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REVIEW ARTICLE

# Use of N-acetyl-cysteine in the Perioperative Period of Liver Transplantation: A Scoping Review

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## ABSTRACT

**Objective:** To find evidence on the use of N-acetyl-cysteine (NAC) in the perioperative period of liver transplantation, since NAC, as it is the acetylated precursor of L-cysteine and reduced glutathione, contributes to the hepatic supply of glutathione, helping the liver to recover from ischemia and reperfusion injury. **Methodology:** This is a scoping review of the PubMed, VHL and Web of Science databases. The descriptors "Liver transplantation", "N-acetyl-cysteine" and "Reperfusion Ischemia" were used, with the Boolean operator "AND", and articles relevant to the topic were selected. Initially, 60 articles were selected, all published in the last 24 years, in Portuguese and/or English. After analysis, eight articles corresponded to the proposed objective. **Results:** The groups that received NAC during TxF showed post-reperfusion hypotension, lower intraoperative pH values, higher plasma concentrations of IL-4 and a significant increase in IL-10 levels five minutes before reperfusion. Inhibition of α-glutathione S-transferase (α-GST) was also observed after reperfusion, unlike the control group, which showed a significant increase in this enzyme. Furthermore, sVCAM-1 and sICAM-1 levels were significantly lower in the NAC group 24 hours after reperfusion compared with the placebo group. The maximum AST value during the first 72 postoperative hours was similar in both groups, although the peak ALT was lower in the NAC group than in the placebo group. In grafts that received NAC in the perfusion solution, survival rates at 3 and 12 months were 93% and 90%, respectively, and in the control group were 82% and 70%, respectively. The incidence of postoperative complications was 23% in the NAC group and 51% in the control group. The incidence of EPD was lower for the NAC group, which was 15% versus 32% in the control group. Regarding the administration of NAC during the intraoperative TxF, the one-year patient survival rate was 78.4% in the NAC group compared to 80.9% in the placebo group. **Conclusion:** Intraoperative administration of NAC during the anhepatic phase was associated with a protective effect against reperfusion injury, however in other studies limitations were observed in protection against liver injury, in biomarkers of oxidative stress, in inflammation and in the functioning of liver enzymes.

**Descriptors:** N-acetyl-cysteine; Liver Transplant; Reperfusion Ischemia.

## *Utilização de N-acetil-cisteína no Perioperatório de Transplante de Fígado: Uma Revisão de Escopo*

## RESUMO

**Objetivo:** Encontrar evidências sobre a utilização de N-acetil-cisteína (NAC) no perioperatório de transplante de fígado, uma vez que a NAC, por ser o precursor acetilado da L-cisteína e da glutationa reduzida, colabora no abastecimento hepático de glutationa ajudando o fígado a se recuperar da lesão de isquemia e reperfusão. **Metodologia:** Trata-se de uma revisão de escopo nas bases de dados *PubMed*, BVS e *Web of Science*. Foram utilizados os descritores "*Liver transplantation*", "*N-acetyl-cysteine*" e "*Reperfusion Ischemia*", com o operador booleano "AND", e selecionados artigos de relevância para o tema. Inicialmente, foram selecionados 60 artigos, todos publicados nos últimos 24 anos, em português e/ou inglês. Após análise, oito artigos corresponderam ao objetivo proposto. **Resultados:** Os grupos que receberam NAC durante o TxF apresentaram hipotensão pós-reperfusão, menores valores de pH intraoperatório, concentrações plasmáticas mais elevadas de IL-4 e aumento significativo dos níveis de IL-10 cinco minutos

antes da reperfusão. Observou-se ainda a inibição da α-glutationa S-transferase (α-GST) após a reperfusão, ao contrário do grupo controle, que apresentou aumento significativo dessa enzima. Além disso, os níveis de sVCAM-1 e sICAM-1 foram significativamente mais baixos no grupo NAC 24 horas após a reperfusão em comparação com o grupo placebo. O valor máximo de AST durante as primeiras 72 horas de pós-operatório foi semelhante em ambos os grupos, embora o pico de ALT tenha sido menor no grupo NAC do que no grupo placebo. Em enxertos que receberam o NAC na solução de perfusão, as taxas de sobrevivência aos 3 e 12 meses foram de 93% e 90%, respectivamente, e no grupo controle foram de 82% e 70%, respectivamente. A incidência de complicações pós-operatórias foi de 23% no grupo com o NAC e de 51% no grupo controle. A incidência de DPE foi menor para o grupo NAC, que apresentou 15% *versus* 32% do grupo controle. Em relação à administração do NAC durante o intraoperatório do TxF, a taxa de sobrevivência dos pacientes em um ano foi de 78,4% no grupo NAC em comparação com 80,9% no grupo placebo. **Conclusão:** A administração intraoperatória de NAC durante a fase anepática esteve associada a um efeito protetor contra a lesão de reperfusão, contudo em outros estudos foram observadas limitações na proteção contra lesões hepáticas, nos biomarcadores de estresse oxidativo, na inflamação e no funcionamento das enzimas hepáticas.

