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Overview of oxidative stress findings in hepatic encephalopathy: From cellular and ammonium-based animal models to human data

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ABSTRACT

Oxidative stress is a natural phenomenon in the body. Under physiological conditions intracellular reactive oxygen species (ROS) are normal components of signal transduction cascades, and their levels are maintained by a complex antioxidants systems participating in the *in-vivo* redox homeostasis. Increased oxidative stress is present in several chronic diseases and interferes with phagocytic and nervous cell functions, causing an up-regulation of cytokines and inflammation. Hepatic encephalopathy (HE) occurs in both acute liver failure (ALF) and chronic liver disease. Increased blood and brain ammonium has been considered as an important factor in pathogenesis of HE and has been associated with inflammation, neurotoxicity, and oxidative stress. The relationship between ROS and the pathophysiology of HE is still poorly understood. Therefore, sensing ROS production for a better understanding of the relationship between oxidative stress and functional outcome in HE pathophysiology is critical for determining the disease mechanisms, as well as to improve the management of patients.

This review is emphasizing the important role of oxidative stress in HE development and documents the changes occurring as a consequence of oxidative stress augmentation based on cellular and ammonium-based animal models to human data.

1. Introduction

Oxidative stress (OS) is believed to play a role in the pathogenesis of hepatic encephalopathy (HE). Under normal physiological conditions reactive oxygen and nitrogen species (ROS and RNS) (Table 1) are balanced by antioxidants scavenging systems, while a permanent increase in ROS and RNS levels results in OS being harmful on cell- and tissue-homeostasis [1–3]. These antioxidants are low molecular weight reductants such as glutathione (GSH) and ascorbate (Asc) which are accompanied by protein antioxidants such as superoxide dismutase (SOD) that reacts with superoxide anion (O_2^{-}), catalase (CAT) and the peroxiredoxins (glutathione peroxidase (GPX)) that catabolize hydrogen peroxide (H_2O_2) [4–6]. There is growing evidence that the modulation of antioxidant levels in cells is inextricably linked to intracellular OS levels [2,6].

ROS are small messenger molecules that are normal components of signal transduction cascades during physiological processes, but when in excess are also involved in neurotoxicity and neurodegeneration [7–9]. The redox homeostasis is maintained by the balance between ROS generation and elimination by antioxidants (Fig. 1).

Ammonium (NH_4^+) is produced by metabolism of amino acid obtained from dietary proteins [10]. Liver plays an important role in ammonium detoxification and in oxidative stress regulation. Therefore, when liver fails, blood ammonium levels increase and its impaired clearance by the diseased liver leads to brain glutamine (Gln) accumulation, causing disturbance of central nervous system (CNS) functions [11–13]. In parallel, a significant decrease in two major liver antioxidant enzymes activity, CAT and SOD, was detected in cirrhotic liver of HE patients [14].

Albumin, which is synthesized by liver hepatocytes and rapidly excreted into the bloodstream, is a very abundant and important circulating antioxidant, accounting for more than 70% of serum's free radical-trapping activity [15,16]. Albumin has been found to be decreased in cirrhotic patients and has been associated with the development of overt HE as well as risk factors associated with death [17–21]. Epidemiological studies have found that decreased albumin level together with irreversibly oxidized albumin (HNA2) are an independent

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Abbreviations

ALF	acute liver failure
Asc	ascorbate
BDL	bile-duct ligation
C HE	type C hepatic encephalopathy
CLD	chronic liver disease
CNS	central nervous system
EPR	electron paramagnetic resonance spectroscopy
ETC	electron transport chain
Gln	glutamine
GPX-1	glutathione peroxidase 1
GR	glutathione reductase
GSH	glutathione
$HO^{\cdot}$	hydroxyl radical
IHC	immunohistochemistry
IMS	mitochondrial intermembrane space
LTD	long-term depression
LTP	long-term potentiation
NAFLD	non-alcoholic fatty liver diseases
NALD	non-alcoholic liver diseases
NBT	Nitroblue Tetrazolium
$ONOO^-$	peroxynitrite
OS	oxidative stress
$O_2^{\cdot -}$	superoxide anion
Oxo-8-dG	8-Oxo-2′-deoxyguanosine
PCA	portocaval anastomosis
PMN	polymorphonuclear neutrophils
ROS/RNS	reactive oxygen and nitrogen species
SOD	superoxide dismutase
WBC	white blood cells
XO	xanthine oxidase

Table 1
ROS and RNS produced during metabolism [5,91].

Reactive oxygen species-ROS			Reactive nitrogen species-RNS		
Molecule	Symbol	Lifetime	Molecule	Symbol	Lifetime
Radicals					
Superoxide anion	$O_2^{\cdot -}$	10^{-6} s	Nitric oxide	$NO^{\cdot}$	s [*]
Hydroxyl radical	$NO^{\cdot}$	10^{-10} s	Nitrogen dioxide	NO_2	s
Alkoxyl radical	$RO^{\cdot}$	10^{-6} s			
Peroxyl Radical	$ROO^{\cdot}$	17 s			
Non-radicals					
Molecular oxygen	O_2	$>10^2$ s	Peroxynitrite	$ONOO^-$	10^{-3} s
Hydrogen peroxide	H_2O_2	10 s	Nitrosyl cation	NO^+	s
Singlet oxygen	$^1\Delta_g$	10^{-6} s	Nitrosyl anion	NO^-	s
Ozone	O_3	s	Dinitrogen trioxide	N_2O_3	s
Organic (lipid) peroxide	ROOH	$> 10^2$	Dinitrogen tetraoxide	N_2O_4	s
Hypochlorous acid	HOCl	Stable (min)	Nitrous acid	HNO_2	s
Hypobromous acid	HOBr	Stable (min)	Peroxynitrous acid	ONOOH	Fairly stable
			Nitryl chloride	NO_2Cl	s

[*] The lifetime of certain radicals is affected by the environment, *i.e.*, the lifetime of $NO^{\cdot}$ in an air saturated solution may be a few minutes.

predictors of mortality [22–26]. The increase of mortality rate of HE patients with hypoalbuminemia has been previously shown and was accounted for 6.7–10.9% for short period of hospitalization and about 42.8–73.9% for the long-term follow up [17–21]. Recent studies demonstrated that long-term human albumin infusion improves the prognosis of cirrhotic patients by lowering the overall mortality and the likelihood of emergent hospitalizations [25,26].

When healthy the environmental/physiological factors, *i.e.,* ROS, trigger immune mechanisms, which positively regulate neuroplasticity and neurogenesis, promoting learning, memory, and cognition [27]. ROS facilitate the response to growth factor activation and the formation of the inflammatory process, as well as playing critical functions in the immune system and microorganisms eradication [28]. Polymorphonuclear neutrophils (PMN) are the most abundant circulating immune cells that participate in immune and inflammatory processes (host defense) by releasing a significant amounts of pro-inflammatory cytokines and ROS/oxidative burst. When in excess, these cause endothelial dysfunction and increase the leakiness of the blood vessels/blood-brain barrier (BBB) [29–31].

Clinical studies have revealed that systemic inflammation is linked to neuroinflammation [32] and leads to cognitive deterioration in HE patients [33–35]. TNF-, IL-18, IL-6, endotoxins (*i.e.,* NH_4^+) levels have been shown to be significantly greater in the plasma of cirrhotic patients with HE [36]. In particular high levels of IL-6, which have been found in brains of BDL rat model of type C HE [2], are associated with ageing [27], the injury response of the CNS [37], impairment of the BBB integrity [38], memory and cognitive functions decline [27,39].

Systemic and brain cytokines, in combination with endotoxins (*i.e.,* NH_4^+), will set up an inflammatory cascade that exacerbates OS [40–43] and OS related activation of the astrocytes, and microglia [13,44]. Therefore, it is becoming increasingly evident that OS, both systemic and CNS is an important feature in the pathogenesis of HE [2,42,45]. In addition, impaired brain NH_4^+ detoxification together with osmotic effect of Gln induces ROS generation [2,7,8,46]. It is worth noting that OS and inflammation are pathophysiological processes that are inextricably linked and interdependent [47]. It is widely accepted that in the presence of OS, inflammatory processes will develop, accelerating the progression of the disease. Similarly, if inflammation is the trigger, an OS response will be activated, contributing to the inflammatory response [11,48–53]. Ammonium, hyponatremia, and inflammatory cytokines have been shown to trigger a self-amplifying cycle between astrocyte osmotic stress and cerebral oxidative/nitrosative stress [41].

2. Oxidative stress

ROS and RNS are common byproducts of normal aerobic cellular metabolism and serve crucial physiological functions in intracellular cell signaling, homeostasis, cell death, immunological response to infections, and mitogenic response induction [3,54–58]. All metabolic processes involve the oxidant-antioxidant equilibrium required to perform routine molecular and biochemical functions [29,59]. The outcome of oxidative stress depends on the number of affected cells and functioning of the antioxidants systems (enzymatic and nonenzymatic antioxidants), *i.e.,* concentrations of GSH, CAT, SOD, GPX, vit. C and vit. E (Fig. 1) [5,60].

Mitochondria respiratory chain form most of the ROS produced in the body. The superoxide anion ($O_2^{\cdot -}$) is one of the most important free radical in biological systems involved in cell signaling. $O_2^{\cdot -}$ is generated at the complexes I and III (Fig. 2), acts as a precursor for the synthesis of many other ROS (*i.e.,* H_2O_2 and $ONOO^-$), and lipid peroxides [61,62]. Approximately 80% of $O_2^{\cdot -}$ is released into the intermembrane space,

with most of remainder going to the mitochondrial matrix [63].

In addition, ROS (ie. $O_2^{\cdot -}$) can be generated while endo/exogenous toxin detoxification by microsomal cytrochrome-p_{450} conciliated hydroxylation. When in pathophysiological states the excess production and accumulation of ROS cause signaling pathways deterioration, overwhelm the antioxidant defense mechanisms capacity, cause the redox homeostasis imbalance, form OS state and became toxic [51,64]. Therefore, ROS can cause nonspecific damage, and participate in degeneration of essential cellular components like lipids, proteins, and DNA, potentially leading to cell senescence and death [51,64].

Elevated OS caused by increased levels of $O_2^{\cdot -}$ induce oxidative damage to proteins, lipids, and nucleic acids, compromising cell health, and has been linked to ageing processes and various human pathogeneses such as the development of cancer, neurodegenerative, and cardiovascular diseases [65–67]. ROS are short-lived [68], they react rapidly with first line antioxidants, like GSH, Asc, and SOD [4,5,69,70]. GSH is a major antioxidant in the brain and its decline decrease the ability of CNS cells to counteract the OS and is a common sign in patients with neurodegenerative disorders [71]. Astrocytes synthesize more GSH than neurons and serve as a source of precursors for neuronal GSH synthesis [11,71].

ROS are critical for hippocampal long-term potentiation (LTP), a synaptic plasticity for learning and memory as well as in aging/disease-related impairment. Therefore, when in excess ROS leads to cellular dysfunction, and long-term depression (LTD) [7,8]. In the CNS the oxidative conditions are essential and play a key role in nerve growth factor (NGF) induced cell differentiation. Despite being the longest-living cell type, CNS cells are more vulnerable to OS-mediated injury because of their physiological and biochemical properties, high energy requirements, and unique redox activities:

(i) Neurons generate the highest rate of ROS and utilize ~20% of the oxygen consumed by the body – the majority of which is used for ATP production (4×10^{12} molecules/min) to maintain neuronal intracellular ion homeostasis.
(ii) Majority of the neuronal cells are nonreplicating therefore more sensitive to OS.
(iii) Neuronal membranes rich in polyunsaturated fatty acids (PUFA) are particularly vulnerable to OS, *ie.* oxidative damage to myelin.
(iv) Modification of ion channels activity, disturbance in Ca^{2+} traffic across neuronal membranes and its intracellular concentration increase often leads to OS.
(v) $O_2^{\cdot -}$ is dismutated to H_2O_2 by SOD
(vi) H_2O_2 in the presence of intracellular iron, copper, or manganese ions, which accumulates in brain as a function of age, favors the Haber–Weiss vicious circle/Fenton chemistry, resulting in the generation of $OH^{\cdot}$, which is among the most active ROS (Fig. 1).
(vii) Antioxidant defense - brain contain low levels of Asc, GSH, CAT, GPX, and vitamin E [29,51,71–73].

2.1. Approaches to measure oxidative stress

The choice of OS biomarkers and methods to assess the oxidative status in biological samples should be based on the aim of the study and the clinical relevance. This article does not intend to be a survey of detailed methods and assays for OS detection in biological samples, which have been described and reviewed elsewhere [2,50,60–62,65,68,74–91].

Oxidative stress presence may be tested in three ways:

(i) direct detection of ROS (*in-vivo, in-vitro* and *ex-vivo* living tissue); Direct detection seem to be the preferred method but depends on local antioxidants concentrations and clearance mechanisms [5,91]. Of note *in-vivo* steady-state concentrations of ROS range from pico-to nanomolar range [92], with the lifetimes span nanoseconds to seconds (Table 1).
(ii) detection of resulting damage to biomolecules (*in-vitro* and *ex-vivo*); Due to some of the challenges encountered by the direct detection some scientists prefer to use techniques based on the detection of final oxidation products and measure the damage on proteins, DNA, RNA, lipids, and other biomolecules.
(iii) detection of antioxidants concentrations, total antioxidants capacity, antioxidants activity (*in-vitro* and *ex-vivo*). This approach measures the activity of specific antioxidant enzymes, like CAT,

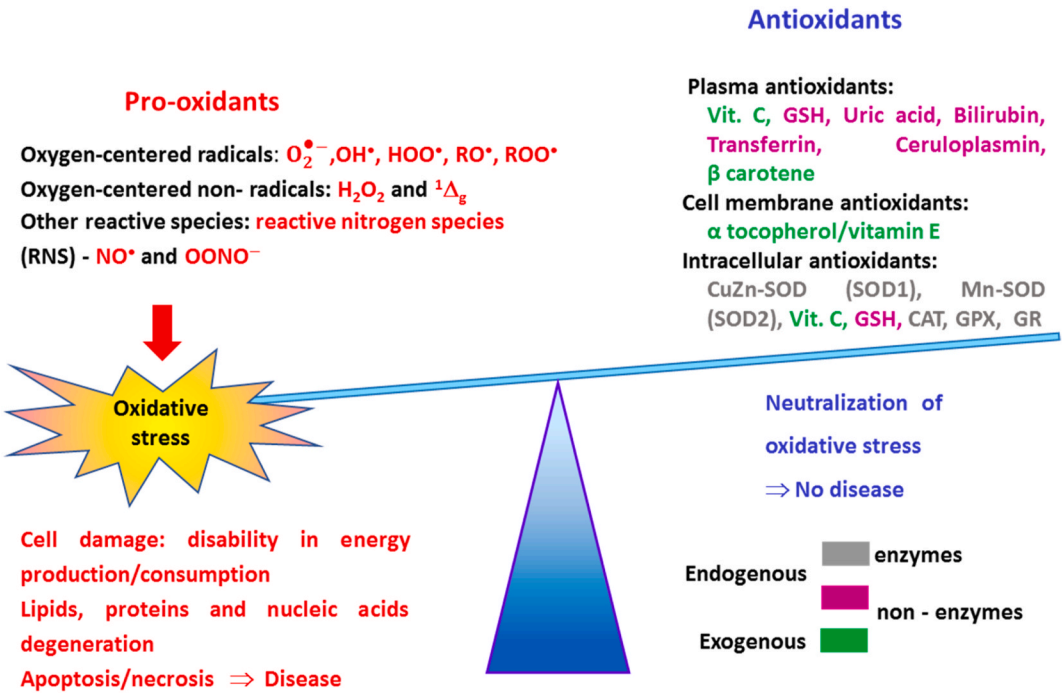

Fig. 1. Balance between ROS generation and elimination by antioxidants.

