Gastrointestinal Tract

REVIEW

Circulating biomarkers predictive of mortality in patients undergoing liver transplantation for hepatocellular carcinoma

Leonardo Lorente*

Intensive Care Unit, Hospital Universitario de Canarias, Ofra s/n, La Laguna, Santa Cruz de Tenerife 38320, Spain

ABSTRACT

There are different macromorphological factors obtained before liver transplantation (LT) to predict the prognosis of patients with hepatocellular carcinoma (HCC) undergoing LT. In addition, the use of blood biomarkers could help to improve the prognostic prediction of these patients. Blood levels of alpha-fetoprotein (AFP) are the first and most studied blood biomarkers of prognosis of HCC patients undergoing LT. Moreover, interesting data on new or already known circulating biomarkers predictive of mortality in patients with HCC undergoing LT have been published in recent years. The aim of this review is to summarize the evidence for circulating biomarkers predicting mortality of HCC patients undergoing LT. A higher mortality rate has been found with high blood levels of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immuninflammation index (SII), C-reactive protein (CRP), albumin bilirubin score (ALBI), de-gamma-carboxy prothrombin (DCP), lactate dehydrogenase (LDH), gamma glutamyl transpeptidase (GGT), fibrinogen, vascular endothelial growth factor (VEGF), homocysteine, Golgi protein 73 (GP73), substance P, soluble CD40 ligand (sCD40L), caspase cleaved cytokeratin (CCCK)-18, caspase-3, malondialdehyde, oxidized guanine species (OGS) and soluble Fas ligand (sFasL). A higher mortality rate has been observed with low blood levels of lymphocyte-to-monocyte ratio (LMR), PD-L1, Galectin-9, total antioxidant capacity (TAC) and melatonin. Furthermore, some studies with small sample sizes have been published associating blood levels of metabolites and microRNAs (MIR) with the prognosis of HCC patients undergoing LT. Therefore, further investigations are needed to determine the potential usefulness of these blood biomarkers in predicting mortality of HCC patients undergoing LT.

Key words: hepatocellular carcinoma, liver transplantation, biomarkers, outcome, survival

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and causes a large number of cancer-related deaths.[1–5] Liver transplantation (LT) is the treatment of choice in some patients with HCC, as liver failure is treated and the tumor is removed.[6–10]

There are different macromorphological factors obtained before HT to predict the prognosis of HCC patients undergoing HT, such as Milan criteria, tumor size, degree of differentiation, number of tumors, infiltration, hepatic microvascular invasion and hepatic macrovascular invasion[11–15]. In addition, the use of blood biomarkers could help to improve the prognostic prediction of these patients. Blood levels of alpha-fetoprotein (AFP) are the first and most studied prognostic blood biomarkers of HCC patients undergoing LT.[16,17] In addition, interesting data on new or already known circulating biomarkers predictive of

*Corresponding Author:
Leonardo Lorente, Intensive Care Unit, Hospital Universitario de Canarias, Ofra s/n, La Laguna, Santa Cruz de Tenerife 38320, Spain. E-mail: lorentemartin@msn.com.
https://orcid.org/0000-0003-4902-4065

Received: 14 March 2023; Revised: 21 April 2023; Accepted: 26 May 2023; Published: 3 August 2023
https://doi.org/10.54844/git.2023.369

mortality in HCC patients undergoing CT have been published in recent years. The aim of this review is to summarize the evidence for circulating biomarkers predicting mortality in HCC patients undergoing CT (see Table 1).

BIOMARKERS

Alpha-fetoprotein

Blood levels of alpha-fetoprotein (AFP), glycoprotein increase with different AFP-producing tumors, such as testicular or ovarian tumors or HCC. High blood levels of AFP have been associated with increased mortality[16] and recurrence of HCC[17] in patients undergoing LT for HCC.

In a review published by Hakeem *et al.* in 2012, 13 observational studies with 12,159 HCC patients undergoing LT for HCC were included to determine the role of circulating AFP levels obtained prior to LT in predicting survival and HCC recurrence.[16] A valid meta-analysis of all these studies was not possible due to the large heterogeneity in the way blood AFP levels were reported. Nine of the 13 studies reported serum AFP levels before LT and survival; however, 4 studies reported absolute serum AFP values, and 5 studies reported serum AFP cut-off levels (and the cut-off values reported in the different studies were very heterogeneous). Most studies found an increased risk of death with elevated pre-TL serum AFP levels and that serum AFP cut-off levels above 1000 ng/mL could predict an increased risk of death. Of the 13 studies that reported data on pre-LT serum AFP values and recurrence, 10 studies found an association between high AFP levels and increased HCC recurrence.

In 2016, a review and meta-analysis by Pommergaard *et al.* examined the potential role of different biomarkers in predicting HCC recurrence in patients undergoing LT.[17] The review included a total of 49 studies with 13,693 patients reporting data on serum AFP levels prior to LT and HCC recurrence. As in the previous review, it was not possible to perform a valid meta-analysis with all these studies due to the large heterogeneity in AFP definitions and cut-off values. However, a meta-analysis was performed that included 17 of 49 studies with AFP cut-off values above 400 ng/mL (although the cut-off values reported in the different studies were very heterogeneous) and found an association between elevated pre-LT serum AFP levels and increased risk of HCC recurrence [hazard ratio (HR): 2.69; 95% confidence interval (CI): 2.08 to 3.47].

Circulating blood cell counts and ratios

Different circulating blood cell count ratios, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), and systemic immunoinflammation index (SII), have been used as biomarkers to assess systemic inflammatory responses and have been studied to establish the prognosis of HCC patients undergoing LT.

In a meta-analysis published by Zheng *et al.* in 2017 involving patients with HCC, the possible associations between NLR and PLR before receiving different treatments (LT, transarterial chemoembolization, radiofrequency ablation or chemotherapy) and overall survival and HCC recurrence were studied.[18] The authors found an association between higher NLR and higher mortality rate with all treatments combined (HR: 1.54, 95% CI: 1.34 to 1.76, $P < 0.001$) in an analysis of 19 studies and 4,889 patients and an association between higher PLR and higher mortality rate with all treatments combined (HR: 1.63, 95% CI: 1.34 to 1.98, $P < 0.001$) in an analysis of 18 studies and 4,867 patients. The authors found an association between higher NLR and higher HCC recurrence rate with all combination therapies (HR: 1.45; 95% CI: 1.16 to 1.82; $P = 0.001$) in an analysis of 11 studies and 2,792 patients and an association between higher PLR and higher HCC recurrence rate with all combination therapies (HR: 1.52; 95% CI: 1.21 to 1.91; $P < 0.001$) in an analysis of 13 studies and 3,308 patients. In the specific analysis of 2 studies with 371 HCC patients undergoing LT, an association was found between a higher NLR and a higher mortality rate (HR: 3.12; 95% CI: 1.62 to 6.00). In the specific analysis of 2 studies with 524 HCC patients undergoing LT, an association was observed between higher NLR and higher mortality rate (HR: 2.28; 95% CI: 1.63 to 3.18). In the specific analysis of 3 studies with 714 patients with HCC undergoing CT, an association was observed between a higher PLR and a higher HCC recurrence rate (HR: 1.83; 95% CI: 1.31 to 2.56). However, in the specific analysis of patients with HCC undergoing LT, no association between LPL and HCC recurrence was observed.

A meta-analysis published in 2016 by Sun *et al.*[19] including 1,687 patients from 10 studies found an association between elevated pre-LT NLR and increased risk of mortality (HR: 2.71, 95% CI: 1.91 to 3.83) in patients with HCC undergoing LT. A meta-analysis published in 2018 by Xu *et al.*,[20] which included 1,936 patients from 13 studies, also found this association between elevated pre-LT NLR and increased mortality risk in patients with HCC undergoing LT (HR: 2.22; 95% CI: 1.34 to 3.68).

