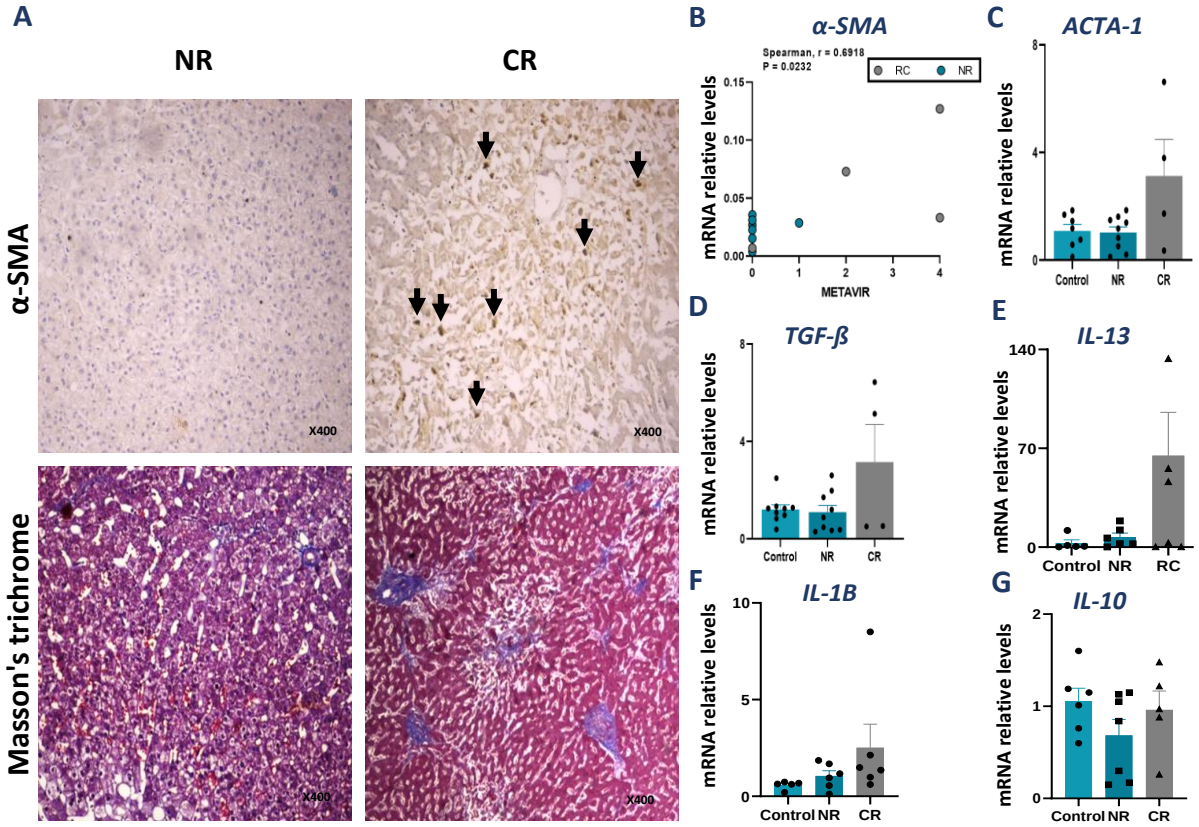


A,B,C. The portal frequency of CD4 cells positively correlates with altered levels of hepatic function markers, such as ALT, AST, and FAL **D.** Increase in collagen deposition in the portal area is directly related to the increase in CD4 cell frequency. **E.** Correlation between CD4 frequency and METAVIR score in transplant recipients, NR and CR. ($p<0.05$, Pearson correlation coefficient).



