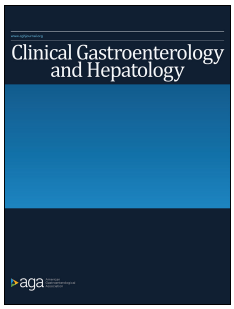
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Complete immunosuppression withdrawal after liver transplantation: An International Multicenter Study

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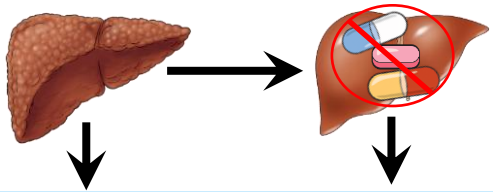
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COMPLETE IMMUNOSUPPRESSION WITHDRAWAL AFTER LIVER TRASPLANTATION: A MULTICENTER, INTERNATIONAL STUDY

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Liver transplant patients who completely weaned immunosuppression for >12 months



International multicenter study
31 centres from 15 countries

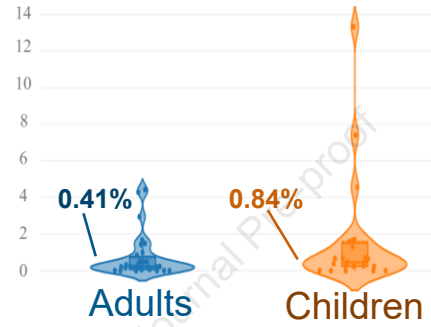


223 adults



64 children

Prevalence of IMS withdrawal





Patient Survival

Adults: 91.2% 5 yrs, 82% at 10 yrs
Children: 100% 5 yrs, 95% 10 yrs



Graft Function

No immunologically mediated graft loss
1.7% rejection, resolved by resuming IS



Histology

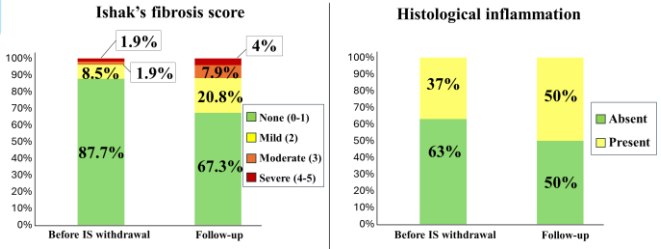
Worsening of fibrosis in 15% or patients but no meaningful impact on overall survival



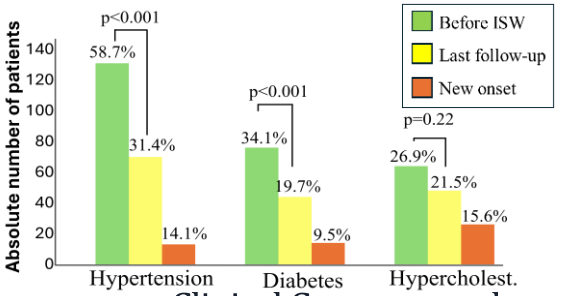
Comorbidities

Regression of hypertension or diabetes in >50% of patients

Graft pathological findings before vs. after



Metabolic Comorbidities in adults



Clinical Gastroenterology and Hepatology

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Complete immunosuppression withdrawal after liver transplantation: An International Multicenter Study

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Bruno Sensi: study coordination, data curation and analysis, drafting the manuscript.

Giuseppe Tisone: acquisition of data and critical revision of the article.

Sandy Feng: study conception and design and critical revision of the article.

Garrett R. Roll: acquisition of data and critical revision of the article.

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Manuel Rodriguez-Perálvarez: study conception and design, obtained funding, data analysis, drafting the manuscript, and study guarantor.

MLR-P and BS had full access to the study data and verified the results. All authors revised and approved the final version of the article and had final responsibility for the decision to submit for publication.

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ABSTRACT

Background & Aims: Immunosuppression withdrawal (ISW) after liver transplantation (LT) is feasible in selected individuals but the risk of chronic graft damage is unknown. We aimed to describe the clinical characteristics and long-term outcomes of patients with successful ISW after LT.

Methods: Retrospective cohort study involving 31 LT centres in 15 countries. Adult and paediatric patients with complete ISW for ≥12 months following LT were eligible. We evaluated long-term outcomes, including evidence of histological graft injury, rejection, and mortality.

Results: In all, 287 patients (223 adults and 64 children) were included, representing 0.46% of the reference LT population. The median time from LT to ISW was 9.4 years, followed by 7.8 years of post-withdrawal follow-up. The most common reasons for ISW were participation in a clinical trial (53%), patient decision (25.1%), and immunosuppression-related side effects (16.7%). ISW was associated with metabolic improvement including reductions in diabetes (34.1% to 19.7%) and hypertension (58.7% to 31.4%). Paired liver biopsies before and after ISW were available in 108 patients (37.6%) and among them 43.5% achieved operational tolerance. Worsening fibrosis (≥1 Ishak's stage) and new-onset inflammation occurred in 30.5% and 22.2% of patients, respectively. Five-year overall survival after ISW was 93%, and no patient experienced immunologically mediated graft loss. Neither worsening fibrosis nor inflammation had an impact on mortality (HR=1.77 [95%CI 0.63-4.96] and HR=1.46 [95%CI 0.39-5.49], respectively).

Conclusions: ISW after LT may improve metabolic comorbidities. Mild histological graft changes may occur, but do not appear to affect long-term outcomes. Future studies should focus on safely expand ISW to a broader subset of LT patients.

Keywords: tolerance, weaning, tacrolimus, calcineurin inhibitors, liver biopsy.

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What you need to know:

Background: Immunosuppression withdrawal after liver transplantation is postulated as a strategy to improve long-term outcomes, but its risks and benefits remain poorly defined. Therefore, ISW is seldomly pursued due to the perceived risk of rejection.

Findings: This study provides robust evidence that, once complete immunosuppression withdrawal is maintained for at least twelve months, the risk of rejection-related graft loss is negligible, whilst survival is excellent and metabolic benefits are substantial.

Implications for patient care: These findings support careful consideration of immunosuppression withdrawal in properly selected patients and highlights its potential to improve long-term outcomes.

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INTRODUCTION

Liver transplantation (LT) is a life-saving procedure for patients with end-stage liver disease, yet it requires lifelong use of immunosuppressive drugs to prevent allograft rejection[1]. Chronic exposure to immunosuppressive drugs is a major source of morbidity and mortality after LT due to increased risk of infection, cardiovascular events, renal impairment, and cancer, including recurrence of hepatic tumors[2-4]. Studies have shown a cumulative effect of immunosuppressive drugs, meaning that higher doses, a larger number of drugs, and prolonged exposure, exert incremental risks of adverse outcomes[3]. As a result, LT patients show 21% excess of mortality at 10 years compared to age- and gender-matched general population, with three out of four early deaths potentially associated with immunosuppression[2]. In children, among whom the expected lifespan is accounted in decades, the survival gap may be even more pronounced[5, 6].

Complete immunosuppression withdrawal (ISW) with or without operational tolerance, often referred to as the "holy grail of transplantation", has been pursued for decades[7-11]. Among solid transplant organs, recipients of a transplanted liver are particularly disposed to immune tolerance due to continuous exposure to dietary and microbial antigens, a wide population of resident lymphocytes, and the expression of Fas-ligands, which induce T-cell apoptosis[10, 12]. Indeed, "operational tolerance", defined as maintenance of normal graft function and histology for at least 12 months after immunosuppression cessation, has been attained in approximately 20% of carefully selected patients[9, 13-16]. These success rates varied widely among studies according to heterogeneous population characteristics[17], baseline immunosuppression[18], the use of protocol biopsies for a canonical definition of operational tolerance, and a myriad of biomarkers, none of them validated[9]. Successful ISW could reduce the incidence of de novo metabolic syndrome and promote the regression of existing metabolic disorders, notably improving arterial

hypertension, diabetes and/or lipid metabolism[19]. Real and perceived barriers limiting ISW in routine clinical practice include a lack of tools to identify patients who have developed spontaneous tolerance, need for stricter clinical and pathological surveillance, and risk of acute rejection during the weaning process, but data on long-term effects of ISW in the real world are surprisingly limited. Specifically, there is a theoretical concern about the development of subclinical histological damage resulting in graft loss, which has not been well elucidated. A better understanding of long-term outcomes after ISW would facilitate informed ISW decisions and reinforce -or discourage- future clinical trials attempting operational tolerance after LT.

In this international cohort study, we aimed to describe clinical features and long-term outcomes of patients with successful ISW after LT, with particular interest in the incidence of immunologically mediated graft damage, graft loss, and survival.

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METHODS

Study design.

