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Clinical use of N-acetyl cysteine during liver transplantation: Implications of oxidative stress and inflammation as therapeutic targets

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ABSTRACT

Currently, liver transplantation is considered as the definitive treatment option for individuals with complete liver failure. However, the detrimental effects of oxidative stress and inflammation remain the predominant feature that drives hepatic ischemia-reperfusion injury during liver transplantation. As such, therapeutic drugs that hinder oxidative stress and attenuate inflammation, have become ideal targets to curb liver injuries during transplantation. The current review analyses available clinical evidence on the importance of using N-acetyl cysteine (NAC) during liver transplantation. Thus, prominent online search engines such as PubMed and Google Scholar were accessed to retrieve literature from randomized clinical trials reporting on the use of NAC during liver transplantation. Overwhelming evidence suggests that established therapeutic properties of NAC, through enhancing endogenous antioxidants like glutathione to block oxidative stress and attenuate inflammation, remain essential to improve liver function in patients undergoing liver transportation. However, to the contrary, some clinical studies did not show any beneficial effects in patients receiving NAC during liver transplantation. Thus, such controversies, in addition to discussing the implications of oxidative stress and inflammation in relation to hepatic ischemia-reperfusion injury remain the major subject of the current review.

1. Introduction

The liver is a vital organ that regulates numerous physiological processes, in particular the metabolism of macronutrient and xenobiotics [1]. Liver detoxification involves a broad spectrum of enzyme systems that can handle diverse chemical structures in the environment to which we are exposed daily [1]. Even worse, undesirable lifestyle choices, including excessive alcohol intake and consumption of a diet rich in fat, contribute directly to hepatic damage. Consistent with the rising trends in obesity and adiposity [2], steatosis is the initial response to heavy drinking and is distinguished by the fat accumulation in hepatocytes [3]. As a result, severe liver disease can greatly impact an individual's quality of life along with their life expectancy. However, exploiting lifestyle interventions such as adhering to regular exercise in combination with maintaining a healthy diet can play a crucial role in improving health outcomes in patients with chronic liver diseases [4]. In fact, exercise can limit the synthesis of fats or enhance their oxidation, in the process, attenuate the detrimental effects of oxidative stress and inflammation, the prominent complications linked with accelerated hepatocellular injury [5,6].

Unfortunately, it is generally accepted that only a few individuals can consistently adhere to necessary lifestyle interventions such as

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; I/R, ischemia-reperfusion injury; α-GST, alpha glutathione S-transferase; GSH, glutathione; NAC, N-acetyl cysteine; RCTs, randomized controlled trials; ROS, reactive oxygen species; TNF-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion molecules.

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regular physical activity. Although, available therapies that could correct against underlying complications linked with liver injury exists, some have limited protective effects and elicit undesirable side effects [4,5,7]. To compensate for liver failure, liver dialysis procedures are usually employed, however, such techniques cannot be applied for long-term [8]. Currently, liver transplantation is the definitive treatment option for individuals with complete liver failure [9]. Liver transplantation or hepatic transplantation describes the replacement of a damaged liver with the healthy one from another person. In recent years, liver transplantation has considerably improved survival rates of patients with chronic liver diseases, as reviewed elsewhere [10]. While such progressive advances are acknowledged, there is still controversy implicating post-operative liver failure. The latter infers to the diminished capability of the liver to sustain its main functions such as to synthesize, excrete, and detoxify different molecules or substances [11]. Thus, it has become imperative to prioritize minimizing post-operative complications, especially hepatic reperfusion injury during liver transplantation.

N-acetyl cysteine (NAC), an accomplished antioxidative agent, is increasingly used to limit hepatic injury during liver transplantation. Certainly, accumulative data from randomized controlled trials (RCTs) suggest that NAC can protect against hepatic injury, by limiting actions related to oxidative stress and inflammation [12], to improve health outcomes in patients undergoing liver transplantation [13]. However, to the contrary, other RCTs have failed to observe any positive outcomes with the use of NAC during hepatic transplantation [14].

Hence, the current study discusses published evidence from RCTs informing on how NAC can be beneficial to patients with chronic liver disease undergoing liver transplantation. A systematic approach was applied to identify relevant studies, using prominent electronic search engines like PubMed and Google Scholar, from inception up to 31 July 2021. Moreover, a brief overview is given on the occurrence of liver injury during transplantation in a clinical setting, as well as the implications of both oxidative stress and inflammation, is given to underscore the therapeutic potential of NAC to limit hepatic injury in patients undergoing transplantation.