A low MRL was associated with mortality in 216 patients with HCC undergoing LT.[21] SII is calculated by multiplying the platelet count by the neutrophil count and dividing by the lymphocyte count. In a study of 189 patients[22] and in another study of 150 patients,[23]

Table 1: Circulating biomarkers predictive of mortality in patients undergoing liver transplantation (LT) for hepatocellular carcinoma

Blood biomarker	Level	Outcome	HCC treatment	Reference, year, study type and number of patients
Alpha-fetoprotein	High High	Mortality Recurrence	LT LT	[16] 2012, review, 12,519 patients [17] 2016, review, 13,693 patients
Neutrophil to lymphocyte ratio	High High High	Mortality Mortality Mortality	LT LT LT	[18] 2017, meta-analysis, 371 patients [19] 2016, meta-analysis, 1,687 patients [20] 2018, meta-analysis ,1,936 patients
Platelet to lymphocyte ratio	High	Mortality	LT	[18] 2017, meta-analysis, 524 patients
Lymphocyte to monocyte ratio	Low	Mortality	LT	[21] 2018, series, 216 patients
Systemic immune-inflammation index	High High	Mortality Mortality	LT LT	[22] 2020, series, 189 patients [23] 2018, series, 150 patients
C-reactive protein	High High High	Mortality Mortality Mortality	LT LT LT	[24] 2012, series, 85 patients [25] 2014, series, 96 patients [27] 2019, series, 119 patients
Albumin-bilirubin score	High High	Mortality Mortality	LT LT	[28] 2019, series, 123 patients [29] 2020, series, 81 patients
Des-gamma-carboxy prothrombin	High High	Mortality Mortality	LT LT	[34] 2016, series, 213 patients [35] 2020, series, 246 patients
Lactate dehydrogenase	High	Mortality	LT	[36] 2021, series, 155 patients
Gamma glutamyl transpeptidase	High High	Mortality Mortality	LT LT	[37] 2016, series, 130 patients [38] 2020, series, 285 patients
Fibrinogen	High High	Mortality Mortality	LT LT	[39] 2016, series, 41 patients [40] 2017, series, 130 patients
Vascular endothelial growth factor	High High High	Mortality Mortality Mortality	LT LT LT	[41] 2013, meta-analysis, 782 patients [42] 2015, series, 164 patients [43] 2020, series, 180 patients
Homocysteine	High	Mortality	LT	[44] 2020, series, 161 patients
Golgi protein 73	High	Mortality	LT	[45] 2017, series, 60 patients
PD-L1	Low	Mortality	LT or resection	[46] 2019, series, 81 patients
Galectin-9	Low	Mortality	LT or resection	[46] 2019, series, 81 patients
Metabolites:phosphatidylcholine (16:0/P-18:1), phosphatidylcholine (18:2/OH-16:0), nutriacholic acid	High	Recurrence	LT	[47] 2019, series, 122 patients
MicroRNA-92b	High	Recurrence	LT	[48] 2019, series, 93 patients
MicroRNA-718	Low	Recurrence	LT	[49] 2015, series, 59 patients
MicroRNA-1246	High	Mortality	LT	[50] 2016, series, 12 patients
MicroRNA-193a-5p	High	Mortality	LT or resection	[51] 2020, series, 41 patients
MicroRNA panel of several MIR	Positive	Mortality	LT	[53] 2021, series, 193 patients
Matrix metalloproteinase-9	High Low	LT rejection Mortality	LT LT or other treatments	[55] 2004, series, 33 patients [56] 2013, series, 134 patients
Substance P	High	Mortality	LT	[60] 2018, series, 142 patients
Soluble CD40 ligand	High	Mortality	LT	[70] 2018, series, 139 patients
Caspase-cleaved cytokeratin-18	High	Mortality	LT	[83] 2016, series, 135 patients
Caspase-3	High	Mortality	LT	[101] 2019, series, 145 patients
Malondialdehyde	High	Mortality	LT	[114] 2016, series, 127 patients
Total antioxidant capacity	Low	Mortality	LT	[129] 2018, series, 142 patients
Melatonin	Low	Mortality	LT	[132] 2019, series, 145 patients

Oxidized guanine species	High	Mortality	LT	[142] 2022, series, 114 patients
Soluble Fas ligand	High	Mortality	LT	[144] 2023, series, 127 patients

elevated SII was associated with mortality in patients with HCC undergoing LT; moreover, the area under the curve for predicting mortality was higher for SII than for PLR, NLR, and MLR.[23]

C-reactive protein

C-reactive protein (CRP) is synthesized by hepatocytes in response to inflammatory processes and subsequently released into the bloodstream. High blood CRP levels have been associated with an increased risk of HCC recurrence in patients undergoing CT.[24–26] In addition, high blood CRP levels have been associated with an increased risk of mortality in patients with HCC undergoing LT (and those studies included 85, 96, and 119 patients, respectively)[24,25,27].

Albumin-bilirubin bilirubin score

A study of 123 patients[28] and another study of 81 patients[29] showed an association between an high blood albumin-bilirubin bilirubin (ALBI) score and mortality in patients with HCC undergoing LT.

De-gamma-carboxy-prothrombin

De-gamma-carboxy-prothrombin (DCP) is a non-functional form of prothrombin produced by the liver. The normal liver converts the glutamic acid residues of the N-terminal portion of prothrombin by carboxylation to gamma-carboxyglutamic acid residues before their release into the peripheral blood. In many HCC cells, the vitamin K-dependent carboxylase that produces this carboxylation is absent; therefore, abnormal prothrombin is secreted.

High blood levels of DCP have been associated with an increased risk of HCC recurrence in patients with HCC undergoing LT.[30–33] In addition, high blood DCP levels were associated with mortality in patients with HCC undergoing LT in a study of 213 patients[34] and in another study of 246 patients.[35]

Lactate dehydrogenase

A study of 155 HCC patients undergoing LT[36] has found an association between high blood lactate dehydrogenase (LDH) and mortality.

Gamma glutamyl transpeptidase

A study of 130 patients[37] and another study of 285 patients[38] showed an association between high blood gamma glutamyl transpeptidase (GGT) levels and mortality in patients with HCC undergoing LT.

Fibrinogen

High blood fibrinogen levels were associated with mortality in patients with HCC undergoing LT in one study of 41 patients[39] and in another study of 130 patients[40].

Vascular endothelial growth factor

In a meta-analysis published in 2013 by Zhang *et al.*, which included 11 studies and 782 HCC patients undergoing LT for HCC, an association was found between high blood vascular endothelial growth factor (VEGF) levels obtained before LT and mortality risk (HR: 1.88; 95% CI: 1.46 to 2.3).[41] After the publication of this meta-analysis, two more studies were published, reporting that high blood VEGF levels obtained before LT in patients with HCC are associated with mortality risk (and these studies included 164 and 180 patients, respectively)[42,43].

Homocysteine

In a study of 161 patients with HCC undergoing LT, high blood homocysteine levels were associated with an increased risk of mortality.[44]

Golgi protein 73

Golgi protein 73 (GP73) is a type II Golgi membrane protein expressed primarily in biliary epithelial cells. GP73 expression is increased in various liver diseases, such as HCC. An association between high blood levels of GP73 and mortality was observed in 60 patients with HCC undergoing HT.[45]

PD-L1 and Galectin-9

PD-L1 and Galectin-9 are two ligands with modulatory effects on T-cell function. When PD-L1 ligand binds to its receptor PD-1 or when Galectin-9 ligand binds to its receptor TIM-3, a negative signal appears on T-cells inhibiting their activation. HCC cells express PD-L1, PD-1, Galectin-9 and TIM-3, and the interaction of these ligands with their receptor impairs the function of effector T-cells in HCC. The authors had previously observed that higher expression levels of PD-L1 and Galectin-9 in HCC are associated with poor survival. Blood levels of the soluble forms of PD-L1 and Galectin-9 have been found in different types of cancer. In a study of 81 patients undergoing resection of hepatocarcinoma or LT for HCC, low blood levels of PD-L1 or galectin-9 were associated with an increased risk of mortality.[46]

Metabolomics

In a study with 122 patients, high blood levels of three

metabolites (phosphatidylcholine 16:0/P-18:1, phosphatidylcholine 18:2/OH-16:0, and nutriacolic acid) obtained before LT in patients with HCC were associated with an increased rate of HCC recurrence.[47]

Cell-free circulating RNA or microRNA molecules

microRNA molecules (MIRs) released from cells by necrosis or apoptosis may appear in the blood. Some studies with small sample sizes have determined the blood levels of different MIRs, which have been associated with the prognosis of HCC patients undergoing LT. High blood levels of miR-92b have been found in 93 patients with HCC compared with control subjects and at 1 month after undergoing LT for HCC in patients with HCC recurrence.[48] Low blood levels of miR-718 obtained in 59 patients prior to LT for HCC have been associated with HCC recurrence.[49] High miR-1246 blood levels obtained in the early phase after LT for HCC (2 hours after portal vein reperfusion) in 12 patients have been associated with higher rates of HCC recurrence and mortality.[50] High blood levels of miR-193a-5p have been found in 41 patients with HCC compared to control subjects and before surgical treatment (tumor resection or LT) for HCC in patients with a higher mortality rate.[51] High blood levels of some MIRs (miR-130b-5p and miR-21-5p) were observed in 46 HCC patients and in patients with poor prognosis HCC; moreover, *in vivo*, tumors treated with miR-130b or miR-21 inhibitors showed significantly less growth.[52] A study of 193 patients with HCC undergoing LT showed that those with positive plama microRNA panel status (miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a and miR-801) prior to LT had a higher rate of tumor recurrence.[53]

Matrix metalloproteinase (MMP)-9

Matrix metalloproteinases (MMPs) are a group of endoproteinases involved in the degradation and remodeling of the extracellular matrix.[54] MMP activity is modulated by different tissue inhibitors of matrix metalloproteinases (TIMPs). MMPs are implicated in physiological functions such as morphogenesis and tissue remodeling and play roles in various diseases such as atherosclerosis, arthritis, tumors, sepsis, stroke and trauma.