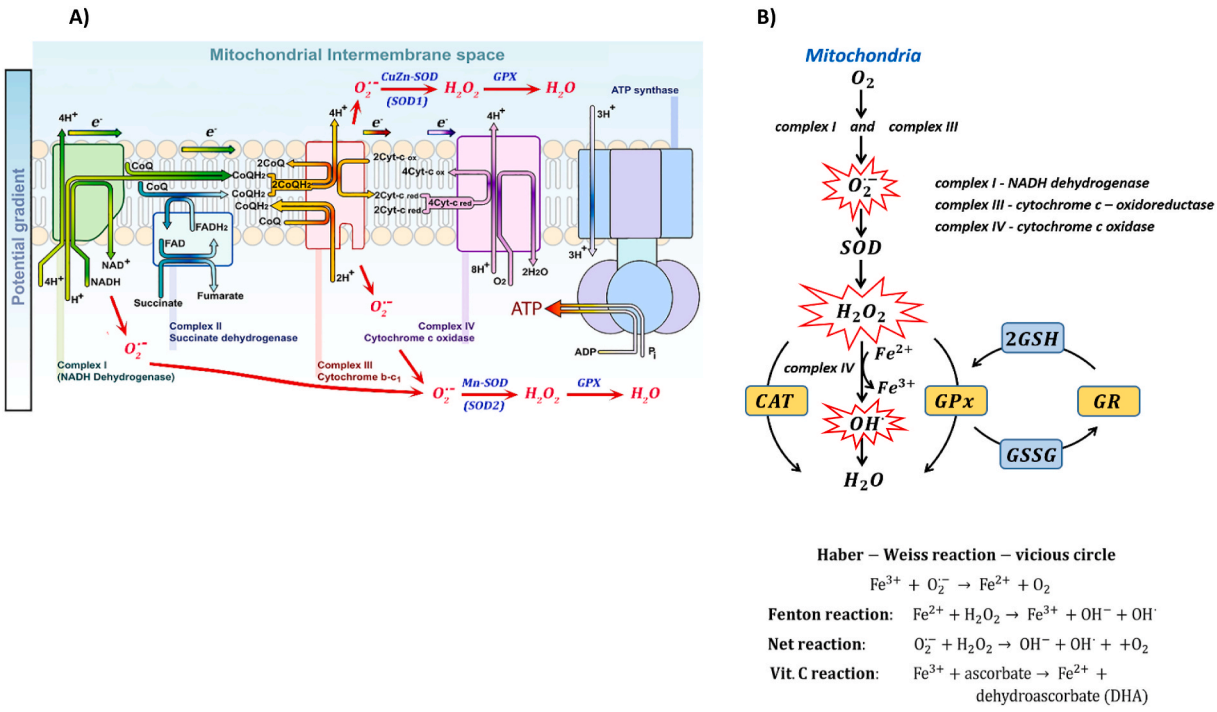

Fig. 2. A) Electron transport chain (ETC) in the mitochondrial intermembrane space (IMS) (adapted from the public domain image, author: LadyofHats). Electrons from NADH and FADH2 pass through the ETC and reduce O_2 to form water at complex IV. ROS ($O_2^{\cdot-}$ & H_2O_2) are produced from the leakage of electrons to form $O_2^{\cdot-}$ at complexes I and III. $O_2^{\cdot-}$ is produced within the matrix at complex I, whereas at complex III it's released towards both the matrix and IMS. $O_2^{\cdot-}$ is dismutated to H_2O_2 by superoxide dismutase 1 (SOD1) in the IMS and by SOD2 in the matrix. H_2O_2 is then fully reduced to water by glutathione peroxidase (GPX). B) Endogenous enzymatic defense systems of all aerobic cells: SOD, GPX, glutathione reductase (GR) and catalase (CAT) - they scavenge directly $O_2^{\cdot-}$ and H_2O_2 converting them to less reactive species. SOD dismutase $O_2^{\cdot-}$ to H_2O_2 which is rapidly converted by Fenton reaction into very reactive $OH^{\cdot}$ radical. GPx neutralizes H_2O_2 by taking hydrogens from 2GSH molecules resulting in $2H_2O$ and one GSSG. GR then regenerates GSH from GSSG. CAT neutralizes H_2O_2 into H_2O.

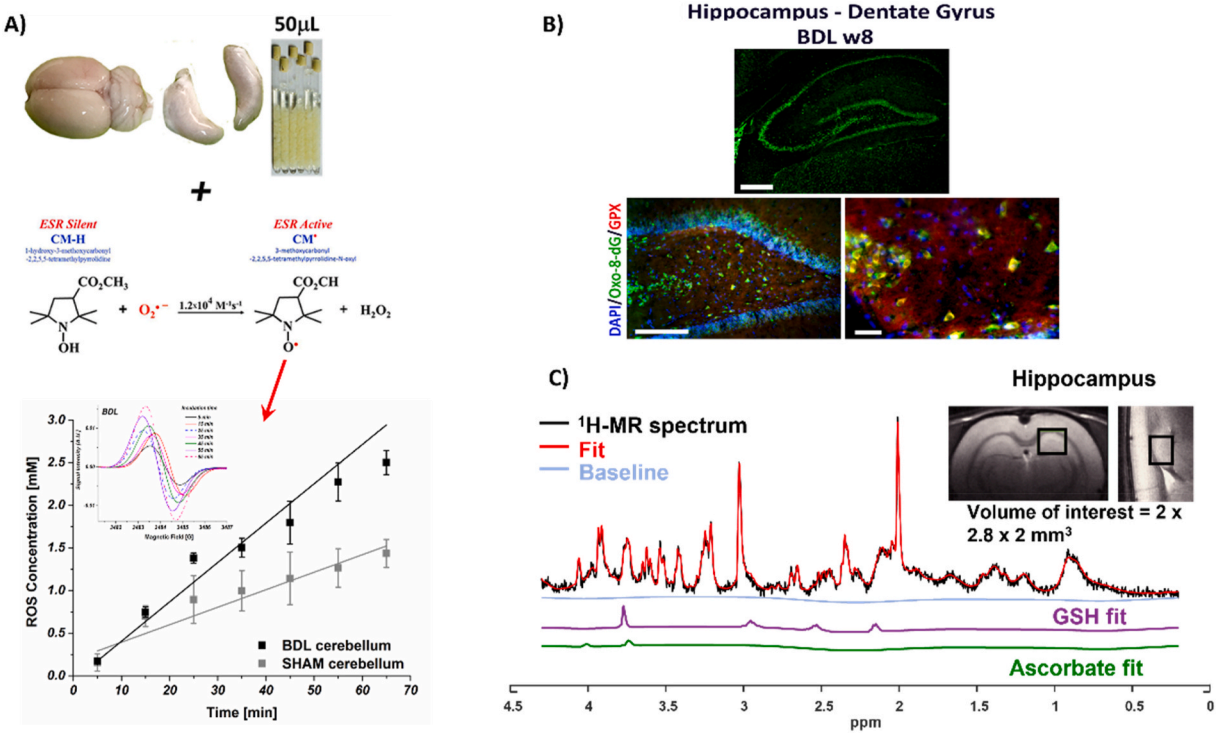

Fig. 3. Oxidative stress detection methods: A) **EPR** in combination with nontoxic cell-permeable CMH spin probe – **direct detection** of $\mathbf{O_2^{\cdot-}}$ in BDL rats [2]. B) **Fluorescent microscopy** - immunohistochemistry (IHC) of BDL rats brain tissue: **Oxo-8-dG** – DNA/RNA damage antibody accumulation– sign of elevated **HO**$^{\cdot}$ and its interaction with a nucleobase. **GPX** antibody build-up - sign of an increased production of $\mathbf{H_2O_2}$ [2]. C) $^{\mathbf{1}}$**H-MRS** – *in-vivo* detection of two main antioxidants **GSH** and **Asc** – alteration of their concentrations represents an **indirect evidence OS** [13].

SOD or GPX, and total antioxidant capacity. Furthermore, each individual marker reflects only partially the oxidative status, and therefore an integrative approach is necessary to achieve comprehensive conclusions.

In-vivo and *ex-vivo* OS detection is a complex task and require probes that are very sensitive, highly selective (*i.e.,* $HO^{\cdot}$, $O_2^{\cdot -}$, $NO^{\cdot}$, $ONOO^-$), and fast to react rapidly with ROS/RNS and create a stable secondary radical, which then can be quantified. Most of experimental assays for OS detection provide relative data. The extensively used approaches are electron paramagnetic resonance (EPR) spectroscopy allowing a real time direct detection of ROS, while fluorescence spectroscopy, microscopy, or flow cytometry can detect the final oxidation products or enzymatic activity [50,61,77]. *In-vivo* antioxidants (GSH and Asc) concentration measurements provide indirect evidence of redox homeostasis imbalance/OS and can be assessed non-invasively using proton magnetic resonance spectroscopy (^{1}H-MRS) [13,93,94].

2.1.1. EPR

Is a method for direct detection of unpaired electrons. Free radicals are chemical molecules that have unpaired electron and are primarily formed from molecular oxygen. EPR in combination with nontoxic cell-permeable and resistant to antioxidants spin-traps (covalent bond with the radical by addition reaction) or spin probes (oxidized by ROS without binding) have been recognized as one of the most powerful and exclusive technique that allow direct and reliable detection of the ROS presence in the system under study being less uncertain as compared to other methods (*i.e.*: immunoassays or UV–Vis spectroscopy) [50,61,75] (Fig. 3A). EPR of sable secondary radicals (paramagnetic/EPR active) formed by adding exogenous spin-traps/probes (diamagnetic/EPR non-active) provides direct information about a variety of biological samples, *i.e.,* living tissue, blood, and other body fluids (*in-vitro*, *ex-vivo* in room temperature and frozen) redox state in an accurate, rapid and quantitative manner by the generation of stable nitroxide radicals [50,65,74,95–97].

Individual traps/probes typically exhibit a high selectivity for specific species, allowing for modification of sensitivity to a given radical (*i.e.,* $HO^{\cdot}$, $O_2^{\cdot -}$, H_2O_2, $NO^{\cdot}$, $ONOO^-$) and identification of the species present in the system [79]. EPR studies of the cyclic hydroxylamine spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH) revealed that it detects a 10-fold lower $O_2^{\cdot -}$ radical level than other spin-traps/probes [50,61,79,82,98]. Furthermore, the high cell permeability allows it to detect both extracellular and intracellular ROS production in a reproducible manner, assess antioxidant and drug effects, providing superior specificity and sensitivity for quantification [2,50,61,75,82,98].

2.1.2. Spectrophotometric assays

Involving ultraviolet and colorimetric assays, are based on the interaction of reactive species with redox compounds and change in absorbance [74,99]. Spectrophotometry has found a widespread use in biomedical research, but, like chemiluminescence probes, it does not provide details about ROS/RNS and its localization within the cell (i.e., extracellular or intracellular) unless various probes and/or inhibitors with differing compartmentalization are used [99].

Remarks: Among most extensively applied spectrophotometric assays are: (i) **GSH/GSSG** as the one of most powerful self-generated antioxidant in the body [100,101] and the (ii) **malonaldehyde (MDA)** as a marker of lipid peroxidation [102,103].

(i) The major issue with blood ***ex-vivo*** **GSH** and **GSSG** concentration measurements is the oxidation of GSH after sample collection, and exaggeration of oxidation by increased formation of ROS while the acid deproteinization process. Therefore, leading to an overestimation of GSSG and an underestimating of GSH concentrations and the GSH/GSSG ratio [81]. To address this issue, the N-Ethylmaleimide (NEM) blocking agent, which forms stable, covalent thioether bonds with sulfhydryls, and prevent the formation of disulfide bond, needs to be added immediately to the blood sample after collection [81,100,101].

(ii) **MDA** is the most commonly used biomarker of OS, which results from lipid peroxidation of polyunsaturated fatty acids [103]. The most often used method for determining MDA in biological fluids is the thiobarbituric acid reactive substances (TBARS) test [68,104]. TBARS test suffer from numbers of limitations. MDA may be produced by other than lipid peroxidation reactions [105] and the false increase could be generated by the heating step of the assay [68]. EDTA treated samples as well as samples stored at −20 °C without addition of antioxidants significantly increase the TBARS levels [102,103,106]. Therefore, the main concerns are: non-specificity of TBA reactivity on MDA (other aldehydes cross-reaction produced from lipid peroxidation), poor reproducibility of analytical results, sample preparation/procedural modifications, storage, and stability of MDA standard solutions [102,103]. Therefore, an expert panel should re-evaluate MDA as an OS biomarker and a validated analytical process should be created [102,103].

2.1.3. The fluorescent redox probes

Are based on sensing mechanisms, and are classified as reaction-based selective probes, and reversible probes which can respond to multiple oxidation-reduction cycles [77]. The advanced fluorescence detection gives the possibility for real-time measurements of large number of samples (plate reader) with high sensitivity, spatial resolution, and specificity [77]. The pattern of oxidation/reduction-induced fluorescence change is crucial in determining the biological significance of a probe. Therefore, it is important to evaluate the probe mechanism: signal enhancement during reduction (nitroxide-based probes - probing of hypoxia or antioxidants effectiveness) or oxidation (most other classes of probes - ROS/RNS activities) [77]. The fluorescence microscopy enables probe localization (cytology/histology - sub-cellular organelles, proteins, and membrane-components) (Fig. 3B).

However, for measuring *in-vitro/ex-vivo living tissue,* the intracellular fluorescent probes concentration is critical for correct interpretation of the results. The fluorescent product formation will depend on experimental conditions and probe internalization [107,108], the formation of multiple nonspecific oxidation products, light sensitivity, and probe redox cycling [78]. Therefore, it is critical to recognize these limitations in order to avoid erroneous interpretations.

2.1.4. Proton magnetic resonance spectroscopy (^{1}H-MRS)

Is the only technique able to measure a large number of metabolites simultaneously *in-vivo* in a non-invasive manner [13,93,94]. Around 19 metabolites can be measured by combining ultra-short echo time localization sequences with an ultra-high magnetic field (≥7T) as also shown in type C HE [13]. As such several processes involved in HE can be studied *in-vivo* and longitudinally: energy metabolism (lactate (Lac), glucose (Glc), alanine (Ala), phosphocreatine (PCr), Cr), osmoregulation (taurine (Tau), Ins, Cr), myelination/cell proliferation (phosphocholine (PCho), glycerophosphocholine (GPC), phosphoethanolamine (PE), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG)) and neurotransmitters and metabolites involved in neurotransmission (glutamate (Glu), Gln, aspartate (Asp), glycine (Gly), γ-aminobutyrate (GABA)) [13].

As shown in Fig. 3C the two main antioxidants **GSH** and **Asc** can also be measured by ^{1}H-MRS in the brain. Of note Asc and GSH are present in low concentration with significant resonance overlap, thus special editing sequences or ultra-high magnetic fields are required [13,70,76,94,109–111]. Changes in these metabolites represent an indirect evidence of OS. Therefore, additional techniques that allows the precise quantification of tissue ROS and identification of different ROS species

would contribute significantly to the understanding of the pathophysiology of type C HE and potentially offer insight into therapeutic options.

As shown above, there are several methods and assays for redox biomarkers detection, making it difficult to select the relevant and most appropriate one. Pros and pitfalls of abovementioned methods are provided in Table 2.

3. Oxidative stress in hyperammonemia

In-vitro (primary cultures), *ex-vivo* (after intraperitoneal (ip) perfusion) and *in-vivo* (microdialysis) studies showed that high concentrations of ammonium (5–12 mM) are involved in oxidative stress related neurotoxicity (Table 3) [45,112–119]. High concentration of ammonium has been associated with the rise in cellular glutamine synthetase (GS) and synthesis of Gln [45,112–119].

Administration of high concentrations of ammonium 5–12 mmol/kg (intraperitoneally) or 60 mM (intrastriatally) in rats caused acute intoxication and increased generation of free radicals [120,121].

Despite the significant increase in cellular GSH levels, but not mitochondrial, both in cultured astrocytes as well as in microdialyzed cerebral cortex, ROS production was not blocked, suggesting that the increased ROS by ammonium reached levels that were beyond the antioxidant capacity [115,120,122]. Therefore, ROS may induce DNA damage mediated by activation of NMDA receptors [123].

Microglial ROS production increased with ammonium time exposure and in a dose-dependent manner (0.25–5 mM). Treatment with apocynin completely abolished the ammonium induced ROS increase, indicating activation of NADPH-oxidase by ammonium, and therefore suggesting that the OS increase is due to ROS not the reactive nitrogen species (RNS) [112,118].

Cortical mitochondria of hyperammonemic rats exhibited significant $O_2^{\cdot -}$, XO and lipid peroxidation increase accompanied by GPX, SOD2, CAT and GSH depletion, while the plasma CAT increased its activity [121,123–125].