**Descritores:** N-acetil-cisteína; Transplante de Fígado; Isquemia de Reperfusão.

## INTRODUCTION

In liver transplantation (LTx), the liver graft is subjected to periods of cold and warm ischemia, and with the reestablishment of blood flow, ischemia and reperfusion injury (IRI) occurs, suffering additional aggression that aggravates the injury already caused by ischemia. IRI impairs postoperative liver function, patient recovery, and clinical outcome. This happens through the interaction of several mechanisms involving edema of endothelial and Kupffer cells, vasoconstriction due to increased endothelin-1 and decreased nitric oxide, upregulation of adhesion molecule expression due to humoral induction of inflammatory mediators and neutrophil infiltration, in addition to platelet aggregation within the sinusoids. The release of oxygen free radicals by Kupffer cells in the early phase of IRI will result in cellular damage due to oxidative stress. The late phase of IRI is related to the production of free radicals through the accumulation of neutrophils, and it is at this time that most liver injuries occur[1,2].

Liver graft IRI represents an unavoidable process that may result in early graft dysfunction (EGD), primary nonfunction, or even graft failure during the acute phase after LTx. Despite efforts to mitigate the effects of graft IRI, such as rapid organ procurement, reduced transport time, continuous machine perfusion, and remote or in situ ischemic preconditioning, the incidence of EGD continues to range from 2% to 23%. The situation is even worse when marginal grafts from extended-criteria donors are used. Using highly steatotic, aged, reduced-size grafts, donors after cardiac death, or HBsAb-positive individuals has significantly expanded the donor pool. However, these conditions are independent risk factors for EGD or graft loss[1,3].

Intracellular enzymatic pathways, mediated by superoxide dismutase and glutathione peroxidase, are crucial in modulating oxidative stress. Glutathione (GSH), found in high concentrations in the liver, is composed of glycine, glutamic acid and cysteine. It acts as an antioxidant, neutralizing oxygen free radicals. The synthesis of GSH depends on the availability of cysteine, whose precursor is N-acetyl-cysteine (NAC)[2].

Under normal conditions of temperature, perfusion and oxygenation, the liver is the primary producer of GSH. However, due to the length of stay of the donor in the intensive care unit (ICU) and the period of ischemia, the potential intrahepatic depletion of GSH may make the liver less capable of overcoming the oxidative stress of reperfusion. Thus, the antioxidant NAC, as the acetylated precursor of L-cysteine and reduced GSH, contributes to the hepatic supply of GSH, helping the liver to recover from IRI[4, 5, 6].

By buffering GSH, NAC promotes antioxidant effects, protecting hepatocytes from oxidative stress; enhances the vasodilatory properties of nitric oxide; inhibits the activation of thrombocytes, neutrophils and monocytes, main components of IRI; in addition to mitigating the increase in circulating adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), present in the inflammatory process[5,7,8,9].

Therefore, this scoping review aimed to find evidence in the literature on using NAC in the perioperative period of LTx.

## METHODOLOGY

The present is a scoping review built on the Joanna Briggs Institute (JBI) methodological framework and the PRISMA Checklist for Scoping Reviews (PRISMA-ScR), which establish the following steps: 1) define and align the objectives and research question; 2) develop and align inclusion criteria with the objectives and questions; 3) describe the planned approach to search,

selection, data extraction and presentation of evidence; 4) search for evidence; 5) select evidence; 6) extract evidence; 7) analyze evidence; 8) present evidence; and 9) summarize the evidence on the purpose of the review, draw conclusions and note any implications of the findings.[10,11].

Given this, the guiding question was formulated according to the PCC strategy, which stands for Population, Concept and Context. Thus, the following research question was developed: What results were obtained using N-acetyl-cysteine in the perioperative period of patients undergoing LTx?

The bibliographic search was carried out systematically in the following databases: PubMed, Web of Science and *Biblioteca Virtual de Saúde* (BVS); scientific articles from Medline and Lilacs were found in the latter. The following descriptors, validated by the Health Sciences Descriptors (Descritores em Ciências da Saúde- DeCS), were used: "Liver transplantation", "N-acetyl-cysteine" and "Reperfusion Ischemia". The Boolean operator permuted the descriptors "AND," and there was a time limit, with articles from the last 24 years being selected. The use of descriptors in English is due to the functioning of the databases and because most indexed articles are available in English, meaning that the search with descriptors in Portuguese limits the results to only articles that provide the Portuguese and English versions.

The descriptors were used in PubMed, BVS, and Web of Science, and the searches were expanded to all fields, resulting in 17, 20, and 23 articles found, respectively. In the end, 60 articles were found. Due to time limitations, other manual sources were not used.

For the systematic selection of articles, the Rayyan – Intelligent Systematic Review tool was used, considering the search strategy of the PRISMA Statement 2020 following the Equator Network CARE Guidelines for Systematic Reviews (Fig. 1)[12].