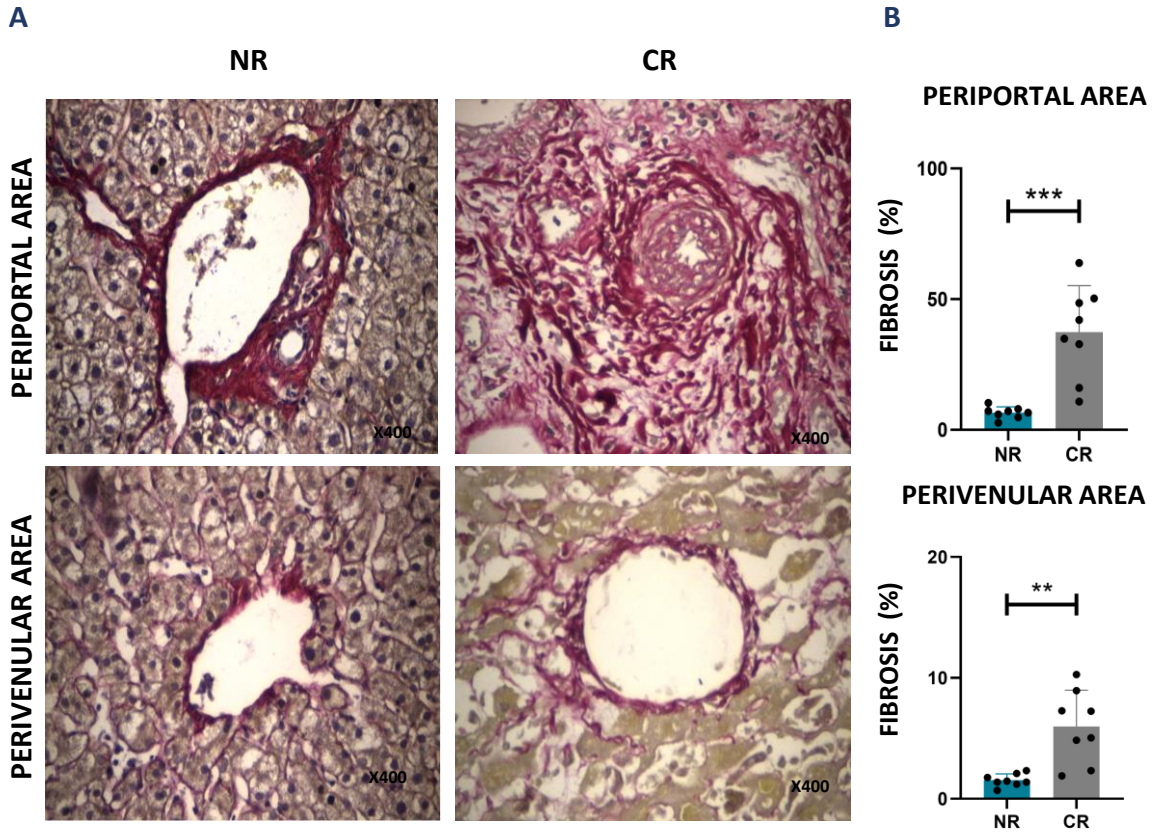
A,B,C. Correlation between CD8 portal frequency with levels of hepatic function markers, such as ALT, AST, and FAL **D.** Increase in collagen deposition in the portal area is not related to CD8 cell frequency. **E.** Correlation between CD8 frequency and METAVIR score in transplant recipients, NR and CR. ($p<0.05$, Pearson correlation coefficient).