Ammonium-induced RNA oxidation confirmed by Oxo-8-dG accumulation [45,113,114]. Among the oxidized RNA species, mRNA coding for the glutamate/aspartate transporter (GLAST) were identified [113].

4. Oxidative stress in type B and C HE animal models

Over the last several years researchers have demonstrated that oxidative stress is a key factor implicated in the pathogenesis of type B and C HE (Table 4) in animal. Indirect detection of OS using biochemical markers on BDL rats brain homogenates [59,127], as well as direct *in-vivo* brain and *ex-vivo* living brain tissue [2,13] have shown the presence of a strong correlation between chronic liver disease induced HE and OS.

The BDL rat model of type C HE has been shown to support the important role of oxidative stress in pathogenesis of HE by increased CNS OS ($O_2^{\cdot -}$) already at week 2 post-surgery [2], and at week 4 post surgery both, CNS and systemic OS [2,13,127]. GSH and ascorbate (Asc) have been shown to be the first line antioxidants which reacts rapidly with ROS to counteract the deleterious effects of OS [4,5]. GSH and Asc decline in brains of BDL rats correlated with OS increase suggesting an increased ROS scavenging in response to increased production [2,13,127]. Elevated levels of SODs have also been observed in response to increased endogenous and exogenous $O_2^{\cdot -}$ in the same animal model at 4 weeks post-surgery [2]. The nuclear translocation of SOD1 for the genomic DNA protection together with SOD2 and GPX-1 upregulation demonstrates a direct evidence of increased need to eradicate ROS [2].

In primary astrocytes, ammonium-induced RNA oxidation has been detected by Oxo-8-dG accumulation [45,84,113,114,128]. Recently a significantly increased formation of Oxo-8-dG, with predominant cytoplasmic localization, was also shown in the hippocampus and cerebellum of BDL rats, indicating interaction of cytosolic and mitochondrial

Table 2
Advantages and drawbacks of methods for OS detection [2,50,60–62,65,68,74–91]. Of note, the concomitant usage of several techniques on the same sample is highly recommended and will lead to complementary information on OS.

Method	+	−
EPR	- direct detection of free radicals - *in- vitro* and *ex-vivo* real-time fresh or pre-incubated frozen sample measurements - *high* quality spin-traps/probes that react with short-lived radicals and convert them to long-lived radicals - cell *permeable* and non-permeable spin probing (cyclic hydroxylamines) – intra vs. extracellular OS - *react* rapidly with ROS/RNS and create a stable radical - direct detection of ROS - *single* chemical reaction - no *redox* cycling = no artificial ROS - very *sensitive*, highly selective - (*i.e.,* $HO^{\cdot}$, $O_2^{\cdot -}$, H_2O_2, $NO^{\cdot}$, $ONOO^-$, GSH/GSSG) - *commercially* available spin probes - *direct* assessment of antioxidant/pro-oxidant property of drugs or food - kinetic analyses and determine the formation and elimination velocities of a free radical - can be quantified	- *ex-vivo* – experimental conditions – tissue must be processed fast and in controlled conditions - *sample* volume - blood sample - influenced by hemolysis **rare:** - if a free radical instantly reacts with a molecule other than the spin-trap/probe agent, no spin adduct is formed - if a spin adduct decomposes, a new spin adduct may be generated
Spectrophotometry and fluorimetry (colorimetric, fluorimetric, and luminescence-based assays)	- *in-vitro* and *ex-vivo* real-time measurements of large number of samples (plate reader) - *commercially* available kits - *easy* to perform - *can* be adapted to automated biochemistry analyzers - *detection* of protein and lipid damage - *detection* of RNA/DNA damage - *assessment* of antioxidant status	- *ex-vivo* - experimental conditions - – tissue must be processed fast and in controlled conditions - sample volume - blood sample - influenced by hemolysis, bilirubin interfere with the reaction - calibration, determination of sensitivity - indirect detection of ROS/RNS - reaction between the radical and a tracer generating a measurable product - detect the final oxidation products or enzymatic activity - nonspecific - difficult to discriminate between the radical species - risk of probe auto-oxidation and instability

(*continued on next page*)

Table 2 (*continued*)

Method	+	−
		- interference from background effects - false higher results - redox cycling - antibody specificity - compromised quantification - mostly relative data
Bright-field and fluorescence microscopy	- tissue histology - IHC - localization of OS markers (*i.e.*, SOD, GPX, Oxo-8-dG) - assessment of antioxidant status	- tissue preservation - antibody specificity - relative data
^{1}H- MRS	- *In-vivo* antioxidants (GSH and Asc) detection - can be quantified - non-invasive - longitudinal tracing of antioxidants over the disease progression - indirect evidence of redox homeostasis imbalance/OS	- *in-vivo* low concentrations - detection limit

nucleic acids rather than nuclear DNA with $HO^{\cdot}$ [2].

It has been shown that several brain regions of BDL rats had impaired enzymes activity of mitochondrial respiratory chain together with increase in mitochondrial reactive ROS generation, and augmentation of lipids and proteins oxidation [127].

However, the PCA model of type B HE has demonstrated conflicting results. At 4 weeks post-surgery no significant differences in plasma ROS, xanthine oxidase (XO), GSH and GSH/GSSG ratio, and CAT levels or in CSF ROS levels have been found between PCA and PCA-sham rats [129,130]. In contrary, in brains of PCA rats at 6 weeks post-surgery ammonium induced an increased brain ROS generation and promoted carbonylation of proteins, together with increased lipid peroxidation [131]. These different results could be related to the use of different animal strains and the length of the experimental design.

In parallel it has been shown that systemic OS correlated significantly with blood NH_4^+ concentrations [2] in BDL rats. Increase of ROS was represented by increased production of $O_2^{\cdot -}$, H_2O_2, increased activity of XO and decreased activity of CAT [2,129,130]. Significantly larger amount of $O_2^{\cdot -}$ production in BDL PMN and LYM as compared to the healthy sham controls [2] indicated high resting oxidative burst and hypo-responsivity to the bacterial challenge [2,132].

5. Oxidative stress in human HE and in patients at risk of HE developing

The patients' diagnoses were determined based on their medical history, physical and clinical examination, laboratory testing (MELD and Child Pugh scores), standardized battery of neuropsychological tests (*i.e.,* the West Haven mental status scale), and histology [112,133–141].

There are few studies on CNS OS in patients with chronic liver disease and chronic liver disease associated HE (Table 5). Evidence of CNS OS presence in HE arose first from the postmortem EM observation of lenticular nuclei [142]. Alzheimer type II astrocytes, the integral neuropathological aspect of HE [143], contain large amounts of lipofuscin pigment aggregates [142] produced by peroxidation of unsaturated fatty acids [144,145]. Further, the IHC of the cerebral cortex of cirrhotic patients with HE supported the presence of oxidative stress in pathogenesis of HE [112]. As such increased expression of neuronal nitric oxide synthase (nNOS) has been accompanied by increased expression of SOD [112], a direct indication of OS presence. The induced RNA oxidation has been detected by increased accumulation of Oxo-8-dG, a direct sign of $HO^{\cdot}$ [84,112,128]. Furthermore, recent studies supported the link between the OS related lipid peroxidation [141,142] and brain atrophy/the grey matter volume (GMV) loss [141].

Liver biopsies of NAFLD patients indicated increased OS accompanied by decreased antioxidant capacity (SOD, GSH and CAT) [14,112].

A significant disorder in redox status was also detected in HE patients' blood, indicating systemic OS involvement in the disease progression, evidenced by significant enhancement of plasma/serum lipid peroxidation, protein carbonylation together with reduction in antioxidant capacity, *i.e.*, significant decrease of albumin, SOD, CAT and GPX enzymatic activity [14,112,134–137,139,140].

Similarly, to the animal model studies the isolated PMN cells of cirrhotic patients demonstrated significantly higher resting state oxidative burst along with reduced phagocytic capacity [132,136], which was correlated with higher risk of multiple infections and organ failure [136].

6. Oxidative stress consequences

It is now well accepted that OS is implicated in various neurodegenerative disorders, causes neurofilaments (NfL) phosphorylation and leads to proteins aggregates formation [147,148]. To date little is known about OS involvement in neurodegeneration in patients with HE.

Excessive lipofuscin pigment aggregates, a sign of lipids peroxidation, have been detected in the Alzheimer type II astrocytes of the lenticular nuclei of HE patients [142]. OS accelerate the lipofuscin accumulation [144,145,149,150]. Lipofuscin is composed primarily of protein, lipid peroxides, and transition metals [150]. Lipofuscin aggregates at high concentration undergo Fenton reaction, resulting in the generation of $OH^{\cdot}$, one of the most active ROS [144], which in consequence may generate additional lipid, protein and RNA/DNA oxidation [141,142,151–153] and therefore lead to brain atrophy [141].

Astrocytes protect neurons against the ROS toxicity, by supplying them with the GSH precursors [71,154,155]. Therefore, it has been shown that inhibition of cystine uptake into astrocytes under chronic ammonium exposure [156] may lead to reduced astrocyte and neuronal GSH levels [14,59,112,127], and place both at risk for oxidative damage.

The loss of glial filaments and dendritic spines of pyramidal neurons, hippocampal and cortical as shown in the BDL rat models [13,96,157], may be related to aforementioned oxidative modification of RNA and may thereby provide another link between OS and ammonium toxicity [2,45,112–114,118,158]. RNA oxidation interferes with translational machinery, gene expression, thereby providing the link between ammonium-induced OS and cognitive decline through impaired protein synthesis [158] and neurotransmission [67].

Filaments loss can be also induced by OS related protein depolymerization [159]. Moreover, ammonium intoxication has been shown to lead to protein nitrotyrosylation and nitric oxide ($NO^{\cdot}$) overproduction in *in-vitro* and human brain studies [45,112,114,116,118,123]. $NO^{\cdot}$ out-compete with SOD and react with $O_2^{\cdot -}$, leading to powerful and toxic peroxinitrite ($ONOO^-$) formation, which may lead to DNA damage [123], NfL and actin nitration and disrupt filaments assembly [160]. Similarly, the actin cytoskeleton is a primary target of OS in hepatocytes [161]. Therefore, OS-induced cytoskeleton morphological alterations in hepatocytes will have a negative impact on hepatobiliary functioning.

Postmortem IHC of cortex of NAFLD patients have demonstrated an increase expression of a small heat shock protein-27 (Hsp27) [112], a direct sign of OS presence. During oxidative stress, Hsp27 has an important antioxidant role lowering the ROS, raising GSH, lowering the intracellular iron concentrations, actin cytoskeleton remodeling, and is capable of inhibiting OS-induced necrosis [162–164]. Because the cytoskeleton is a network of filaments linked together by crosslinking proteins, damage to one component affects the entire cytoskeletal network.

Table 3
Summary of some published work highlighting the presence of oxidative stress in hyperammonemia models.

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
Rat – **hyperammonemia** - $NH_4CH_3CO_2$ (7 mmol/kg)(coma in ~30 min) Rat (Wistar rats - 200–220 g) – **hyperammonemia** - $NH_4CH_3CO_2$ (12 mmol/kg) (coma in ~12 min)	**Ex-vivo:** 15 min **after ip perfusion** **Mitochondria** extraction from **forebrain** or hemisphere and **liver**, cytosolic fraction **Brain tissue extracts** **Blood RBC and plasma**	UV–Vis and fluorescence spectroscopy -Enzyme activity	GPX GSH SOD CAT MAO XDH, XO Aldehyde oxidase (AO) H_2O_2 $O_2^{\cdot-}$ NAD^+ nuclear protein nitrotyrosylation DNA damage Lipid peroxidation	*Acute ammonium intoxication* **Brain mitochondria** (non-specific to region) # - $O_2^{\cdot-}$ ↑118%, (a.u.): HA 3.64 ± 0.55 vs. control 1.635 ± 0.15, $p < 0.01$ - H_2O_2 ↓ 31%, (a.u.): HA 0.18 ± 0.06 vs. control 0.26 ± 0.03, $p < 0.01$ - GPX↓ activity 47%, (nmol/min/mg protein): HA 31.5 ± 9.8 vs. control 61.25 ± 7, $p < 0.001$ - GR↓ activity (nmol/min/mg protein): HA 17.04 ± 0.9 vs. control 20 ± 5.2, ns - SOD2↓ 32%, (U/mg protein): HA 1.85 ± 0.4 vs. control 1.25 ± 0.4, $p < 0.05$ - CAT↓ 32%, (s^{-1}/mg protein x 10^4): HA 1.85 ± 0.4 vs. control 1.25 ± 0.4, $p < 0.001$ - XO ↑ activity, (a.u.): HA 3.9 ± 0.5 vs. control 2.93 ± 0.24, $p < 0.001$ - XDH↓ activity, (a.u.): HA 7.5 ± 0.6 vs. control 10.66 ± 0.6, $p < 0.001$ - XDH/XO ↓ ratio indicating conversion of XDH to XO, (a.u.): HA 2.1 ± 0.36 vs. control 3.75 ± 0.45, $p < 0.001$ - AO ↑ activity, (a.u.): HA 72 ± 7.5 vs. control 42.5 ± 5, $p < 0.001$ - MAO-A + B ↑ activity, (a.u.): HA 1.53 ± 0.07 vs. control .096 ± 0.01, $p < 0.001$ **Brain cells nuclei** - NAD^+ ↓ 55%, (pmol/mg protein): 13.7 ± 2.9 before and 14.9 ± 1.7, 11.7 ± 2.5 and 6.2 ± 1.5 at 5, 8 and 11 min after ammonium injection - $O_2^{\cdot-}$ ↑ (nmol/min/mg protein): HA 0.46 ± 0.08 vs. control 0.27 ± 0.55, $p < 0.05$ **Brain** - GPX↓, 38% decrease in cytosol and 47% in mitochondria, (nmol/min/mg protein): cytosol - HA 81 ± 9 vs. control 130 ± 30 $p < 0.05$, mitochondria - HA 16 ± 0.8 vs. control 30 ± 4 $p < 0.05$ - GR- no change, (nmol/min/mg protein): cytosol - HA 41 ± 5 vs. control 45 ± 7, mitochondria - HA 14 ± 2 vs. control 19 ± 1 - SOD↓ 40% in cytosol and 37% in mitochondria, (U/mg protein): cytosol - HA 6 ± 0.9 vs. control 10 ± 0.9 $p < 0.05$, mitochondria - HA 2.7 ± 0.1 vs. control 4.3 ± 0.0 ns - CAT↓ 52% in cytosol and 58% in mitochondria, (s^{-1}/mg protein): cytosol - HA 5.5 ± 0.42 vs. control 11 ± 1.8 $p < 0.05$, mitochondria - HA 1.55 ± 0.15 vs. control 3.7 ± 1.8 $p < 0.05$ **Liver** - GPX↓, 24% in cytosol and 35% in mitochondria, (nmol/min/mg protein): cytosol - HA 1040 ± 100 vs. control 1370 ± 160 $p < 0.05$, mitochondria - HA 212 ± 32 vs. control 328 ± 22 $p < 0.05$ - GR- no change, (nmol/min/mg protein): cytosol - HA 103 ± 6 vs. control 109 ± 10, mitochondria - HA 51 ± 11 vs. control 51 ± 7 - SOD↓ 32% in cytosol and 41% in mitochondria, (U/min/mg protein): cytosol - HA 36 ± 3 vs. control 53 ± 46 $p < 0.05$, mitochondria - HA 4.7 ± 0.8 vs. control 8 ± 0.9 $p < 0.05$	[121] [123] [124] [125] [126]

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Table 3 (*continued*)