This is a retrospective international cohort study conducted by the Immunology Special Interest Group of the International Liver Transplant Society (ILTS). ILTS members were invited to participate in the study via e-mail, provided they had at least one patient meeting study criteria. Thirty-one LT centers from 15 countries across 4 continents (Europe, America, Asia, and Africa) participated (Figure 1). Study investigators reviewed the historical LT cohort at each center from inception to January 2025, to identify potential study candidates. Data was recorded in an anonymous, non-identifiable, and encrypted Research Electronic Data Capture (REDCap) platform.

The study was approved by the Internal Review Board (IRB) of the University of Rome Tor Vergata, Rome, Italy [R.S. 106.23 CET2 PTV 14/12/2023] and by the local IRB of each center when required. The study was conducted in accordance with the international ethical recommendations on clinical research established by the Helsinki and Istanbul declarations.

Study population and outcomes.

All adult and pediatric LT recipients, from both deceased and living donors, who underwent complete ISW and remained off immunosuppression for at least 12 months were eligible. We did not restrict the study to patients with operational tolerance because it requires biopsy adjudication, which may not be available in all patients. ISW for any reason was accepted, including participation in a clinical trial, immunosuppression-related adverse effects, or spontaneous discontinuation by the patient. Exclusion criteria were as follows: history of retransplantation or multiorgan transplant prior to ISW, minimization of immunosuppression without complete drug withdrawal, and

discontinuation of medication due to a severe medical condition associated with short life expectancy (<6 months) in the judgment of the investigator.

The primary outcome of the study was overall survival after ISW. In addition, we recorded biopsy-proven acute rejection, chronic rejection, graft loss (defined as death or retransplantation), incidence of metabolic comorbidities (diabetes, dyslipidemia), cardiovascular disease (arterial hypertension, coronary artery disease, major cardiac event, cerebrovascular accident), and malignancies (recurrent and de novo).

Definitions and variables.

Data collected for this study were divided into three domains: at transplantation, at ISW initiation, and during follow-up after ISW. Transplant data comprised recipient demographics and medical history, as well as donor and transplant-related variables. ISW initiation data referred to the time of drug weaning or discontinuation, including anthropometric measurements, induction agents (comprising depleting and not depleting antibodies used in the perioperative period), maintenance immunosuppression therapy, serum liver and renal function tests, donor-specific antibodies (DSAs), and metabolic parameters: obesity defined as body mass index >30, diabetes, arterial hypertension and dyslipidemia defined according to international guidelines[20]. During follow-up, we recorded biopsy-proven acute rejection episodes, chronic rejection, de-novo diabetes, cardiovascular events, retransplantation, and mortality, together with relevant dates of each outcome. Regarding pathological surveillance, information from liver biopsy was recorded before ISW and during follow-up whenever available. Given the heterogeneous reporting of graft biopsies, we recorded only the presence and grade of inflammation, evidence of acute or chronic rejection according to Banff recommendations[21], and fibrosis according to Ishak's stage[22]. Operational tolerance was defined as persistently

normal liver tests and absence of histopathological damage in the follow-up biopsy at least 12 months after ISW as defined by the Banff working group[23].

Statistical analyses are detailed as supplementary material.

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RESULTS

There were 31 participating transplant centers from 15 countries located in Europe (n=22), America (n=5), Asia (n=3), and Africa (n=1). Participating centers accounted for 62,399 patients who underwent liver transplantation (LT) from inception to January 2025, and among them, 287 patients (223 transplanted as adults and 64 transplanted as children) were identified as having achieved complete ISW for a period longer than 12 months. The prevalence of complete ISW in the reference LT population was 0.41% in adults (range 0%-4.4%) and 0.84% in children (range 0%-13.3%) (supplementary Figure S1).

Demographics and clinical characteristics.

Demographic and clinical features of the study cohort are presented in Table 1. Adults (n=223) with complete ISW were 51±12 years old at the time of LT, and 74.9% (n=167) were males. Most participants were Caucasian (87.4%). The main indication for LT was decompensated cirrhosis (71.8%), followed by hepatocellular carcinoma (11.2%). Five patients (2.2%) had underlying autoimmune liver disease. Most patients received a donation after brain death graft (88.3%). Only 3.1% of patients received a transplantation after living partial liver donation. A history of treated acute rejection was reported in 29 patients (13%), which was biopsy-proven in 22 cases (75.9%). Regarding metabolic comorbidities prior to ISW, 131 patients (58.7%) had hypertension, 76 patients (34.1%) had diabetes, 60 patients (26.9%) had hypercholesterolemia, and 19 patients (8.5%) had hypertriglyceridemia.

Children (n=64) had a median age of 2 years old at LT (interquartile range -[IQR] 0.6-9.4), 53.1% were girls, and 75% were Caucasian. The most common etiology of liver disease was biliary atresia (41.2%; n=27), followed by acute liver failure in 18.8% of patients. Living donation was performed in 22 children (34.4%). Thirty-six children

(56.3%) received partial or reduced grafts. A history of acute rejection before ISW occurred in 23 children (35.9%), most of them biopsy-proven (91.3%). Metabolic comorbidities before ISW were uncommon in children.

Exposure to immunosuppressants and assessment before withdrawal is presented in Table 2, supplementary text and supplementary Table S1. The median time between LT and ISW was 9.36 years (IQR 5.09-14.61), without significant differences between adults and children (9.11 years vs. 10.46 years; p=0.53). The most frequent indication for ISW was enrolment in a clinical trial (53%), followed by the patient's decision (25.1%), side effects of immunosuppression (16.7%) and other reasons (5.2%).

Outcomes after immunosuppression withdrawal.

Patient and graft survival

Median follow-up after ISW was 7.4 years (IQR 3.9-12.5) in adults and 8.2 years (IQR 4.3-15.3) in children. Kaplan-Meier curves showing overall survival after ISW are depicted in Figure 2. In adults, overall survival rates after ISW were 94.6% at 3 years, 91.2% at 5 years, and 82% at 10 years. In children, overall survival rates were 100% at 3 and 5 years, and 95% at 10 years. Causes of death were unrelated to immunologically mediated graft damage (supplementary Table S2).

Cumulative incidence of graft loss in adults was 1.5% at 3 years, 2% at 5 years, and 3% at 10 years. Graft loss episodes occurred at 15 months, 18 months, 23 months, 45 months, 95 months, 143 months, and 203 months, respectively. No child experienced graft loss within the first 10 years after ISW. Two children developed graft loss at 170 months and 176 months, respectively. None of the episodes of graft loss in adults or children were immunologically mediated (supplementary Table S2).

Liver function, rejection and histological features

Liver tests remained within normal range for most patients during follow-up, with no significant increase in the proportion of patients who showed elevations >1.5 or >2 times the upper normal range at ISW (supplementary Table S1).

Five patients developed acute rejection episodes (1.7%, 4 adults and one child), after a median of 16 months post-ISW. One of them had developed de-novo DSAs. One adult patient with acute rejection developed chronic rejection after 20 months but maintained a preserved graft function. All patients restarted calcineurin inhibitor-based immunosuppression and none of them experienced graft loss. No other patient resumed immunosuppression during follow-up in the study cohort.

One hundred and eight patients (37.6%), including 86 adults and 22 children, had paired liver biopsies before ISW and during follow-up after ISW, with a median interval between both biopsies of 44 months (IQR 26-97) (Figure 3). Ishak's fibrosis stage improved by one stage in 5 patients (4.6%), was stable in 70 patients (64.9%), worsened by one stage in 17 patients (15.7%) and worsened by two or three stages in 16 patients (14.8%). Histological inflammation improved in 10 patients (9.3%) remained stable in 74 patients (31.5%) and worsened in 24 patients (22.2%). Criteria of operational tolerance were met in 47 patients with paired liver biopsies (43.5%), including 39 adults and 8 children. Worsening inflammation or fibrosis had no impact on mortality (supplementary Figures S2-S3), after controlling for potential confounders (HR=1.47 [95%CI 0.39-4.49] and HR=1.77, [95%CI 0.63-4.96], respectively) (supplementary Table S3).

Comorbidities

The evolution of metabolic comorbidities during follow-up is summarized in Figure 4 and supplementary Table S4. The prevalence of hypertension in adults decreased from 58.7% to 31.4% despite the occurrence of 14.1% of de-novo hypertension cases

($p<0.001$). Similarly, new-onset diabetes occurred in 9.5 % of adults and diabetes regression was observed in 60.5% of patients, with prevalence declining from 34.1% to 19.7% ($p<0.001$). Hypercholesterolemia and hypertriglyceridemia developed in 15.6% and 7.4% of adults, respectively, with corresponding regression rates of 63.3% and 52.6%. The cumulative incidence of major cardiovascular events after ISW was 3.1% at 3 years, 4.9% at 5 years, and 12.3% at 10 years. Hepatocellular carcinoma was present in 47 (21.1%) adults at transplant and recurred only in 1 patient (2.1%), 8 years after transplant and 2 years after ISW.