2. An overview of acute and chronic liver failure

Acute liver failure is a life-threatening rare illness that occurs most often in patients with no pre-existing liver disease. Clinical presentations of acute liver failure include hepatic dysfunction, abnormal liver biochemical values, as well as the development of coagulopathy and encephalopathy [15]. Due to its rare unpredictable nature, acute liver failure is very difficult to identify during its early stages which in turn delays treatment initiation [15]. In a developed country like the United States, drug-induced liver injury is responsible for approximately 50% of cases of acute liver failure [15]. On the other hand, acute-on-chronic liver failure, is a combination of acute deterioration in liver function in an individual with pre-existing chronic liver disease, which may lead to multisystem organ failure due to increases in inflammatory cytokines [16–18]. The most precipitating factors of acute-on-chronic liver failure include infections caused by either bacteria or viruses, alcoholic hepatitis, as well as surgery. The precipitating events of this syndrome are known to vary among countries as has been seen from multicenter studies [16,19]. Moreau and co-workers reported that bacterial infections and active alcohol intake, presumably alcoholic hepatitis, were identifiable factors in Western countries [16]. While Shi and co-workers, found that hepatic insult such as exacerbation of hepatitis B virus, hepatitis A/E virus infections, and active alcoholism were confounding precipitating triggers [20]. Be that as it may, in more than 40% of patients who developed acute-on-chronic liver failure, there were no precipitating events that were noticed [16]. Although different definitions of what acute-on-chronic liver failure is according to societies (the Asia-Pacific Association for the Study of Liver Disease and the European and American associations for the study of liver disease), a consensus has been reached, stating that acute-on-chronic liver failure is a distinguished syndrome characterized by organ failure with a high morbidity and mortality. Given the high mortality correlated with acute-on-chronic liver failure, liver transplantation remains a condemnatory option in the treatment of patients suffering from this condition. This of course is an option aside from treating the underlying precipitating factors etiologies with corticoids and or antibiotics. A simple definition of liver transplantation or hepatic transplantation, as scientifically renowned refers to a medical procedure where a diseased/dysfunctional liver organ becomes replaced with a healthy liver from another person. Liver donors are usually cadavers for whole liver organ transplant or healthy living donors where only a small portion of the liver can be donated. Although, solid liver transplantation is an established lifesaving procedure, however, ischemia-reperfusion injury (I/R) is an inevitable problem because of the technical nature of transplantation.

3. An overview of ischemic-reperfusion liver injury

Ischemia-reperfusion injury, sometimes known as reoxygenation injury, is a tissue damage which involves multifactorial processes that are triggered when blood supply returns to the liver organ following a transient oxygen deprivation (anoxia) or lack of oxygen (hypoxia) [21–23]. A period of oxygen deprivation and reoxygenation are likely to occur during surgical procedures, for instance, liver surgery, transplantation and during hemorrhagic shock. This consequence is characterized by a significant rise in free radical species which are subsequently accompanied by the depletion of endogenous antioxidants. Fig. 1 gives an overview of pathophysiological mechanisms that link a state of I/R with enhanced liver injury. In a vicious circle, reduction of cellular ATP levels during ischemia causes acidification in both intra and extracellular media, in part through the aberrations in the control of calcium ion (Ca^{2+}) effluxes and intracellular sodium accumulation. As a consequence, calcium overload leads to the activation of calcium dependent proteases which in turn disrupts cell membrane structure leading to cell death by necrosis, apoptosis, and autophagic mechanisms, which is believed to be a crucial step in irreversible damage [23,24]. Abnormally enhanced production of superoxide radicals, pro-inflammatory factors and impaired nitric oxide levels can lead to chain activation of other free radical molecules, which through activation of hypoxanthine-xanthine oxidase system, can facilitate increased generation of oxidative stress and lead to hepatocellular injury [25,26]. Nonetheless, it is known that although ischemia causes significant injury to tissue and cells, the injury during reperfusion is more severe. Indeed, oxygen levels are actually restored upon reperfusion, however, a surge in the generation of reactive oxygen species (ROS) is still very much possible. The key cells that initiate I/R injury are the Kupffer cells. Evidence has shown that ROS and reactive nitrogen species (RNS) are important mediators in liver I/R [25]. Collectively, ROS includes but not limited to hydrogen peroxide (H_2O_2), superoxide anion, and hydroxyl radicals, while the most biologically relevant RNS is nitric oxide (NO), are generated when Kupffer cells “resident liver macrophages” become activated [25]. Kupffer cells are also activated by ROS, a process that may prompt chain activation of other free radicals and perpetuate a vicious cycle of self-activation and destruction [25,27,28]. In addition to the generation of ROS, the activation of Kupffer cells leads to structural changes and the production of cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)− 1 [29]. Moreover, evidence exists suggesting that during hepatic I/R there is generation and release of ROS which causes oxidative stress in the liver and promotes endothelial dysfunction, DNA damage, and local inflammatory responses [24]. Recognized sources for ROS are hepatocyte derived xanthine oxidase, and Kupffer cells, sinusoidal endothelial cells and mitochondria [30,31]. Interestingly, the role of nitric oxide in liver I/R injury has been described by many [32–34]. Briefly, nitric oxide synthase exists in two major isoforms, namely endothelial NOS (expressed constitutively, and