The screening criteria (inclusion and exclusion) were used to screen the articles, excluding duplicate articles and those that did not fit the focus of the research on the use of NAC in the perioperative period of LTx. Therefore, the priority of the search was to consider only scientific articles that described the mechanism of liver injury due to I/R, the mechanism of action of N-acetyl-cysteine in IRI, the positive and negative results from the use of NAC during LTx, and the limitations in the evidence regarding the benefits of using NAC in the perioperative period of LTx.

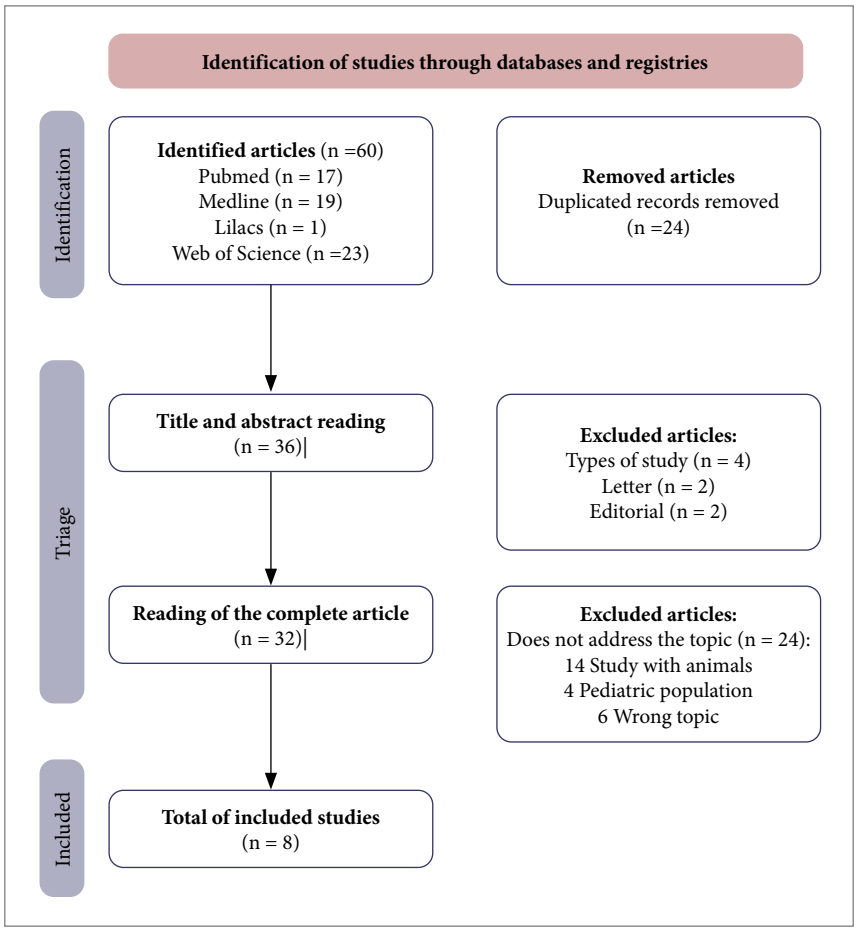
Finally, the articles that fit the theme were analyzed and included in a data extraction table with information that contributed to answering the question and objectives of the research. The results are described in Table 1.

## RESULTS

Figure 1 describes screening the selected articles based on strict inclusion and exclusion criteria. First, the articles were identified in the databases by one author. These were added to the Rayyan software, and duplicate articles were eliminated. After this step, the articles were screened by two other authors based on the reading of titles and abstracts, and those that did not fit the methodological design of the research, such as letters and editorials, were excluded. Then, the same two authors performed a new screening based on the titles and abstracts, and studies that did not specifically address the use of N-acetyl-cysteine in the perioperative period of LTx were excluded. Finally, the full text was evaluated by two researchers individually, according to the eligibility criteria. If there was disagreement between the evaluators, the text was discussed according to the criteria, and a consensus was sought. In the end, eight studies were included for the qualitative synthesis.

A double-blind, randomized clinical trial included 115 donors, divided into 49 donors who received 2 grams of NAC in addition to the standard preservation solution. In comparison, a control group of 66 donors received only the standard solution in the perfusion. The two groups of recipients were comparable in age, sex, patient weight, Model for End-Stage Liver Disease (MELD) score, and graft weight. There was no significant difference in intraoperative bleeding between the NAC and control groups, averaging 1810.15 mL and 2104.08 mL, respectively. Hospital stay was approximately 12 days in both groups. The mean norepinephrine dosage in the control and experimental groups before reperfusion was 14.52 mcg and 16.08 mcg, respectively.

