

Supplementary Materials

Donor Hepatic Occult Collagen Deposition Predisposes to Peri-transplant Stress and Impacts Human Liver Transplantation

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Figure S1

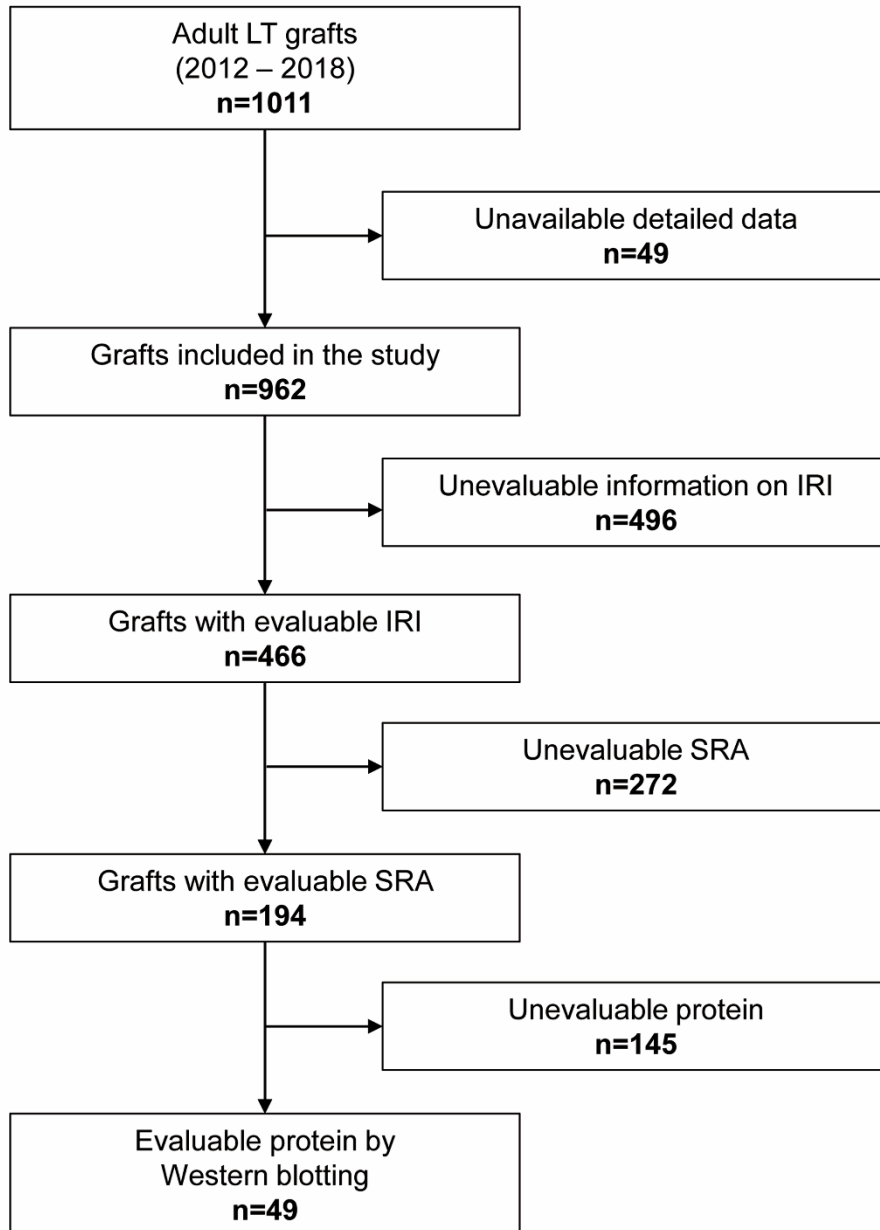
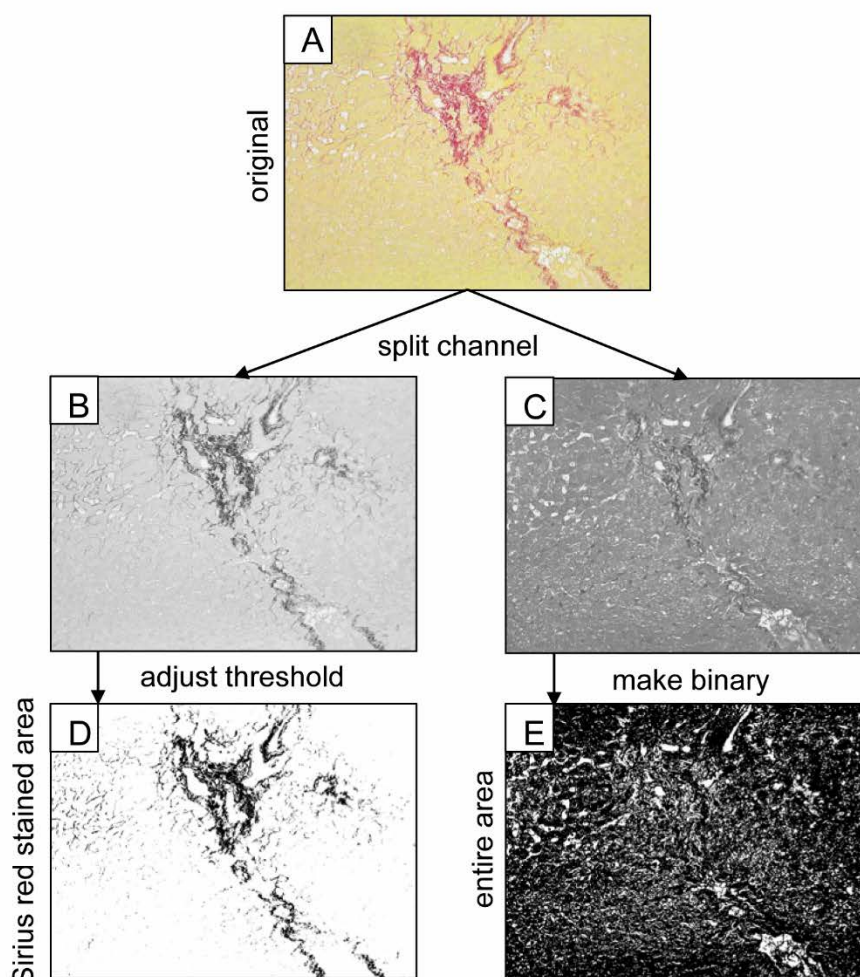