In a study of 33 patients with HCC or cirrhosis without HCC undergoing LT, high serum levels of MMP-9 one week after LT were associated with an increased rate of LT rejection.[55] On the other hand, in another study of 134 HCC patients undergoing different treatments (LT, curative resection, thermoablation or transarterial chemoembolization), low serum levels of MMP-9 and high serum levels of TIMP-1 were found to be associated with a higher mortality rate; however, patients undergoing LT were not specifically examined due to the small sample size of this group of patients.[56] Therefore, further investigations are needed to determine the possible prognostic role of blood MMP-9 levels in HCC patients undergoing LT.

Substance P

Tachykinins are distributed throughout the respiratory system, central and peripheral nervous systems, intestine and urinary system. They are involved in various biological processes, such as transmission of nociceptive responses, inflammation, vasodilation, smooth muscle contraction, salivary secretion and airway contraction.[57] Substance P, a member of the tachykinin family, has been associated with diseases such as asthma, inflammatory bowel disease, and diseases of the central and peripheral nervous systems.

High blood levels of substance P have been found in patients with liver disease, especially in patients with severe liver disease.[58,59] In a study performed by our team with 142 patients with HCC undergoing LT, higher levels of substance P in blood before LT were observed in patients who died during the first year of LT than in survivors at one year.[60] These findings are consistent with those of other studies reporting an association between high blood levels of substance P and increased risk of mortality in patients with head injury,[61] ischemic stroke[62] or cerebral hemorrhage.[63]

The use of agents that reduce substance P activity in animal models of traumatic brain injury[64,65] or ischemic stroke[66,67] has reduced the inflammatory process and edema formation. Therefore, it would be interesting to explore the potential benefits of administering agents that reduce substance P activity in HCC patients undergoing LT.

Soluble CD40 ligand

CD40 ligand (CD40L) is a protein belonging to the tumor necrosis factor superfamily (TNFSF) group of ligands, and its receptor CD40 belongs to the tumor necrosis factor receptor superfamily (TNFRSF).[68] CD40L is mainly restored on platelets, but also on lymphocytes, monocytes, endothelial cells, smooth muscle cells and microglia. CD40L is released into the blood in soluble form (sCD40L). CD40L exhibits proinflammatory and prothrombotic effects when bound to CD40.

An association was found between high blood levels of sCD40L and poor prognosis in patients with HCC.[69] In a study by our team of 139 patients with HCC undergoing LT, higher levels of sCD40L in blood before LT were observed in patients who died during the first year of LT than in survivors at 1 year.[70] These results

are consistent with those of other studies reporting an association between elevated sCD40L blood levels and an increased risk of mortality in patients with acute coronary syndrome,[71] sepsis,[72] traumatic brain injury[73] or cerebral infarction.[74]

A reduction in CD40 expression was observed when cerebral vascular endothelial cells were preincubated with statins.[75] In addition, the use of statins in rats with traumatic brain injury decreased cerebral edema and intravascular thrombosis and impaired cognitive and motor functions.[76] In addition, a reduction in sCD40L blood levels has been observed in patients treated with statins.[77–79] A meta-analysis of patients with ischemic stroke found a lower risk of death and less neurological deterioration in patients treated with statins.[80] Therefore, it would be interesting to explore the potential benefits of administering agents that reduce sCD40L levels in patients with HCC undergoing LT.

Caspase-cleaved cytokeratin-18

Cytokeratin-18 is the major intermediate filament protein in the liver, epithelial cells and parenchymal cells. Cytokeratin-18 is cleaved by caspases during hepatocyte apoptosis and released into the blood.[81]

An association was found between high blood levels of caspase-cleaved cytokeratin (CCCK)-18 and mortality in patients with HCC.[82] In a study performed by our team with 135 HCC patients undergoing LT, higher levels of CCCK-18 in blood before LT were observed in patients who died during the first year of LT than in survivors at 1 year.[83] These findings are consistent with those of other studies reporting an association between high blood CCCK-18 levels and poor prognosis in patients with various tumor diseases,[84–88] sepsis,[89] cerebral infarction,[90] cerebral hemorrhage[91] or traumatic brain injury.[92]

The use of different agents decreased apoptosis and improved prognosis in animal models of sepsis,[93] traumatic brain injury,[94] cerebral infarction[95] and cerebral hemorrhage.[96] Therefore, it would be interesting to explore the potential benefits of administering agents that reduce apoptosis in HCC patients undergoing LT.

Caspase-3

Caspase-3 is the main caspase executioner that produces cellular changes during apoptosis[97]. There are two main pathways (extrinsic and intrinsic) for cell death by apoptosis. The extrinsic apoptotic pathway is activated when a TNFRSF member binds to its ligand (TNFSF), generating a death signal responsible for the activation of caspase-8. The intrinsic apoptotic pathway is activated when oxygen free radicals, proinflammatory cytokines or genetic mutations cause the release of cytochrome c from the mitochondria into the cytosol, generating an apoptosome responsible for caspase-9 activation. Subsequently, both apoptotic pathways, due to caspase-8 and caspase-9 activation, lead to caspase-3 activation, resulting in cell death.

A higher degree of hepatic apoptosis and hepatic caspase-3 activity has been found in patients with different liver diseases (chronic alcoholic hepatitis or chronic hepatitis C virus infection).[98–100] In a study performed by our team with 145 patients with HCC undergoing LT, higher levels of caspase-3 in blood before LT were found in patients who died during the first year of LT than in survivors at 1 year.[101] These findings are in line with those of other studies reporting an association between high blood caspase-3 levels and an increased risk of mortality in patients with sepsis,[102] cerebral infarction,[103] traumatic brain injury[104] or cerebral hemorrhage.[105]

The use of different agents decreased apoptosis and improved prognosis in animal models of sepsis,[106] traumatic brain injury,[107] cerebral infarction[108] and cerebral hemorrhage.[109] Therefore, it might be interesting to explore the potential benefits of administering caspase-3-lowering agents in HCC patients undergoing LT.

Malondialdehyde

Malondialdehyde is a low molecular weight aldehyde and is one of the end products that appear during lipid peroxidation of cell membrane phospholipids by free radical action. Malondialdehyde is released into the extracellular space and can reach the bloodstream.[110]

High blood levels of malondialdehyde have been reported in patients with HCC compared to control subjects.[111–113] In a study performed by our team with 127 patients with HCC undergoing LT, higher levels of malondialdehyde in blood before LT were observed in patients who died during the first year of LT than in survivors at 1 year.[114] These findings are in line with those of other studies reporting an association between high blood malondialdehyde levels and increased risk of mortality in patients with sepsis,[115] traumatic brain injury,[116] cerebral infarcts[117] or spontaneous intracerebral hemorrhage.[118]

Melatonin administration (with antioxidant effects) has been associated with improved liver function in animals with different liver diseases[119–123] and in patients with HCC.[124–127] Therefore, it would be interesting to explore the possible benefits of the administration of antioxidant agents to reduce oxidative damage in HCC patients undergoing LT.

Total antioxidant capacity

Total antioxidant capacity (TAC) provides information on the overall antioxidant status that protects us from free radicals.[128] Higher blood levels of malondialdehyde have been reported in patients with HCC than in control subjects.[111–113] In a study performed by our team with 142 patients with HCC undergoing LT, lower pre-LT blood TAC levels were observed in patients who died during the first year of LT than in survivors at 1 year.[129] Furthermore, we found a negative association between serum TAC levels and malondialdehyde; thus, non-surviving patients showed lower antioxidant status and higher lipid peroxidation. As mentioned above, melatonin administration (with antioxidant effects) has been associated with improved liver function in animals with different liver diseases[119–123] and in patients with HCC.[124–127] Therefore, it would be interesting to explore the possible benefits of administration of antioxidant agents to increase antioxidant status in HCC patients undergoing HT.

Melatonin

Melatonin is released mainly by the pineal gland with a circadian rhythm (high production during the night and low production during the day) and by other organs (bone marrow, gastrointestinal tract, lymphocytes and thymus) without a circadian rhythm.[130] Melatonin, in addition to playing an important role in sleep regulation, also has anti-inflammatory, anti-apoptotic and antioxidant effects.[131]

In a study performed by our team with 145 HCC patients undergoing LT, lower pre-LT blood melatonin levels were observed in patients who died during the first year of LT than in survivors at 1 year.[132] Furthermore, we found a positive association between serum melatonin levels and TAC and a negative association between serum melatonin levels and malondialdehyde; thus, non-surviving patients showed lower melatonin levels, lower antioxidant status and higher lipid peroxidation.