INCREMENT OF FIBROGENIC FACTORS AND INFLAMMATORY GENES DURING CHRONIC REJECTION



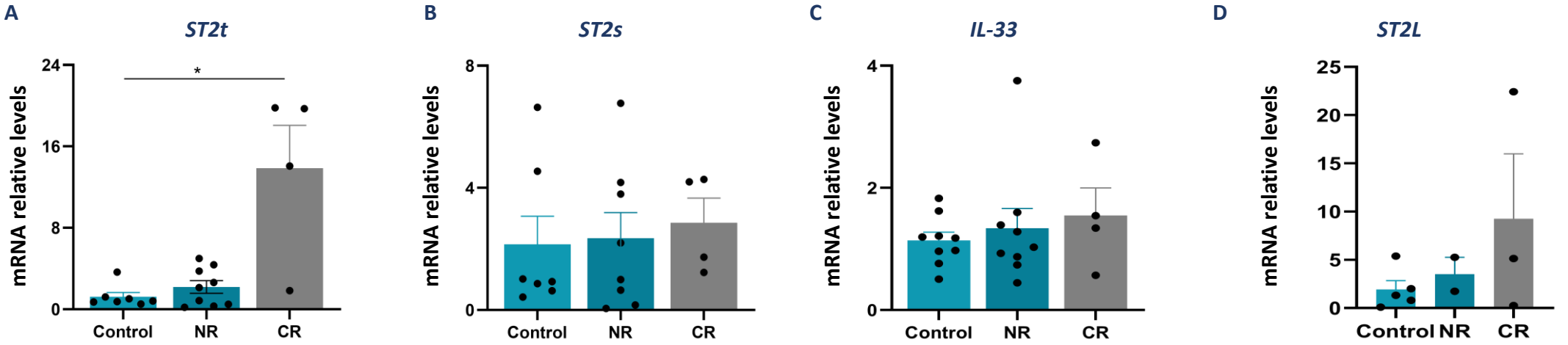
The levels of α-SMA and TGF-ß increased in the CR group compared to controls and the NR group (p>0.05). The expression of α-SMA positively correlated with the METAVIR score in patients with RC (p<0.05). A. Detection of α-SMA by immunohistochemistry and evaluation of collagen deposition by Masson's trichrome histological staining. **B.** Correlation between α-SMA expression levels and METAVIR score in transplanted patients, RC versus NR. **C, D, E, F and G.** Gene expression levels of profibrotic genes (ACTA-1, TGF-β and IL-13), inflammatory (IL-1B) and regulatory (IL-10) genes in transplanted NR and RC patients vs. healthy patients (control). Gene expression levels were evaluated by RT-qPCR. p<0.05, Kruskal-Wallis test with post-Dunn test.

ELEVATED COLLAGEN DEPOSITION DURING CHRONIC REJECTION

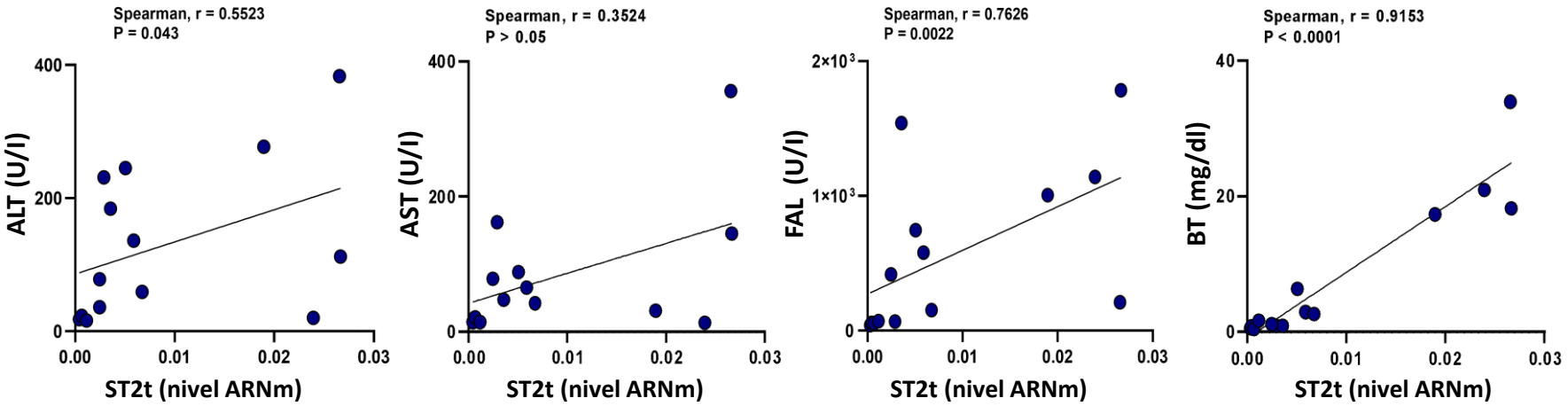


Increase in collagen deposition in both periportal and lobular areas in liver tissue of transplant patients. A. Representative images of collagen fibers using Sirius Red histological staining (×400). **B.** Percentage of fibrosis determined by quantifying collagen in relation to the periportal and lobular areas (×400). p<0.05, Mann-Whitney test.