Fig. S1. Flowchart of study population

Among 962 grafts included in this study, IRI severity was evaluated in 466 grafts. Of 466,

194 grafts were available for SRA evaluation. 49 grafts were usable for western blot analysis.

Figure S2**Fig. S2. Analysis of SRA with Image J software**

To calculate the SRA, the original picture A was split into B and C by 'channel split' function. The threshold in picture B was adjusted to extract Sirius red stained area D. To calculate the entire area of the picture, C was converted into E with 'make binary' function. Finally, the black area (D: Sirius red positive area, E: entire area) in each picture was quantified and the SRA (%) was determined by the division of D by E.

Figure S3

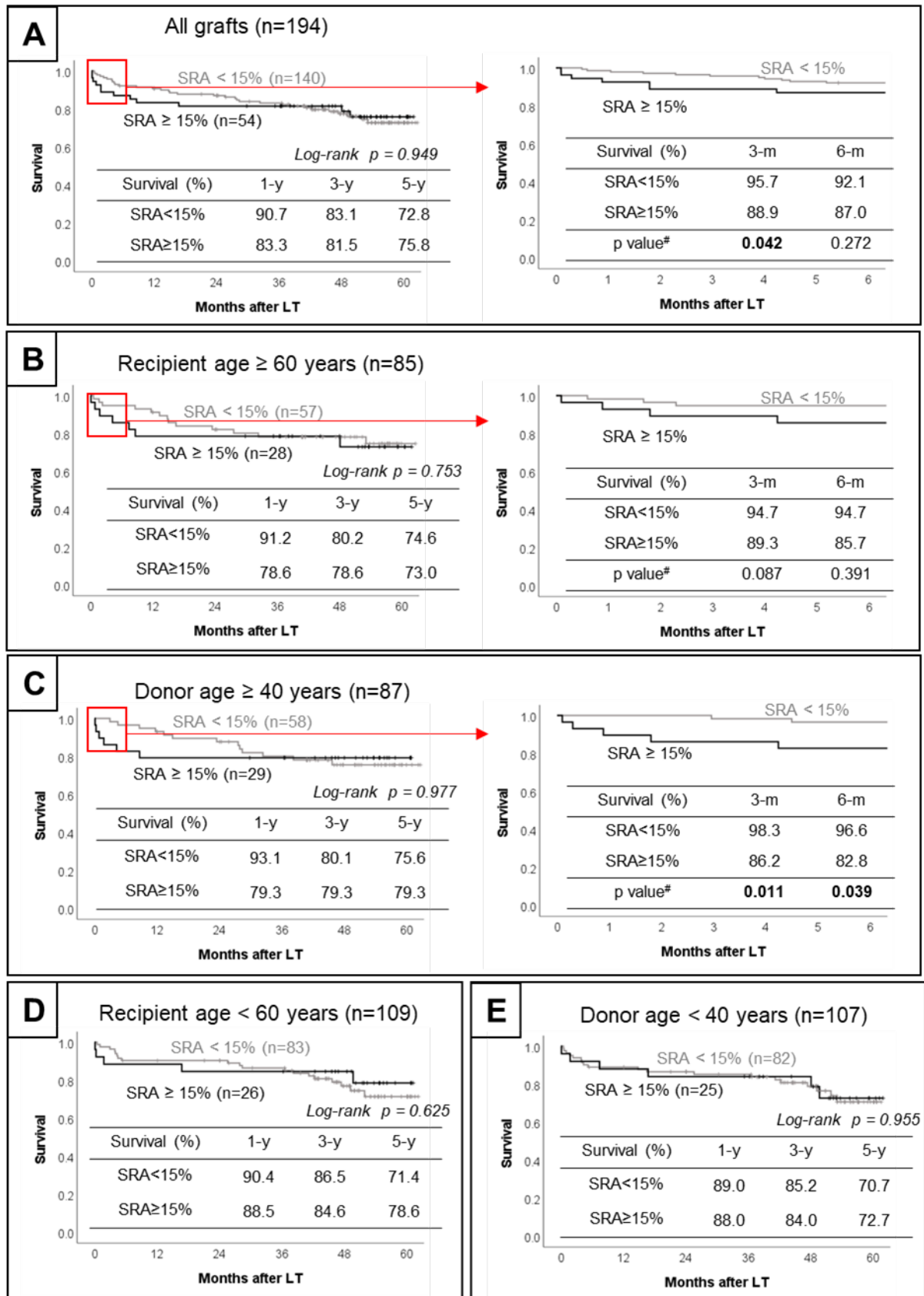
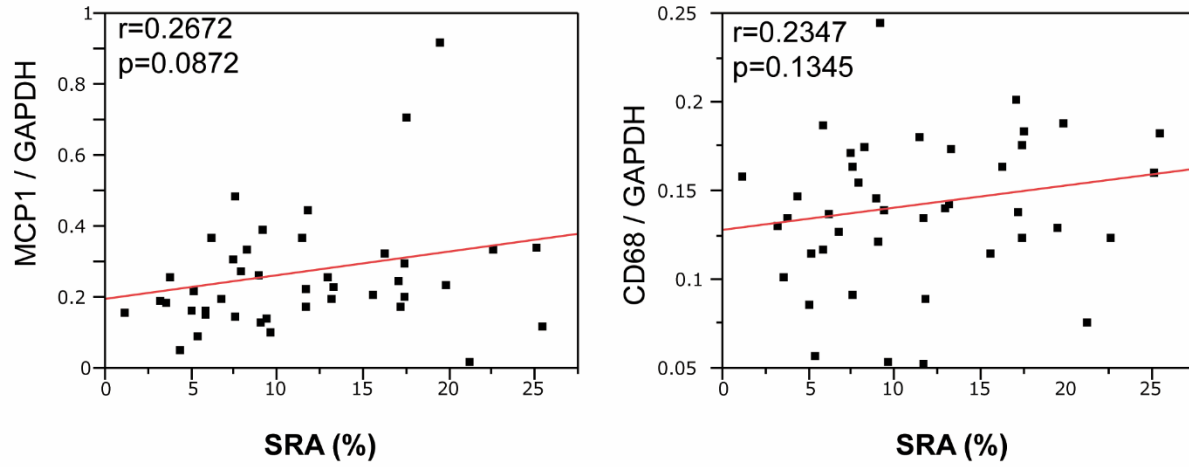


Fig. S3. Graft survival after LT by SRA

(A) On Log-rank test in 194 patients, there were no significant differences in graft survival after LT between the two groups; SRA <15% and SRA ≥15%. Survival rate at 3 months after LT showed a significantly higher rate of graft failure in the SRA ≥15% group compared to SRA <15% group ($p=0.042$) by comparing the number of graft failure within each time as binary outcomes. (B) In the group of the recipients older than 60 years, the recipients implanted with high SRA (15% ≥) grafts showed inferior trend of 3 months survival ($p=0.087$). (C) In the sub-group analysis with donor age older than 40 years, the recipients implanted with high SRA (15% ≥) exhibited significantly worse 3 and 6 months graft survival ($p<0.05$). (D, E) There were no differences in short and long term graft survival in sub-group analysis by donor (< 40 years) and recipient (< 60 years) age.