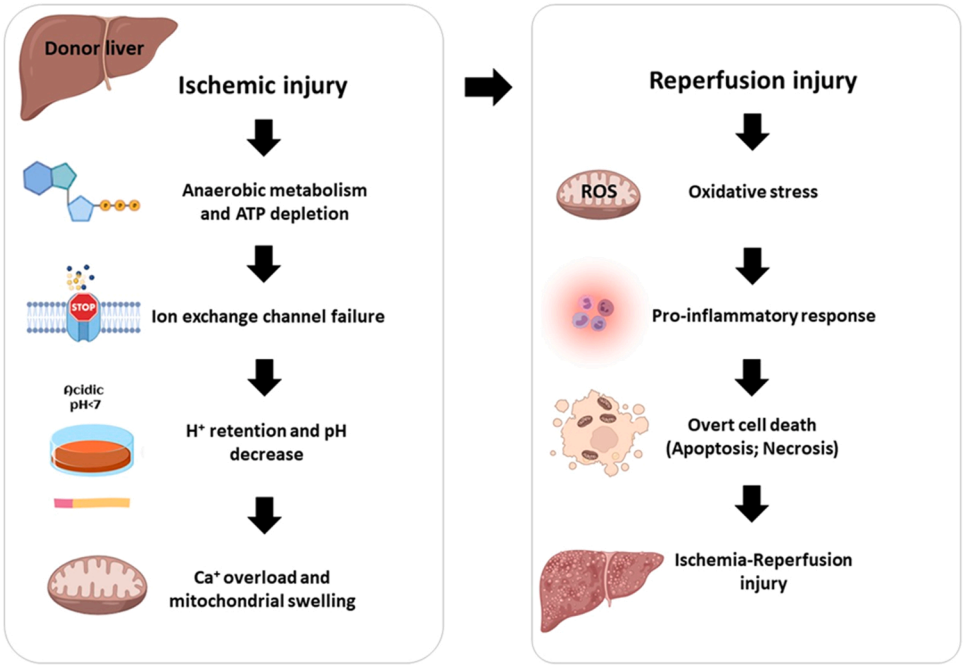

Fig. 1. A summary of pathophysiological mechanisms underlying hepatic ischemic-reperfusion injury. Briefly, a period of oxygen deprivation and reoxygenation are likely to occur during surgical procedures, for instance liver surgery, transplantation and during hemorrhagic shock. This consequence is characterized by a significant rise in free radical species which are subsequently accompanied by the depletion of endogenous antioxidants. Abbreviations: ATP, Adenosine Triphosphate; ROS, reactive oxygen species; calcium ion, Ca^{+}.

its activity is dependent on Ca^{2+} and calmodulin) and an inducible form which is synthesized by endothelial cells, hepatocytes and Kupffer cells and its activity is Ca^{2+} independent [30,35]. Induction of the latter has either toxic or protective effects depending on the type of insult, level and duration of its activation. The effects are further dependent on the type of insult, the level and duration of its expression as well as the simultaneous production of superoxide anion [32–34]. Additionally, it has been reported that inflammatory cascades coupled with oxidative stress subsequently induces a cytokine storm, leading to cell death due to damage of cellular structures [36]. Thus, I/R is a major problem in liver transplantation, that poses an important therapeutic challenge as physicians must control cell damage and preserve organ function even after surgery. An understanding of the mechanisms of I/R has a potential to provide a strong foundation that will not only prompt for novel therapeutic opportunities, but also for prevention of this injury.

4. Implications of oxidative stress and systemic inflammation during ischemia-reperfusion injury

Although liver transplantation remains a challenge for patients with end-stage liver failure, the inevitable I/R has become an utmost concern during this procedure [37,38]. Meanwhile, a shred of cumulative evidence outlined from published research [39], shows that oxidative stress and inflammation are critically involved in the pathophysiology of hepatic I/R [40]. Apart from ischemic preconditioning, there is no definitive therapy to avoid hepatic I/R, particularly during the reperfusion phase. As discussed in this section, it is imperative to understand the pathological mechanisms of hepatic I/R in order to develop novel therapeutic interventions.

4.1. Ischemic phase

In the ischemic phase, low oxygenated blood supply induces mitochondrial oxidative phosphorylation defect and anaerobic metabolism, which causes ATP depletion, increase lactate and decrease pH levels in hepatocytes [41]. Recently, Gan et al. [42] have demonstrated that acidic microenvironment accentuates severity of liver I/R in mice and humans. Here, the mild liver injury was observed with a significantly lower pH after ischemia while the most serious injury and abnormal liver pH appeared at 6 h after reperfusion [42]. The extent of liver damage was determined by decrease dysregulations in the modulation of regulatory T (CD4 +CD25 +Foxp3 +) cells, which are responsible for immune tolerance [41,42]. This occurred concurrent to the elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are conventional biomarkers of liver injury. Thus, measuring prudent biomarkers such as ALT, AST, pH, lactate and bilirubin prior to reperfusion has proved very helpful to predict early graft performance after the liver transplantation [43].

4.2. Reperfusion phase

Upon reperfusion, paradoxically restoration of oxygenated blood supply aggravates liver injury by inducing oxidative stress and inflammation which exacerbates apoptosis and necrosis [44]. Under this circumstance, the liver-resident macrophages Kupffer cells are activated by CD4 + T cells, which causes the increased production ROS and the release of pro-inflammatory cytokines, including TNF-α and IL-1β [44]. According to a recent review by Bhogal and co-workers [45], circulating levels of pro-inflammatory cytokines such as TNF-α and some ILs can be used as biomarkers for the liver injuries that occur during transplantation. Inversely, anti-inflammatory cytokines such as IL-4 and IL-10 play a major role in macrophage polarization and attenuating hepatic I/R, possibly through regulating the apoptosis [46]. In parallel, the depletion of endogenous antioxidants, particularly glutathione (GSH) and other antioxidant enzymes systems such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and alpha glutathione S-transferase (α-GST) have been associated with many liver complications including I/R [47]. In fact, the measurement of α-GST has long been considered as an early biomarker to predict hepatocellular injury [48]. The worst-case scenario is association of GST null genotype with the new onset of diabetes in liver transplant patients [49]. Fig. 2 gives an overview of mechanisms underlying oxidative stress and inflammation are implicated in the pathophysiology of the ischemic-reperfusion (reoxygenation) injury.