Furthermore, the mean post-reperfusion norepinephrine dosage was approximately 12.05 mcg and 18.44 mcg in the control and experimental groups. Norepinephrine use was 78.92 min for the control group and 97.33 min for the experimental group. Hypotension after portal reperfusion was significantly more common in the experimental group compared to the control group, with 4 cases in the control group and 12 episodes in the experimental group. However, no difference was found in the duration of post-reperfusion hypotension between the groups. Retransplantation due to primary dysfunction was performed in two cases in the NAC group, whereas no patient in the control group required retransplantation. Both nonfunctioning livers were marginal grafts with prolonged cold ischemia time of approximately 12 hours. In-hospital mortality was comparable, with two cases in the control group and three in the NAC group[2].




Source: Elaborated by the authors

**Figure 1.** Article screening using the PRISMA Statement 2020 flowchart for systematic reviews.

Patients were randomized to NAC or placebo in a clinical trial of 50 LTx recipients, with each group comprising 25 patients. Intraoperative plasma concentrations of IL-4 were significantly higher in NAC-treated LTx recipients than in the placebo group. The differences were significant 5 minutes before reperfusion and 5 minutes after reperfusion. Plasma levels of IL-10 were similar in both groups, although there was a considerable increase 5 minutes before reperfusion in the NAC-treated group[8].

In another clinical study, 22 recipients were included and divided into two groups: one group received grafts from donors treated with NAC. In contrast, the control group received grafts from donors who did not receive any intervention during organ procurement surgery. Intravenous infusion of NAC was started approximately 15 minutes before cardiac arrest and through the portal vein in the in situ perfusion solution and ex-situ perfusion before bagging. In laboratory tests of the recipients post-LTx, serum transaminase levels during the first seven days were similar in both groups. Patients selected to receive marginal grafts, characterized by donor age over 50 years or macrovesicular steatosis, totaled seven in the NAC group and five in the control group. Laboratory tests of this group of post-transplant patients revealed elevated transaminase levels. One-hour post-reperfusion biopsy showed no statistical difference based on the number of necrotic foci/hepatocyte lobule[9].

In another study, 140 patients were divided into a group that received the graft with NAC added to the perfusion solution and a control group that did not receive NAC in the perfusion. The graft survival rates at 3 and 12 months were 93% and 90% in the NAC group and 82% and 70% in the control group, respectively. The incidence of postoperative complications was 23% in the NAC group and 51% in the control group. The incidence of primary liver dysfunction was lower for the NAC group, which presented 15% versus 32% in the control group[4].

In a study of 20 patients undergoing LTx who were randomized into a group receiving NAC and a control group receiving placebo immediately before and during liver reperfusion, graft reperfusion led to a significant increase in α-GST. This increase, however, was significantly inhibited by NAC during reperfusion. Both soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble intercellular adhesion molecule-1 (sICAM-1) in the placebo group increased 24 hours after donor

liver reperfusion. In marked contrast, sVCAM-1 and sICAM-1 levels were significantly lower in the NAC group 24 hours after reperfusion compared with the placebo group. The peak serum aspartate aminotransferase (AST) value during the first 72 hours postoperatively was similar in the placebo and NAC groups. However, the peak alanine aminotransferase (ALT) was lower in the NAC group (494 U/L) than in the placebo group (739 U/L)[5].

Regarding intraoperative pH values, in a study conducted with 50 LTx recipients, one group was treated with NAC, and the other was treated with a placebo. pH values were lower among patients treated with NAC than those in the placebo group. The differences were significant at 5 and 20 minutes after reperfusion, with a decrease in intraoperative pH observed 5 minutes before reperfusion in the group treated with NAC[13].

In one study, 93 patients underwent LTx, 46 of whom received NAC intraoperatively and 47 in the placebo group. The one-year survival rate was 78.4% in the NAC group compared with 80.9% in the placebo group. Severe post-reperfusion syndrome was reported in 26 patients in the NAC group and 25 in the control group, and no significant effects of NAC on postoperative recovery of liver function were detected. Postoperative serum levels of ALT, AST, INR, and total bilirubin were similar in both groups during the first three months of the study period. In the first 14 days, 18 patients in the NAC group developed acute kidney injury (AKI), while 16 in the placebo group developed AKI. GSH levels increased in the NAC group, while levels remained close to baseline in the placebo group. GSH subsequently returned to baseline values for the NAC group. The subgroup of individuals receiving NAC who exhibited an increase in GSH levels above five μM showed no significant difference in allograft survival at one year compared with those with an increase in GSH of less than five μM. Furthermore, no beneficial effects of increased GSH on liver function tests were demonstrated in the first-week post-LTx in the NAC group[14].