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
				- CAT↓ 42% in cytosol and 27% in mitochondria, (s^{-1}/mg protein): cytosol - HA 0.18 ± 0.016 vs. control 0.31 ± 0.03 p < 0.05, mitochondria - HA 0.8 ± 0.09 vs. control 1.1 ± 0.08 p < 0.05 **RBC** - GPX↓ 25%, (μmol/min/ml of cells): HA 94 ± 23 vs. control 125 ± 23 p < 0.05 - GR- no change, (μmol/min/ml of cells): HA 66 ± 16 vs. control 67 ± 12 - SOD↓ 69%, (U/min/ml of cells): HA 286 ± 90 vs. control 932 ± 62 p < 0.05 - CAT↓ 29%, (s^{-1}/ml of cells): HA 17 ± 1.7 vs. control 24 ± 2.2 p < 0.05 **Plasma** - GPX no change, (nmol/min/ml): HA 5690 ± 600 vs. control 5330 ± 200 - GR- no change, (nmol/min/ml): HA 38 ± 4 vs. control 38 ± 5 - SOD- no change, (nmol/min/ml): HA 1.6 ± 0.1 vs. control 1.5 ± 0.1 - CAT↑ 88%, (s^{-1}/ml x 10^5): HA 3.0 ± 0.8 vs. control 1.6 ± 0.1 p < 0.05 **Lipid peroxidation** – MDA ↑, mediated by activation of NMDA receptors: cytosol - HA 70 ± 4 vs. control 55 ± 2 (nmol/g tissue) p < 0.001, mitochondria - HA 3.8 ± 0.3 vs. control 2.8 ± 0.2 (nmol/mg protein)p < 0.001 - Blocking NMDA receptors with MK-801 prevents ammonium-induced oxidative stress, XDH to XO conversion and MAO-A activation	
Rat - **hyperammonemia** - NH_4Cl (5 mM and 10 mM)	**In-vitro:** Primary cultures of **astrocytes** from **neonatal cerebral cortex** **In-vivo:** brain microdialysis - **cortex**	UV–Vis and fluorescence spectroscopy Enzyme activity	ROS GSH GSSG SOD	*Acute ammonium intoxication* - Ammonium → ROS ↑ - dose dependent - Ammonium → GSH ↑[#]: HA – 3 days NH_4Cl 5 mM 21.4 ± 3.8 nmol/mg protein in cells and 24.1 ± 3.6 μmol/mg cell protein in medium vs. control 13.9 ± 2.6 nmol/mg protein in cells and 11.6 ± 2.7 μmol/mg cell protein in medium, the increase is facilitated by activation of the uptake of the GSH precursor cystine - GSH ↑- 10 mM NH_4Cl for 48 h–80% - SOD supplementation → ROS ↓ in astrocytes exposed to NH_4Cl 5 mM - In vivo, intracerebral administration of ammonium via a microdialysis probe → GSH ↑ in the brain extracellular space → dependent on undisturbed GSH synthesis in astrocytes - basal GSH[#] concentrations in microdialysates from rat's striatum were about 10-fold higher than from prefrontal cortex: 1.32 ± 0.54 μM vs. 0.097 ± 0.04 μM and 40 min after infusion 3.75 ± 0.53 μM vs. 0.185 ± 0.05 μM - Ammonium promotes GSH synthesis, export from astrocytes, increases its extracellular degradation → supporting GSH synthesis in neurons. - GSH/GSSG ratio ↓	[115] [117, 122]
Rat - **hyperammonemia** - NH_4Cl (60 mM) – microdialysis → extracellular concentration ~5 mM	**In-vivo:** brain microdialysis - **cortex**	Liquid chromatography	$HO^{\cdot}$	*Acute ammonium intoxication* - $HO^{\cdot}$ ↑ contents in microdialysates – HA: 2.5-fold ↑ - $HO^{\cdot}$ ↑ contents in microdialysates – NMDA 1 mM infusion: 2-fold ↑ - Taurine (85 mM) co-administration (''ammonium + Tau'') → the NMDA-induced $HO^{\cdot}$ ↓	[120]

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Table 3 (*continued*)

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
Rat/mice – **hypoosmolality, hyperammonemia** - NH_4Cl (5 mM)	**In-vitro:** cultured rat **astrocytes** - from the **cortices** of cerebral hemispheres of newborn Wistar rats **Ex-Vivo:** brain slices (**cortex**) from male mice (adult)	Immunostaining Fluorescence microscopy Western Blot Analysis	ROS NOX	*Acute ammonium intoxication* - ammonium stimulates ROS ↑ - ROS ↑ → NH_4Cl 5 mM ~3-fold ↑[#] - ROS ↑ → NH_4Cl 5 mM + apomycin (300 μM) ~1.5-fold ↑[#] - involvement of NADPH oxidase in hypoosmotic ROS production - NADPH oxidase inhibitor apocynin almost completely prevents hypoosmotic ROS production - ROS ↑ with decreasing osmolarity ~1-fold ↑[#]→ increased cellular O_2^-, absent in presence of apocynin - astroglia ROS may affect the function of neighboring neurons	[116]
Rat, mouse – **hyperammonemia** - NH_4Cl (0.2–5 mM)	**In-vitro:** cultured rat **astrocytes** - from the **cortices** of cerebral hemispheres of newborn rats Isolated RNA **In-vivo/Ex-vivo:** rats brain **Ex-vivo:** mice brain slices 400–500 μm	UV–Vis and fluorescence spectroscopy RNA Oxidation IHC - confocal laser-scanning microscopy North-Western and Slot-Blot Analysis - isolated RNA	RNA oxidation	*Acute ammonium intoxication* - RNA oxidation ↑ in primary astrocytes - 6 h NH_4Cl 0.5 mM → ~1.5-fold ↑[#] and 6 h NH_4Cl 1 mM → ~2.5-fold ↑[#], $p \leq 0.05$ - RNA oxidation ↑ - cortical mouse brain slices - 6 h NH_4Cl 5 mM → ~1.6-fold ↑[#], $p \leq 0.05$ - Ammonium-induced RNA oxidation is reversible in-vivo - Among the oxidized RNA species, 18S-rRNA and the messenger RNA (mRNA) coding for the glutamate/aspartate transporter (GLAST) were identified	[113]
Rat – **hyperammonemia** - NH_4Cl (5 mM)	**In-vitro:** cultured rat **astrocytes** - from the **cortices** of cerebral hemispheres of newborn Wistar rats	Slot Blot UV–Vis and fluorescence spectroscopy Confocal laser-scanning and epifluorescence microscopy	RNA oxidation NO and RNOS production/ inhibition	*Acute ammonium intoxication* - RNA oxidation ↑ - Oxo-8-dG ↑ in cytosolic mRNA and ribosomal RNA - NH_4Cl 1 mM for 1 h → 2.2-fold ↑[#], $p \leq 0.05$, pretreatment with TPEN 25 μM → no RNA oxidation - NH_4Cl 5 mM for 6 h → astroglia ↑ of Zn^{2+} ~1.16-fold ↑[#], $p \leq 0.05$ → levels of ROS/ RNOS ↑ and RNA oxidation ↑ - ↑$[Zn^{2+}]_i$ - effect of NOS inhibition - NH_4Cl 5 mM for 6 h → intracellular Ca^{2+} ↑ ~1.17-fold ↑[#], $p \leq 0.01$ - ammonium increases cytosolic and mitochondrial levels of Zn^{2+}in response to an ammonium induced Ca^{2+} and NMDA receptor-dependent NO synthesis - ROS/RNS ↑ in cultured rat brain cells 3 min after addition of NH_4Cl 5 mM → astrocytes ~1.08-fold ↑[#], $p \leq 0.05$ and neurons ~1.09-fold ↑[#], $p \leq 0.05$	[45] [114]
Rat – **hyperammonemia** - NH4CH3CO2 (4.5 mmol/kg) (coma in ~15 min) **Astrocytes/microglia -** NH_4Cl (0.25–5 mM)	**In-vitro:** cultured rat **astrocytes/microglia** - from the **cortices** of cerebral hemispheres of newborn Wistar rats **Ex-Vivo:** brain slices (**cortex**)	Fluorescence microscopy Western Blot	ROS production/ inhibition iNOS	*Acute ammonium intoxication* - microglia ROS ↑: time/dose-dependent manner - basal/control microglia ROS – not reported - microglia ROS ↑ NH_4Cl 1 mM vs. 5 mM (a.u.): 3 h - 0.99 ± 0.05 vs. 1.12 ± 0.01, 6 h–1.36 ± 0.02 vs. 1.67 ± 0.05, 20 h–1.56 ± 0.15 vs. 2.92 ± 0.05, $p \leq 0.05$ - ammonium up-regulates ionized Ca^{2+}-binding adaptor protein-1 - pretreatment of microglia with apocynin completely abolished the ammonium induced ROS increase → activation of NADPH-oxidase by ammonium - oxidative stress ↑ is due to ROS ↑, but not RNS - iNOS ↑ in cultured astrocytes - iNOS was not affected by ammonium acetate treatment in vivo in the cerebral cortex	[118]

HA – hyperammonemia.

[#] Values approximation – estimation from graphs.

Table 4
Summary of some published work highlighting the presence of oxidative stress in type B and C HE animal models.

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
Wistar rats, weighing 250–320 g - **BDL** – 5- and 10-days post-op Plasma ammonium: NA	**Ex-vivo: cerebral cortex** from the frontal lobe, **midbrain**, and the **cerebellum**	UV–Vis spectroscopy enzymatic activity	Thiol Redox State: GSH, GSSG Lipid Peroxidation (MDA)	**Cortex** (μmol TRS component/gram protein): GSH ↓: Sham 7.25 ± 0.35 vs. 5d 5.16 ± 0.28 vs. 10d 2.13 ± 0.45, $p < 0.05$; GSSG ↑: Sham 0.34 ± 0.02 vs. 5d 0.61 ± 0.09 vs. 10d 0.20 ± 0.02, $p < 0.05$; and the GSH:GSSG ratio ↓ ~50% - lipid peroxidation (pmol MDA/mg protein) ↑: Sham 0.130 ± 0.05 vs. 5d 0.134 ± 0.00 vs. 10d 0.246 ± 0.02, $p < 0.05$ **Midbrain** (μmol TRS component/gram protein): GSH ↓: Sham 3.13 ± 0.13 vs. 5d 2.85 ± 0.29 vs. 10d 1.59 ± 0.28, $p < 0.05$; GSSG: Sham 0.23 ± 0.01 vs. 5d 0.39 ± 0.06 vs. 10d 0.25 ± 0.15, $p < 0.05$; GSH:GSSG ratio ↓~46% - lipid peroxidation (pmol MDA/mg protein) ↑: Sham 0.34 ± 0.02 vs. 5d 0.61 ± 0.09 vs. 10d 0.20 ± 0.2, $p < 0.05$ **Cerebellum** (μmol TRS component/gram protein): GSH: Sham 3.63 ± 0.41 vs. 5d ↑ 5.04 ± 0.36 vs. 10d ↓ 1.32 ± 0.23, $p < 0.05$; GSSG Sham 0.25 ± 0.05 vs. 5d ↑ 0.73 ± 0.10 vs. 10d ↓ 0.18 ± 0.3, $p < 0.05$; GSH:GSSG ratio ↓ ~42% - lipid peroxidation (pmol MDA/mg protein) ↑: Sham 0.122 ± 0.00 vs. 5d 0.141 ± 0.01 vs. 10d 0.277 ± 0.02, $p < 0.05$ **Brain regional difference: control/Sham GSH value was higher by 50% in the cortex as opposed to cerebellum and midbrain**	[59]
Rats - **ammonium-fed** (weight 230–280 g) Plasma ammonium*: 200.1 ± 16.7 μM	**In-vitro: neutrophils**	Flow cytometry	Systemic OS	- spontaneous oxidative burst ↑ in neutrophils (%): control 8 ± 1.9 vs. ammonium-fed 31 ± 5.3, $p < 0.001$	[132]
PCA rat model - 6 weeks post-op Plasma ammonium*: 312 ± 32.0 μM	**Ex-vivo:** brain tissue extracts	HPLC Western blot	ROS Lipid peroxidation (MDA)	- brain ROS ↑: 2.5-fold - lipid peroxidation ↑ (μmol/mg protein): Sham 0.020 ± 0.010 vs. PCA 0.060 ± 0.020, $p < 0.01$ - protein oxidation ↑ (nmol/mg protein): Sham 2.7 ± 0.4 vs. PCA 3.9 ± 0.4, $p < 0.01$	[131]
Two rat models of HE: **PCA** at week 4, and **BDL** at 6 weeks post-op Plasma ammonium PCA*: 142.3 ± 12.0 μM CSF ammonium PCA*: 142.3 ± 12.0 μM	**Ex-vivo:** tissue extracts - **frontal cortex** In-vitro: **plasma**	UV–Vis and fluorescence spectroscopy	ROS H_2O_2 XO SSAO MAO A + B CAT SOD GR GPX GSH/GSSG Lipid peroxidation (MDA)	**BDL** **Plasma:** - ROS↑(RFU): Sham 0.15 ± 0.04 vs. BDL 5.49 ± 1.93, ($p < 0.001$) - H_2O_2↑ 2.4-fold, (μM): Sham 3.35 ± 0.53 vs. BDL 8.02 ± 1.20, ($p < 0.001$) - CAT ↓ 40% → increased activity of XO, (U/mL): Sham 323.4 ± 56.2 vs. BDL 126.5 ± 19.7, ($p < 0.01$) - lipids peroxidation ↑ (μM): Sham 9.87 ± 0.62 vs. BDL 41.91 ± 7.40, ($p < 0.001$) - antioxidant defense ↓ - GSH ↓ ~44% (mM): Sham 1.37 ± 0.04 vs. BDL 0.77 ± 0.09, ($p < 0.001$) - GSH/GSSG ratio ↓ 2.3-fold ($p < 0.05$) - GR ↑ 4.5-fold, (mU/mL): Sham 9.23 ± 1.02 vs. BDL 42.2 ± 8.94, ($p < 0.001$) - XO ↑(mU/mL): Sham 6.46 ± 0.75 vs. BDL 30.47 ± 5.04, ($p < 0.001$) **Brain:** - GR ↑ 2-fold (mU/100 μg protein): Sham 0.39 ± 0.03 vs. BDL 0.79 ± 0.03, ($p < 0.001$) **PCA:** - brain GR ↑ 2.6-fold, (mU/100 μg protein): Sham 0.33 ± 0.02 vs. PCA 0.88 ± 0.08, ($p < 0.001$) - no significant difference in plasma and brain ROS and XO, CAT levels was found between PCA and PCA-sham rats or in CSF ROS levels - no significant difference in plasma GSH and GSH/GSSG ratio	[129] [130]

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Table 4 (*continued*)