In the late phase of reperfusion, the ROS and pro-inflammatory mediators can activate sinusoidal endothelial cells (HSEC) and CD4 + T cells and trigger the recruitment of neutrophils [44]. Upon activation, HSEC release adhesion molecules such as intercellular

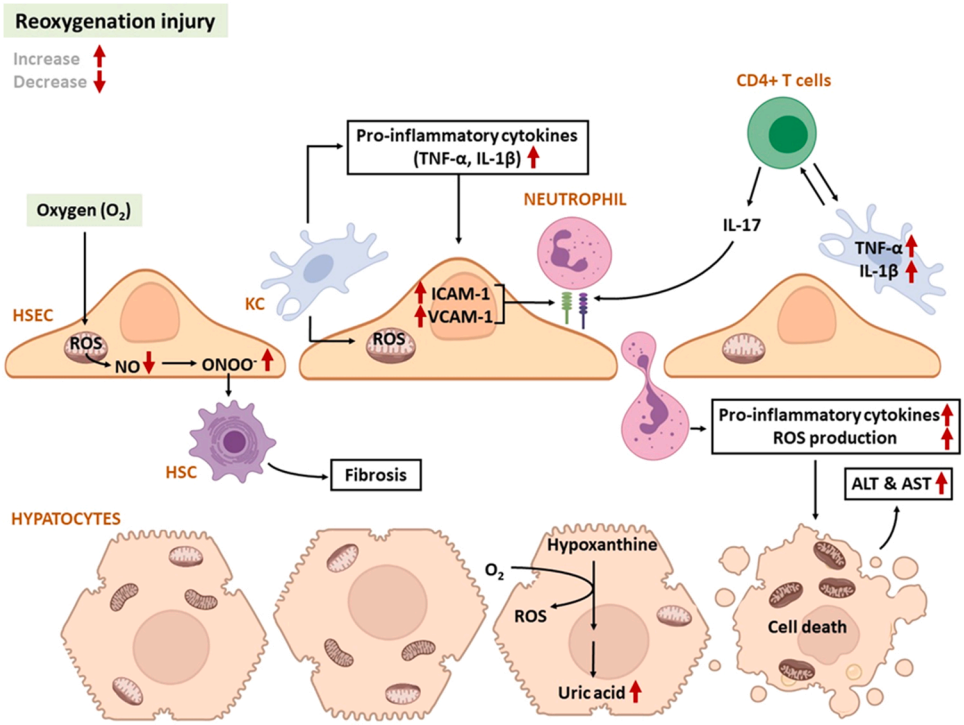

Fig. 2. The mechanisms underlying oxidative stress and inflammation are implicated in the pathophysiology of the ischemic-reperfusion (reoxygenation) injury. Briefly, abnormally enhanced production of superoxide radicals, pro-inflammatory factors and impaired nitric oxide levels, can lead to chain activation of other free radical molecules, which through activation of hypoxanthine-xanthine oxidase system, can facilitate increased generation of oxidative stress and lead to hepatocellular injury. Abbreviations: HSEC, hepatic sinusoidal endothelial cells; KC, Kupffer cells; HSC, hepatic stellate cells; $NO^{\cdot}$, nitric oxide; $ONOO^{-}$, peroxynitrite TNF-α, tumor necrosis factor alpha; IL, interleukin, ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecules; AL, alanine aminotransferase; AST, aspartate aminotransferase.

adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecules (VCAM-1) for infiltration of neutrophils [44]. On the other hand, ROS generated in sinusoidal endothelial cells can reduce the levels of nitric oxide, in the process prompt hepatic stellate cells contraction and the release of pro-inflammatory and profibrogenic cytokines such as IL6, IL-8, monocyte chemoattractant protein-1 and ICAM-1 that induce hepatic fibrosis and apoptosis of hepatocytes [44]. It has been strongly suggested that hepatic stellate cells play a significant functional role in exacerbating the degree of hepatic injury [50]. While there is a reciprocal interaction between KC and $CD4^{+}$ T cells during hepatic I/R [51], the CD4 + T cells are also important regulators of hepatic neutrophil recruitment during hepatic I/R via the release of IL-17 [52]. Altogether, these cascades of events may provoke the release of proteases and other cytotoxic enzymes that promote cellular degradation and death, which underlie the hepatic I/R.

5. An overview of the potential therapeutic mechanisms by which NAC protects against hepatic ischemia reperfusion injury

Various studies have reported on the beneficial effects of antioxidants against I/R [40,53]. NAC, being a strong antioxidant agent among its varied effects is highly associated with protecting against I/R injuries in different organs [26,54,55]. The major driving force of I/R liver injury has been its ameliorative effects against oxidative stress. Apparently, enhancing intracellular antioxidative systems remains a feasible strategy to alleviate cellular damage. In a nutshell, NAC has been reported to promote the reduction of disulfide bonds in proteins, consequently disrupting their ligand bonding and correcting their structures. Moreover, NAC exerts its antioxidant effects by directly scavenging and detoxifying oxidizing radicals and by serving as a precursor of cysteine for GSH synthesis. Our recent work highlighted the mechanisms by which NAC protects against oxidative stress drug induced liver injury [56], a potential mechanism by which NAC may exert its protective effects against I/R injury [54,57]. Briefly, the proposed mechanism is centered around increasing the expression of a potent antioxidant coenzyme Q_{10} [58], as well as activating the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, an essential regulator of cellular resistance to oxidants. Briefly, activation or enhanced expression of Nrf2 has been associated with increased expression of genes and enzymes that encode for cytoprotective defense antioxidants in response to oxidative stress exposure including SOD, GPx, GSH, heme oxigenase-1 (HO-1), and NADPH quinone oxidoreductase 1 (Nqo1) [56,59–61]. The importance of this mechanism is particularly evident in the effective use of NAC against overdose of acetaminophen (paracetamol) induced liver injury which causes an abrupt depletion of GSH levels in the liver [62].