In a single-center study, 214 liver grafts for LTx were randomized to receive NAC or to the standard protocol without NAC, which served as the control group. EGD was defined as the presence of one or more of the following postoperative laboratory tests reflecting liver injury and function: bilirubin ≥ 10 mg/dL on day 7, INR ≥ 1.6 on day 7, and ALT or AST ≥ 2,000 IU/L in the first seven days, according to the Olthoff criteria15. In the control group, 37.4% of patients had EGD compared with 31% in the NAC treatment group. When cold ischemia time exceeded 6 hours, a significant increase in peak postoperative ALT levels was observed in the control group. However, NAC administration resulted in a reduction in ALT levels over the same cold ischemia time interval. The overall incidence of postoperative complications was 57.4% and 34.5% of patients in the control and treatment groups, respectively. Graft survival rates at 12 and 36 months were 89% and 87%, respectively, in the study group and 85% and 84%, respectively, in the control group. In addition, the GSH/glutathione disulfide (GSSG) ratio tended to be higher in the treatment group. The level of ophthalmic acid – also known as ophthalmate, a tripeptide analog of glutathione linked to increased oxidative stress conditions – was higher in the control group[6].

**Table 1.** Articles included in the scoping review.

| Author | Periodical/Year | Objective | Type of Study | Results |
|---|---|---|---|---|
| ALIAKBARIAN, M. et al.[2] | *Experimental and Clinical Transplantation*/2017 | To investigate whether NAC can decrease the rate of I/R syndrome and improve short-term outcomes in LTx recipients. | Prospective, randomized, double-blind clinical trial. | Post-perfusion hypotension was significantly higher in the experimental group, but the duration of hypotension was comparable between groups. The exact mechanism to explain this difference needs to be better understood. The question is whether the hypotension is related to using NAC or simply due to the small sample size. |
| SANTIAGO, FM. et al.[8] | *Transplantation Proceedings*/2008 | To evaluate the effect of NAC administration on intraoperative plasma levels of the anti-inflammatory cytokines IL-4 and IL-10 during LTx. | Prospective, randomized, double-blind clinical trial. | Intraoperative plasma concentrations of IL-4 were much higher among NAC-treated LTx recipients than in the placebo group. Differences were significant 5 minutes before and 5 minutes after reperfusion. Plasma levels of IL-10 were similar in both groups, although a substantial increase was observed 5 minutes before reperfusion in the NAC-treated group. |

continue...

**Table 1.** Continuation...

| Author | Periodical/Year | Objective | Type of Study | Results |
|---|---|---|---|---|
| KHAN, AW. et al.[9] | *Annals of Hepatology*/2005 | To study the effect of NAC administered to the organ donor before cold preservation. | Prospective and randomized clinical trial. | Peak serum AST levels were similar, and postreperfusion biopsy showed moderate to severe reperfusion injury in 3 recipients in the NAC group and 4 in the Control group. NAC administered during donor operation showed no protective effect on IRI. |
| D'AMICO, F. et al.[4] | *Liver Transplantation*/2012 | To test the impact of systemic and locoregional NAC infusions during liver procurement on post-LTx outcomes. | Prospective and randomized study. | Graft survival rates at 3 and 12 months were 93% and 90% in the NAC group and 82% and 70% in the control group, respectively. The incidence of postoperative complications was lower in the NAC group (23%) compared with the control group (51%). The incidence of primary liver dysfunction was lower for the NAC group (15%) versus the control group (32%). |
| WEIGAND, MA. et al.[5] | *Transplantation*/2001 | To investigate the effects of NAC on α-GST, plasma cytokines, and circulating and cell adhesion molecules during LTx. Furthermore, since changes in surface expression of CD18 and CD62L on neutrophils were associated with increased serum liver enzymes postoperatively, we investigated whether NAC directly affected the expression of the stimulated adhesion molecule on human neutrophils in vitro. | Prospective and randomized study. | NAC inhibits the increase in α-GST and circulating ICAM-1 and VCAM-1 after graft reperfusion. This may point to a possible protective function of NAC during LTx. |
| SANTIAGO, FM. et al.[13] | *Transplantation Proceedings*/2010 | To evaluate the effect of NAC administration on intraoperative pH values during LTx. | Prospective, randomized, double-blind study. | Intraoperative administration of NAC during the anhepatic phase of LTx significantly decreased receptor pH values 5 and 20 minutes after reperfusion, a decrease detected 5 minutes before reperfusion. |
| HILMI, IA. et al.[14] | *Nephrology dialysis transplantation*/2010 | To evaluate the efficacy of NAC in improving liver graft performance and reducing the incidence of AKI post-LTx. Secondary objectives were to investigate the effect of NAC on GSH levels and to examine the relationship between GSH and AKI. | Prospective, randomized, double-blind study. | NAC did not affect liver graft survival and function. However, GSH levels were highly variable, with only 50% of patients receiving NAC exhibiting increased levels. |
| GÓMEZ-GAVARA, C. et al.[6] | *Transplantation*/2021 | To compare the impact of NAC infusion during liver procurement on post-LTx outcomes. | Single-center clinical trial. | The incidence of primary dysfunction was 34%, 31% in the NAC group and 37.4% in the control group. NAC administration reduced ALT levels when cold ischemia time was longer than 6 hours. Both groups had similar oxidative metabolites (GSH/oxidized GSH and ophthalmic acid). Graft and patient survival rates at 12 months and three years were comparable between groups. |