As mentioned above, melatonin administration in animals subjected to hepatic ischemia/reperfusion and injection of a HCC-inducing agent or injection of hepatoma cells was associated with less impairment of liver function, less oxidative damage, and a higher survival rate.[119–123] Furthermore, melatonin administration in patients undergoing hepatectomy or in patients with unresectable HCC was associated with less deterioration of liver function.[124–127] Therefore, it would be interesting to explore the potential benefits of melatonin administration in HCC patients undergoing HT.

Oxidized guanine species

Higher blood levels of oxidative damage products of nucleic acids have been found in patients with cardiovascular disease, heart failure and periodontal disease than in healthy subjects,[133–135] and in patients who died after sepsis,[136] traumatic brain injury,[137] cerebral infarction[138] or spontaneous intracerebral hemorrhage.[139]

Reactive oxygen species can damage deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Guanine is one of the nucleobases present in DNA and RNA. The three oxidized guanine species (OGS) species are 8-hydroxy-2'-deoxyguanosine (8-OHdG) from DNA, 8-hydroxyguanosine (8-OHGua) from DNA or RNA, and 8-hydroxyguanosine (8-OHG) from RNA.

Greater oxidative DNA damage (assessed by 8-OHdG concentration in liver biopsy samples) has been found in patients with chronic liver disease with HCC than without.[140,141] In a study performed by our team with 114 patients with HCC undergoing LT, a higher blood OGS concentration prior to LT was found in patients who died during the first year of LT than in survivors at 1 year.[142] These findings are in line with those of other studies reporting an association between high blood GOS levels and an increased risk of mortality in patients with sepsis,[136] traumatic brain injury,[137] cerebral infarction[138] or spontaneous intracerebral hemorrhage.[139] Therefore, it would be interesting to explore the potential benefits of administering antioxidant agents to reduce oxidative damage to nucleic acids in HCC patients undergoing LT.

Soluble Fas ligand

Soluble Fas ligand (sFasL) is one of the main ligands that activate apoptosis by extrinsic pathways[97]; upon binding of FasL to Fas (its receptor), a death signal appears that will activate caspase 8. Subsequently, caspase 8 will activate caspase 3, causing cell death. Subsequently, caspase 8 will activate caspase 3, causing cell death.

A higher lymphocyte expression of FasL[143] has been found in patients with acute LT rejection than in patients without rejection. In a study by our team of 127 patients with HCC undergoing LT, higher pre-LT blood levels of sFasL were observed in patients who died during the first year of LT than in survivors at 1 year.[144] These findings are in line with those of other studies reporting an association between high blood sFasL levels and increased risk of mortality in patients with sepsis,[145] head injury[146] or cerebral hemorrhage.[147]

The administration of different agents that reduce Fas/FasL activity in animal models of ischemia-reperfusion liver injury has reduced hepatocyte apoptosis and increased animal survival.[148–150] Therefore, it might be interesting to explore the potential benefits of administering agents that reduce the activity of the Fas/FasL

system in HCC patients undergoing LT.

CONCLUSION

A higher mortality rate has been observed in HCC patients undergoing LT with high blood levels of AFP, NLR, PLR, SII, CRP, ALBI score, DCP, LDH, GGT, fibrinogen, VEGF, homocysteine, GP73, substance P, sCD40L, CCCK-18, caspase-3, malondialdehyde, OGS, and sFasL. A higher mortality rate has been observed in HCC patients undergoing LT with low blood levels of MRL, PD-L1, galectin-9, TAC and melatonin. In addition, some studies with small sample sizes have been published associating blood levels of metabolites and MIR and prognosis of HCC patients undergoing LT.

DECLARATIONS

Conflicts of interest

The author has no financial or other conflicts of interest to declare related to the submitted manuscript.

Data sharing statement

No additional data is available.

REFERENCES

1. Santopaolo F, Lenci I, Milana M, Manzia TM, Baiocchi L. Liver transplantation for hepatocellular carcinoma: Where do we stand? *World J Gastroenterol*. 2019; 25:2591–2602.
2. Mullath A, Krishna M. Hepatocellular carcinoma - time to take the ticket. *World J Gastrointest Surg*. 2019; 11:287–295.
3. Lorente L. New prognostic biomarkers of mortality in patients undergoing liver transplantation for hepatocellular carcinoma. *World J Gastroenterol*. 2018; 24:4230–4242.
4. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, *et al*. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology*. 2018; 68:723–750.
5. Castanedo S, Toledo E, Fernández-Santiago R, Castillo F, Echeverri J, Rodríguez-Sanjuán JC. Influence of postoperative complications on long-term survival in liver transplant patients. *World J Gastrointest Surg*. 2020; 12:336–345.
6. Xu X, Chen J, Wei Q, Liu ZK, Yang Z, Zhang M, *et al*. Clinical practice guidelines on liver transplantation for hepatocellular carcinoma in China (2018 edition). *Hepatobiliary Pancreat Dis Int*. 2019; 18:307–312.
7. Alqahtani SA, Sanai FM, Alolayan A, Abaalkhail F, Alsuhaibani H, Hassanain M, *et al*. Saudi Association for the Study of Liver diseases and Transplantation practice guidelines on the diagnosis and management of hepatocellular carcinoma. *Saudi J Gastroenterol*. 2020; 26(Supplement):S1–S40.
8. Mehta N, Bhangui P, Yao FY, Mazzaferro V, Toso C, Akamatsu N, *et al*. Liver Transplantation for Hepatocellular Carcinoma. Working Group Report from the ILTS Transplant Oncology Consensus Conference. *Transplantation*. 2020; 104:1136–1142.
9. Kumar A, Acharya SK, Singh SP, Arora A, Dhiman RK, Aggarwal R, *et al*. 2019 Update of Indian National Association for Study of the Liver Consensus on Prevention, Diagnosis, and Management of Hepatocellular Carcinoma in India: The Puri II Recommendations. *J Clin Exp Hepatol*. 2020; 10:43–80.
10. Vogel A, Cervantes A, Chau I, Daniele B, Llovet JM, Meyer T, *et al*. Hepatocellular carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2018; 29(Suppl 4):iv238–iv255.
11. Citores MJ, Lucena JL, de la Fuente S, Cuervas-Mons V. Serum biomarkers and risk of hepatocellular carcinoma recurrence after liver transplantation. *World J Hepatol*. 2019; 11:50–64.
12. Filgueira NA. Hepatocellular carcinoma recurrence after liver transplantation: Risk factors, screening and clinical presentation. *World J Hepatol*. 2019; 11:261–272.
13. Ma KW, She WH, Chan ACY, Cheung TT, Fung JYY, Dai WC, *et al*. Validated model for prediction of recurrent hepatocellular carcinoma after liver transplantation in Asian population. *World J Gastrointest Oncol*. 2019; 11:322–334.
14. Yaprak O, Acar S, Ertugrul G, Dayangac M. Role of pre-transplant 18F-FDG PET/CT in predicting hepatocellular carcinoma recurrence after liver transplantation. *World J Gastrointest Oncol*. 2018; 10:336–343.
15. Hoffman D, Mehta N. Recurrence of hepatocellular carcinoma following liver transplantation. *Expert Rev Gastroenterol Hepatol*. 2020:1–12.
16. Hakeem AR, Young RS, Marangoni G, Lodge JP, Prasad KR. Systematic review: the prognostic role of alpha-fetoprotein following liver transplantation for hepatocellular carcinoma. *Aliment Pharmacol Ther*. 2012; 35:987–999.
17. Pommergaard HC, Burcharth J, Rosenberg J, Rasmussen A. Serologic and molecular biomarkers for recurrence of hepatocellular carcinoma after liver transplantation: A systematic review and meta-analysis. *Transplant Rev (Orlando)*. 2016; 30:171–177.
18. Zheng J, Cai J, Li H, Zeng K, He L, Fu H, *et al*. Neutrophil to Lymphocyte Ratio and Platelet to Lymphocyte Ratio as Prognostic Predictors for Hepatocellular Carcinoma Patients with Various Treatments: a Meta-Analysis and Systematic Review. *Cell Physiol Biochem*. 2017; 44:967–981.
19. Sun XD, Shi XJ, Chen YG, Wang CL, Ma Q, Lv GY. Elevated Preoperative Neutrophil-Lymphocyte Ratio Is Associated with Poor Prognosis in Hepatocellular Carcinoma Patients Treated with Liver Transplantation: A Meta-Analysis. *Gastroenterol Res Pract*. 2016; 2016:4743808.
20. Xu ZG, Ye CJ, Liu LX, Wu G, Zhao ZX, Wang YZ, *et al*. The pretransplant neutrophil-lymphocyte ratio as a new prognostic predictor after liver transplantation for hepatocellular cancer: a systematic review and meta-analysis. *Biomark Med*. 2018; 12:189–199.
21. Mano Y, Yoshizumi T, Yugawa K, Ohira M, Motomura T, Toshima T, *et al*. Lymphocyte-to-Monocyte Ratio Is a Predictor of Survival After Liver Transplantation for Hepatocellular Carcinoma. *Liver Transpl*. 2018; 24:1603–1611.
22. Ren A, Li Z, Zhang X, Deng R, Ma Y. Inflammation-Based Prognostic Scores in Patients with Hepatitis B Virus-Related Hepatocellular Carcinoma After Liver Transplantation. *J Hepatocell Carcinoma*. 2020; 7:101–106.
23. Fu H, Zheng J, Cai J, Zeng K, Yao J, Chen L, *et al*. Systemic Immune-Inflammation Index (SII) is Useful to Predict Survival Outcomes in Patients After Liver Transplantation for Hepatocellular Carcinoma within Hangzhou Criteria. *Cell Physiol Biochem*. 2018; 47:293–301.
24. An HJ, Jang JW, Bae SH, Choi JY, Yoon SK, Lee MA, *et al*. Serum C-reactive protein is a useful biomarker for predicting outcomes after liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl*. 2012; 18:1406–1414.
25. Mori S, Choi Y, Park MS, Kim H, Hong G, Yi NJ, *et al*. Usefulness of preoperative C-reactive protein and alpha-fetoprotein levels for prognostication of patients with hepatocellular carcinoma after living donor liver transplantation. *Hepatogastroenterology*. 2014; 61:2353–2358.
26. Meischl T, Rasoul-Rockenschaub S, Györi G, Sieghart W, Reiberger T, Trauner M, *et al*. C-reactive protein is an independent predictor for hepatocellular carcinoma recurrence after liver transplantation. *PLoS*