Children and young adults were minimally affected by metabolic comorbidities, and *de novo* cases were few, ranging from 1.6% for hypertriglyceridemia to 8% for hypertension. When metabolic comorbidities were pre-existent, regression rates were also high in the pediatric population (supplementary Table S4). Children showed a trend to lower risk of major cardiovascular events compared to adults: 0% at 5 years and 2.6% at 10 years ($p=0.054$).

There were 32 patients with de novo malignancy after ISW, 30 among adults and 2 among children. The cumulative incidence of cancer at 3, 5, and 10 years in adults was 4.7%, 6.5%, and 14.8%, respectively. In children, cases occurred at 87 months and 104 months post-ISW, respectively.

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DISCUSSION

In the present study, we analyzed the largest cohort of LT patients with complete ISW published hitherto aiming to describe long-term outcomes and to provide a real-world picture of the immunosuppression-free practice after LT. Patients were recruited from 15 countries in four continents with heterogeneous protocols and practices. Although immunosuppression-free patients account for a small minority among the LT population, they exhibit a meaningful improvement in their metabolic profile with a favorable long-term patient and graft survival. Moreover, our data support intuitive beliefs that reducing immunosuppression exposure mitigates well-known drug toxicities. These excellent long-term outcomes warrant further investigations on ISW under optimal safety conditions, both in children and adults.

The liver is a privileged organ in terms of tolerance owing to its wide functional reserve, dual vascular inflow, and unique immune microenvironment[4]. Small prospective clinical trials have shown that complete ISW is safe in highly selected LT patients under close surveillance. Some patients experience acute rejection episodes while reducing immunosuppression or shortly after complete withdrawal. Although refractory rejection leading to graft loss has not been reported so far in ISW trials, both transplant physicians and patients may harbor concerns. Another barrier precluding a broader adoption of ISW in clinical practice is the need for closer surveillance after ISW, which demands patient commitment and staff resources. Previous prospective clinical trials including stable patients at least 3 years after LT with histologically healthy allografts achieved complete ISW in 13% to 60% of patients[13-15, 24]. We showed that approximately 0.5% of LT patients are currently immunosuppression-free, but with prevalences as high as 4.4% in adults and 13.3% in children in some centers. These contrasting figures suggest that some

LT patients may be unnoticeably tolerant and could be receiving lifelong immunosuppression needlessly.

Adults and children transplanted for non-autoimmune disease who are stable five or more years after transplant could be eligible to consider ISW but refined selection criteria, including non-invasive surrogate biomarkers, are needed. In our cohort, most patients were receiving stable immunosuppression monotherapy before attempting ISW. In fact, the use of higher doses and more potent immunosuppression drugs, particularly early after LT, could erode the probability of minimal dependence on immunosuppression or even complete withdrawal in the long run[25]. It may well be that early recognition of the graft by the host immune system, sometimes expressed as self-limited acute rejection episodes, triggers tolerance mechanisms[26]. Aligning with this hypothesis, the current study cohort was characterized by infrequent use of induction therapies and a higher proportion of calcineurin inhibitor-free maintenance immunosuppression than that reported for the general LT population[27].

A liver biopsy to rule out subclinical rejection is highly recommended before attempting minimization or ISW[28]. Elevated ALT, particularly if associated with *de novo* high titer Class II DSAs or abnormal elastography findings are associated with histopathology such that ISW should not be attempted[29]. In our cohort, a liver biopsy was more often performed in adults, probably because they were more frequently involved in clinical trials, but mild histological findings (inflammation or fibrosis) were not prohibitive ISW if ALT was normal.

The most relevant contribution of this study was to show that complete ISW, once achieved for a period longer than 12 months, is safe in the long-term. In this study, a median interval between LT and ISW of almost a decade was observed. Long-term outcomes after ISW were excellent, particularly in younger patients. We did not observe

immunologically mediated graft failure, and the causes of death were not attributable to ISW. Besides waiving the need to take immunosuppressive drugs, which likely offers quality of life benefit, we found meaningful rates of regression in metabolic comorbidities, ranging from 57% to 64%. Although some patients did develop de novo metabolic comorbidities, the incidence aligned with that described for the general population[30, 31] and was lower than reported for LT patients receiving maintenance immunosuppression [32-34]. This appears in line with previous findings from the largest single-center experience so far[19]. Among children, the metabolic impact of ISW is reduced, but previous studies have shown benefits in terms of growth, puberty and fertility[35].

After ISW, follow-up biopsies frequently showed persistence of mild parenchymal inflammation, and one in three patients experienced worsening fibrosis by at least one Ishak's stage. In previous trials with shorter surveillance, it has been suggested that one third of patients not meeting histological criteria of operational tolerance in the first surveillance biopsy, experienced histological improvement afterwards[9], aligning with longitudinal changes in the literature for patients treated with standard of care maintenance immunosuppression[13, 36, 37]. Indeed, worsening fibrosis or inflammation do not result in graft loss, even 10 years after ISW. Complete ISW may be a viable long-term strategy that could be considered for carefully selected patients after thorough discussion about individual risks and benefits, and patient commitment to adhere prescribed monitoring.

A crucial question is how these patients should be monitored. As a general and intuitive recommendation, patients achieving ISW should be followed up more intensively than patients receiving standard of care immunosuppression. The role of protocol biopsies has been long debated because they might detect the development of subclinical graft damage

and fibrosis, as described in previous studies, but exposes patients to invasive procedures[36]. However, we showed that these histological findings, when accompanied by normal transaminases, may not translate into clinically relevant consequences. Liver tests combined with non-invasive tools, such as elastography and DSAs, may be sufficient follow up in adult patients[4]. In children, given their longer life expectancy, further studies are needed with several decades of follow-up to extract solid conclusions. The optimal monitoring strategy is unknown, but protocol biopsies may still have a role.

Recent investigations have shown that T-reg lymphocytes may hold the key to operational tolerance. Indeed, Tregs circulate from lymphoid organs to the bloodstream and then to peripheral tissues, including the liver, where these engage in a bidirectional crosstalk with endothelial cells to counteract rejection[38]. *Ex vivo* manipulation of Tregs by transduction with human leukocyte antigen A2-specific chimeric antigen receptor (CAR-Tregs) provided a strong suppression of alloimmune responses and prolonged graft survival in a mouse model[39]. Clinical studies in humans have suggested that CAR-Tregs could be safe, but further investigations are required to refine manufacturing and to explore their potential to allow ISW early after LT in a meaningful proportion of patients[40].

The present study was limited by its retrospective design. There were heterogeneous policies and practices among participating centers regarding the selection of candidates for ISW, baseline assessment, and follow-up. Uniform pathological surveillance was lacking, and histological reports were heterogeneous, without granular data on the specific location of findings and insufficient time point observations to fully elucidate dynamic graft changes. Some relevant outcomes such as growth, puberty, fertility, or chronic kidney disease could not be systematically analyzed. An intention-to-treat analysis, including all patients who attempted ISW and those remaining on lifelong immunosuppression, would have allowed a wider range of comparisons and

interpretations regarding clinical benefits after ISW. Finally, although the study investigators performed a careful review of the LT population in each center to identify candidates meeting the inclusion criteria, selection bias cannot be ruled out.

In conclusion, ISW is seldom attempted after LT and mostly within clinical trials. After successful ISW for at least one-year, acute rejection episodes are anecdotal and, if occurred, fully resolved after reintroducing calcineurin inhibitors. Although some patients may experience histological changes during follow-up, including inflammation and fibrosis, graft function is preserved in the long term. Patients showed excellent overall survival rates with fewer metabolic comorbidities. Future prospective investigations should focus on innovative strategies to safely induce tolerance in a wider proportion of patients and earlier after LT to favorably impact long-term outcomes.

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ABBREVIATIONS.

- 95%CI: 95% confidence interval.

- DSAs: donor-specific antibodies.

- HR: hazard ratio.

- ILTS: International Liver Transplant Society.

-IQR: interquartile range

- ISW: immunosuppression withdrawal

- LT: liver transplantation.

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ACKNOWLEDGEMENTS

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FIGURE LEGENDS.

Figure 1. Geographical distribution of thirty-one liver transplant institutions participating in the study. "N." indicates the number of patients recruited at each centre.

Figure 2. Kaplan Meier curve showing overall survival in 287 liver transplant patients after complete immunosuppression withdrawal. Patients were categorized as adults or children at the time of transplantation.

Figure 3. Stacked bar diagrams comparing biopsy findings in 108 liver transplant patients before and after complete immunosuppression withdrawal with a median interval between both biopsies of 44 months (IQR 26-97). Panel A: Ishak's fibrosis score; Panel B: histological inflammation.

Figure 4. Prevalence of metabolic comorbidities before immunosuppression withdrawal and at the end of follow-up among 287 liver transplant patients: 223 adults, Panel A, and 64 children, Panel B. HyperTGC: hypertrigliceridemia; Hypercolest.: hypercholesterolemia.