6. Evidence on the use of NAC during liver transplantation and characteristic features of included RCTs

Briefly, a systematic search of literature through major electronic databases identified 41 eligible RCTs. Overall, 34 studies met the inclusion criteria, published in peer-reviewed journals between 2001 and 2020. Encompassed RCTs were predominantly from countries in Europe, and the United States, with some studies from Canada, China, India and Iran. All included studies were RCTs reporting on the clinical use of NAC during liver transplantation. The subsequent sections discuss evidence from RCTs, based on the different outcomes with the NAC use in settings of liver transplantation. In addition to implications of dose and treatment duration, other essential information such as the effect of NAC on oxidative stress and inflammation markers, including the overall impact on the transplant-free survival is discussed.

6.1. Evidence on the favorable outcomes with NAC use during liver transplantation

Table 1 summarizes evidence from RCTs supporting the beneficial effects of NAC during liver transplantation. Approximately, fourteen RCTs have been published thus far supporting the use of NAC in patients undergoing liver transplantation, and these studies have predominantly featured adult patients, but some have also encompassed children (Table 1). Briefly, the year 2001 marked an important period where two research groups, from Germany and the United States, reported on the usefulness of NAC during liver transplantation. During this time, Bucuvalas et al., 2001 [63] showed that 3-month patient survival rate was

100%, including reduced peak serum ALT levels and allograft rejection, in children undergoing liver transplantation. Weigand et al., 2001 [64] went further to confirm significantly reduced plasma levels of α-GST, a phase II metabolic isozyme, 24 hr after reperfusion in patients undergoing orthotopic liver transplantation. These patients further presented with lower concentrations of circulating ICAM-1 and VCAM-1. Importantly, these early clinical trials clearly highlighted an important role NAC plays in combating complications linked with oxidative stress and inflammation.

Indeed, Santiago et al., [13] also reported on markedly elevated levels of plasma IL-4 and IL-10 in liver transplant recipients. Consistently, Stravitz et al., 2013 [65] demonstrated that NAC could improve transplant-free survival by ameliorating the production of IL-17, which is associated with progression of hepatic encephalopathy and poor outcome, in patients with non-acetaminophen-induced acute liver failure. Whereas Li et al., [66] showed that the use of NAC was linked with a reduced incidence of postoperative pulmonary complications after orthotopic liver transplantation. Here, the levels of prominent markers of systematic inflammation such as TNF-α, IL-8, Clara cell secretory protein-16, and ICAM-1 were significantly lower, while a well-known antioxidant, SOD activity was higher with NAC treatment. These results are of interest since it has already been recorded that

Table 1
An overview of randomized clinical trials supporting the beneficial effects of n-acetyl cysteine (NAC) during liver transplantation.

Study	Country	NAC dosage and duration	Participants	Main findings
Treatment duration (≤ 24 h)				
Bucuvalas et al., 2001 [63]	United States	Received 70 mg/kg over 1 h following reperfusion and then every 12 h for 6 days	12 children undergoing liver transplantation, average age 3 years	The 3-month patient survival rate was 100%. Peak serum alanine aminotransferase (ALT) and allograft rejection was improved with the intervention compared to controls
Weigand et al., 2001 [64]	Germany	Received 150 mg/kg over 15 min shortly before reperfusion, followed by a continuous infusion of 50 mg/kg over the next 4 h. Then 100 mg/kg was administered in 16 h	10 patients undergoing orthotopic liver transplantation, age range 18–69 years	Significantly reduced alpha-glutathione S-transferase, as well as circulating intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) 24 hr after reperfusion but did not affect L- and P-selectin. Also did not affect serum aspartate transaminase (AST) or peak ALT levels
Santiago et al., 2008 [13]	Spain	Received 100 mg/kg over 15 min during the anhepatic phase, followed by a continuous infusion of 50 mg/kg over the next 24 h	25 liver transplant recipients, age of patients not disclosed	Significantly increased recipient interleukin (IL)− 4 plasma levels before and after reperfusion, and IL-10 plasma values before reperfusion
Lee et al., 2009 [68]	United States	Received 150 mg/kg/h of NAC over 1 h, followed by 12.5 mg/kg/h for 4 h	81 patients with non-acetaminophen-related acute liver failure, average age 18–71 years	Improved transplant-free survival in patients. However, patients with advanced coma grades did not benefit from NAC
Santiago et al., 2010 [75]	Spain	Received 100 mg/kg over 15 min during the anhepatic phase, followed by a continuous infusion of 50 mg/kg during the next 24 h	25 liver transplant recipients, age of patients not disclosed	Significantly decreased recipient pH values at 5 and 20 min after reperfusion, a decrease that was detected at 5 min before reperfusion
D'Amico et al., 2013 [71]	Italy	Received 30 mg/kg 1 h before the beginning of liver procurement and a locoregional infusion of 300 mg through the portal vein just before cross-clamping	69 adult candidates with chronic liver disease, average age 54 years	Improved the graft survival rates at 3 and 12 months. Also, the incidence of postoperative complications, including primary dysfunction of the liver was reduced with NAC treatment
Li et al., 2018 [66]	China	Received atomization inhalation of 3 ml NAC (10%) (Fluimucil) for 30 min before surgery and 3 h after reperfusion	30 patients undergoing orthotopic liver transplantation, average age 43 years	Significantly reduced the incidence of postoperative pulmonary complications after orthotopic liver transplantation. The levels of tumor necrosis factor-α, IL-8, Clara cell secretory protein-16, and ICAM-1 were significantly lower, while superoxide dismutase activity was higher with NAC treatment
Kakaei et al., 2020 [12]	Iran	Received 200 mg/kg/h in the first 8 h, followed by 100 mg/kg/h for another 16 h, the same dose for another 24 h	15 patients with obstructive jaundice, average age 64 years	Significantly preserved liver function after bypass surgery. This was validated by a decrease in mean serum levels of ALT, AST, Alkaline phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), and bilirubin
Treatment duration (> 24 h)				
Singh et al., 2013 [73]	United States	Received 150 mg/kg/h of NAC for 1 h, followed by 12.5 mg/kg/h for 4 h, then continuous infusions of 6.25 mg/kg NAC for the remaining 67 h	81 patients with non-acetaminophen induced acute liver failure, average age 42 years	Reduced risk of transplantation or death in early coma grade patients. This was reflected in improvement in parameters related to hepatocyte necrosis and bile excretion including ALT and bilirubin, but not in creatinine or AST
Squires et al., 2013 [74]	United States	Received 150 mg/kg/day infusions over 24 h, for up to 7 days	47 children with paediatric acute liver failure, average age 3 years	One-year liver transplantation-free survival was significantly lower with NAC, particularly among those < 2 years old
Stravitz et al., 2013 [65]	United States	Received intravenous NAC for 72 h, NAC dose not given	78 patients with non-acetaminophen-induced acute liver failure, average age 42 years	Improved transplant-free survival by ameliorating the production of IL-17, which is associated with progression of hepatic encephalopathy and poor outcome
Donadon et al., 2016 [70]	Italy	Received 150 mg/kg as a bolus and then as a continuous infusion of 50 mg/kg/h	16 patients undergoing hepatic resection, average age 63 years	There was a favourable outcome with NAC as designated by lower postoperative aberration in liver function tests (ALT/AST) compared with placebo
Nabi et al., 2017 [69]	India	Received 150 mg/kg over 1 h, followed by 12.5 mg/kg/h for 4 h and continuous infusion of 6.25 mg/kg/h for remaining 67 h	40 patients with non-acetaminophen-induced acute liver failure, average age 30 years	Reduced overall mortality by 28% versus 53% in the control group. NAC was associated with shorter length of hospital stay in survived patients. Moreover, drug-induced acute liver function showed improved outcome compared to other etiologies
Onk et al., 2018 [72]	Turkey	Received 900 mg/ for 7 days before the operation	35 patients with chronic obstructive pulmonary disease, average age 43 years	Improved renal and hepatic functions through regulation of ammonia and nitrogen metabolism and reduction of lactate