One. 2019; 14:e0216677.

27. Kornberg A, Schernhammer M, Kornberg J, Friess H, Thrum K. Serological Risk Index Based on Alpha-Fetoprotein and C-Reactive Protein to Indicate Futile Liver Transplantation Among Patients with Advanced Hepatocellular Carcinoma. *Dig Dis Sci*. 2019; 64:269–280.
28. Kornberg A, Witt U, Schernhammer M, Kornberg J, Müller K, Friess H, *et al*. The role of preoperative albumin-bilirubin grade for oncological risk stratification in liver transplant patients with hepatocellular carcinoma. *J Surg Oncol*. 2019; 120:1126–1136.
29. Tai K, Kuramitsu K, Kido M, Tanaka M, Komatsu S, Awazu M. Impact of Albumin-Bilirubin Score on Short- and Long-Term Survival After Living-Donor Liver Transplantation: A Retrospective Study. *Transplant Proc*. 2020; 52:910–919.
30. Fujiki M, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, *et al*. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. *Am J Transplant*. 2009; 9:2362–2371.
31. Taketomi A, Sanefuji K, Soejima Y, Yoshizumi T, Uhciyama H, Ikegami T, *et al*. Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. *Transplantation*. 2009; 87:531–537.
32. Lee JH, Cho Y, Kim HY, Cho EJ, Lee DH, Yu SJ, *et al*. Serum tumor markers provide refined prognostication in selecting liver transplantation candidate for hepatocellular carcinoma patients beyond the milan criteria. *Ann Surg*. 2016; 263:842–850.
33. Xu Y, Wang PX, Cheng JW, Huang XW, Zhou J, Fan J, *et al*. Study on the value of serum des-γ-carboxy prothrombin in predicting hepatocellular carcinoma recurrence after liver transplantation. *Zhonghua Gan Zang Bing Za Zhi*. 2020; 28:918–923.
34. Harimoto N, Yoshizumi T, Shimagaki T, Nagatsu A, Motomura T, Harada N, *et al*. Inflammation-based Prognostic Score in Patients with Living Donor Liver Transplantation for Hepatocellular Carcinoma. *Anticancer Res*. 2016; 36:5537–5542.
35. Yonemura Y, Yoshizumi T, Inokuchi S, Kosai-Fujimoto Y, Harada N, Itoh S, *et al*. Predictor of outcome after living donor liver transplantation for patients with hepatocellular carcinoma beyond the Japan criteria. Ann Gastroenterol Surg 2020; 4: 413-421.
36. Zhang Q, Jiao X. LDH and GGT/ALT ratio as novel prognostic biomarkers in hepatocellular carcinoma patients after liver transplantation. *Comput Math Methods Med*. 2021; 2021:9809990.
37. Fu SJ, Zhao Q, Ji F, Chen MG, Wu LW, Ren QQ, *et al*. Elevated preoperative serum gamma-glutamyltranspeptidase predicts poor prognosis for hepatocellular carcinoma after liver transplantation. *Sci Rep*. 2016; 6:28835.
38. Ince V, Carr BI, Bag HG, Koc C, Usta S, Ersan V, *et al*. Gamma glutamyl transpeptidase as a prognostic biomarker in hepatocellular cancer patients especially with > 5 cm tumors, treated by liver transplantation. *Int J Biol Markers*. 2020; 35:91–95.
39. Wang GY, Jiang N, Yi HM, Wang GS, Zhang JW, Li H, *et al*. Pretransplant elevated plasma fibrinogen level is a novel prognostic predictor for hepatocellular carcinoma recurrence and patient survival following liver transplantation. *Ann Transplant*. 2016; 21:125–130.
40. Fu SJ, Ji F, Han M, Chen MG, Wang XP, Ju WQ, *et al*. Prognostic value of combined preoperative fibrinogen and neutrophil-lymphocyte ratio in patients with hepatocellular carcinoma after liver transplantation. *Oncotarget*. 2017; 8:4301–4312.
41. Zhan P, Qian Q, Yu LK. Serum VEGF level is associated with the outcome of patients with hepatocellular carcinoma: a meta-analysis. *Hepatobiliary Surg Nutr*. 2013; 2:209–215.
42. Zhang W, Kim R, Quintini C, Hashimoto K, Fujiki M, Diago T, *et al*. Prognostic role of plasma vascular endothelial growth factor in patients with hepatocellular carcinoma undergoing liver transplantation. *Liver Transpl*. 2015; 21:101–111.
43. Duda DG, Dima SO, Cucu D, Sorop A, Klein S, Ancukiewicz M, *et al*. Potential Circulating Biomarkers of Recurrence after Hepatic Resection or Liver Transplantation in Hepatocellular Carcinoma Patients. *Cancers (Basel)*. 2020; 12:1275.
44. Yang M, Tan W, Yang X, Zhuo J, Lin Z, Cen B, *et al*. Homocysteine: A novel prognostic biomarker in liver transplantation for alpha-fetoprotein- negative hepatocellular carcinoma. *Cancer Biomark*. 2020; 29:197–206.
45. Wang Y, Zhang Z, Zou D, Hao Q, Ma X, Wang C, *et al*. Serum Golgi protein 73 is a prognostic biomarker of liver transplantation patients. *Int J Clin Exp Pathol*. 2017; 10:8626–8632.
46. Sideras K, de Man RA, Harrington SM, Polak WG, Zhou G, Schutz HM, *et al*. Circulating levels of PD-L1 and Galectin-9 are associated with patient survival in surgically treated Hepatocellular Carcinoma independent of their intra-tumoral expression levels. *Sci Rep*. 2019; 9:10677.
47. Lu D, Yang F, Lin Z, Zhuo J, Liu P, Cen B, *et al*. A prognostic fingerprint in liver transplantation for hepatocellular carcinoma based on plasma metabolomics profiling. *Eur J Surg Oncol*. 2019; 45:2347–2352.
48. Nakano T, Chen IH, Wang CC, Chen PJ, Tseng HP, Huang KT, *et al*. Circulating exosomal miR-92b: Its role for cancer immunoediting and clinical value for prediction of posttransplant hepatocellular carcinoma recurrence. *Am J Transplant*. 2019; 19:3250–3262.
49. Sugimachi K, Matsumura T, Hirata H, Uchi R, Ueda M, Ueo H, *et al*. Identification of a bona fide microRNA biomarker in serum exosomes that predicts hepatocellular carcinoma recurrence after liver transplantation. *Br J Cancer*. 2015; 112:532–538.
50. Ng KT, Lo CM, Wong N, Li CX, Qi X, Liu XB, *et al*. Early-phase circulating miRNAs predict tumor recurrence and survival of hepatocellular carcinoma patients after liver transplantation. *Oncotarget*. 2016; 7:19824–19839.
51. Loosen SH, Wirtz TH, Roy S, Vucur M, Castoldi M, Schneider AT, *et al*. Circulating levels of microRNA193a-5p predict outcome in early stage hepatocellular carcinoma. *PLoS One*. 2020; 15:e0239386.
52. Zhang N, Hu Z, Qiang Y, Zhu X. Circulating miR-130b- and miR-21-based diagnostic markers and therapeutic targets for hepatocellular carcinoma. *Mol Genet Genomic Med*. 2019; 7:e1012.
53. Huang A, Guo DZ, Wang YP, Yang GH, Sun QM, Zhang X, *et al*. Plasma microRNA panel predicts early tumor recurrence in patients with hepatocellular carcinoma after liver transplantation. *J Cancer*. 2021; 12:7190–7200.
54. Brinckerhoff CE, Matrisian LM. Matrix metalloproteinases: a tail of a frog that became a prince. *Nat Rev Mol Cell Biol*. 2002; 3:207–214.
55. Kuyvenhoven JP, Verspaget HW, Gao Q, Ringers J, Smit VT, Lamers CB, *et al*. Assessment of serum matrix metalloproteinases MMP-2 and MMP-9 after human liver transplantation: increased serum MMP-9 level in acute rejection. *Transplantation*. 2004; 77:1646–1652.
56. Lempinen M, Lyytinen I, Nordin A, Tervahartiala T, Mäkisalo H, Sorsa T, *et al*. Prognostic value of serum MMP-8, -9 and TIMP-1 in patients with hepatocellular carcinoma. *Ann Med*. 2013; 45:482–487.
57. Almeida TA, Rojo J, Nieto PM, Pinto FM, Hernandez M, Martín JD, *et al*. Tachykinins and tachykinin receptors: structure and activity relationships. *Curr Med Chem*. 2004; 11:2045–2081.
58. Lee FY, Lin HC, Tsai YT, Chang FY, Lu RH, Hou MC, *et al*. Plasma substance P levels in patients with liver cirrhosis: relationship to systemic and portal hemodynamics. *Am J Gastroenterol*. 1997; 92:2080–2084.
59. Hörtnagl H, Singer EA, Lenz K, Kleinberger G, Lochs H. Substance P is markedly increased in plasma of patients with hepatic coma. *Lancet*. 1984; 1:480–483.
60. Lorente L, Rodriguez ST, Sanz P, Pérez-Cejas A, Padilla J, Díaz D, *et al*. Patients with high serum substance P levels previously to liver transplantation for hepatocellular carcinoma have higher risk of one-year liver transplantation mortality. *Oncotarget*. 2018; 9:21552–9.
61. Lorente L, Martín MM, Pérez-Cejas A, González-Rivero AF, Argueso M, Ramos L, *et al*. Persistently High Serum Substance P Levels and