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Table 1. Demographics and clinical characteristics of 287 patients who achieved complete immunosuppression withdrawal for at least 12 months after liver transplantation in 31 centres from 15 countries. Continuous variables are expressed in mean ± standard deviation. Categorical variables are expressed in absolute numbers and percentages.

Variable	Overall (n=287)	Adults (n=223)	Children (n=64)
Age at LT, years old	40.10±22.22	50.55±12.25	4.66±5.07
Sex (female), n (%)	90 (31.4%)	56 (25.1%)	34 (53.1%)
Ethnicity, n (%)			
Caucasian	243 (84.7%)	195 (87.4%)	48 (75%)
Asian	18 (8.3%)	15 (6.7 %)	3 (4.7%)
Hispanic	14 (4.9%)	9 (4%)	5 (7.8%)
African American	3 (1%)	1 (0.5%)	2 (3.1%)
Other	6 (2.1%)	1 (0.5%)	5 (7.8%)
Unknown	3 (1%)	2 (0.9%)	1 (1.6%)
Indication for LT, n (%)			
Decomp. Cirrhosis	170 (59.2%)	160 (71.8%)	10 (15.6%)
Special indications	60 (20.9%)	19 (8.5%)	41 (64.2%)
Acute liver failure	29 (10.1%)	17 (7.6%)	12 (18.8%)
HCC	25 (8.7%)	25 (11.2%)	0 (0%)
Non-HCC tumors	3 (1%)	2 (0.9%)	1 (1.6%)
HCC, n (%)	47 (16.4%)	47 (21.1%)	0 (0%)
Aetiology of liver disease, n (%)*			
Biliary atresia	27 (9.4%)	0 (0%)	27 (42.2%)
Alcohol consumption	57 (19.9%)	57 (25.6%)	0 (0%)
Hepatitis B	55 (19.2%)	54 (24.2%)	1 (1.6%)
Hepatitis C	54 (18.8%)	54 (24.2%)	0 (0%)
MASLD	11 (3.8%)	11 (4.9%)	0 (0%)
Autoimmune	5 (1.7%)	5 (2.2%)	0 (0%)
Others	87 (30.3%)	51 (22.9%)	36 (56.2%)
Date of LT, n (%)			
Before 1995	68 (23.7%)	49 (22%)	19 (29.7%)
1996-2005	132 (46%)	103 (46.2%)	29 (45.3%)
2006-2015	75 (26.1%)	64 (28.7%)	11 (17.2%)
2016-2022	12 (4.2%)	7 (3.1%)	5 (7.8%)
Modality of donation, n (%)			
Deceased (DBD)	233 (81.2%)	197 (88.3%)	36 (56.2%)
Deceased (DCD)	4 (1.4%)	3 (1.3%)	1 (1.6%)
Deceased (unknown)	17 (5.9%)	13 (5.8%)	4 (6.2%)
Living donor	29 (10.1%)	7 (3.1%)	22 (34.4%)
Unknown	4 (1.4%)	3 (1.3%)	1 (1.6%)
Donor age, years old	40.48 ± 21.12	44.91±19.90	24.21±17.29
Type of graft, n (%)			
Whole	218 (76%)	197 (88.3%)	21 (32.8%)
Partial/split	45 (15.7%)	15 (6.7%)	30 (46.9%)
Reduced	8 (2.8%)	2 (1%)	6 (9.4%)
Domino	1 (0.3%)	0 (0%)	1 (1.6%)
Unknown	15 (5.2%)	9 (4%)	6 (9.4%)
History of acute rejection, n (%)	52 (11.1%)	29 (13%)	23 (35.9%)
Diagnosis of acute rejection, n (%)			
Biopsy-proven	43 (82.7%)	22 (75.9%)	21 (91.3%)
Treated empirically	9 (17.3%)	7 (24.1%)	2 (8.7%)
Diabetes at ISW, n (%)	77 (26.8%)	76 (34.1%)	1 (1.6%)
Hypertension at ISW, n (%)	133 (46.3%)	131 (58.7%)	2 (3.1%)
Hypercholesterolemia at ISW, n (%)	60 (20.9%)	60 (26.9%)	0 (0%)
Hypertriglyceridemia at ISW, n (%)	22 (7.7%)	19 (8.5%)	3 (4.7%)

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Cardiovascular disease at ISW, n (%)	30 (10.5%)	25 (11.2%)	5 (7.8%)
Liver biopsy at ISW, n (%)	157 (54.9%)	131 (59%)	26 (40.6%)
Ishak fibrosis stage at ISW, n (%)			
None (0-1)	134 (87%)	111 (86%)	23 (92%)
Mild (2)	12 (7.8%)	10 (7.8%)	2 (8%)
Moderate (3)	2 (1.3%)	2 (1.6%)	0 (0%)
Severe (4-5)	6 (3.9%)	6 (4.7%)	0 (0%)
Investigation of DSAs at ISW, n (%)	18 (6.3%)	4 (1.8%)	14 (21.9%)

* Each patient could account for more than one cause of liver disease.

DBD: donation after brain death; DCD: donation after circulatory death; DSAs: donor-specific antibodies; HCC: hepatocellular carcinoma; ISW: immunosuppression withdrawal; LT: liver transplantation; MASLD: metabolic associated steatotic liver disease.

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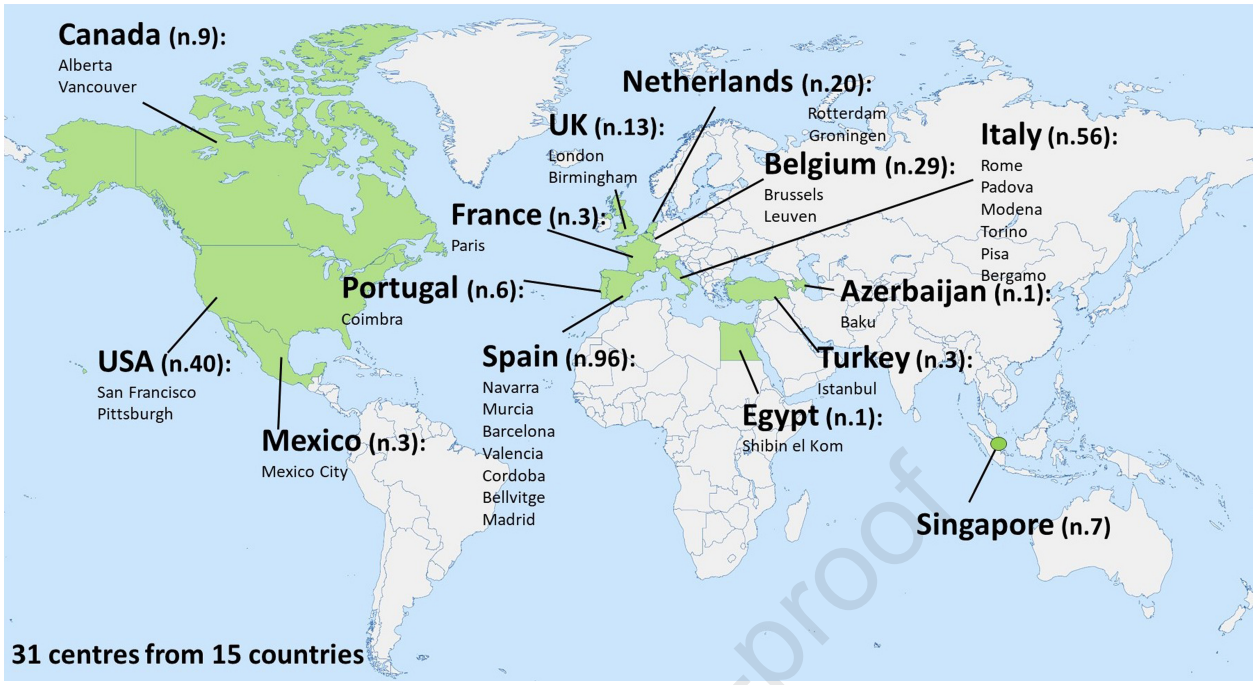
Table 2. Immunosuppression-related features of 287 patients who achieved complete immunosuppression withdrawal for at least 12 months after liver transplantation. Continuous variables are expressed in mean ± standard deviation except those with asymmetric distribution, which are displayed as median and interquartile range (IQR). Categorical variables are expressed in absolute numbers and percentages.