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
				- no significant differences in levels of lipid peroxidation **Both:** - SOD activity, in both plasma and frontal cortex, showed no significant difference in either of the experimental groups - plasma GR activity ↑ only in of BDL - frontal cortex GR activity ↑ in both BDL and PCA - No changes in GP and MAO A + B activity between the two experimental groups - in both plasma and frontal cortex.	
Male Wistar rats weighing between 220 and 250 g – **BDL -** 4 weeks post-op Plasma ammonium[*]: 194.45 ± 4.89 μM	**Ex-vivo:** mitochondria – 4w post-op, brains were removed - **cortex**, **hippocampus**, **striatum**, and **cerebellum** - homogenized	UV–Vis and fluorescence spectroscopy	NADH Succinate Dehydrogenase Cytochrome *c* Oxidase Lipid peroxidation (MDA) Protein carbonyls ROS GSH/GSSG	**Mitochondria** **Cortex** - ROS ↑, (pmol DCF/mg protein)[#]: Sham 1.69 ± 0.12 vs. BDL 4.31 ± 0.37, $p < 0.05$ - lipid peroxidation ↑, (nmol of MDA/mg protein)[#]: Sham 29.54 ± 1.23 vs. BDL 49.85 ± 0.61, $p < 0.05$ - protein carbonyls ↑, (nmol/min/mg protein)[#]: Sham 105.97 ± 12.46 vs. BDL 227.53 ± 9.35, $p < 0.05$ - GSH ↓ 43%, (nmol/mg protein): Sham 0.30 ± 0.03 vs. BDL 0.17 ± 0.02, $p < 0.05$ - GSSG ↑ 100%, (nmol/mg protein): Sham 0.02 ± 0.002 vs. 0.04 ± 0.004, $p < 0.05$ - GSH/GSSG ↓ 67%, Sham 11.80 ± 1.35 vs. BDL 3.91 ± 0.25, $p < 0.05$ - NADH dehydrogenase ↓ 26%, (nmol NADH oxidized/min/mg protein): Sham 50.53 ± 2.74 vs. BDL 37.11 ± 4.78, $p < 0.05$ - Succinate dehydrogenase ↓ 46%, (nmol succinate oxidized/min/mg protein): Sham 24.70 ± 1.42 vs. BDL 13.40 ± 1.11, $p < 0.05$ - Cytochrome *c* oxidase ↓ 71%, (nmol cytochrome *c* oxidized/min/mg protein): Sham 4.81 ± 0.44 vs. BDL 1.40 ± 0.43, $p < 0.05$ **Hippocampus** - ROS ↑, (pmol DCF/mg protein)[#]: Sham 1.12 ± 0.087 vs. BDL 4.56 ± 0.44, $p < 0.05$ - lipid peroxidation ↑, (nmol of MDA/mg protein)[#]: Sham 25.85 ± 2.46 vs. BDL 45.53 ± 2.46, $p < 0.05$ - protein carbonyls ↑, (nmol/min/mg protein): Sham 65.45 ± 4.36 vs. BDL 143.38 ± 12.45, $p < 0.05$ - GSH ↓ 41%, (nmol/mg protein): Sham 0.29 ± 0.03 vs. BDL 0.17 ± 0.01, $p < 0.05$ - GSSG ↑ 150%, (nmol/mg protein): Sham 0.02 ± 0.002 vs. BDL 0.05 ± 0.003, $p < 0.05$ - GSH/GSSG ↓ 79%, Sham 16.45 ± 3.83 vs. BDL 3.45 ± 0.40, $p < 0.05$ - NADH dehydrogenase ↓ 44%, (nmol NADH oxidized/min/mg protein): Sham 60.33 ± 2.74 vs. BDL 33.58 ± 1.56, $p < 0.05$ - Succinate dehydrogenase ↓ 33%, (nmol succinate oxidized/min/mg protein): Sham 13.34 ± 1.61 vs. BDL 8.91 ± 1.13, $p < 0.05$ - Cytochrome *c* oxidase ↓ 49%,(nmol cytochrome *c* oxidized/min/mg protein): Sham 3.67 ± 0.50 vs. BDL 3.67 ± 0.50, $p < 0.05$ **Striatum** - ROS ↑, (pmol DCF/mg protein)[#]: Sham 1.56 ± 0.19 vs. BDL 4.56 ± 0.25, $p < 0.05$ - lipid peroxidation ↑, (nmol of MDA/mg protein)[#]: Sham 24 ± 2.46 vs. BDL 49.23 ± 2.46, $p < 0.05$ - protein carbonyls ↑, (nmol/min/mg protein)[#]: Sham 81.03 ± 3.12 vs. BDL 202.6 ± 28.05, $p < 0.05$	[127]

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Table 4 (*continued*)

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
				- GSH ↓ 27%, (nmol/mg protein): Sham 0.26 ± 0.02 vs. BDL 0.19 ± 0.01, $p < 0.05$ - GSSG ↑ 150%, (nmol/mg protein): Sham 0.02 ± 0.002 vs. BDL 0.05 ± 0.004, $p < 0.05$ - GSH/GSSG ↓ 70%, Sham 11.86 ± 1.46 vs. BDL 3.59 ± 0.38, $p < 0.05$ - NADH dehydrogenase ↓ 35%, (nmol NADH oxidized/min/mg protein): Sham 44.95 ± 2.23 vs. BDL 29.21 ± 5.18, $p < 0.05$ - Succinate dehydrogenase ↓ 53%, (nmol succinate oxidized/min/mg protein): Sham 20.23 ± 2.56 vs. BDL 9.42 ± 1.92, $p < 0.05$ - Cytochrome *c* oxidase ↓ 64%, (nmol cytochrome *c* oxidized/min/mg protein): Sham 3.97 ± 0.54 vs. BDL 1.41 ± 0.51, $p < 0.05$ **Cerebellum** - ROS ↑, (pmol DCF/mg protein)[#]: Sham 1.56 ± 0.12 vs. BDL 5.19 ± 0.25, $p < 0.05$ - lipid peroxidation ↑, (nmol of MDA/mg protein)[#]: Sham 28.92 ± 0.61 vs. BDL 52.92 ± 3.69, $p < 0.05$ - protein carbonyls ↑, (nmol/min/mg protein)[#]: Sham 118.44 ± 1.87 vs. BDL 183.9 ± 15.58, $p < 0.05$ - GSH ↓ 38%, (nmol/mg protein): Sham 0.37 ± 0.02 vs. BDL 0.23 ± 0.01$p < 0.05$ - GSSG ↑ 67%, (nmol/mg protein): Sham 0.03 ± 0.002 vs. BDL 0.05 ± 0.002, $p < 0.05$ - GSH/GSSG ↓ 63%, Sham 12.62 ± 1.14 vs. BDL 4.60 ± 0.44, $p < 0.05$ - NADH dehydrogenase ↓ 43%, (nmol NADH oxidized/min/mg protein): Sham 60.07 ± 7.89 vs. BDL 34.35 ± 1.54, $p < 0.05$ - Succinate dehydrogenase ↓ 51%, (nmol succinate oxidized/min/mg protein): Sham 32.79 ± 1.87 vs. BDL 15.88 ± 1.44, $p < 0.05$ - Cytochrome *c* oxidase ↓64%, (nmol cytochrome *c* oxidized/min/mg protein): Sham 6.11 ± 0.83 vs. BDL 2.19 ± 0.61, $p < 0.05$	
Male Wistar rats weighing between 220 and 250 g – **BDL** Plasma ammonium[*]: 133 ± 75 μM	**In vivo:** BDL – 2-, 4-, 6- and 8-weeks post-op - **hippocampus**	^{1}H-MRS	Asc	**Hippocampus** - Asc ↓ → significant decrease already at 4 weeks post-BDL, (mmol/kg ww): week-0 2.76 ± 0.36 vs. week-2 2.61 ± 0.34 vs. week-4 vs. 2.43 ± 0.43 ($p < 0.05$) vs. week-6 2.27 ± 0.51 ($p < 0.001$) vs. week-8 2.35 ± 0.47 ($p < 0.001$) - Asc ↓ over time correlated mainly with the rise in plasma ammonium	[13]
Male Wistar rats weighing between 220 and 250 g – **BDL** Plasma ammonium[*]: 88.2 ± 40.8 μM	BDL – 2-, 4-, 6- and 8-weeks post-op **Ex-vivo:** brain tissue - **cortex**, **hippocampus**, **striatum**, **thalamus,** and **cerebellum** **In-vitro: whole blood** and **neutrophils**	ESR UV–Vis and fluorescence Spectroscopy Epifluorescence microscopy - IHC	$O_2^{\cdot-}$ SOD1 SOD2 GPX-1 NBT RNA oxidation	**CNS OS is an early event in type C – 2 weeks post-op** **CNS OS precede systemic OS** - CNS OS ↑ is due to enhanced formation of intra- and extra-cellular ROS rather than due to reduced antioxidant capacity - a pattern of intracellular $O_2^{\cdot-}$ increase in SHAM rats confirms a substantial increase of OS with age **Hippocampus** - $O_2^{\cdot-}$ production ↑, (μmol/g/min): week-2 Sham 39.68 ± 11.31 vs. BDL 64.82 ± 13.34 (+63%, $p < 0.01$); week-4 Sham 66.84 ± 15.19 vs. 96.05 ± 22.98 BDL (+43%, $p < 0.001$); week-6 Sham 73.49 ± 16.31 vs. BDL 103.64 ± 19.09 (+41%, $p < 0.01$); week-8 Sham 81.51 ± 7.29 vs. BDL 111.01 ± 26.07 (+36%, $p < 0.05$) - SOD1 ↑ - granular layer: week-4 +87.36% ($p < 0.001$), week-8 +114.41% ($p < 0.001$)	[2]

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Table 4 (*continued*)

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
				- SOD1 ↑ - hilus: week-4 +165.02% ($p < 0.001$), week-8 +207.42% ($p < 0.001$) - SOD2 - granular layer: week-4 +12.65% ($p < 0.001$), week-8 +14.93% ($p < 0.001$) - SOD2 ↑ - hilus: week-4 +4.9% (ns), week-8 +50.91% ($p < 0.001$) - Oxo-8-dG ↑ granular layer: week-8 +213.87% ($p < 0.001$) - Oxo-8-dG ↑ hilus: week-8 +153.06% ($p < 0.001$) - GPX-1 ↑ granular layer: week-8 +116.39% ($p < 0.001$) - GPX-1 ↑ hilus: week-8 +95.75% ($p < 0.001$) **Cerebellum** - $O_2^{\cdot -}$ production ↑, (μmol/g/min): week-6 Sham 96.2 ± 27.4 vs. BDL 136.8 ± 22.7 ± 19.09 (+42%, $p < 0.001$) - SOD1 ↑ - granular layer: week-4 +33.69% ($p < 0.001$), week-8 +15.64% ($p < 0.001$) - SOD1 ↑ - Pj cells: week-4 +451.92% ($p < 0.001$), week-8 +94.69% ($p < 0.001$) - SOD2 - granular layer: ns - SOD2 ↑ - Pj cells: week-4 +41.85% ($p < 0.001$), week-8 +34.74% ($p < 0.001$) - Oxo-8-dG ↑ granular layer: week-8 +177.25% ($p < 0.001$) - Oxo-8-dG ↑ Pj cells: week-8 +153.06% ($p < 0.001$) - Oxo-8-dG ↑ DN: week-8 +73.21% ($p < 0.001$) - GPX-1 ↑ granular layer: week-8 +84.85% ($p < 0.001$) - GPX-1 ↑ Pj cells: week-8 +91.72% ($p < 0.001$) - GPX-1 ↑ DN: week-8 +96.56% ($p < 0.001$) **Cortex** - SOD1 ↑: week-4 +99.05% ($p < 0.001$), week-8 +249.31% ($p < 0.001$) - SOD2 ↑: week-4 +22.69% ($p < 0.001$), week-8 +31.31% ($p < 0.001$) **Striatum** - SOD1 ↑: week-4 +66.29% ($p < 0.001$), week-8 +93.55% ($p < 0.001$) - SOD2 ↑: week-4 +44.40% ($p < 0.001$), week-8 +96.99% ($p < 0.001$) **Thalamus** - SOD1 ↑: week-4 +73.71% ($p < 0.001$), week-8 +115.62% ($p < 0.001$) - SOD2 ↑: week-4 -4.81% (ns), week-8 +41.35% ($p < 0.001$) - **Protective role of SOD1 – translocation into the nucleus** **Blood** -Systemic OS ↑ at week 4 post-BDL - $O_2^{\cdot -}$ production ↑ → Resting oxidative burst ↑ - $O_2^{\cdot -}$ production in blood↑ (μmol/mL/min): Sham 11.07 ± 0.74 vs. week-2 12.85 ± 2.58 (ns) vs. week-4 13.44 ± 2.89 (ns) vs. week-6 16.52 ± 2.89 ($p < 0.01$) vs. week-8 17.52 ± 3.38 ($p < 0.01$) - $O_2^{\cdot -}$ production ↑ at week-8 post-BDL by peripheral PMN ~300%, ($p < 0.001$), LYM ~112%, ($p < 0.006$) compromise their functions → continuous neutrophil activation cause hypo-responsivity → phagocytic capacity ↓	
Male Sprague-Dawley rats weighing between 300 and 350 g – **BDL -** 3 weeks post-op Plasma ammonium*: 194.45 ± 4.89 μM	BDL – 3-weeks post-op **Ex-vivo: brain, heart, kidney** **In-vitro: plasma**	UV–Vis spectroscopy enzymatic activity	Lipid peroxidation (MDA) and lipid peroxides (LP) Conjugated dienes (CD) GSH, GSSG	**Plasma:** # - GSSG ↓, (μM): BDL 0.76 ± 0.16 vs. Sham 2.42 ± 0.38, $p < 0.005$ - GSH ↓, (μM): BDL 3.48 ± 0.47 vs. Sham 7.10 ± 1.07, $p < 0.005$	[133]

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Table 4 (*continued*)

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
				- GSSG/GSH, BDL 0.26 ± 0.04 vs Sham 0.22 ± 0.05, ns **Brain:** [#] CD (nmol/mg protein): BDL 4.74 ± 0.26 vs. Sham 4.49 ± 0.26, ns LP↓ (nmol/mg protein): BDL 1.45 ± 0.14 vs. Sham 2.07 ± 0.286, ns MDA↑(nmol/mg protein): BDL 0.6 ± 0.08 vs. Sham 0.3 ± 0.02, $p < 0.001$ **Heart:** [#] CD (nmol/mg protein): BDL 0.73 ± 0.15 vs. Sham 0.66 ± 0.14, ns LP↑ (nmol/mg protein): BDL 0.32 ± 0.07 vs. Sham 0.21 ± 0.04, $p < 0.05$ MDA↑(nmol/mg protein): BDL 0.09 ± 0.005 vs. Sham 0.06 ± 0.003, $p < 0.01$ **Kidney:** [#] CD↑ (nmol/mg protein): BDL 0.68 ± 0.03 vs. Sham 0.38 ± 0.04, $p < 0.05$ LP↑ (nmol/mg protein): BDL 1.43 ± 0.33 vs. Sham 0.63 ± 0.66, $p < 0.05$ MDA↑(nmol/mg protein): BDL 0.6 ± 0.06 vs. Sham 0.33 ± 0.03, $p < 0.05$	

[*] Ammonium measured before sacrifice.
[#] Values approximation – estimation from graphs, DN - dentate nucleus.

In-vitro studies of cultured astrocytes exposed to Gln revealed an increase in OS [165]. Increased ammonium and Gln concentration together with Asc decline correlated significantly with OS increase in BDL rats [2,13]. In addition, the same study demostrated an elevated $O_2^{\cdot -}$ production and overexpression of SOD1 and SOD2 in CNS accompanied by SOD1 nuclear translocation to protect the genomic DNA [2]. Similarly, an increase of SOD expression was found in postmortem brain tissue of HE patients [112]. Increased IL-6 expression in activated microglia and astrocytes [2] can trigger the proinflammatory cascade as well as increased ROS formation [34,37,49,53,118,166]. SOD2 overexpression [2,112] acts as a switch to modulate microglial activation/inactivation during inflammation [167]. Increase of $O_2^{\cdot -}$ production and overexpression of SOD will lead to an increase of H_2O_2 concentrations having a negative impact on LTP [7,168,169].

OS and inflammation are strongly linked and interdependent pathophysiological processes [47,170]. In the presence of OS, inflammatory processes will occur, hence assisting in the progression of OS. In parallel, if inflammation is the trigger, an OS response will be generated, contributing to the immune response [48,49]. Previous studies have demonstrated the synergistic participation of CNS OS which precedes the systemic OS [2], and inflammation in the progression of HE [2,96,135,138].

Bacterial infection and especially spontaneous bacterial peritonitis is a frequent precipitating factor in HE that contributes to the systemic inflammation [171,172]. Leukocytosis, a hallmark of systemic inflammation and indirect sign of OS, associated with an increased risk of mortality, was identified in both, the BDL rat model [2] and in HE patients [173,174]. Accelerated immune response (increased levels of TNF-, IL-18, IL-6) [24,36] and the oxidative metabolism/oxidative burst of phagocytic cells (neutrophil dysfunction and increased ROS emission) [2,33,129] at the inflamed site will advance tissue injury and immunopathology [175,176]. Furthermore, studies have indicated that high levels of IL-6 have a deleterious impact on the BBB integrity [38]. As a result, high levels of IL-6 in both the peripheral [36] and CNS [2] may increase the BBB permeability, allowing neurotoxic components (ROS/RNS, cytokines, NH_4^+, bile acids, bilirubin) to enter the brain and affect neurological functions.

Bile acids have the potential and ability of altering the gut microbiota, resulting in modifications of the total bile acid pool [177,178]. Increased bile acid concentrations have been shown to disrupt tight junctions, permeabilize the BBB via detergent-like cytolytic actions on cell membranes, gain access to the CNS, and contribute to neurological decline [179,180]. Bile acids have been also recognized as a pro-oxidants causing ROS release (interrupt electron transport chain at complex III), which may lead to depletion of antioxidants, thiol groups oxidation and lipid peroxidation [181,182]. Furthermore, bile acids may cause indirectly increase of OS through resident macrophages activation/oxidative burst [183].