Early Mortality in Patients with Severe Traumatic Brain Injury. *World Neurosurg*. 2019; 132:e613–e617.

62. Lorente L, Martín MM, Pérez-Cejas A, González-Rivero AF, Argueso M, Ramos L, *et al*. High serum substance P levels and mortality after malignant middle cerebral artery infarction. *J Crit Care*. 2020; 57:1–4.
63. Lorente L, Martín MM, González-Rivero AF, Pérez-Cejas A, Sabatel R, Ramos-Gómez L, *et al*. Serum substance P levels and early mortality of spontaneous intracerebral haemorrhage patients. *J Stroke Cerebrovasc Dis*. 2020; 29:104893.
64. Ameliorate JL, Ghabriel MN, Vink R. Magnesium enhances the beneficial effects of NK1 antagonist administration on blood-brain barrier permeability and motor outcome after traumatic brain injury. *Magnes Res*. 2017; 30:88–97.
65. Li Q, Wu X, Yang Y, Zhang Y, He F, Xu X, *et al*. Tachykinin NK1 receptor antagonist L-733,060 and substance P deletion exert neuroprotection through inhibiting oxidative stress and cell death after traumatic brain injury in mice. *Int J Biochem Cell Biol*. 2019; 107:154–165.
66. Turner RJ, Vink R. NK1 tachykinin receptor treatment is superior to capsaicin pre-treatment in improving functional outcome following acute ischemic stroke. *Neuropeptides*. 2014; 48:267–272.
67. Kaur J, Sharma S, Sandhu M, Sharma S. Neurokinin-1 receptor inhibition reverses ischaemic brain injury and dementia in bilateral common carotid artery occluded rats: possible mechanisms. *Inflammopharmacology*. 2016; 24:133–143.
68. Zhang B, Wu T, Chen M, Zhou Y, Yi D, Guo R. The CD40/CD40L system: A new therapeutic target for disease. *Immunol Lett*. 2013; 153:58–61.
69. Eltaher SM, El-Gil R, Fouad N, Mitwali R, El-Kholy H. Evaluation of serum levels and significance of soluble CD40 ligand in screening patients with hepatitis C virus-related hepatocellular carcinoma. *East Mediterr Health J*. 2016; 22:603–610.
70. Lorente L, Rodriguez ST, Sanz P, Pérez-Cejas A, Padilla J, Díaz D, *et al*. High serum soluble CD40L levels previously to liver transplantation in patients with hepatocellular carcinoma are associated with mortality at one year. *J Crit Care*. 2018; 43:316–320.
71. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ, Kaski JC. Soluble CD40 ligand:interleukin-10 ratio predicts in-hospital adverse events in patients with ST-segment elevation myocardial infarction. *Thromb Res*. 2007; 121:293–299 [PMID: 17521712 DOI: 10.1016/j.thromres.2007.04.007].
72. Lorente L, Martín MM, Pérez-Cejas A, Ferreres J, Solé-Violán J, Labarta L, *et al*. Non-survivor septic patients have persistently higher serum sCD40L levels than survivors. *J Crit Care*. 2017; 41:177–182.
73. Lorente L, Martín MM, González-Rivero AF, Ramos L, Argueso M, Cáceres JJ, *et al*. Nonsurviving patients with severe traumatic brain injury have maintained high serum sCD40L levels. *World Neurosurg*. 2019; 126:e1537–e1541.
74. Lorente L, Martín MM, González-Rivero AF, Sabatel R, Ramos L, Argueso M, *et al*. High Serum sCD40L Levels During the First Week of Malignant Middle Cerebral Artery Infarction and Mortality. *World Neurosurg*. 2019; 132:e630–e636.
75. Lin R, Liu J, Peng N, Gan W, Wang W, Han C, *et al*. Lovastatin reduces apoptosis and downregulates the CD40 expression induced by TNF-alpha in cerebral vascular endothelial cells. *Curr Neurovasc Res*. 2006; 3:41–47.
76. Wible EF, Laskowitz DT. Statins in traumatic brain injury. *Neurotherapeutics*. 2010; 7:62–73.
77. Li J, Zhao SP, Peng DQ, Xu ZM, Zhou HN. Early effect of pravastatin on serum soluble CD40L, matrix metalloproteinase-9, and C-reactive protein in patients with acute myocardial infarction. *Clin Chem*. 2004; 50:1696–1699.
78. Hamdan R, Hajj F, Kadry Z, Kassab R, Salame E, Aboujaoude S, *et al*. Benefit and tolerability of the coadministration of ezetimibe and atorvastatin in acute coronary syndrome patients. *J Med Liban*. 2011; 59:65–69.
79. Han SH, Koh KK, Quon MJ, Lee Y, Shin EK. The effects of simvastatin, losartan, and combined therapy on soluble CD40 ligand in hypercholesterolemic, hypertensive patients. *Atherosclerosis*. 2007; 190:205–211.
80. Ní Chróinín D, Asplund K, Åsberg S, Callaly E, Cuadrado-Godia E, Díez-Tejedor E, *et al*. Statin therapy and outcome after ischemic stroke: systematic review and meta-analysis of observational studies and randomized trials. *Stroke*. 2013; 44:448–456.
81. Luedde T, Kaplowitz N, Schwabe RF. Cell death and cell death responses in liver disease: Mechanisms and clinical relevance. *Gastroenterology*. 2014; 147:765–783.e4.
82. Morris KL, Tugwood JD, Khoja L, Lancashire M, Sloane R, Burt D, *et al*. Circulating biomarkers in hepatocellular carcinoma. *Cancer Chemother Pharmacol*. 2014; 74:323–332.
83. Lorente L, Rodriguez ST, Sanz P, Pérez-Cejas A, Padilla J, Díaz D, *et al*. Prognostic value of serum caspase-cleaved cytokeratin-18 levels before liver transplantationfor one-year survival of patients with hepatocellular carcinoma. *Int J Mol Sci*. 2016; 17:pii: E1524.
84. Ulukaya E, Yilmaztepe A, Akgoz S, Linder S, Karadag M. The levels of caspase-cleaved cytokeratin 18 are elevated in serum from patients with lung cancer and helpful to predict the survival. *Lung Cancer*. 2007; 56:399–404.
85. Yaman E, Coskun U, Sancak B, Buyukberber S, Ozturk B, Benekli M. Serum M30 levels are associated with survival in advanced gastric carcinoma patients. *Int Immunopharmacol*. 2010; 10:719–722.
86. de Haas EC, di Pietro A, Simpson KL, Meijer C, Suurmeijer AJ, Lancashire LJ, *et al*. Clinical evaluation of M30 and M65 ELISA cell death assays as circulating biomarkers in a drug-sensitive tumor, testicular cancer. *Neoplasia*. 2008; 10:1041–1048.
87. Koelink PJ, Lamers CB, Hommes DW, Verspaget HW. Circulating cell death products predict clinical outcome of colorectal cancer patients. *BMC Cancer*. 2009; 9:88.
88. Wu YX, Wang JH, Wang H, Yang XY. Study on expression of Ki-67, early apoptotic protein M30 in endometrial carcinoma and their correlation with prognosis. *Zhonghua Bing Li Xue Za Zhi*. 2003; 32:314–318.
89. Lorente L, Martín MM, Pérez-Cejas A, López RO, Ferreres J, Solé-Violán J, *et al*. Higher serum caspase-cleaved cytokeratin-18 levels during the first week of sepsis diagnosis in non-survivor patients. *Clin Chem Lab Med*. 2017; 55:1621–1629.
90. Lorente L, Martín MM, Pérez-Cejas A, González-Rivero AF, Sabatel R, Ramos L, *et al*. Non-survivor ischemic stroke patients maintain high serum caspase-cleaved cytokeratin-18 levels. *Brain Sci*. 2020; 10:132.
91. Lorente L, Martín MM, Pérez-Cejas A, Ramos L, Argueso M, Solé-Violán J, *et al*. Association between serum levels of caspase-cleaved cytokeratin-18 and early mortality in patients with severe spontaneous intracerebral hemorrhage. *BMC Neurosci*. 2018; 19:23.
92. Lorente L, Martín MM, González-Rivero AF, Pérez-Cejas A, Argueso M, Ramos L, *et al*. High serum caspase-cleaved cytokeratin-18 levels and mortality of traumatic brain injury patients. *Brain Sci*. 2019; 9:269.
93. Chung CS, Song GY, Lomas J, Simms HH, Chaudry IH, Ayala A. Inhibition of Fas/Fas ligand signaling improves septic survival: differential effects on macrophage apoptotic and functional capacity. *J Leukoc Biol*. 2003; 74:344–351.
94. Xue Z, Song Z, Wan Y, Wang K, Mo L, Wang Y. Calcium-sensing receptor antagonist NPS2390 attenuates neuronal apoptosis though intrinsic pathway following traumatic brain injury in rats. *Biochem Biophys Res Commun*. 2017; 486:589–594.
95. Cho YS, Shin MS, Ko IG, Kim SE, Kim CJ, Sung YH, *et al*. Ulinastatin inhibits cerebral ischemia-induced apoptosis in the hippocampus of gerbils. *Mol Med Rep*. 2015; 12:1796–1802.
96. Liu X, Yuan D, Nie X, Shen J, Yan Y, Zhang D, *et al*. BTEB2 prevents neuronal apoptosis via promoting bad phosphorylation in rat intracerebral hemorrhage model. *J Mol Neurosci*. 2015; 55:206–216.