Variable	Overall (n=287)	Adults (n=223)	Children (n=64)	*p*
Induction therapy, n (%)				**0.003**
Yes	35 (12.2%)	22 (9.9%)	13 (20.3%)	
No	214 (74.6%)	180 (80.7%)	34 (53.1%)	
Unknown	38 (13.2%)	21 (9.4%)	17 (26.6%)	
Type of induction, n (%)				0.68
Basiliximab	25 (71.4%)	19 (72.7%)	9 (69.2%)	
Thymoglobulin	9 (25.7%)	5 (22.7%)	4 (30.8%)	
Alemtuzumab	1 (2.9%)	1 (4.6%)	0 (0%)	
Intraoperative steroids, n (%)				0.10
Yes	194 (67.6%)	152 (78.4%)	42 (65.6%)	
No	65 (22.6%)	57 (25.6%)	8 (12.5%)	
Unknown	28 (9.8%)	14 (6.3%)	14 (21.9%)	
CNI, n (%)*				**0.027**
Tacrolimus	158 (55.1%)	116 (52%)	42 (65.6%)	
Cyclosporine	74 (25.8%)	57 (25.6%)	17 (26.6%)	
No CNI	55 (19.2%)	50 (22.4%)	5 (7.8%)	
Mycophenolate, n (%)*	65 (22.6%)	60 (26.9%)	5 (7.8%)	**0.001**
Azathioprine, n (%)*	16 (5.6%)	9 (4%)	7 (10.9%)	**0.041**
mTOR inhibitors, n (%)*	12 (4.2%)	12 (5.4%)	0 (0%)	**0.045**
Everolimus, n (%)*	8 (2.8%)	8 (3.6%)	0 (0%)	--
Sirolimus, n (%)*	4 (1.4%)	4 (1.8%)	0 (0%)	--
Corticosteroids, n (%)*	22 (7.7%)	12 (5.4%)	10 (15.6%)	**0.010**
IMS protocol, n (%)*				**0.043**
CNI alone	190 (67.1%)	141 (63.8%)	49 (79%)	
Antimetabolite alone	44 (15.5%)	41 (18.6%)	3 (4.8%)	
mTORi alone	7 (2.5%)	7 (3.2%)	0 (0%)	
CNI + antimetabolite	37 (13.1%)	27 (12.2%)	10 (16.1%)	
Others	5 (1.8%)	5 (2.3%)	0 (0%)	
Interval transplant to IMS withdrawal (years)	9.36 (IQR 5.09-14.61)	9.11 (IQR 4.97-14.20)	10.46 (IQR 6.80-17.43)	0.53
Reason for IMS withdrawal, n (%)				**<0.001**
Clinical trial	152 (53%)	131 (58.7%)	21 (32.8%)	
Patient's decision	72 (25.1%)	42 (18.8%)	30 (46.9%)	
Side effects	48 (16.7%)	37 (16.6%)	11 (17.2%)	
Others	15 (5.2%)	13 (5.8)	2 (3.1%)	
Side effects, n (%)**				
Cancer	16 (33.3%)	11 (29.7%)	5 (45.5%)	0.27
Infections	7 (14.6%)	7 (18.9%)	0 (0%)	0.14
Renal impairment	15 (31.2%)	13 (35.1%)	2 (18.2%)	0.25
Cardiovascular event	3 (6.2%)	3 (8.1%)	0 (0%)	0.45
Metabolic syndrome	2 (4.2%)	2 (5.4%)	0 (0%)	0.59
Others	11 (22.9%)	6 (16.2%)	5 (45.5%)	0.06
Duration of IMS weaning (months)	8.12 (IQR 2.73-11.92)	8.48 (IQR 4.62-12.56)	3.70 (IQR 0-8.64)	0.093

CNI: calcineurin inhibitors; IMS: immunosuppression; mTOR: mammalian target of rapamycin.

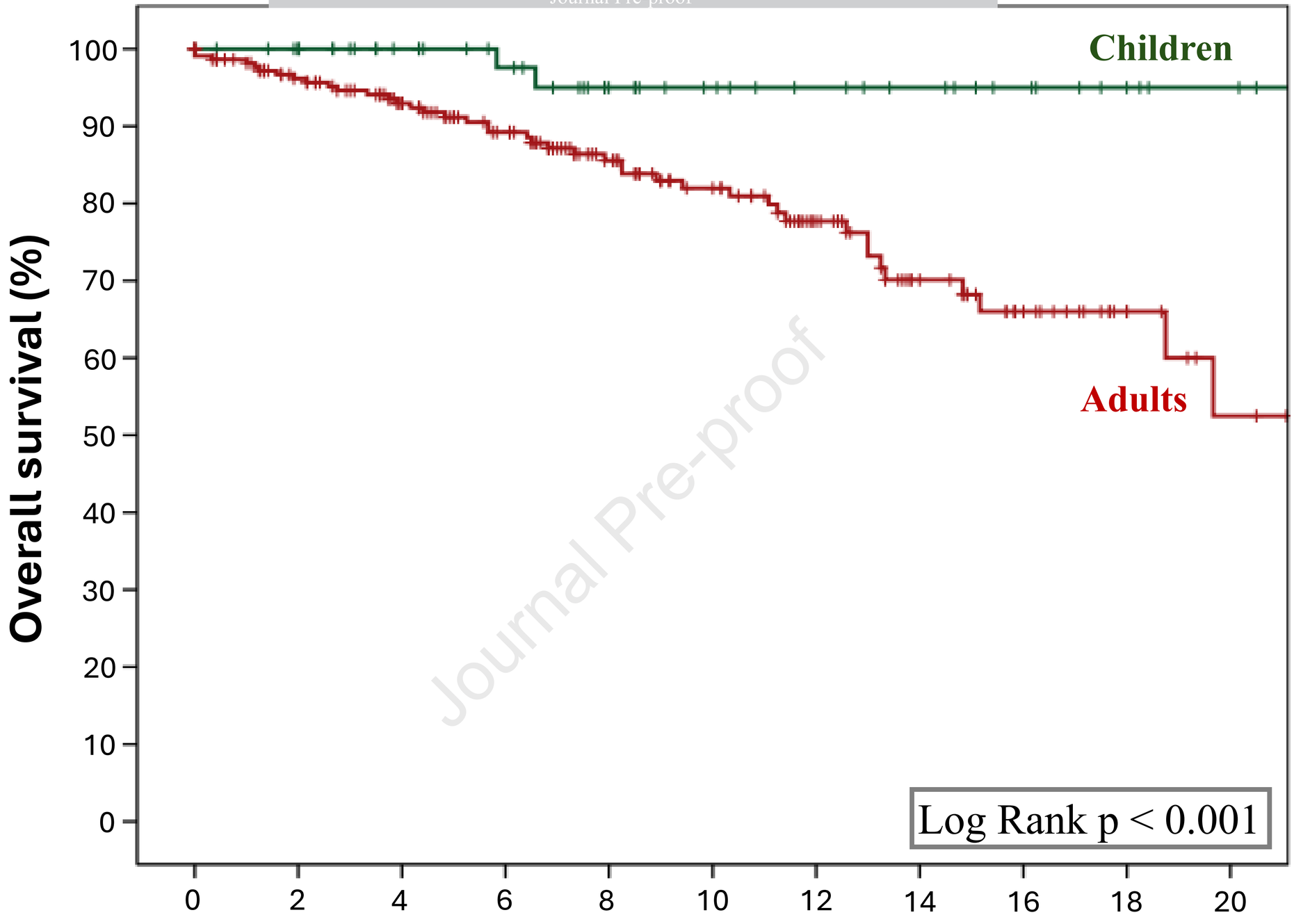
* At starting immunosuppression weaning

** Only in 48 patients who discontinued immunosuppression due to side effects. Each patient could account for more than one side effect.