Figure S4**Fig. S4. Correlation between SRA and inflammatory markers**

SRA showed positive trend of correlation with mRNA expressions of MCP1 and CD68 in post-reperfused livers.

Figure S5

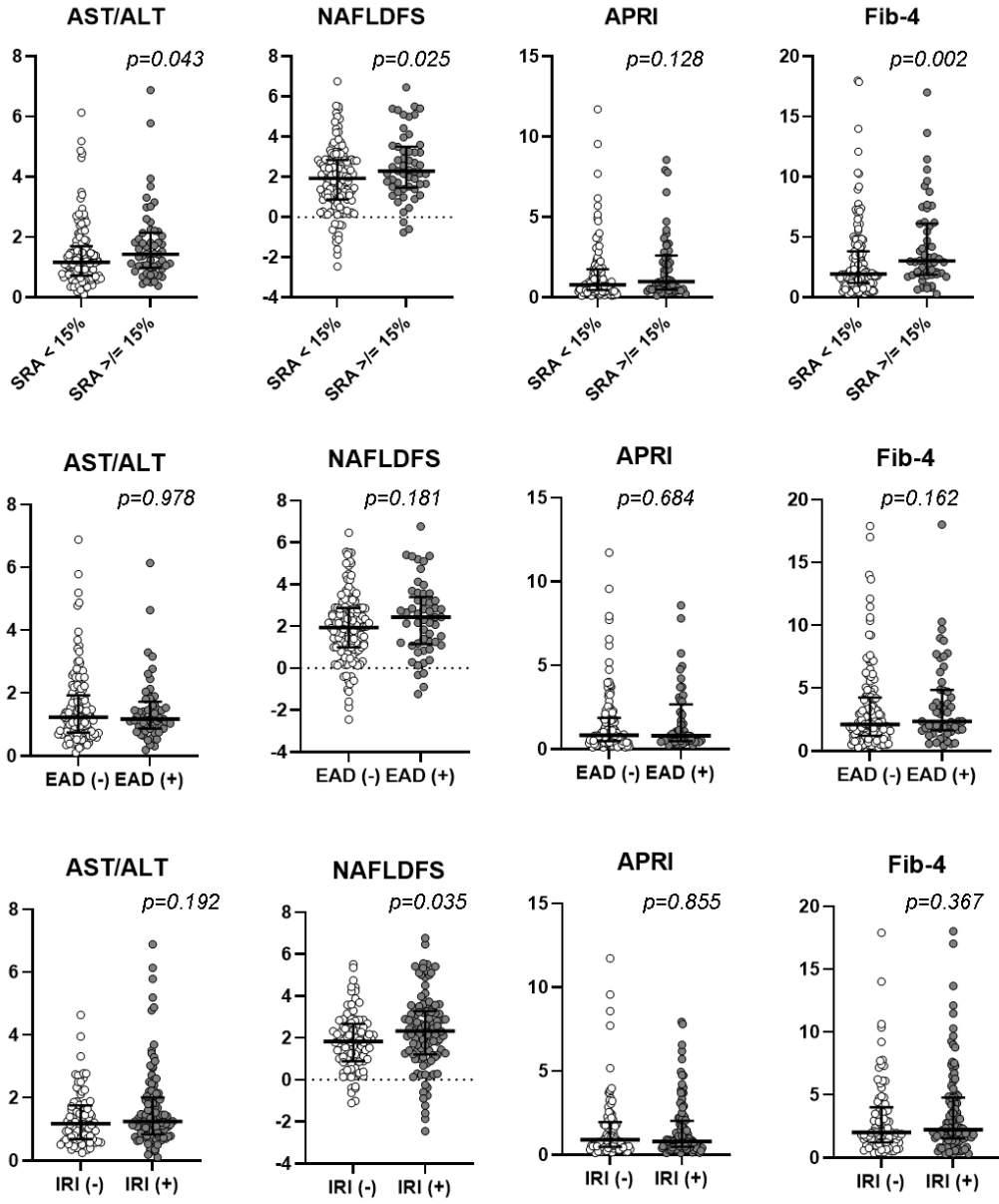


Fig. S5. Relationship between other liver fibrosis scores and SRA, EAD, and IRI in 194 grafts

When comparing grafts with SRA <15% vs SRA \geq 15%, AST/ALT ratio, NAFLD fibrosis score (NAFLDFS), and Fibrosis-4 (Fib-4) were significantly higher in grafts with SRA \geq 15%.