97. Fischer U, Schulze-Osthoff K. Apoptosis-based therapies and drug targets. *Cell Death Differ.* 2005; 12 Suppl 1:942–961.
98. Valva P, De Matteo E, Galoppo MC, Gismondi MI, Preciado MV. Apoptosis markers related to pathogenesis of pediatric chronic hepatitis C virus infection: M30 mirrors the severity of steatosis. *J Med Virol.* 2010; 82:949–957.
99. Bantel H, Lügering A, Poremba C, Lügering N, Held J, Domschke W, *et al.* Caspase activation correlates with the degree of inflammatory liver injury in chronic hepatitis C virus infection. *Hepatology.* 2001; 34:758–767.
100. Natori S, Rust C, Stadheim LM, Srinivasan A, Burgart LJ, Gores GJ. Hepatocyte apoptosis is a pathologic feature of human alcoholic hepatitis. *J Hepatol.* 2001; 34:248–253.
101. Lorente L, Rodriguez ST, Sanz P, González-Rivero AF, Pérez-Cejas A, Padilla J, *et al.* High serum caspase-3 levels in hepatocellular carcinoma prior to liver transplantation and high mortality risk during the first year after liver transplantation. *Expert Rev Mol Diagn.* 2019; 19:635–640.
102. Lorente L, Martín MM, Pérez-Cejas A, González-Rivero AF, López RO, Ferreres J, *et al.* Sustained high serum caspase-3 concentrations and mortality in septic patients. *Eur J Clin Microbiol Infect Dis.* 2018; 37:281–288.
103. Lorente L, Martín MM, Pérez-Cejas A, González-Rivero AF, Sabatel R, Ramos L, *et al.* Serum Caspase-3 Levels and Early Mortality of Patients with Malignant Middle Cerebral Artery Infarction. *Neurocrit Care.* 2019; 31:486–493.
104. Lorente L, Martín MM, Pérez-Cejas A, González-Rivero AF, Argueso M, Ramos L, *et al.* Serum caspase-3 levels during the first week of traumatic brain injury. *Med Intensiva.* 2019:S0210–5691(19)30205.
105. Lorente L, Martín MM, Pérez-Cejas A, González-Rivero AF, Sabatel R, Ramos L, *et al.* High serum levels of caspase-3 and early mortality in patients with severe spontaneous intracerebral hemorrhage. *Neurocrit Care.* 2020.
106. Hotchkiss RS, Chang KC, Swanson PE, Tinsley KW, Hui JJ, Klender P, *et al.* Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. *Nat Immunol.* 2000; 1:496–501.
107. Abrahamson EE, Ikonomovic MD, Ciallella JR, Hope CE, Paljug WR, *et al.* Caspase inhibition therapy abolishes brain trauma-induced increases in Abeta peptide: implications for clinical outcome. *Exp Neurol.* 2006; 197:437–450.
108. Chen B, Wang G, Li W, Liu W, Lin R, Tao J, *et al.* Memantine attenuates cell apoptosis by suppressing the calpain-caspase-3 pathway in an experimental model of ischemic stroke. *Exp Cell Res.* 2017; 351:163–172.
109. Rodrigues CM, Sola S, Nan Z, Castro RE, Ribeiro PS, Low WC, *et al.* Tauroursodeoxycholic acid reduces apoptosis and protects against neurological injury after acute hemorrhagic stroke in rats. *Proc Natl Acad Sci USA.* 2003; 100:6087–6092.
110. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem.* 2006; 52:601–623.
111. Yahya RS, Ghanem OH, Foyouh AA, Atwa M, Enany SA. Role of interleukin-8 and oxidative stress in patients with hepatocellular carcinoma. *Clin Lab.* 2013; 59:969–976.
112. Zhao J, Zhao Y, Wang H, Gu X, Ji J, Gao C. Association between metabolic abnormalities and HBV related hepatocelluar carcinoma in Chinese: A cross-sectional study. *Nutr J.* 2011; 10:49.
113. Tsai SM, Lin SK, Lee KT, Hsiao JK, Huang JC, Wu SH, *et al.* Evaluation of redox statuses in patients with hepatitis B virus-associated hepatocellular carcinoma. *Ann Clin Biochem.* 2009; 46:394–400.
114. Lorente L, Rodriguez ST, Sanz P, Abreu-González P, Díaz D, Moreno AM, *et al.* Association between Pre-Transplant Serum Malondialdehyde Levels and Survival One Year after Liver Transplantation for Hepatocellular Carcinoma. *Int J Mol Sci.* 2016; 17:500.
115. Lorente L, Martín MM, Abreu-González P, Domínguez-Rodriguez A, Labarta L, Díaz C, *et al.* Sustained high serum malondialdehyde levels are associated with severity and mortality in septic patients. *Crit Care.* 2013; 17:R290.
116. Lorente L, Martín MM, Abreu-González P, Ramos L, Cáceres JJ, Argueso M, *et al.* Maintained high sustained serum malondialdehyde levels after severe brain trauma injury in non-survivor patients. *BMC Res Notes.* 2019; 12:789.
117. Lorente L, Martín MM, Abreu-González P, Sabatel R, Ramos L, Argueso M, *et al.* Non-survivor patients with malignant middle cerebral artery infarction showed persistently high serum malondialdehyde levels. *BMC Neurol.* 2019; 19:238.
118. Lorente L, Martín MM, Abreu-González P, Sabatel R, Ramos L, Argueso M, *et al.* Serum malondialdehyde levels and mortality in patients with spontaneous intracerebral hemorrhage. *World Neurosurg.* 2018; 113:e542–e547.
119. Kireev R, Bitoun S, Cuesta S, Tejerina A, Ibarrola C, Moreno E, *et al.* Melatonin treatment protects liver of Zucker rats after ischemia/reperfusion by diminishing oxidative stress and apoptosis. *Eur J Pharmacol.* 2013; 701:185–193.
120. Li JY, Yin HZ, Gu X, Zhou Y, Zhang WH, Qin YM. Melatonin protects liver from intestine ischemia reperfusion injury in rats. *World J Gastroenterol.* 2008; 14:7392–7396.
121. Zaoualí MA, Reiter RJ, Padrissa-Altés S, Boncompagni E, García JJ, Ben Abnennebi H, *et al.* Melatonin protects steatotic and nonsteatotic liver grafts against cold ischemia and reperfusion injury. *J Pineal Res.* 2011; 50:213–221.
122. Filatova NA, Kirpichnikova KM, Aksenov ND, Vakhromova EA, Gamaleĭ IA. Decrease in tumorigenic activity of murine hepatoma cells after treatment with antioxidants and melatonin. *Tsitologiia.* 2011; 53:404–410.
123. Dakshayani KB, Subramanian P, Manivasagam T, Essa MM, Manoharan S. Melatonin modulates the oxidant-antioxidant imbalance during N-nitrosodiethylamine induced hepatocarcinogenesis in rats. *J Pharm Pharm Sci.* 2005; 8:316–321.
124. Solanas E, Sostres C, Serrablo A, García-Gil A, García JJ, Aranguren FJ, *et al.* Effect of Dimethyl Sulfoxide and Melatonin on the Isolation of Human Primary Hepatocytes. *Cells Tissues Organs.* 2015; 200:316–325.
125. Nickkholgh A, Schneider H, Sobirey M, Venetz WP, Hinz U, Pelzl LH, *et al.* The use of high-dose melatonin in liver resection is safe: first clinical experience. *J Pineal Res.* 2011; 50:381–388.
126. Yan JJ, Shen F, Wang K, Wu MC. Patients with advanced primary hepatocellular carcinoma treated by melatonin and transcatheter arterial chemoembolization: a prospective study. *Hepatobiliary Pancreat Dis Int.* 2002; 1:183–186.
127. Aldeghi R, Lissoni P, Barni S, Ardizzoia A, Tancini G, Piperno A, *et al.* Low-dose interleukin-2 subcutaneous immunotherapy in association with the pineal hormone melatonin as a first-line therapy in locally advanced or metastatic hepatocellular carcinoma. *Eur J Cancer.* 1994; 30:167–170.
128. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radic Biol Med.* 2000; 29:1106–1114.
129. Lorente L, Rodriguez ST, Sanz P, Pérez-Cejas A, Abreu-González P, Padilla J, *et al.* Serum total antioxidant capacity prior to liver transplantation for hepatocellular carcinoma is associated with 1-year liver transplantation survival. *J Int Med Res.* 2018; 46:2641–2649.
130. Mortezaee K. Human hepatocellular carcinoma: Protection by melatonin. *J Cell Physiol.* 2018; 233:6486–6508.
131. Mortezaee K, Khanlarkhani N. Melatonin application in targeting oxidative-induced liver injuries: A review. *J Cell Physiol.* 2018; 233:4015–4032.
132. Lorente L, Rodriguez ST, Sanz P, Abreu-González P, González-