The enteric nervous system connects the gut microbiome to the CNS and acts as a key communication route for the gut-liver-brain axis mediated by the activity of the vagal nerves [184]. Therefore, bacterial infection and systemic inflammation may also impact brain function through afferent vagal nerves activation by cytokines/chemokines release at the inflammatory sites [185,186] and impact the cognitive and motor functions [184]. Therefore, OS being related to innate inflammation is a common denominator and therapeutic target for many neurodegenerative disorders associated with cognitive deficits [11,50–53].

Serum albumin accounts for a significant component of total extracellular antioxidant capacity (~70%). The reduced cysteine residue (Cys34) and a thiol group in serum albumin allow to scavenge the $HO^{\cdot}$ and $ONOO^-$ respectively [16,24,171,187–190]. The hypoalbuminemia was shown to increase the mortality risk in HE patients [17–21]. Therefore, a significant decrease of plasma albumin concentrations, an important extracellular antioxidant, and increase in the percentage of oxidized albumin [25,26,140,190] in HE patients will lead to decreased systemic antioxidative capacity and contribute to increase of OS. Furthermore, albumin binds several molecules reversibly, allowing for solubilization and transport (*i.e.*, bilirubin, bile acids, hormones, and endotoxins), controls the immune system, and protects the endothelium [16,24,171,187–190]. In liver cirrhosis bilirubin and bile acids concentrations rise [191,192], while binding capacity of albumin is impaired [140,190,193]. Therefore, decreased albumin synthesis by the diseased liver together with decreased binding capacity will decrease toxin clearance [190], increase of lipid peroxidation [171] and therefore lead to increase of OS, both systemic and CNS, and patient clinical status deterioration.

7. Conclusions

Taken together, the presented findings show that OS is a critical

Table 5
Summary of some published work highlighting the presence of oxidative stress in humans with chronic liver disease and chronic liver disease associated HE or at risk to develop HE. In these studies, HE has been assessed by standardized battery of neuropsychological tests.

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
NAFLD patients (steatosis (S) and steatohepatitis (SH)) (age 18–55 y) Plasma ammonium: NA	**Liver** biopsies $2cm^3$/liver homogenates and plasma	UV-VIS Spectroscopy	SOD CAT GPX GSH Protein carbonyls	**Liver** - protein carbonyls ↑, (nmol carbonyls/mg protein)[#]: S 4.88 ± 0.96 (+403%) and SH 1.77 ± 1.12 (188%) vs. healthy 1.04 ± 0.18, $p < 0.05$ - 1280% increase in the ratio of hepatic protein carbonyl/GSH content (nmol carbonyls/nmol): S 0.48 ± 0.10 vs. control 0.0375 ± 0.007, $p < 0.05$ - SOD ↓ (U/mg protein)[#]: S 12.9 ± 1.49 (−48%) and SH 8.68 ± 0.5 (−64%) vs. healthy 24.8 ± 1.49, $p < 0.05$ - CAT ↓ (U/mg protein)[#]: S 0.56 ± 0.054 and SH 0.32 ± 0.04 (−48%) vs. healthy 0.62 ± 0.01, $p < 0.05$ - GSH ↓ (nmol/mg protein)[#]: S 11.65 ± 2.28 (+57%) and SH 0.32 ± 0.04 (+27%) vs. healthy 19.1 ± 1.49, $p < 0.05$ - GPX (nmol/mg protein)[#]: S 0.084 ± 0.011 vs. SH 0.067 ± 0.06 vs. healthy 0.08 ± 0.03, ns **Plasma** - total antioxidant capacity of plasma ↓: S 310.1 ± 9.3 (−21%) and SH 263.6 ± 6.2 (−33%) vs. control 393.8 ± 12.4 - activity of plasma GSH ↓57%, SOD ↓ 48% and ferric reducing ability of plasma ↓ 21%, ($p < 0.05$)	[14]
Postmortem Brain Tissue - **liver cirrhosis and HE** Plasma ammonium: NA	**Ex-vivo: Brain** -intersection parietal to occipital **cortex** (17 ±8 h (5–35 h))	IHC - confocal laser-scanning Microscopy Western blot	RNA oxidation NOS SOD	- HE but not cirrhosis triggers oxidative stress in the cerebral cortex - iNOS mRNA levels in the cerebral cortex → not significantly different between and patients with cirrhosis - Oxidized RNA ↑ - 2-fold increase vs. control and a small heat shock protein-27 (Hsp27) ↑ - 4-fold increase vs. control → marker for oxidative stress - neuronal nNOS ↑ and SOD ↑ in the cerebral cortex – non significantly - GS activity ↓, but not the GS protein level	[112]
Neutrophils from patients blood (age 55.3 ± 7.5y) Plasma ammonium: 74 ± 6 μM	**In-vitro: neutrophils** Whole blood incubated with 75 μM NH_4Cl for 90 min and then **neutrophils** were isolated	Flow cytometry	Oxidative burst	- Spontaneous oxidative burst activity ↑ in neutrophils incubated with 75 μM ammonium chloride → +10.7 ± 1.8% ($p < 0.0005$)	[132]
Patients-**hepatogenic coma** (male, age 67 -biliary obstruction; male, age 42 -alcoholic cirrhosis) Plasma ammonium: NA	Ex-vivo**: Brain** tissue postmortem **- lenticular nuclei**	Electron microscopy	Lipid peroxidation	- Lipid peroxidation ↑ - excess of lipofuscin aggregates accumulated in astrocytes	[142]
Patients - **liver cirrhosis** (alcoholic) (age 50y (range 25–65)) Plasma ammonium: NA	**In-vitro: blood** samples – serum		CAT GPX Lipid peroxidation	- CAT ↓ (pmol/mg protein): CLD 1.5 ± 0.2 vs. healthy 2.2 ± 0.2, $p < 0.032$ - GPX ↓ (nmol/mg protein): CLD 0.011 ± 0.002 vs. healthy 0.020 ± 0.003, $p < 0.04$ - Lipid peroxidation ↑ (nmol/mg protein): CLD 0.074 ± 0.007 vs. healthy 0.046 ± 0.005, $p < 0.035$ - proteins content (Apo A1 and Apo B_{100})↓ (mg/mL): CLD 200 ± 88 vs. healthy 415 ± 64, $p < 0.0024$ OS plays a critical negative role in the process of lipid liver metabolism, causing liver injury	[134]
Patients - **liver cirrhosis and HE** with high and normal systemic ammonemia (age 56.0 ± 10.4 y) Plasma ammonium: 35 ± 4 μM	**In-vitro: blood** samples – serum	UV-VIS Spectroscopy	SOD GPX Lipid peroxidation (MDA)	- Oxidative stress ↑ in HE → significant decrease of serum antioxidant enzymatic activity - SOD ↓: healthy 1.35 ± 0.08 vs. CLD 0.90 ± 0.08 U/mL - GPX ↓: healthy 0.093 ± 0.06 vs. CLD 0.006 ± 0.008 U/mL - Lipid peroxidation ↑: healthy 35.94 ± 1.37 vs. CLD 68.90 ± 5.68 nmol/mL - Systemic ammonemia ↑ in HE associated with the present antioxidant enzymes activity ↓ - Compensatory increase in the activity of SOD and GPX in high ammonemia, as compared with HE patients bearing normal ammonemia	[139]

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Table 5 (*continued*)

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
Patients - **liver cirrhosis** (alcoholic (A) and viral (V)) (Age 38–75 y) Plasma ammonium: NA	**In-vitro: blood** – plasma/ serum and blood cell fractions were separated	UV-VIS Spectroscopy	Lipid peroxidation (MDA) Protein carbonylation TAC	- Oxidative stress ↑ - Protein carbonylation ↑(nmol carbonyls/mg protein)[#]: A 0.23 ± 0.05 vs. V 0.14 ± 0.012, $p < 0.05$ - Lipid peroxidation ↑ (μM/mL)[#]: A 0.77 ± 0.06 ($p < 0.01$ vs. healthy) and V 0.57 ± 0.04 ($p < 0.05$ vs. A) vs. healthy 0.48 ± 0.05 - No significant differences were noted in plasma total antioxidant capacity	[135]
Patients - **liver cirrhosis** and HE (non-alcoholic and alcoholic) (age 59 ± 6 y) Plasma ammonium: 95–345 μM	**In-vitro: blood** – plasma	UV-VIS Spectroscopy Fluorescence spectroscopy	Lipid peroxidation (MDA) Protein carbonylation NBT Tyrosine dimers	- oxidative stress ↑ - lipid peroxidation ↑(μmol/L): CLD 16.69 ± 6.19 vs. healthy 6.58 ± 3.11 - protein carbonylation ↑ (nmol/mg protein): CLD 3.93 ± 1.73 vs. healthy 0.75 ± 0.38 - formazan formation ↑(nmol/mg protein): CLD 9.07 ± 2.24 vs. healthy 4.93 ± 0.73 - dityrosines↑(pmol/mg protein): CLD 393 ± 133 vs. healthy 126 ± 47	[137]
Patients with **cirrhosis** and **alcoholic hepatitis (AH)** (Age 50.3 ± 1.3y) Plasma ammonium: NA	**In-vitro: blood**, **neutrophils** in a whole blood or after isolation	UV–Vis spectroscopy Fluorescence-activated cell sorting	Oxidative burst Lipid peroxidation (MDA) Albumin	- resting oxidative burst ↑, indicating neutrophil activation,(%)[#]: CLD 32.7 ± 1.2 and CLD + AH ± 7.58 ($p < 0.001$ vs. healthy and CLD) vs. healthy 10.6 ± 1.52 - resting oxidative burst → predictive of 90-day mortality ($p < 0.005$) and organ failure ($p < 0.001$) - resting burst of >55% → sensitivity of 77% and specificity of 69% for predicting death - phagocytic capacity ↓ → predictive of death ($p < 0.05$) and organ failure ($p < 0.0001$) - phagocytic capacity <42% of normal → sensitivity of 86% and specificity of 70% to predict mortality - presence of a transmissible factor in patients plasma promoting neutrophil activation - albumin ↓ (g/L): CLD 37.12 ± 5.3 and CLD + AH 27.6 ± 1.4 ($p < 0.001$ vs. healthy) vs. healthy 33.9 ± 1.3 - Lipid peroxidation ↑ (μM/mL): CLD 2.7 ± 0.5 and CLD + AH 4.3 ± 0.8 vs. healthy 2.3 ± 0.5, $p < 0.05$	[136]
Patients – **chronic NALD** (age 37.61 ± 2 y) Plasma ammonium: NA	**Blood** - RBC	UV-VIS Spectroscopy	SOD	- No significant differences were noted	[146]
Patients with **MHE** (Age 63 ± 10 y) Plasma ammonium: 117 μM	**In-vitro: blood** samples – serum	UV-VIS Spectroscopy	GSH GSSG/GSH GPX Protein carbonylation RNA/DNA oxidation Lipid peroxidation (MDA)	- Oxidative stress ↑ - Protein carbonylation ↑(nmol/mg protein): MHE 0.186 ± 0.013 vs. healthy 0.150 ± 0.008 - Lipid peroxidation ↑ (nmol/mL): MHE 16.69 ± 6.19 vs. healthy 0.20 ± 0.01 - GPX and GR activity↑ - GSH ↓ (μM): MHE 427 ± 36 vs. healthy 794 ± 49 - GSSG (μM): MHE 427 ± 36 vs. healthy 27 ± 3 - GSSG/GSH ↑ (%): MHE 25 ± 2 vs. healthy 4.7 ± 0.5 - RNA/DNA oxydation - Oxo-8-dG ↑(ngl/mL): MHE 12 ± 0.7 vs. healthy 9.2 ± 0.4 - 3-NTyr ↑ (nM): MHE 24 ± 4 vs. healthy 7 ± 0.6	[138]
Patients with **cirrhosis** (Age 54 ± 12y) Plasma ammonium: NA	**In-vitro: blood** samples – plasma	Chromatography Mass spectrometry	Albumin: HMA, HNA1, HNA2	- non-oxidized human mercaptalbumin – HMA (g/dL): CLD 3.2 ± 0.6 vs. healthy 4.8 ± 0.2 - HMA fraction (%) ↓: CLD 46 ± 13 vs. healthy 65 ± 4 - reversibly oxidized human non-mercaptalbumin-1 (HNA1) fraction (%) ↑: CLD 44 ± 12 vs. healthy 31 ± 4 - irreversibly oxidized human non-mercaptalbumin-2 (HNA2) fraction (%) ↑: CLD 9 ± 4 vs. healthy 4 ± 1 - HNA2 levels >12% → CLD 30-day mortality 24% and CLD 90-day mortality 33%	[140]
Patients with **cirrhosis** (Age 49.29 ± 9.48 y) Plasma ammonium: 35.53 ± 10.09 μM	**In-vivo: MRI** **In-vitro: blood** samples – serum	MRI - voxel-based morphometry (VBM)	Grey matter volume (GMV) Lipid peroxidation - serum MDA Albumin	- lipid peroxidation ↑ (nmol/mL): CLD 0.36 ± 0.04 vs. healthy 0.17 ± 0.04, $p < 0.001$ - normalized GMV ↓: CLD 0.43 ± 0.02 vs. healthy 0.44 ± 0.01, $p = 0.042$ - increased serum MDA levels were associated with GMV loss in patients with CLD → negative correlation $r = -0.378$, $p = 0.036$	[141]

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Table 5 (*continued*)

Subject	Type of measurements	Method	Findings OS detection	Comments	Ref
				- oxidative damage may be involved in GMV loss → OS adversely affects GMV - albumin ↓ (g/L): CLD 34.85 ± 7.08vs. healthy 46.50 ± 8.35	

[#] Values approximation – estimation from graphs.

component of HE pathogenesis even at an early stage and that a lack of defense exacerbates CNS status. According to recent studies, CNS OS occurs before systemic OS, suggesting that increased BBB permeability in the latter stage of disease progression may play a substantial role and contribute significantly to the increase of ROS/RNS in the brain. Therefore, further work on cell culture, animal models and postmortem brain tissue of patients with type C HE is required to elucidate the relationship/synergy between OS, ammonium, Gln, inflammation and the pathogenesis of HE. Moreover, additional longitudinal, multi-parametric, and multimodal studies combining direct/indirect with *in-vivo/ex-vivo/in-vitro* techniques to study OS, brain regional differences in parallel with the relationship between OS, brain metabolic/functional alterations, cellular changes, and neurological manifestations in HE are needed. Because of the complexity of oxidative damage within the CNS, identifying OS biomarkers in clinical samples of HE patients is critical for a better understanding of OS-induced processes (molecular mechanisms) and to develop an appropriate diagnostic strategies.

Despite significant research on oxidative stress related damage to the cells biomolecules (RNA/DNA, lipids, and proteins) and antioxidant status in biological samples, the literature on direct measurement of OS in clinical samples is limited, and screening and surveillance for OS biomarkers are not yet routine procedures in the healthcare sector. Furthermore, sample preparation should be done with caution to ensure sample stability and to reduce the possibility of oxidative damage to tissue/cells/biomolecules during collection. Because there is no gold standard for defining redox status, complementary techniques must be used when screening for OS biomarkers to eliminate methodological biases and to obtain clinically comprehensive (diagnostic and prognostic) information with high sensitivity and specificity to pathological alterations. It is critical to conduct a comprehensive panel analysis of both pro- and antioxidants, as well as inflammation biomarkers, which should be defined by the study's goal and provide an overall redox state in specific conditions.

Authors' contributions

KP, CC and DS have participated in conceptualization and manuscript preparation and have read and approved the final manuscript.