patients with chronic liver disease already display reduced levels of antioxidants SOD, GSH and catalase [67].

Furthermore, independently of assessing markers of oxidative stress and inflammation, others have also supported the beneficial effects of NAC in improved transplant-free survival in patients with non-acetaminophen-related acute liver failure [68,69], subjects undergoing hepatic resection [70], or those with chronic liver disease [71]. Recently, Onk et al., [72] and Kakaei et al., [12] reported on the impact of NAC in improving renal and hepatic functions through regulation of ammonia and nitrogen metabolism and reduction of lactate in patients with chronic obstructive pulmonary disease or with obstructive jaundice. Hence, suggesting that diverse therapeutic mechanisms may be at play in the protective effects of NAC against hepatic damage in a setting of liver disease. Another important aspect worth mentioning, Singh et al., [73] found that intravenous application of NAC in early coma grade patients with non-acetaminophen induced acute liver injury could reduce the risk of transplantation or death. This was reflected by improved parameters related to hepatocyte necrosis and bile excretion including ALT and bilirubin. Perhaps inferring that NAC may be more useful for patients with early coma grades. However, indicating some limitations on the beneficial effects of NAC, Squires and colleagues showed that one-year liver transplantation-free survival was significantly lower with NAC, particularly among those < 2 years old [74]. With authors indicating that the small sample size could have affected the statistical power to find statistical significance. Nonetheless, the summary of evidence from RCTs (Table 1) suggests that the beneficial effects of NAC extend beyond protecting against oxidative stress and inflammation. In fact, Santiago et al., 2010 [75] showed NAC decreased recipient pH values at 5 and 20 min after reperfusion, leading to improved outcome in transplant recipients. Further inferring that NAC may protect against plasma acidosis by decreasing pH values, an aspect that needs further evaluation in future research.

6.2. *Evidence on unfavorable outcomes with NAC use during liver transplantation*

Despite growing research advances in understand the therapeutic effects of NAC in protecting against diverse disease complications [76,77], certainty in available evidence is currently lacking. For example, while Table 1 presents evidence supporting the usefulness of NAC in protecting against hepatic damage in patients undergoing liver transplantation, other published RCTs have reported on the opposite outcomes with the use of this thiol containing antioxidant. Notably, from information presented in Table 2, NAC may have limited protective effects against ischemia reperfusion injury. As an example, Khan et al., [14] reported that NAC did not affect serum peak AST levels post-reperfusion in patients during donor operation. Aliakbarian et al., [78] did not see significant changes in hepatic artery reperfusion, hospital stay, vascular complications, inotrope requirement before and after portal declamping, and blood gas analysis in patients with orthotopic liver transplant. Alternatively, in those with veno-occlusive liver disease, Barkholt et al., [79] saw that NAC did not affect bilirubin levels nor the recovery of ALT and AST levels. Belgaumkar et al., [80] revealed that NAC did not reduce intraoperative liver injury, as there no differences in ALT/AST levels, white cell count, cytokines and cytokeratin-18 fragments in patients undergoing laparoscopic sleeve gastrectomy.

Even worse, Grendar et al., [81] observed there was a trend toward a higher rate of overall complications and a significantly higher rate of

Table 2
An overview of randomized clinical trials not supporting the beneficial effects of n-acetyl cysteine (NAC) during liver transplantation.