Rivero AF, Pérez-Cejas A, *et al*. Low Serum Melatonin Levels Prior to Liver Transplantation in Patients with Hepatocellular Carcinoma are Associated with Lower Survival after Liver Transplantation. *Int J Mol Sci*. 2019; 20:1696.

133. Di Minno A, Turnu L, Porro B, Squellerio I, Cavalca V, Tremoli E, *et al*. 8-Hydroxy-2-Deoxyguanosine Levels and Cardiovascular Disease: A Systematic Review and Meta-Analysis of the Literature. *Antioxid Redox Signa*. l 2016; 24: 548-555.
134. Di Minno A, Turnu L, Porro B, Squellerio I, Cavalca V, Tremoli E, *et al*. 8-Hydroxy-2-deoxyguanosine levels and heart failure: A systematic review and meta-analysis of the literature. *Nutr Metab Cardiovasc Dis*. 2017; 27:201–208.
135. Paredes-Sánchez E, Montiel-Company JM, Iranzo-Cortés JE, Almerich-Torres T, Bellot-Arcís C, Almerich-Silla JM. Meta-Analysis of the Use of 8-OHdG in Saliva as a Marker of Periodontal Disease. *Dis Markers*. 2018; 2018:7916578.
136. Lorente L, Martín MM, González-Rivero AF, Pérez-Cejas A, Abreu-González P, Ortiz-López R, *et al*. Association between DNA and RNA oxidative damage and mortality in septic patients. *J Crit Care*. 2019; 54:94–98.
137. Lorente L, Martín MM, González-Rivero AF, Pérez-Cejas A, Abreu-González P, Ramos L, *et al*. Association Between DNA and RNA Oxidative Damage and Mortality of Patients with Traumatic Brain Injury. *Neurocrit Care*. 2020; 32:790–795.
138. Lorente L, Martín MM, González-Rivero AF, Pérez-Cejas A, Abreu-González P, Ramos L, *et al*. DNA and RNA oxidative damage are associated to mortality in patients with cerebral infarction. *Med Intensiva*. 2021; 45:35–41.
139. Lorente L, Martín MM, González-Rivero AF, Pérez-Cejas A, Abreu-González P, Sabatel R, *et al*. High Serum DNA and RNA Oxidative Damage in Non-surviving Patients with Spontaneous Intracerebral Hemorrhage. *Neurocrit Care*. 2020; 33:90–96.
140. Schwarz KB, Kew M, Klein A, Abrams RA, Sitzmann J, Jones L, *et al*. Increased hepatic oxidative DNA damage in patients with hepatocellular carcinoma. *Dig Dis Sci*. 2001; 46:2173–2178.
141. Tanaka H, Fujita N, Sugimoto R, Urawa N, Horiike S, Kobayashi Y, *et al*. Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. *Br J Cancer*. 2008; 98:580–586.
142. Lorente L, Rodriguez ST, Sanz P, González-Rivero AF, Pérez-Cejas A, Padilla J, *et al*. DNA and RNA oxidative damage in hepatocellular carcinoma patients and mortality during the first year of liver transplantation. *World J Hepatol*. 2022; 14:1182–1189.
143. Yu LX, Han SL, Miao Y. Detection of CD95 and CD95L mRNA expression after liver transplantation using SYBR real-time PCR during acute rejection. *Nanfang Yike Daxue Xuebao*. 2006; 26:185–188.
144. Lorente L, Rodriguez ST, Sanz P, González-Rivero AF, Pérez-Cejas A, Padilla J, *et al*. Patients with hepatocellular carcinoma that die during the first year of liver transplantation have high blood sFasL concentrations. *World J Clin Cases*. 2023; 11:1753–1760.
145. Lorente L, Martín MM, Ortiz-López R, González-Rivero AF, Pérez-Cejas A, Cabrera J, *et al*. Association between serum sFasL concentrations and sepsis mortality. *Infect Dis (Lond)*. 2021; 53:38–43.
146. Lorente L, Martín MM, Pérez-Cejas A, González-Rivero AF, Ramos-Gómez L, Solé-Violán J, *et al*. High Serum Soluble Fas Ligand Levels in Non-survivor Traumatic Brain Injury Patients. *Neurocrit Care*. 2021; 35:249–254.
147. Lorente L, Martín MM, Pérez-Cejas A, González-Rivero AF, Ramos-Gómez L, Solé-Violán J, *et al*. Mortality prediction of patients with spontaneous intracerebral hemorrhage by serum soluble Fas ligand concentrations. *Expert Rev Mol Diagn*. 2022; 22:233–238.
148. Al-Saeedi M, Steinebrunner N, Kudsi H, Halama N, Mogler C, Büchler MW, *et al*. Neutralization of CD95 ligand protects the liver against ischemia-reperfusion injury and prevents acute liver failure. *Cell Death Dis*. 2018; 9:132.
149. Nakajima H, Mizuta N, Fujiwara I, Sakaguchi K, Ogata H, Magae J, *et al*. Blockade of the Fas/Fas ligand interaction suppresses hepatocyte apoptosis in ischemia-reperfusion rat liver. *Apoptosis*. 2008; 13:1013–1021.
150. Wang J, Li W, Min J, Ou Q, Chen J. Fas siRNA reduces apoptotic cell death of allogeneic-transplanted hepatocytes in mouse spleen. *Transplant Proc*. 2003; 35:1594–1595.