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References

[1] S.K. Bardaweel, et al., Reactive oxygen species: the dual role in physiological and pathological conditions of the human body, Eurasian J. Med. 50 (3) (2018) 193–201.
[2] K. Pierzchala, et al., Central nervous system and systemic oxidative stress interplay with inflammation in a bile duct ligation rat model of type C hepatic encephalopathy, Free Radic. Biol. Med. 178 (2022) 295–307.
[3] J. Roy, et al., Physiological role of reactive oxygen species as promoters of natural defenses, Faseb. J. 31 (9) (2017) 3729–3745.
[4] J. C.A, P.W. A, D.T. E, Glutathione and ascorbate during ischemia and postischemic reperfusion in rat brain, J. Neurochem. 35 (5) (1980) 4.
[5] A. Phaniendra, D.B. Jestadi, L. Periyasamy, Free radicals: properties, sources, targets, and their implication in various diseases, Indian J. Clin. Biochem. 30 (1) (2015) 11–26.
[6] T. Finkel, Signal transduction by reactive oxygen species, J. Cell Biol. 194 (1) (2011) 7–15.
[7] L.T. Knapp, E. Klann, Role of reactive oxygen species in hippocampal long-term potentiation: contributory or inhibitory? J. Neurosci. Res. 70 (1) (2002) 1–7.
[8] L.T.K.E. Knapp, Potentiation of hippocampal synaptic transmission by superoxide requires the oxidative activation of protein kinase C, J. Neurosci. 22 (3) (2002) 674–683.
[9] M. Schieber, N.S. Chandel, ROS function in redox signaling and oxidative stress, Curr. Biol. 24 (10) (2014) R453–R462.
[10] D.S. D, Ammonia metabolism and the urea cycle function and clinical, J. Vet. Intern. Med. 8 (2) (1994) 73–78. Mar-Apr.
[11] M. Skowrońska, J. Albrecht, Oxidative and nitrosative stress in ammonia neurotoxicity, Neurochem. Int. 62 (5) (2013) 731–737.
[12] V. Lachmann, et al., Precipitants of hepatic encephalopathy induce rapid astrocyte swelling in an oxidative stress dependent manner, Arch. Biochem. Biophys. 536 (2) (2013) 143–151.
[13] O. Braissant, et al., Longitudinal neurometabolic changes in the hippocampus of a rat model of chronic hepatic encephalopathy, J. Hepatol. 71 (3) (2019) 505–515.
[14] L.A. Videla, et al., Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients, Clin. Sci. (Lond.) 106 (3) (2004) 261–268.
[15] M.A. Rothschild, M. Oratz, S.S. Schreiber, Albumin synthesis, Int. Rev. Physiol. 21 (1980) 249–274.
[16] M. Roche, et al., The antioxidant properties of serum albumin, FEBS Lett. 582 (13) (2008) 1783–1787.
[17] Z. Bai, et al., Association of serum albumin level with incidence and mortality of overt hepatic encephalopathy in cirrhosis during hospitalization, Therap Adv Gastroenterol 12 (2019), 1756284819881302.
[18] J. Bustamante, et al., Prognostic significance of hepatic encephalopathy in patients with cirrhosis, J. Hepatol. 30 (5) (1999) 890–895.
[19] J. Fichet, et al., Prognosis and 1-year mortality of intensive care unit patients with severe hepatic encephalopathy, J. Crit. Care 24 (3) (2009) 364–370.
[20] Y. Cui, et al., Establishment and evaluation of a model for predicting 3-month mortality in Chinese patients with hepatic encephalopathy, Metab. Brain Dis. 34 (1) (2019) 213–221.
[21] J. Cordoba, et al., Characteristics, risk factors, and mortality of cirrhotic patients hospitalized for hepatic encephalopathy with and without acute-on-chronic liver failure (ACLF), J. Hepatol. 60 (2) (2014) 275–281.
[22] P. Goldwasser, J. Feldman, Association of serum albumin and mortality risk, J. Clin. Epidemiol. 50 (6) (1997) 693–703.
[23] F.H. Yap, et al., Association of serum albumin concentration and mortality risk in critically ill patients, Anaesth. Intensive Care 30 (2) (2002) 202–207.
[24] M. Bernardi, et al., Albumin in decompensated cirrhosis: new concepts and perspectives, Gut 69 (6) (2020) 1127–1138.
[25] M. Di Pascoli, et al., Long-term administration of human albumin improves survival in patients with cirrhosis and refractory ascites, Liver Int. 39 (1) (2019) 98–105.
[26] P. Caraceni, et al., Long-term albumin administration in decompensated cirrhosis (ANSWER): an open-label randomised trial, Lancet 391 (10138) (2018) 2417–2429.
[27] R. Yirmiya, I. Goshen, Immune modulation of learning, memory, neural plasticity and neurogenesis, Brain Behav. Immun. 25 (2) (2011) 181–213.
[28] K.H. Krause, K. Bedard, NOX enzymes in immuno-inflammatory pathologies, Semin. Immunopathol. 30 (3) (2008) 193–194.
[29] M. Mittal, et al., Reactive oxygen species in inflammation and tissue injury, Antioxidants Redox Signal. 20 (7) (2014) 1126–1167.
[30] R. Pahwa, et al., Chronic inflammation, in: StatPearls. 2021 StatPearls Publishing Copyright ©, StatPearls Publishing LLC, Treasure Island (FL, 2021.
[31] J.M. Robinson, Reactive oxygen species in phagocytic leukocytes, Histochem. Cell Biol. 130 (2) (2008) 281–297.
[32] J. Wang, et al., Connection between systemic inflammation and neuroinflammation underlies neuroprotective mechanism of several phytochemicals in neurodegenerative diseases, Oxid. Med. Cell. Longev. 2018 (2018), 1972714.
[33] T.H. Tranah, et al., Systemic inflammation and ammonia in hepatic encephalopathy, Metab. Brain Dis. 28 (1) (2013) 1–5.
[34] I. Coltart, T.H. Tranah, D.L. Shawcross, Inflammation and hepatic encephalopathy, Arch. Biochem. Biophys. 536 (2) (2013) 189–196.

[35] T. Balzano, et al., Chronic hyperammonemia induces peripheral inflammation that leads to cognitive impairment in rats: reversed by anti-TNF-alpha treatment, J. Hepatol. 73 (3) (2020) 582–592.
[36] L. Jain, et al., Serum endotoxin and inflammatory mediators in patients with cirrhosis and hepatic encephalopathy, Dig. Liver Dis. 44 (12) (2012) 1027–1031.
[37] M. Erta, A. Quintana, J. Hidalgo, Interleukin-6, a major cytokine in the central nervous system, Int. J. Biol. Sci. 8 (9) (2012) 1254–1266.
[38] S.S. Cohen, et al., Effects of interleukin-6 on the expression of tight junction proteins in isolated cerebral microvessels from yearling and adult sheep, Neuroimmunomodulation 20 (5) (2013) 264–273.
[39] H. Wei, et al., Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors, Biochim. Biophys. Acta 1822 (6) (2012) 831–842.
[40] D.R. Aldridge, E.J. Tranah, D.L. Shawcross, Pathogenesis of hepatic encephalopathy: role of ammonia and systemic inflammation, J. Clin. Exp. Hepatol. 5 (Suppl 1) (2015) S7–S20.
[41] B. Görg, F. Schliess, D. Häussinger, Osmotic and oxidative/nitrosative stress in ammonia toxicity and hepatic encephalopathy, Arch. Biochem. Biophys. 536 (2) (2013) 158–163.
[42] D. Haussinger, Low grade cerebral edema and the pathogenesis of hepatic encephalopathy in cirrhosis, Hepatology 43 (6) (2006) 1187–1190.
[43] A.R. Jayakumar, K.V. Rama Rao, M.D. Norenberg, Neuroinflammation in hepatic encephalopathy: mechanistic aspects, J. Clin. Exp. Hepatol. 5 (Suppl 1) (2015) S21–S28.
[44] C.R. Bosoi, C.F. Rose, Oxidative stress: a systemic factor implicated in the pathogenesis of hepatic encephalopathy, Metab. Brain Dis. 28 (2) (2013) 175–178.
[45] F. Schliess, B. Görg, D. Häussinger, RNA oxidation and zinc in hepatic encephalopathy and hyperammonemia, Metab. Brain Dis. 24 (1) (2009) 119–134.
[46] M.D. Norenberg, A.R. J, K.V. Rama Rao, Oxidative stress in the pathogenesis of hepatic Encephalopathy, Metab. Brain Dis. 19 (3–4) (2004) 313–329.
[47] P. Castellani, E. Balza, A. Rubartelli, Inflammation, DAMPs, tumor development, and progression: a vicious circle orchestrated by redox signaling, Antioxidants Redox Signal. 20 (7) (2014) 1086–1097.
[48] N.D. Vaziri, B. Rodriguez-Iturbe, Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension, Nat. Clin. Pract. Nephrol. 2 (10) (2006) 582–593.
[49] S.K. Biswas, Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? Oxid. Med. Cell. Longev. 2016 (2016), 5698931.
[50] S.I. Dikalov, Y.F. Polienko, I. Kirilyuk, Electron paramagnetic resonance measurements of reactive oxygen species by cyclic hydroxylamine spin probes, Antioxidants Redox Signal. 28 (15) (2018) 1433–1443.
[51] G.H.H. Gadoth N, Oxidative Stress and Free Radical Damage in Neurology, Humana Press, 2011.
[52] E. Niedzielska, et al., Oxidative stress in neurodegenerative diseases, Mol. Neurobiol. 53 (6) (2016) 4094–4125.
[53] G. Aguilera, et al., Redox signaling, neuroinflammation, and neurodegeneration, Antioxidants Redox Signal. 28 (18) (2018) 1626–1651.
[54] M. Genestra, Oxyl radicals, redox-sensitive signalling cascades and antioxidants, Cell. Signal. 19 (9) (2007) 1807–1819.
[55] G. Pizzino, et al., Oxidative Stress: Harms and Benefits for Human Health, Oxid Med Cell Longev, 2017, 8416763, 2017.
[56] W. Droge, Free radicals in the physiological control of cell function, Physiol. Rev. 82 (1) (2002) 47–95.
[57] M. Valko, et al., Free radicals and antioxidants in normal physiological functions and human disease, Int. J. Biochem. Cell Biol. 39 (1) (2007) 44–84.
[58] P. Rajendran, et al., Antioxidants and human diseases, Clin. Chim. Acta 436 (2014) 332–347.
[59] E. Chroni, et al., Brain oxidative stress induced by obstructive jaundice in rats, JNEN (J. Neuropathol. Exp. Neurol.) 65 (2) (2006) 193–198.
[60] M. Katerji, M. Filippova, P. Duerksen-Hughes, Approaches and methods to measure oxidative stress in clinical samples: research applications in the cancer field, Oxid. Med. Cell. Longev. 2019 (2019), 1279250.
[61] S.I. Dikalov, D.G. Harrison, Methods for detection of mitochondrial and cellular reactive oxygen species, Antioxidants Redox Signal. 20 (2) (2014) 372–382.
[62] I. Marrocco, F. Altieri, I. Peluso, Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans, Oxid Med Cell Longev, 2017, 6501046, 2017.
[63] E. Cadenas, Mitochondrial free radical production and cell signaling, Mol. Aspect. Med. 25 (1–2) (2004) 17–26.
[64] S. Cardaci, M.R. Ciriolo, TCA cycle defects and cancer: when metabolism tunes redox state, Int. J. Cell. Biol. 2012 (2012), 161837.
[65] S.I.K. Dikalov, I. A, M. Voinov, I.A. Grigor'ev, EPR detection of cellular and mitochondrial superoxide using cyclic hydroxylamines, Free Radic. Res. 45 (4) (2011) 417–430.
[66] K.H. Krause, Aging: a revisited theory based on free radicals generated by NOX family NADPH oxidases, Exp. Gerontol. 42 (4) (2007) 256–262.
[67] A. Kumar, B. Yegla, T.C. Foster, Redox signaling in neurotransmission and cognition during aging, Antioxidants Redox Signal. 28 (18) (2018) 1724–1745.
[68] C.P. Rubio, J.J. Ceron, Spectrophotometric assays for evaluation of Reactive Oxygen Species (ROS) in serum: general concepts and applications in dogs and humans, BMC Vet. Res. 17 (1) (2021) 226.
[69] B. Halliwell, Biochemistry of oxidative stress, Biochem. Soc. Trans. 35 (5) (2007) 1147–1150.
[70] M. Terpstra, et al., Quantification of vitamin C in the rat brain in vivo using short echo-time 1H MRS, Magn. Reson. Med. 55 (5) (2006) 979–983.
[71] K. Aoyama, Glutathione in the brain, Int. J. Mol. Sci. 22 (9) (2021).
[72] M.A. Sharpe, S.J. Robb, J.B. Clark, Nitric oxide and Fenton/Haber-Weiss chemistry: nitric oxide is a potent antioxidant at physiological concentrations, J. Neurochem. 87 (2) (2003) 386–394.
[73] H.J.H. F, Oxidation of tartaric acid in presence of iron, J. Chem. Soc. 65 (1894) 899–910.
[74] R. Olowe, et al., Approaches for reactive oxygen species and oxidative stress quantification in epilepsy, Antioxidants 9 (10) (2020).
[75] M.C.I. Lee, Assessment of oxidative stress and antioxidant property using electron spin resonance (ESR) spectroscopy, J. Clin. Biochem. Nutr. 52 (1) (2013) 1–8.
[76] M. Terpstra, et al., Detection of an antioxidant profile in the human brain in vivo via double editing with MEGA-PRESS, Magn. Reson. Med. 56 (6) (2006) 1192–1199.
[77] A. Kaur, Fluorescent Tools for Imaging Oxidative Stress in Biology, Springer Theses, 2018.
[78] B. Kalyanaraman, et al., Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations, Free Radic. Biol. Med. 52 (1) (2012) 1–6.
[79] L. Valgimigli, G.F. Pedulli, P. M, Measurement of oxidative stress by EPR radical-probe technique, Free Radic. Biol. Med. 31 (6) (2001) 708–716.
[80] K.K. Griendling, et al., Measurement of reactive oxygen species, reactive nitrogen species, and redox-dependent signaling in the cardiovascular system: a scientific statement from the American heart association, Circ. Res. 119 (5) (2016) e39–75.
[81] A.S. Veskoukis, et al., Spectrophotometric assays for measuring redox biomarkers in blood, Biomarkers 21 (3) (2016) 208–217.
[82] S. Mrakic-Sposta, et al., A quantitative method to monitor reactive oxygen species production by electron paramagnetic resonance in physiological and pathological conditions, Oxid. Med. Cell. Longev. 2014 (2014), 306179.
[83] S. Suzen, H. Gurer-Orhan, L. Saso, Detection of reactive oxygen and nitrogen species by electron paramagnetic resonance (EPR) technique, Molecules 22 (1) (2017).
[84] K.S.D.B. Korkmaz, D. Roggenbuck, Detection of 8-OHdG as a diagnostic biomarker, J. Lab. Precis. Med. 3 (2018) 95. B.
[85] R.P. Brandes, M. Janiszewski, Direct detection of reactive oxygen species ex vivo, Kidney Int. 67 (5) (2005) 1662–1664.
[86] M. K, Applications of electron spin resonance spectrometry for reactive oxygen species and reactive nitrogen species research, J. Clin. Biochem. Nutr. 47 (1) (2010) 1–11.
[87] B. Halliwell, M. Whiteman, Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br. J. Pharmacol. 142 (2) (2004) 231–255.
[88] C.L. Hawkins, M.J. Davies, Detection, identification, and quantification of oxidative protein modifications, J. Biol. Chem. 294 (51) (2019) 19683–19708.
[89] A. Dasgupta, K. Klein, Methods for measuring oxidative stress in the laboratory, in: Antioxidants in Food, Vitamins and Supplements, 2014, pp. 19–40.
[90] S. H, Methods for measurements of increased release of free oxygen radicals, Forsch Komplemetärmed 2 (5) (1995) 254–258.
[91] B. Palmieri, V. Sblendorio, Oxidative stress tests: overview on reliability and use - Part I, Eur. Rev. Med. Pharmacol. Sci. 11 (5) (2007) 309–342.
[92] E. Cadenas, K.J. Davies, Mitochondrial free radical generation, oxidative stress, and aging, Free Radic. Biol. Med. 29 (3–4) (2000) 222–230.
[93] D.H.K. Matsuzawa, Magnetic resonance spectroscopy study of the antioxidant defense system in schizophrenia, Antioxidants Redox Signal. 15 (7) (2011) 2057–2065.
[94] F. Bottino, et al., In Vivo brain GSH: MRS methods and clinical applications, Antioxidants 10 (9) (2021).
[95] G. Cheng, et al., Detection of mitochondria-generated reactive oxygen species in cells using multiple probes and methods: potentials, pitfalls, and the future, J. Biol. Chem. 293 (26) (2018) 10363–10380.
[96] K.S. Pierzchala, A. Sienkiewicz, D. Sessa, S. Mitrea, O. Braissant, V. McLin, R. Gruetter, C. Cudalbu, Hippocampal and Systemic Oxidative Stress in a Rat Model of Chronic Hepatic Encephalopathy, a Multimodal Approach, ISMRM, 2020, p. 2040. D.
[97] V.Y.U. Rani, Free Radicals in Human Health and Disease, Springer, New Delhi, 2015.
[98] S.I. Dikalov, et al., Mitochondrial reactive oxygen species and calcium uptake regulate activation of phagocytic NADPH oxidase, Am. J. Physiol. Regul. Integr. Comp. Physiol. 302 (10) (2012) R1134–R1142.
[99] Chapter 6 - UV–vis absorption and chemiluminescence techniques, in: Frederick A. Villamena (Ed.), Reactive Species Detection in Biology, Elsevier, 2017, pp. 203–251.
[100] P. Di Simplicio, et al., Blood glutathione disulfide: in vivo factor or in vitro artifact? Clin. Chem. 48 (5) (2002) 742–753.
[101] D. Giustarini, et al., Pitfalls in the analysis of the physiological antioxidant glutathione (GSH) and its disulfide (GSSG) in biological samples: an elephant in the room, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1019 (2016) 21–28.
[102] Khoubnasab Jafari M, Ansarin K, and J. A, *Comments on "Use of Malondialdehyde as a Biomarker for assessing oxidative Stress in different disease pathologies: a review"*. Iran. J. Public Health. . 44(5): p. 714-715.
[103] M. Khoubnasabjafari, K. Ansarin, A. Jouyban, Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders, Bioimpacts 5 (3) (2015) 123–127.