Source: Elaborated by the authors. NAC = N-acetyl-cysteine; I/R = ischemia-reperfusion; LTx = liver transplant; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; α-GST = α-glutathione S-transferase; AKI = acute kidney injury; GSH = glutathione

## DISCUSSION

Organ preservation is one of the most critical factors in reducing transplant complications and increasing graft and patient survival. Cold ischemia time may increase the risk of primary nonfunction or delayed graft function. At the same time, using an appropriate preservative fluid during transport may decrease the risk of liver injury. However, metabolic changes occurring

during LTx may cause severe complications to the liver graft due to IRI. N-acetyl-cysteine, in turn, is a potential combatant of oxidative stress generated during transplantation due to its action on GSH and other metabolic pathways involved during the process[2, 16].

## Mechanism of liver injury due to ischemia and reperfusion

IRI, or reoxygenation injury, is tissue damage involving multifactorial processes triggered when the blood supply returns to the organ after transient oxygen deprivation or insufficiency[16, 17].

Due to the lack of oxygen, the final electron acceptor of oxidative phosphorylation, the synthesis of adenosine triphosphate (ATP) is restricted. The reduction in cellular ATP levels during ischemia causes acidification in the intra- and extracellular environment due to changes in the control of calcium ion (Ca2+) efflux and the accumulation of intracellular sodium. Consequently, the calcium overload activates calcium-dependent proteases that will unbalance the structure of the cell membrane, causing cell death by necrosis, apoptosis and autophagic mechanisms. The increased production of superoxide radicals, pro-inflammatory factors and impaired levels of nitric oxide, resulting from the cell death process, can lead to the chain activation of other free radical molecules and contribute to hepatocellular damage[1].

The process of liver reperfusion exacerbates injury by transient portal hypertension and hyperdynamic stress. Elevated portal pressure causes direct damage to liver sinusoidal endothelial cells and exposes the vessel wall to the adhesion of circulating platelets and leukocytes. Platelet aggregation and activation narrow the venule and release large amounts of cytokines, chemokines, and vasoactive molecules. The imbalance of vasoconstriction and vasodilation factors further aggravates microcirculatory dysfunction. Decreased microcirculation worsens hyperdynamic stress, resulting in sinusoidal congestion and collapse of the space of Disse between endothelial cells and hepatocytes, thus prolonging hypoxia[17,1].

After liver graft reperfusion, the restoration of oxygen levels activates the hypoxanthine-xanthine oxidase system, increasing the production of reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion, and hydroxyl radicals. These ROS activate Kupffer cells, resident macrophages of the liver, triggering a vicious cycle of self-destruction through the cascade activation of other free radicals. In addition, this process also occurs with the activation of polymorphonuclear neutrophil macrophages, together with injured liver sinusoidal endothelial cells that secrete large amounts of cytokines and chemokines into the sinusoid, exacerbating the inflammatory reaction and recruiting more innate and adaptive immune cells to the graft. Within two hours after liver graft revascularization, a cytokine storm is observed and peaks at 6 to 24 hours after reperfusion. In the pro-inflammatory microenvironment, the cytokines involved are interleukin (IL) 1β, IL2, IL6, IL15, interferon-γ (IFNγ) and tumor necrosis factor-α (TNF-α), and the chemokines are CCL2, CXCL8 and CXCL10[1, 18].

The release of damage suppressor molecules activates the first wave of resident Kupffer cells and adhesive neutrophils by binding to pattern recognition receptors on their cell surfaces. Toll-like 4 (TLR4) is the predominant pattern recognition receptor expressed on nearly all cells of the innate immune system, and its increased expression not only increases the pro-inflammatory activity of macrophages but also recruits more polymorphonuclear neutrophils from the circulation. Evidence shows reactive nitrogen species (RNS) are important liver I/R mediators. Concerning nitric oxide (NO), the most relevant RNS are characterized by endothelial NO synthase (NOS), whose activity is dependent on Ca2+ and calmodulin, and by an inducible form synthesized by endothelial cells, hepatocytes and Kupffer cells, whose activity is independent of Ca2+. The induction of this last isoform can have toxic or protective effects, depending on the type of insult and the level and duration of its activation[16,1].

The process of liver graft recognition occurs with the activation of adaptive immunity, which makes the organ more susceptible to allograft rejection. Natural killer T cells (NKT) and T cells are primarily responsible for allograft rejection, with CD4+ T cells being the leading players. The Complement System is also activated and promotes graft damage after LTx. The deposition of the membrane attack complex composed of C5-9 on cell membranes contributes to parenchymal damage[1].