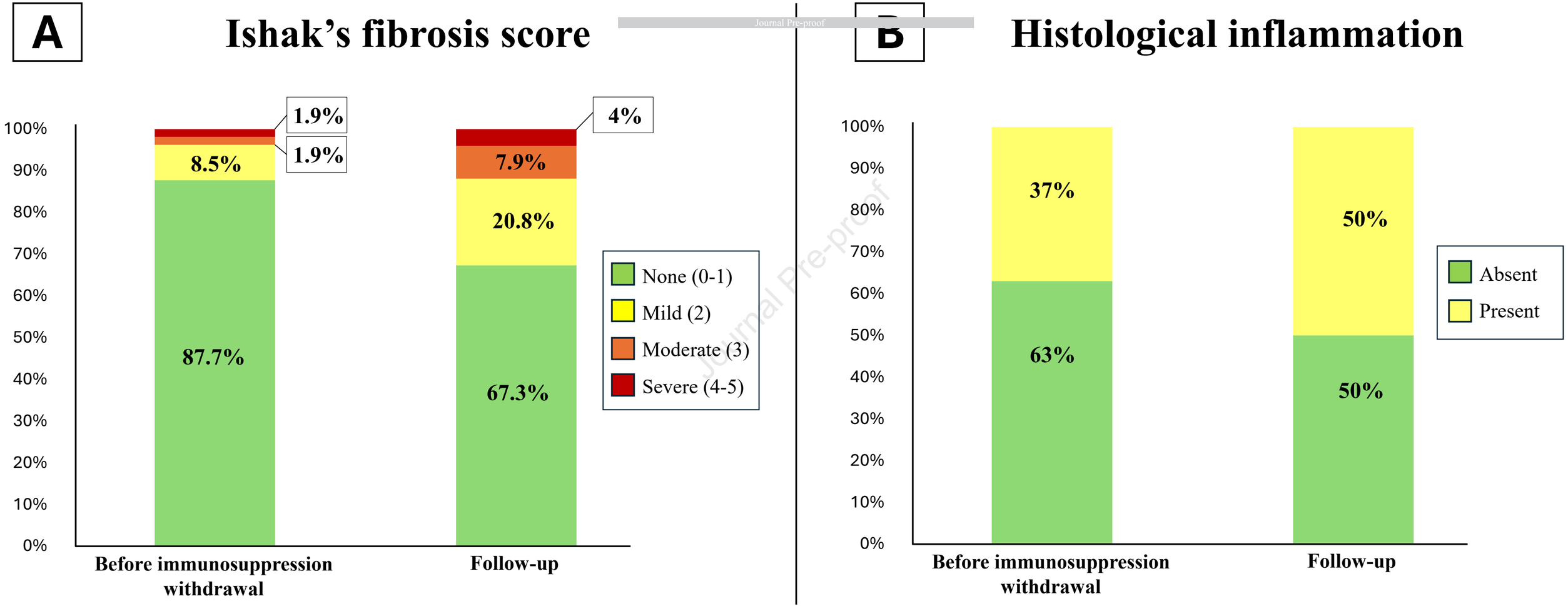
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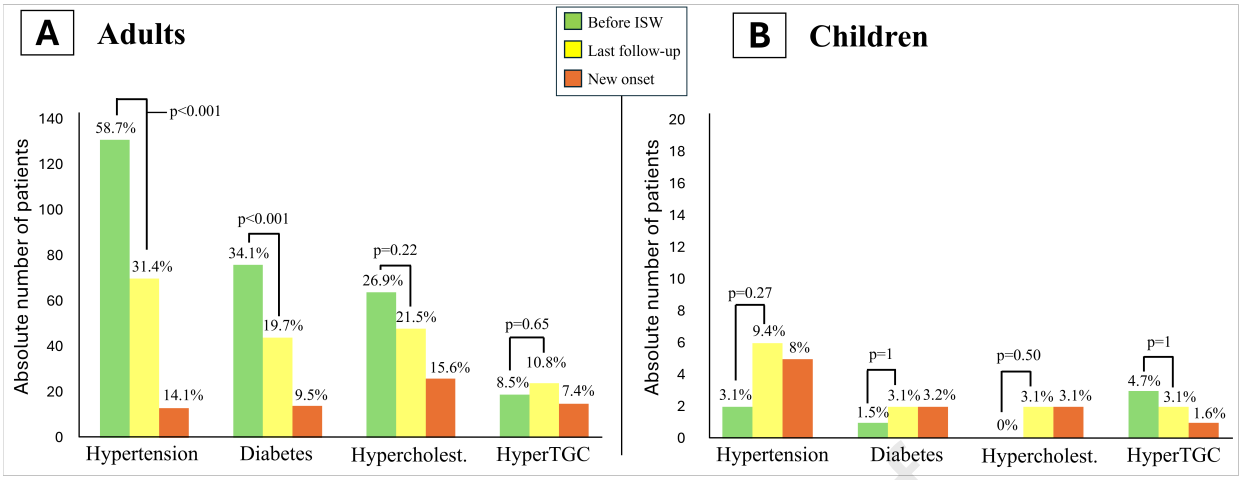


Number at risk	1 year	3 years	5 years	10 years	20 years
Children (n=64)	62	51	43	25	7
Adults (n=223)	220	178	142	82	7

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What you need to know:

Background: Immunosuppression withdrawal after liver transplantation is postulated as a strategy to improve long-term outcomes, but its risks and benefits remain poorly defined. Therefore, ISW is seldomly pursued due to the perceived risk of rejection.

Findings: This study provides robust evidence that, once complete immunosuppression withdrawal is maintained for at least twelve months, the risk of rejection-related graft loss is negligible, whilst survival is excellent and metabolic benefits are substantial.

Implications for patient care: These findings support careful consideration of immunosuppression withdrawal in properly selected patients and highlights its potential to improve long-term outcomes.

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SUPPLEMENTARY MATERIAL

Complete immunosuppression withdrawal after liver transplantation: An International Multicenter Study

Supplementary text to Methods: Statistical analysis.

Continuous variables were described as means and standard deviation or as medians and interquartile range (IQR) for normal or asymmetric distributions, respectively. Student T-test and Mann–Whitney U test were applied for parametric and non-parametric comparisons, respectively, for continuous variables. For comparisons involving more than two categories, ANOVA or Kruskal-Wallis tests were used. Categorical variables were presented as absolute values and percentages. The chi-squared test was used to compare frequencies.

The starting observation point for outcome analyses was the initiation of ISW. Survival analyses were performed using Kaplan–Meier curves, with the log-rank test for group comparisons. Cox proportional hazards regression models (both univariable and multivariable) were used to identify risk factors associated with overall mortality, with particular emphasis on the effect of worsening histological inflammation and fibrosis (in the subgroup of patients with paired biopsies available). Variables with $p < 0.20$ in the univariate analysis entered the initial multivariable model. Covariables that did not reach statistical significance were eliminated in a backward stepwise process, except those considered potential confounding factors. All possible interactions were tested. The strength of association was expressed as a hazard ratio (HR) and 95% confidence intervals (95%CI). All analyses were performed using SPSS Version 27.0 (IBM Inc., Chicago, USA). Every hypothesis tested was two-tailed and considered significant if $p < 0.05$.

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Supplementary text to Results: Exposure to immunosuppressants and assessment before withdrawal.

Adults and children differed in immunosuppression history. Children received induction therapy more frequently than adults (20.3% vs. 9.9%; p=0.003). The predominant induction agent was basiliximab, both in adults and children (72.7% vs. 69.2%; p=0.68). At initiation of ISW, the maintenance regime was monotherapy in 85.6% of adults and 83.8% of children. Calcineurin inhibitor-free maintenance immunosuppression was more frequent in adults (22.4%) than in children (7.8%) (p=0.027). Mycophenolate was more frequent among adults (26.9% vs. 7.8%; p=0.001) and azathioprine among children (10.9% vs. 4%; p=0.041). Inhibitors of the mammalian target of rapamycin were part of maintenance immunosuppression in 5.4% of adults and in no children (p=0.045).

Liver tests were within normal range or marginally elevated in most cases at ISW initiation: an elevation >1.5 times the upper range was found in 2.4% of patients for alanine aminotransferase, 8% for gamma-glutamyl transferase, and 5.2% for bilirubin (Table S1). Before attempting ISW, 157 patients (54.9%) underwent liver biopsy. Liver biopsy was more frequently performed in adults (59%) than in children (40.6%) before initiating ISW (p=0.009). Moderate-severe histological liver fibrosis according to the Ishak staging system (ie. stage 3-5) was present in 8 adults (6.3%) but in no children. Inflammation at any grade was also more frequent in adults (n=59; 45%) than in children (n=4; 15.4%) (p=0.005). Mild bile duct damage was described in 13.1% of adults and in 0% of children (p=0.076). Investigation of DSAs before immunosuppression weaning was performed in 18 patients overall (6.3%), including 4 adults (1.8%), half of them class II, and 14 children (21.9%), 35.7% class II (p<0.001).

The median time between LT and ISW was 9.36 years (IQR 5.09-14.61), without significant differences between adults and children (9.11 years vs. 10.46 years; p=0.53). The shortest interval between LT and ISW observed in the study cohort was 10 months. The most frequent indication for ISW was enrolment in a clinical trial (53%), followed by the patient's decision (25.1%), side effects of immunosuppression (16.7%) and other reasons (5.2%). In adults, the most frequent reason for ISW was inclusion in a clinical trial (58.7%) while in children, the most frequent indication for ISW was the patient's decision (46.9%) (p<0.001). Among 48 patients with side effects of immunosuppression motivating weaning, the most frequent were malignancies (33.3%), renal impairment (31.2%), infections (14.6%), and cardiovascular events (6.2%).

The interval between start and completion of immunosuppression weaning was 8.48 months in adults (IQR 4.62-12.56) and 3.70 (IQR 0-8.64) months in children (p=0.093).

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Table S1. Demographics and clinical characteristics of 287 patients who achieved complete immunosuppression withdrawal for at least 12 months after liver transplantation according to the date of transplantation (before 2000 vs. 2000 onwards).