Study	Country	NAC dosage and duration	Participants	Main findings
Taut et al., 2001 [82]	Germany	Received 150 mg/kg of body weight during 15 min after reperfusion, 50 mg/kg during the following 4 h, and 100 mg/kg during the following 16 h	10 patients undergoing orthotopic liver transplantation, average age 54 years	Induced loss of amino acids and increased urea nitrogen release from the liver graft. Further increased net protein catabolism, hepatic urea, and glutamine production rate
Khan et al., 2005 [14]	United Kingdom	Received 150 mg/kg, started about 15 min before cardiac arrest	9 patients during donor operation, average age 48 years	Serum peak AST levels were similar and post-reperfusion biopsy showed moderate to severe reperfusion injury. Did not show a protective effect on ischemia reperfusion injury or on acute cellular rejection
Barkholt et al., 2008 [79]	Sweden	Received 100 mg/kg bodyweight was given as a continuous 6-h infusion	72 patients with elevated bilirubin, average age 38 years	NAC did not affect bilirubin levels nor the increased and recovery of ALT and AST levels There were two patients in the NAC group who developed veno-occlusive liver disease
Hilmi et al., 2010 [84]	United States	Received a loading dose of 140 mg/kg of intravenous over 1 h followed by 70 mg/kg IV repeated every 4 h for a total of 12 doses	50 patients undergoing cadaveric orthotopic liver transplantation for the first time, average age 59 years	Did not affect survival, graft function or risk of acute kidney injury. However, glutathione (GSH) levels were highly variable with only 50% of patients receiving NAC exhibiting increased levels and fewer patients developed acute kidney injury when GSH levels were increased
Bastin et al., 2016 [83]	United Kingdom	Received 240 mg/kg over 12 h preoperative infusion	23 patients undergoing lung resection, average age 68 years	Postoperative plasma thiol concentration was significantly higher in the NAC group but there was no significant difference in any of the measured biomarkers of inflammation or oxidative damage
Belgaumkar et al., 2016 [80]	United Kingdom	Received 150 mg/kg over 15 min at induction of anaesthesia followed by an infusion of 50 mg/kg during surgical retraction of the liver for a maximum of 4 h	20 patients undergoing laparoscopic sleeve gastrectomy, average age 48 years	NAC did not reduce intraoperative liver injury, as there no differences in ALT/AST levels, white cell count, cytokines and cytokeratin-18 fragments
Grendar et al., 2016 [81]	Canada	Received 150 mg/kg over 45 min upon arrival in the postanaesthesia recovery room. Then 50 mg/kg over 4 hr, followed by 100 mg/kg over the next 16 hr. Then 100 mg/kg per day over the next 3 days	96 patients scheduled to undergo liver resection, average age 59 years	Observed no benefit. Moreover, there was a trend toward a higher rate of overall complications and a significantly higher rate of delirium in the NAC group
Aliakbarian et al., 2016 [78]	Iran	2 g of NAC was added to UW solution as a preservative, and the liver was perfused through the superior mesenteric vein with 1 liter of solution containing 2 g of NAC	49 patients with orthotopic liver transplant, average age 63 years	Did not change the rate of ischemia reperfusion injury and short-term outcome in liver transplant recipients. This included no significant changes time to hepatic artery reperfusion, hospital stay, vascular complications, inotrope requirement before and after portal declamping, and blood gas analysis

delirium in the NAC group in patients scheduled to undergo liver resection. Apparently, there are fairly few factors that could contribute to the limited favourable outcomes seen with NAC, and these encompass the fact that some patients may present with other underlying conditions that might interfere with the therapeutic of this antioxidant. Beyond that, Taut et al., [82] showed that NAC may induce loss of amino acids and promote urea nitrogen release from the liver graft in patients undergoing orthotopic liver transplantation. Further indicating that research into NAC metabolism is crucial to undertake, especially in relation to its impact on net protein catabolism, hepatic urea, and glutamine production rate.

In relation to its impact on oxidative stress-related parameters, Bastin et al., [83] demonstrated that postoperative plasma thiol concentration was markedly higher in the NAC group (following 240 mg/kg over 12 h preoperative infusion) but there was no significant difference in any of the measured biomarkers of inflammation or oxidative damage in patients undergoing lung resection. Clearly, although these patients presented with increased thiols, which translates to increased GSH synthesis, but this effect could not compensate for increased levels of oxidative damage. Here, several factors could contribute to the limited protective effects of NAC, with restricted treatment/infusion time being the major culprit, as some other studies show the hepatoprotective potential could be reached with infusions lasting 67 h [69] or even 7 days [72]. Similarly, Hilmi et al., [84] reported that although plasma GSH levels were elevated after patients received NAC, but this did not affect survival, graft function or risk of acute kidney injury. In contrary, this study also showed that GSH levels were highly variable with only 50% of patients receiving NAC displaying augmented levels and fewer patients developed injury when GSH levels were high. From these results, its deducible that GSH is vital for protecting against cellular stress during liver transplantation. However, more efforts should be made that an appropriate dose of NAC is administered to promote GSH synthesis, also this has to adjusted with different patient setup.

7. Summary and future perspective

Based on the current state of knowledge, I/R appears to be the predominant feature driving the detrimental effects of oxidative stress and inflammation during liver transplantation. For instance, transplanting marginal liver grafts is a challenge because of their susceptible to an I/R, which also poses a threat to patient survival rate after the transplantation [85,86]. Hence, controlling I/R during liver transplantation can increase the donor pool and reduce the burden of organ shortage [87]. Ideally, identification of pathophysiological patterns and designing targeted therapies is necessary to curb liver injuries during the process of transplantation. Alternatively, improving transplant free-survival or liver transplantation can help to prevent or avoid liver transplantation. Recent data suggests that emerging therapies such as ursodeoxycholic acid and bezafibrate are tested for their effect on improving transplant free survival in primary biliary cholangitis, a likely cause of liver failure [88]. For example, Tanaka et al. [89] showed that there is need to enhance transplant free survival in patients with an incomplete response to ursodeoxycholic acid and bezafibrate display synergistic effect. Nonetheless, the effort has been made to develop a model and identified new drug targets to predict transplant-free survival in acute liver failure [90,91].