IRI is measured by elevated alanine aminotransferase and aspartate aminotransferase levels, total bilirubin and international normalized ratio, as well as clinical symptoms such as the onset of ascites and encephalopathy between seven and ten days after LTx. Prothrombin time and international normalized ratio have been used as valuable indicators of EGD and graft injury, but their elevation is a late indicator and may delay identification. The inflammatory response begins two hours after reperfusion. Still, its application as a diagnostic tool for EGD is limited due to its low specificity and the influence of the immunosuppressive regimen. In addition, GSH, which is synthesized mainly in liver cells, requires cysteine to be produced, but cysteine is not available during the oxidative stress caused by liver graft revascularization. Therefore, the GSH level is depleted during this process[1].

In summary, evidence suggests that during hepatic I/R, there is the generation and release of ROS and RNS that cause oxidative stress in the liver and promote endothelial dysfunction, DNA damage and local inflammatory responses. The key

cells that initiate IRI are Kupffer cells, which promote releasing these reactive species. Hepatocyte-derived xanthine oxidase and Kupffer cells, sinusoidal endothelial cells and mitochondria are also known sources of ROS. Furthermore, it has been reported that inflammatory cascades and oxidative stress subsequently induce a cytokine storm, leading to cell death due to damage to their structure. Considering that histological analysis is the gold standard for the identification of liver injuries resulting from I/R, it is possible to identify hepatic microvesicular steatosis, foci of parenchymal neutrophilic infiltration, cholestasis, hepatocyte ballooning, necrosis and apoptosis. These changes are commonly observed in routine reperfusion biopsies, albeit to a lesser extent, and are usually most prominent in the centrilobular regions. However, the utility of this analysis is limited by the fact that only a tiny area of the liver can be examined, which makes it difficult to assess the condition of the entire organ. This challenge can be partially mitigated by combining circulating biomarker results with intrahepatic findings[16,1,9].

## Mechanism of action of N-acetyl-cysteine in ischemia and reperfusion injury

IRI in the liver triggers the expression of inducible nitric oxide synthase (iNOS), which promotes the release of large amounts of NO. The most favorable reaction of NO occurs with ROS, which forms hydroxyl radicals and peroxynitrites, potent oxidants. The antioxidant effect of NAC acts by slowing the oxidative stress IRI created and attenuating the liver graft's injury. In addition, NAC is a precursor of cysteine for the synthesis of GSH and acts by replenishing intrahepatic GSH reserves and increasing the activity of GSH reductase between LTx receptors – which usually presents with decreased activity, facilitating liver IRI[8,7].

Another relevant action of NAC is the reduction of disulfide bonds in proteins, which increases the expression of coenzyme Q10 with antioxidant properties. It activates the erythroid factor 2 (Nrf2) signaling pathway, a crucial regulator of cellular resistance to oxidants. Activation or increased expression of Nrf2 has been associated with increased expression of genes and enzymes encoding cytoprotective defense antioxidants in response to exposure to oxidative stress. The importance of this mechanism is particularly evident in the effective use of NAC against an overdose of acetaminophen-induced liver injury, popularly known as paracetamol, which causes an abrupt depletion of GSH levels in the liver[19].

Thus, NAC reduces oxidative stress by increasing intracellular GSH and reducing the release of IL-6, IL-1β and TNF-α. It also generates effects on molecular signaling, gene expression and common transcription factors, intending to accelerate repair mechanisms for liver graft tissue damage caused by IRI.

## Positive results of the use of N-acetyl-cysteine during liver transplantation

In a study using NAC in LTx recipients, significantly reduced plasma levels of α-GST were reported 24 hours after liver graft reperfusion. α-GST is found predominantly in the liver and, unlike ALT and AST, which have higher concentrations in periportal hepatocytes, is equally distributed in the periportal and centrilobular regions. Because centrilobular hepatocytes are more susceptible to hypoxic damage, α-GST was a more sensitive indicator of hepatocellular injury than ALT and AST. This may be why NAC significantly inhibited peak α-GST levels in the immediate reperfusion period and not peak ALT or AST levels, although peak ALT levels were lower in the NAC group than in the placebo group. These patients exhibited reduced circulating ICAM-1 and VCAM-1 concentrations and elevated plasma IL-4 and IL-10 levels. These low concentrations result in the inhibition of neutrophil chemotaxis, reduced leukocyte adhesion via interaction with VCAM-1, decreased platelet aggregation, and improved endothelium-dependent vasodilation[5, 20].

In a clinical trial, peak plasma IL-4 and IL-10 levels were observed 20 and 60 minutes after reperfusion, respectively, in both the placebo and NAC groups. IL-4 and IL-10 act as anti-inflammatories, blocking the synthesis of IL-1, TNF-α, IL-6, and macrophage inflammatory proteins. A significant increase in IL-4 and IL-10 was observed among LTx recipients treated with NAC 5 minutes before and 5 minutes after reperfusion, with IL-10 reaching its maximum peak at 60 minutes after liver revascularization, indicating a positive modulatory effect of NAC on the anti-inflammatory response mediated by these cytokines[8].