Variable	Transplanted before 2000 (n=138)	Transplanted 2000 onwards (n=149)	p
Age group, n (%)			0.95
Adults	107 (77.5%)	116 (77.9%)	
Children	31 (22.5%)	33 (22.1%)	
Sex (female), n (%)	42 (30.4%)	48 (32.2%)	0.75
Indication for LT, n (%)			0.004
Decomp. Cirrhosis	92 (66.8%)	78 (52.3%)	
Special indications	30 (21.7%)	30 (20.1%)	
Acute liver failure	10 (7.2%)	19 (12.8%)	
HCC	4 (2.9%)	21 (14.1%)	
Non-HCC tumors	2 (1.4%)	1 (0.7%)	
HCC, n (%)	13 (9.4%)	34 (22.8%)	0.002
Modality of donation, n (%)			0.30
Deceased (DBD)	116 (84.2%)	117 (78.5%)	
Deceased (DCD)	1 (0.7%)	3 (2%)	
Deceased (unknown)	10 (7.2%)	7 (4.7%)	
Living donor	10 (7.2%)	19 (12.8%)	
Unknown	1 (0.7%)	3 (2%)	
Donor age, years old	32.61 ± 18.92	46.05 ± 20.89	<0.001
Type of graft, n (%)			0.30
Whole	111 (80.5%)	107 (71.8%)	
Partial/split	20 (14.5%)	25 (16.8%)	
Reduced	2 (1.4%)	6 (4%)	
Domino	0 (0%)	1 (0.7%)	
Unknown	5 (3.6%)	10 (6.7%)	
Induction therapy, n (%)			0.034
Yes	10 (7.2%)	25 (16.8%)	
No	111 (80.4%)	103 (69.1%)	
Unknown	17 (12.3%)	21 (14.1%)	
IMS protocol, n (%)*			0.001
CNI alone	88 (63.8%)	102 (68.6%)	
Antimetabolite alone	32 (23.1%)	12 (8%)	
mTORi alone	4 (2.9%)	3 (2%)	
CNI + antimetabolite	11 (8%)	26 (17.4%)	
Others	3 (2.2%)	6 (4%)	
Interval transplant to IMS withdrawal (years)	12.91 (IQR 9.01-18.62)	6.79 (IQR 4.09-10.20)	<0.001
Reason for IMS withdrawal, n (%)			0.13
Clinical trial	75 (54.3%)	77 (51.6%)	
Patient's decision	29 (21%)	43 (28.9%)	
Side effects	23 (16.7%)	25 (16.8%)	
Others	11 (8%)	4 (2.7%)	
History of acute rejection, n (%)	25 (18.1%)	27 (18.1%)	0.96
Liver biopsy at ISW, n (%)	77 (56.2%)	80 (53.7%)	0.67
Investigation of DSAs at ISW, n (%)	1 (0.7%)	17 (11.7%)	<0.001

CNI: calcineurin inhibitors; DBD: donation after brain death; DCD: donation after circulatory death; DSAs: donor-specific antibodies; HCC: hepatocellular carcinoma; IMS: immunosuppression; ISW: immunosuppression withdrawal; LT: liver transplantation; MASLD: metabolic associated steatotic liver disease; mTOR: mammalian target of rapamycin.

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Table S2. Liver tests of 287 patients who achieved complete immunosuppression withdrawal for at least 12 months after liver transplantation.

Variable	At withdrawal	At last follow-up	*p*
ALT (U/L)			
Overall	23 (17-32)	23 (17-34)	0.99
Adults	22 (17-31)	22 (16-34)	0.29
Children	26 (18-37)	24 (18-31)	0.26
ALT (x1.5 normal range)			
Overall	7 (2.4%)	9 (3.1%)	0.80
Adults	6 (2.7%)	8 (3.6%)	0.78
Children	1 (1.6%)	1 (0.3%)	1
ALT (x2 normal range)			
Overall	1 (0.3%)	5 (1.7%)	0.22
Adults	1 (0.4%)	4 (1.8%)	0.37
Children	0	1 (1.6%)	1
GGT (U/L)			
Overall	28 (17-51)	34 (18-65)	0.010
Adults	31 (18-58)	38 (21-68)	0.019
Children	19 (13-33)	23 (15-34)	0.48
GGT (x1.5 normal range)			
Overall	23 (8%)	37 (12.9%)	0.08
Adults	23 (10.3%)	33 (14.8%)	0.20
Children	0	4 (6.3%)	0.12
GGT (x2 normal range)			
Overall	16 (5.6%)	24 (8.4%)	0.25
Adults	16 (7.2%)	21 (9.4%)	0.49
Children	0	3 (4.7%)	0.24
Total Bilirubin (mg/dL)			
Overall	0.7 (0.5-0.9)	0.7 (0.5-1)	0.84
Adults	0.7 (0.5-1)	0.7 (0.5-1.1)	0.68
Children	0.6 (0.5-0.8)	0.5 (0.4-0.8)	0.58
Total Bilirubin (x1.5 normal range)			
Overall	15 (5.2%)	25 (8.7%)	0.14
Adults	14 (6.3%)	20 (9%)	0.37
Children	1 (1.6%)	5 (7.8%)	0.21
Total bilirubin (x2 normal range)			
Overall	6 (2.1%)	12 (4.2%)	0.23
Adults	5 (2.2%)	10 (4.5%)	0.29
Children	1 (1.6%)	2 (3.1%)	1

ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase.

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Table S3. Causes of death and graft loss.

Cause of Death (general)	**N (%)**
Non-immunologically related graft failure	11 (24%)
Ischemic type biliary lesions	*3 (7%)*
Recurrent alcohol related liver disease	*4 (9%)*
Recurrent HCV	*2 (4%)*
Recurrent PSC	*1 (2%)*
Recurrent secondary biliary cirrhosis (graft vs host disease)	*1 (2%)*
Cardiovascular	10 (22%)
Cardiac failure	*6 (13%)*
Myocardial infarction	*3 (7%)*
Stroke	*1 (2%)*
Tumors	8 (18%)
Cholangiocarcinoma	*1 (2%)*
Multiple myeloma	*1 (2%)*
Renal cell carcinoma	*1 (2%)*
Colorectal cancer	*1 (2%)*
Ovarian cancer	*1 (2%)*
Breast cancer	*1 (2%)*
Lung cancer	*1 (2%)*
Melanoma	*1 (2%)*
Infections	5 (11%)
Urinary sepsis	*2 (4%)*
Covid-19	*2 (4%)*
Pneumonia	*1 (2%)*
Others	10 (22%)
Chronic kidney disease	*3 (7%)*
Alzheimer's disease	*2 (4%)*
Idiopathic pulmonary fibrosis	*2 (4%)*
Motor vehicle accident	*2 (4%)*
Ruptured peri-splenic aneurysm	*1 (2%)*
Unknown	1 (2%)
Cause of Graft Loss	**N (%)**
Disease recurrence	5 (55.6%)
Alcohol related liver disease	*4 (44.5%)*
HCV	*1 (11.1%)*
Ischemic type biliary lesions	3 (33.3%)
Congenital heart disease	1 (11.1%)
Immunologically mediated	0 (0%)

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Table S4. Multivariable Cox´s regression analysis of clinical predictors of overall mortality after immunosuppression withdrawal in the subset of patients with paired liver biopsies available (n=108).

Variable	β coefficient	HR	95%CI	p
Age at ISW	0.068	1.070	1.007-1.138	0.029
Sex (female)	0.059	1.061	0.299-3.769	0.93
Hepatocellular carcinoma before liver transplantation	0.155	1.168	0.242-5.638	0.85
Diabetes at ISW	0.276	1.318	0.416-4.172	0.64
Hypertension at ISW	0.238	1.269	0.363-4.439	0.71
Reason for ISW (side effects of immunosuppression vs. others)	0.813	2.255	0.449-11.323	0.32
Worsening histological inflammation*	0.382	1.465	0.391-5.485	0.57
Worsening histological fibrosis**	0.571	1.770	0.631-4.962	0.28

ISW: immunosuppression withdrawal

* Absence of inflammation in the baseline biopsy and presence of inflammation at any grade in the follow-up biopsy.