Comparisons between EAD vs no EAD grafts and mild to severe IRI vs no or minimal IRI grafts

showed no statistical differences between the two groups ($p > 0.05$), except for NAFLDFS for IRI ($p = 0.035$). P values were from Mann–Whitney U test.

Table S1: Severity of ischemia-reperfusion injury (IRI)

Severity of IRI	
No	None
Minimal	PMNs in sinusoidal zone 3 with rare hepatocellular necrosis (A)
Mild	PMNs in sinusoidal zone 3 with ≥ 1 cluster of necrotic hepatocytes (B)
Moderate	Clusters of hepatocellular necrosis in $> 50\%$ of lobules (C)
Severe	60% of parenchyma or panlobular necrosis in > 1 lobule (D)

Table S2: Comparison of grafts with EAD vs Non-EAD

Variables	Non-EAD n=141	EAD n=53	p value
Recipient factors			
Age (years)	59 (18-78)	59 (24-73)	0.601
Gender (Female / Male)	48 / 93	19 / 34	0.814
BMI (kg/m ²)	26.8 (16.9-47.5)	26.8 (16.1-41.1)	0.978
Comorbidity			
DM	48 (34.0)	17 (32.1)	0.796
HTN	62 (44.0)	22 (41.5)	0.758
CAD	25 (17.7)	10 (18.9)	0.854
Underlying liver disease			
HBV	5 (3.5)	4 (7.5)	
HCV	61 (43.3)	16 (30.2)	
EtOH	26 (18.4)	8 (15.1)	
NASH	15 (10.6)	9 (17.0)	0.237
ALF	8 (5.7)	4 (7.5)	
PBC	5 (3.5)	0	
Others	21 (14.9)	12 (22.6)	
Preoperative hospital stay (days)	5 (0-168)	3 (0-136)	0.399
Preoperative ICU admission	79 (56.0)	22 (41.5)	0.071
Laboratory data at LT			
AST (IU/l)	66 (18-6705)	62 (24-2614)	0.793
ALT (IU/l)	34 (8-5025)	38 (9-6850)	0.440
Laboratory MELD	31.7 (6.4-45.5)	29.0 (6.4-46.2)	0.400
Donor factors			
Age (years)	35 (13-74)	38 (18-72)	0.101
Gender (Female / Male)	58 / 83	22 / 31	0.962
BMI (kg/m²)	25.7 (17.3-53.0)	29.2 (18.1-38.4)	0.013
Comorbidity			
DM	9 (6.4)	7 (13.2)	0.124
HTN	31 (22.0)	19 (35.8)	0.049
CAD	9 (6.4)	1 (1.9)	0.293
Cause of death			
Trauma	51 (36.2)	20 (37.7)	
CVS	44 (31.2)	22 (41.5)	0.352
Anoxia	43 (30.5)	11 (20.8)	

Others	3 (2.1)	0	
Laboratory data at procurement			
AST (IU/l)	36 (9-483)	37 (9-403)	0.139
ALT (IU/l)	31 (8-730)	37 (9-403)	0.209
DCD	5 (3.5)	5 (3.5)	0.098
HCV Ab positive	5 (3.5)	0	0.326
Cold ischemia time (min)	419 (138-794)	500 (181-878)	0.010
Warm ischemia time (min)	48 (23-79)	50 (35-78)	0.107
Large droplet steatosis \geq 30%	2 (1.4)	6 (11.3)	0.006
High-SRA \geq 15%	33 (23.4)	21 (39.6)	0.025

ALF: acute liver failure, BMI: body mass index, CAD: coronary artery disease, CVS: cerebrovascular stroke, DCD: donor after cardiac death, DM: diabetes mellitus, EtOH: alcoholic (ethanol) related liver disease, HBV: hepatitis B virus, HCV: hepatitis C virus, HTN: hypertension, MELD: model for end-stage liver disease NASH: Non-alcoholic steatohepatitis, PBC: primary biliary cholangitis