Additionally, NAC is a prodrug of L-cysteine used in acetaminophen and carbon monoxide toxicity, contrast-induced nephropathy, chronic bronchitis, radiation-induced alveolitis, and neonatal thick bile syndrome. In addition to its antioxidant effects, NAC inhibits leukocyte adhesion and decreases TNF-α[7].

In one study, intraoperative pH was evaluated during LTx using NAC. Minimum pH values in both study groups, placebo and NAC, were observed five minutes after reperfusion. A significant reduction was detected among liver graft recipients treated with NAC at 5 and 20 minutes after reperfusion as an indication of a modulating effect of NAC on acid-base balance. The reduction in pH values among LTx recipients treated with NAC may be related to the hydrolysis of NAC into cysteine and acetic acid, which may react further with bicarbonate anions, reducing their values in the NAC group and consequently increasing carbon dioxide[13].

### Negative results on the use of N-acetyl-cysteine during liver transplantation

NAC may have limited protective effects against ischemia-reperfusion injury. One study reported that NAC did not affect peak AST levels post-reperfusion of liver graft[9]. In another study, no significant changes were observed in hepatic artery reperfusion, hospital stay, need for inotropes before and after portal clamping in LTx or vascular complications. It was also described that NAC can induce amino acid loss and promote the release of urea nitrogen from the liver graft in patients undergoing LTx[21].

In one study, NAC was administered to patients undergoing LTx. In the group receiving NAC, GSH levels were elevated when NAC was being administered. Still, later, levels had fallen to baseline, suggesting that the effect of NAC on GSH levels was not sustained. However, despite the increase in GSH levels, which lasted for approximately 48 hours, there was no significant change in the recovery of graft function as measured by liver function tests[14].

In another study, it was observed that EGD, according to the Olthoff classification, did not differ between the group receiving NAC and the control group not receiving NAC. NAC was administered at the graft harvesting stage. In this study, the incidence of EGD was 34%, compared with 23% reported by Olthoff et al. One possible reason for this is that the mean age of the donors in this series was 63 years, and the mean age of the donors in the study by Olthoff et al. was 48 years. Of the patients who presented EPD, 27% had eventual graft loss. In 90% of the included recipients, the criteria for EGD were due to ALT/AST on the first postoperative day. However, ALT/AST levels normalized to less than 2,000 IU/L from the second day onwards at 85%[15,6].

### Limitations in the evidence regarding the benefits of using N-acetyl-cysteine in the perioperative period of liver transplantation

From studies investigating the protective effect of NAC against liver injury due to I/R in the context of liver transplantation, clinical evidence of the benefit of this drug has yet to be definitive. These results must be more consistent due to the small sample size and insufficient power to detect minor differences. The heterogeneous results of these studies also prevent the pooling of data to draw more robust conclusions in the format of a scoping review article. The limitation of this study is due to the large number of studies conducted in animals that demonstrate the efficacy of NAC against ischemia and reperfusion injury. In contrast, the number of studies in humans is smaller. However, clinical trials conducted with patients undergoing human LTx demonstrated that intravenous NAC administration is associated with a lower incidence of EGD and improved liver function. Still, studies in the literature have yet to demonstrate any benefit of NAC in IRI.

## FINAL CONSIDERATIONS

IRI drives the detrimental effects of oxidative stress and inflammatory response during LTx. Dysregulation of hepatic microcirculation, homeostasis, and metabolism causes early injury and initiates an immunologic cascade. Both innate and adaptive immunity prolong graft injury through the adhesion and recruitment of macrophages, neutrophils, and dendritic cells and by activating interleukins, NKT cells, and cytotoxic T lymphocytes. Administration of N-acetylcysteine during liver procurement has not demonstrated significant improvement in EGD, but when cold ischemia time is greater than six hours, NAC positively influences postoperative ALT levels. Intraoperative administration of NAC during the anhepatic phase in LTx recipients was associated with a protective effect against reperfusion injury; however, in other studies, limitations were observed in protection against liver injury, in biomarkers of oxidative stress, inflammation and in the functioning of liver enzymes.

## CONFLICT OF INTEREST

Nothing to declare.

## AUTHOR'S CONTRIBUTION

**Substantive scientific and intellectual contributions to the study:** Silva HRS, Lima MI and Fonseca Neto OCL; **Conception and design:** Santos FAM, Muniz GVC, Rocha MERT, Diógenes SFG, Tôrres DGB, Lima CM, Bermond BC, Lima MI and Fonseca Neto OCL; **Data analysis and interpretation:** Santos FAM, Muniz GVC, Rocha MERT, Diógenes SFG, Tôrres DGB, Lima CM and Bermond BC; **Article writing:** Santos FAM, Muniz GVC, Rocha MERT, Diógenes SFG, Tôrres DGB, Lima CM and Bermond BC; **Critical revision:** Lima MI and Fonseca Neto OCL; **Final approval:** Fonseca Neto OCL.

## DATA AVAILABILITY STATEMENT

All datasets were generated/analyzed in the current study.

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