** Worsening at least one Ishak's fibrosis stage between baseline and follow-up biopsies.

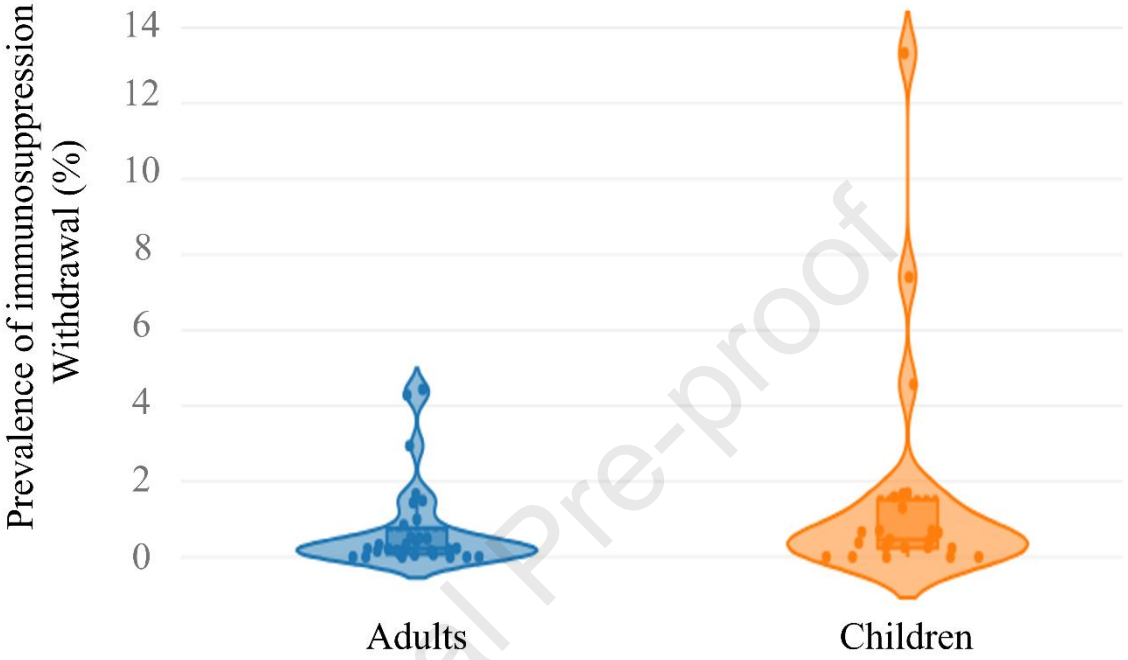
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Table S5. Metabolic comorbidities of 287 patients who achieved complete immunosuppression withdrawal for at least 12 months after liver transplantation. The presence of comorbidities was assessed at immunosuppression withdrawal and at last follow-up.

Variable	At Withdrawal	At last Follow-up	*p*
Hypertension			
Overall	133 (46.3%)	76 (26.4%)	< 0.001
Adults	131 (58.7%)	70 (31.4%)	< 0.001
Children	2 (3.1%)	6 (9.4%)	0.27
Hypertension Regression			
Overall		75 (56.4%)	
Adults		74 (56.5%)	
Children	---	1 (50%)	
New onset Hypertension			
Overall		18 (11.7%)	
Adults		13 (14.13%)	
Children		5 (8%)	
Diabetes Mellitus			
Overall	77 (26.8%)	46 (20.6%)	0.002
Adults	76 (34.1%)	44 (19.7%)	<0.001
Children	1 (1.5%)	2 (3.1%)	1
Diabetes Mellitus Regression			
Overall		47 (61%)	
Adults		46 (60.5%)	
Children		1 (50%)	
New onset Diabetes Mellitus			
Overall		16 (7.6%)	
Adults		14 (9.5%)	
Children		2 (3.2%)	
Hypercholesterolemia			
Overall	60 (20.9%)	50 (17.4%)	0.34
Adults	60 (26.9%)	48 (21.5%)	0.22
Children	0 (0%)	2 (3.1%)	0.50
Hypercholesterolemia Regression			
Overall		38 (63.3%)	
Adults		38 (63.3%)	
Children		-	
New onset hypercholesterolemia			
Overall		28 (12.3%)	
Adults		26 (15.6%)	
Children		2 (3.1%)	
Hypertriglyceridemia			
Overall	22 (7.7%)	26 (9.1%)	0.52
Adults	19 (8.5%)	24 (10.8%)	0.65
Children	3 (4.7%)	2 (3.1%)	1
Hypertriglyceridemia Regression			
Overall		12 (55%)	
Adults		10 (52.6%)	
Children		2 (66.7%)	
New onset hypertriglyceridemia			
Overall		16 (6%)	
Adults		15 (7.4%)	
Children		1 (1.6%)	

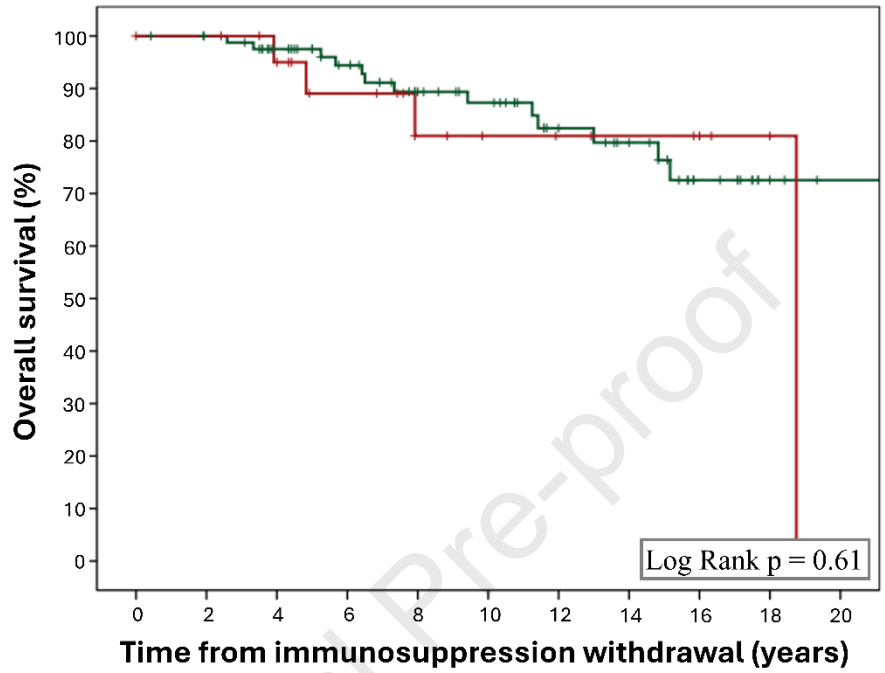
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Figure S1. Violin plot depicting the prevalence of immunosuppression withdrawal in the reference liver transplant population of 54,832 adults and 7,567 children. Each dot represents the prevalence for a certain participating centre. The box in each plot and the line within indicates the interquartile range and the overall prevalence, respectively.



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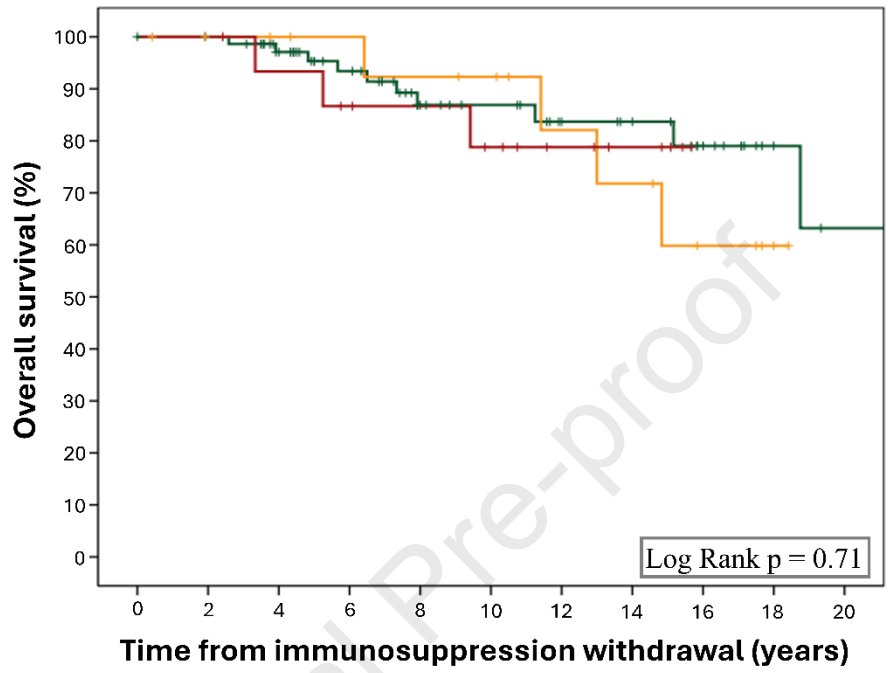
Figure S2. Kaplan-Meier curves showing cumulative overall survival after immunosuppression withdrawal in 108 patients with paired liver biopsies. Patients were stratified according to histological worsening of inflammation during follow-up.



Number at risk	1 year	3 years	5 years	10 years	20 years
Stable or improvement (n=84)	84	80	66	42	3
Worsening (n=23)	23	21	14	7	0

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Figure S3. Kaplan-Meier curves showing cumulative overall survival after immunosuppression withdrawal in 108 patients with paired liver biopsies. Patients were stratified according to histological worsening of graft fibrosis during follow-up: stable or regression of fibrosis, worsening of one Ishak's stage, or worsening of two or more Ishak's fibrosis stages.



Number at risk	1 year	3 years	5 years	10 years	20 years
Stable or improvement (n=75)	75	71	53	29	3
Worsening 1 stage (n=17)	17	15	13	11	0
Worsening ≥ 2 stages (n=16)	16	15	14	9	0

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