INCREASE ST2T EXPRESSION CORRELATES WITH IMPAIRED LIVER FUNCTION



E



PARAMETER	NR	CR
ALT (U/l)	108,7 ± 34,9	152,3 ± 78,47
AST (U/l)	37,2 ± 26,1	164,5 ± 65,73
BT (mg/dl)	1,9 ± 0,6	17,63 ± 6,7
GGT (U/l)	616,6 ± 306,8	565,7 ± 282,3
FAL (U/l)	367,8 ± 170,3	854 ± 352,8
Albumina (g/dl)	3,5 ± 0,4	2,3 ± 0

A. The total expression of ST2 (ST2t) significantly increased in the RC group compared to the control group ($p<0.05$). **B.** The expression levels of ST2s, the soluble form of the receptor that acts as a decoy factor, showed no differences between the groups. **C.** A trend towards increased levels of IL-33 was observed in the R group. **D.** The levels of ST2L expression, the transmembrane form of the receptor, showed a trend towards increased levels in the CR group. **E.** The levels of ST2t in liver tissue positively correlated with altered levels of liver function markers, such as ALT, AST, FAL, and Total Bilirubin ($p<0.05$, Pearson correlation coefficient). Gene expression levels were evaluated by RT-qPCR, and biochemical parameters were recorded during the 24 hours following sample collection.

CONCLUSION

Our results indicate that the IL-33/ST2 axis is modulated during chronic rejection following liver transplantation. To complete our study, we will conduct various experiments, including the neutralization of molecules involved in this axis, with the aim of considering them as potential therapeutic targets. Inhibition of the signaling of this axis could represent a novel therapeutic approach to reduce the development of fibrosis in patients with chronic rejection. Additionally, we plan to increase the number of cases in each experimental group to clarify the effects of this axis.

REFERENCES

1. *Tan Z, Sun B. IL-33/ST2 signaling in liver transplantation. Cell Mol Immunol [Internet]. 2021 Mar 1 [cited 2023 Oct 30];18(3):761. Available from: : https://pubmed.ncbi.nlm.nih.gov/pmc/articles/PMC8026639*
2. *Rabindranath M, Zaya R, Prayitno K, Orchanian-Cheff A, Patel K, Jaeckel E, et al. A Comprehensive Review of Liver Allograft Fibrosis and Steatosis: From Cause to Diagnosis. Transplant Direct [Internet]. 2023 Oct 16 [cited 2023 Oct 30];9(11):e1547. Available from: https://pubmed.ncbi.nlm.nih.gov/37854023/*
3. *Choudhary NS, Saigal S, Bansal RK, Saraf N, Gautam D, Soin AS. Acute and Chronic Rejection After Liver Transplantation: What A Clinician Needs to Know. J Clin Exp Hepatol [Internet]. 2017 Dec 1 [cited 2023 Oct 30];7(4):358–66. Available from: https://pubmed.ncbi.nlm.nih.gov/29234201/*
4. *Tan Z, Liu Q, Jiang R, Lv L, Shoto SS, Maillet I, et al. Interleukin-33 drives hepatic fibrosis through activation of hepatic stellate cells. Cell Mol Immunol [Internet]. 2018 Apr 1 [cited 2023 Oct 30];15(4):388–98. Available from: https://pubmed.ncbi.nlm.nih.gov/28194023/*
5. *Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nature Reviews Drug Discovery 2008 7:10 [Internet]. 2008 [cited 2023 Oct 30];7(10):827–40. Available from: https://www.nature.com/articles/nrd2660*