Research into therapeutic capabilities of NAC has certainly increased over the years, looking at its clinical use within different medical settings [76,92–94]. The strong antioxidant properties, mostly related to its ability to enhance endogenous GSH levels, make it an attractive molecule to protect against oxidative stress and cellular damage. In relation to the liver transplantation, groundbreaking work from Bucuvalas [63] and Weigand [64] was instrumental in reporting on the beneficial effects of using NAC during liver transplantation. In addition to reducing peak serum markers indicating liver damage such as ALT, patients receiving NAC also presented with lower levels of oxidative stress and inflammation, as demonstrated by effective modulation of pathological markers such as α-GST, circulating ICAM-1 and VCAM-1. These effects

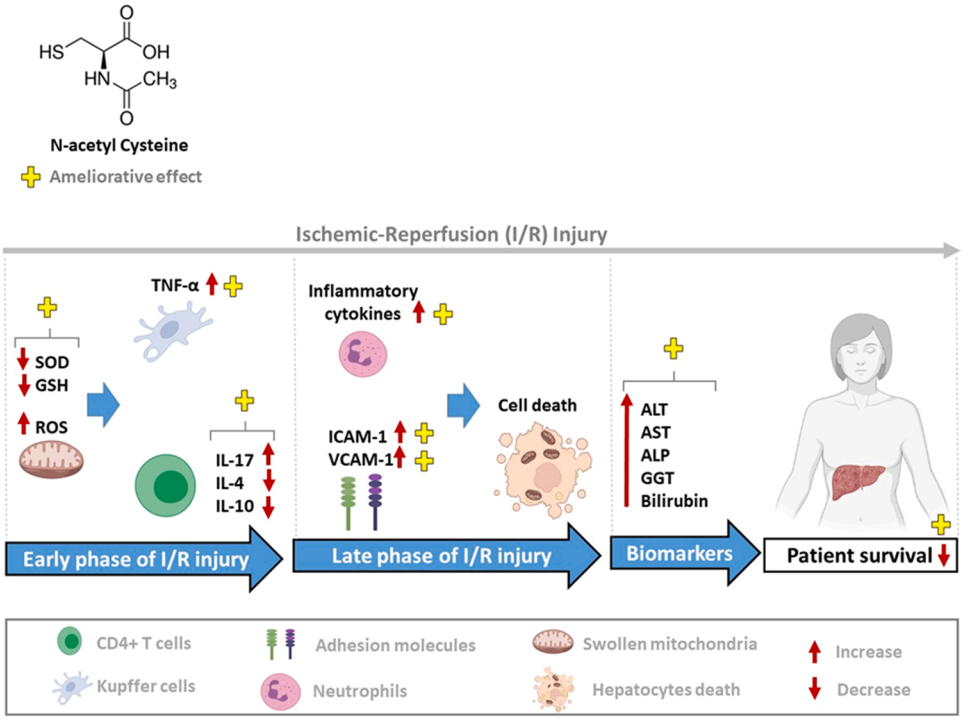

Fig. 3. A summary of potential mechanisms underlying the ameliorative effect of N-acetyl cysteine (NAC) on ischemic-reperfusion injury during liver transplantation. Briefly, the overwhelming clinical evidence summarized suggests NAC improves liver function and displays beneficial effects by reducing biomarkers of oxidative stress and inflammation, as demonstrated by effective modulation of pathological markers such as SOD, GSH, circulating ICAM-1 and VCAM-1, as well as others. Abbreviations: ALP, alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT, Gamma-glutamyl transferase; GSH, glutathione; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; ROS: reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion molecules.

were consistently demonstrated by average doses of 70 mg/kg in children and 150 mg/kg in adult patients, though through different treatment times. Table 1 summarizes supportive findings on the beneficial effects of NAC in patients with non-acetaminophen-related acute liver failure, through regulation of other therapeutic mechanisms such as improving ammonia and nitrogen metabolism, as well as decreasing the levels of lactate.

Although the beneficial effects of NAC during transplantation were noted, other RCTs reported on the contrary (Table 2), with limitations in protecting against liver injury or affecting the biomarkers of oxidative stress, inflammation, or even liver function enzymes. Notably, although circulating thiol or GSH levels were raised in response to some patients receiving NAC, but this did not affect survival, graft function or risk of acute kidney injury [84]. Further, indicating the need for additional RCTs investigating other therapeutic mechanisms related to the protective effects of NAC against liver injury during transplantation, this also includes understanding how patients with different underlying disease conditions could benefit from receiving NAC during liver transplantation.

improving ammonia and nitrogen metabolism, as well as decreasing the levels of lactate. (Fig. 3).

Ethics approval and consent to participate

This is a review of already published studies and thus it does not require ethical approval.

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CRediT authorship contribution statement

YN, KZ and PVD: concept and original draft. **YN, KZ and PVD**: literature search and data extraction. **YN, KZ, NC, BBN, TMN, SEM-M, KBG, PO, LT and PVD:** writing and final approval of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data related to search strategy, study selection, and extraction items will be made available upon request after the manuscript is published.

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Consent for publication

Not applicable. No individual person's data has been included in this manuscript.

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