[104] C. Ceconi, et al., Evaluation of phospholipid peroxidation as malondialdehyde during myocardial ischemia and reperfusion injury, Am. J. Physiol. 260 (4 Pt 2) (1991) 1057–1061.
[105] J. Liu, et al., Assay of aldehydes from lipid peroxidation: gas chromatography-mass spectrometry compared to thiobarbituric acid, Anal. Biochem. 245 (2) (1997) 161–166.
[106] H.H. Draper, M. Hadley, Malondialdehyde determination as index of lipid-peroxidation, Methods Enzymol. 186 (1990) 421–431.
[107] B. K, Pitfalls of reactive oxygen species (ROS) measurements by fluorescent probes and mitochondrial superoxide determination using MitoSOX, in: L. J. Berliner, N.L. Parinandi (Eds.), *Measuring Oxidants and Oxidative Stress in Biological Systems*. Biological Magnetic Resonance, vol. 34, 2020.
[108] J. Zielonka, B. Kalyanaraman, Hydroethidine- and MitoSOX-derived red fluorescence is not a reliable indicator of intracellular superoxide formation: another inconvenient truth, Free Radic. Biol. Med. 48 (8) (2010) 983–1001.
[109] C.D. Rae, S.R. Williams, Glutathione in the human brain: review of its roles and measurement by magnetic resonance spectroscopy, Anal. Biochem. 529 (2017) 127–143.
[110] M. Terpstra, R. Rao, I. Tkac, Region-specific changes in ascorbate concentration during rat brain development quantified by in vivo (1)H NMR spectroscopy, NMR Biomed. 23 (9) (2010) 1038–1043.
[111] D.K. Deelchand, et al., Sensitivity and specificity of human brain glutathione concentrations measured using short-TE (1)H MRS at 7 T, NMR Biomed. 29 (5) (2016) 600–606.
[112] B. Gorg, et al., Oxidative stress markers in the brain of patients with cirrhosis and hepatic encephalopathy, Hepatology 52 (1) (2010) 256–265.
[113] B. Gorg, et al., Ammonia induces RNA oxidation in cultured astrocytes and brain in vivo, Hepatology 48 (2) (2008) 567–579.
[114] C. Kruczek, et al., Ammonia increases nitric oxide, free Zn2+and metallothionein mRNA expression in cultured rat astrocytes, Biol. Chem. (2011) -.
[115] C.R. Murthy, A.S. Bender, R.S. Dombro, G. Bai, M.D. Norenberg, Elevation of glutathione levels by ammonium ions in primary cultures of rat astrocytes, Neurochem. Int. 37 (2000) 255–268.
[116] R. Reinehr, et al., Hypoosmotic swelling and ammonia increase oxidative stress by NADPH oxidase in cultured astrocytes and vital brain slices, Glia 55 (7) (2007) 758–771.
[117] M. Wegrzynowicz, et al., Upregulation of cerebral cortical glutathione synthesis by ammonia in vivo and in cultured glial cells: the role of cystine uptake, Neurochem. Int. 50 (7–8) (2007) 883–889.
[118] I. Zemtsova, et al., Microglia activation in hepatic encephalopathy in rats and humans, Hepatology 54 (1) (2011) 204–215.
[119] G.Y. Subbalakshmi, C.R. Murthy, Acute metabolic effects of ammonia on the enzymes of glutamate metabolism in isolated astroglial cells, Neurochem. Int. 5 (5) (1983) 593–597.
[120] W. Hilgier, et al., Taurine reduces ammonia- and N-methyl-d-aspartate-induced accumulation of cyclic GMP and hydroxyl radicals in microdialysates of the rat striatum, Eur. J. Pharmacol. 468 (1) (2003) 21–25.
[121] E. Kosenko, et al., Sources of oxygen radicals in brain in acute ammonia intoxication in vivo, Brain Res. 981 (1–2) (2003) 193–200.
[122] C.R.K. Murthy, Bai Ge, M.D. Norenberg, Ammonia-induced production of free radicals in primary cultures of rat, J. Neurosci. Res. 66 (2001) 282–288. R.R.K.V.
[123] E. Kosenko, et al., Acute ammonia intoxication induces an NMDA receptor-mediated increase in poly(ADP-ribose) polymerase level and NAD metabolism in nuclei of rat brain cells, J. Neurochem. 89 (5) (2004) 1101–1110.
[124] E. Kosenko, et al., Effects of acute hyperammonemia in vivo on oxidative metabolism in nonsynaptic rat brain mitochondria, Metab. Brain Dis. 12 (1) (1997) 69–82.
[125] E. Kosenko, et al., Superoxide production and antioxidant enzymes in ammonia intoxication in rats, Free Radic. Res. 27 (6) (1997) 637–644.
[126] E. Kosenko, K. Y, O. Lopata, N. Muravyov, V. Felipo, Blocking NMDA receptors prevents the oxidative stress induced by acute ammonia intoxication, Free Radic. Biol. Med. 26 (11–12) (1999) 1369–1374. Jun.
[127] S. Dhanda, et al., Mitochondrial dysfunctions contribute to energy deficits in rodent model of hepatic encephalopathy, Metab. Brain Dis. 33 (1) (2018) 209–223.
[128] M. Giorgio, et al., On the epigenetic role of guanosine oxidation, Redox Biol. 29 (2020), 101398.
[129] C.R. Bosoi, et al., Systemic oxidative stress is implicated in the pathogenesis of brain edema in rats with chronic liver failure, Free Radic. Biol. Med. 52 (7) (2012) 1228–1235.
[130] X. Yang, et al., Portacaval anastomosis-induced hyperammonemia does not lead to oxidative stress, Metab. Brain Dis. 25 (1) (2010) 11–15.
[131] P. Carbonero-Aguilar, et al., Brain biomolecules oxidation in portacaval-shunted rats, Liver Int. 31 (7) (2011) 964–969.
[132] D.L. Shawcross, et al., Ammonia impairs neutrophil phagocytic function in liver disease, Hepatology 48 (4) (2008) 1202–1212.
[133] P. Ljubuncic, Z. Tanne, A. Bomzon, Evidence of a systemic phenomenon for oxidative stress in cholestatic liver disease, Gut 47 (5) (2000) 710–716.
[134] A. Lemberg, et al., Involvement of serum apolipoprotein AI and B100 and lecithin cholesterol acyl transferase in alcoholic cirrhotics, Ann. Hepatol. 6 (4) (2007) 227–232.
[135] M.M. Pomacu, et al., Interrelation of inflammation and oxidative stress in liver cirrhosis, Exp. Ther. Med. 21 (6) (2021) 602.
[136] R.P. Mookerjee, et al., Neutrophil dysfunction in alcoholic hepatitis superimposed on cirrhosis is reversible and predicts the outcome, Hepatology 46 (3) (2007) 831–840.
[137] D.H. Montes-Cortes, et al., Characterization of oxidative stress and ammonia according to the different grades of hepatic encephalopathy, Dig. Dis. 38 (3) (2020) 240–250.
[138] C. Gimenez-Garzo, et al., Is cognitive impairment in cirrhotic patients due to increased peroxynitrite and oxidative stress? Antioxidants Redox Signal. 22 (10) (2015) 871–877.
[139] C. Sfarti, et al., Systemic oxidative stress markers in cirrhotic patients with hepatic encephalopathy: possible connections with systemic ammoniemia, Medicina (Kaunas) 56 (4) (2020).
[140] K. Oettl, et al., Oxidative albumin damage in chronic liver failure: relation to albumin binding capacity, liver dysfunction and survival, J. Hepatol. 59 (5) (2013) 978–983.
[141] W. Zheng, et al., Increased serum malondialdehyde levels are associated with grey matter volume loss in patients with non-alcoholic cirrhosis, Quant. Imag. Med. Surg. 9 (2) (2019) 230–237.
[142] A. Martinez, Electron microscopy in human hepatic encephalopathy, Acta Neuropathol. 11 (1968) 82–86.
[143] M. Norenberg, in: S. Fedoroff, L. Hertz (Eds.), The Astrocyte in Liver Disease. in: Advances in Cellular Neurobiology, vol. 2, Academic, New York, 1981, pp. 303–352.
[144] S. Cho, E.S. Hwang, Fluorescence-based detection and quantification of features of cellular senescence, Methods Cell Biol. 103 (2011) 149–188.
[145] U.T. Brunk, On the origin of lipofuscin - the iron content of residual bodies, and the relation of these organelles to the lysosomal vacuome - a study on cultured human glial-cells, Lipofuscin and Ceroid Pigments 266 (1989) 313–322.
[146] T.C. Ozenirler S, O. Ongun, N. Altan, U. Kandilci, Activities of superoxide dismutase in erythrocyte of nonalcoholic chronic liver diseases, Gen. Pharmacol. 25 (7) (1994) 1349–1351.
[147] J. Gardiner, R. Overall, J. Marc, The nervous system cytoskeleton under oxidative stress, Diseases 1 (1) (2013) 36–50.
[148] S. Grimm, et al., Protein oxidative modifications in the ageing brain: consequence for the onset of neurodegenerative disease, Free Radic. Res. 45 (1) (2011) 73–88.
[149] R.S. Sohal, U.T. Brunk, Lipofuscin as an indicator of oxidative stress and aging, Lipofuscin and Ceroid Pigments 266 (1989) 17–29.
[150] J.N. Keller, et al., Autophagy, proteasomes, lipofuscin, and oxidative stress in the aging brain, Int. J. Biochem. Cell Biol. 36 (12) (2004) 2376–2391.
[151] G.R. Buettner, The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate, Arch. Biochem. Biophys. 300 (2) (1993) 535–543.
[152] A. Hacışevki, An overview of ascorbic acid biochemistry, J. Fac. Pharm. 38 (3) (2009) 233–255.
[153] G.J. Halliwell B, Free Radicals in Biology and Medicine, fifth ed., Oxford University Press, New York, 2015.
[154] E. Hatem, et al., Glutathione is essential to preserve nuclear function and cell survival under oxidative stress, Free Radic. Biol. Med. 75 (Suppl 1) (2014) S25–S26.
[155] R.G. Dringen, J. M, J. Hirrlinger, Glutathione metabolism in brain. Metabolic interaction between astrocytes and neurons in the defense againstreactive oxygen species, Eur. J. Biochem. 267 (2000) 4912–4916.
[156] A.S.R. Bender, M.D. Norenberg, Characterization of cystine uptake in cultured astrocytes, Neurochem. Int. 37 (2–3) (2000) 269–276. W.
[157] J.R. Chen, et al., Morphological changes of cortical pyramidal neurons in hepatic encephalopathy, BMC Neurosci. 15 (2014) 15.
[158] A. Nunomura, et al., Consequences of RNA oxidation on protein synthesis rate and fidelity: implications for the pathophysiology of neuropsychiatric disorders, Biochem. Soc. Trans. 45 (5) (2017) 1053–1066.
[159] C.F. Lee, et al., Oxidative stress-induced depolymerization of microtubules and alteration of mitochondrial mass in human cells, Ann. N. Y. Acad. Sci. 1042 (2005) 246–254.
[160] J.S. Beckman, W.H. Koppenol, Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly, Am. J. Physiol. 271 (5 Pt 1) (1996) C1424–C1437.
[161] M.G. Roma, E.J. Sanchez Pozzi, Oxidative stress: a radical way to stop making bile, Ann. Hepatol. 7 (1) (2008) 16–33.
[162] A.W. Vidyasagar, N. A, A. Djamali, Heat shock protein 27 (HSP27): biomarker of disease and therapeutic target, Fibrogenesis Tissue Repair 5 (1) (2012).
[163] J.H. Huot, F. Marceau, J. Landry, Oxidative stress-induced actin reorganization mediated by the p38 mitogen-activated protein Kinase_Heat shock protein 27 pathway in vascular endothelial cells, Circ. Res. 80 (3) (1997) 383–392. F.
[164] I.S.-d.F. Guénal, S. Gaumer, B. Mignotte, Bcl-2 and Hsp27 act at di€erent levels to suppress programmed cell death, Oncogene 15 (3) (1997) 347–360. C.
[165] A.R. Jayakumar, et al., Glutamine-induced free radical production in cultured astrocytes, Glia 46 (3) (2004) 296–301.
[166] W.Y. Wang, et al., Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease, Ann. Transl. Med. 3 (10) (2015) 136.
[167] Y. Ishihara, et al., Dual role of superoxide dismutase 2 induced in activated microglia: oxidative stress tolerance and convergence of inflammatory responses, J. Biol. Chem. 290 (37) (2015) 22805–22817.
[168] J.M. Auerbach, M. Segal, Peroxide modulation of slow onset potentiation in rat hippocampus, J. Neurosci. 17 (22) (1997) 8695–8701.
[169] T.C. Pellmar, G.E. Hollinden, J.M. Sarvey, Free radicals accelerate the decay of long-term potentiation in field CA1 of Guinea-pig hippocampus, Neuroscience 44 (2) (1991) 353–359.
[170] D.S.A. Simpson, P.L. Oliver, ROS generation in microglia: understanding oxidative stress and inflammation in neurodegenerative disease, Antioxidants 9 (8) (2020).

[171] V. Arroyo, R. Garcia-Martinez, X. Salvatella, Human serum albumin, systemic inflammation, and cirrhosis, J. Hepatol. 61 (2) (2014) 396–407.
[172] N.S. Poudyal, et al., Precipitating factors and treatment outcomes of hepatic encephalopathy in liver cirrhosis, Cureus 11 (4) (2019) e4363.
[173] J. Vaquero, et al., Infection and the progression of hepatic encephalopathy in acute liver failure, Gastroenterology 125 (3) (2003) 755–764.
[174] N. Weiss, et al., Cerebrospinal fluid metabolomics highlights dysregulation of energy metabolism in overt hepatic encephalopathy, J. Hepatol. 65 (6) (2016) 1120–1130.
[175] P. Kruger, et al., Neutrophils: between host defence, immune modulation, and tissue injury, PLoS Pathog. 11 (3) (2015), e1004651.
[176] T. Narasaraju, et al., Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis, Am. J. Pathol. 179 (1) (2011) 199–210.
[177] E. Williams, C. Chu, S. DeMorrow, A critical review of bile acids and their receptors in hepatic encephalopathy, Anal. Biochem. 643 (2022), 114436.
[178] K.B.M.S. Islam, et al., Bile acid is a host factor that regulates the composition of the cecal microbiota in rats, Gastroenterology 141 (5) (2011) 1773–1781.
[179] M. McMillin, et al., Bile acid signaling is involved in the neurological decline in a murine model of acute liver failure, Am. J. Pathol. 186 (2) (2016) 312–323.
[180] M. Quinn, et al., Bile acids permeabilize the blood brain barrier after bile duct ligation in rats via Rac1-dependent mechanisms, Dig. Liver Dis. 46 (6) (2014) 527–534.
[181] R.J. Sokol, et al., Evidence for involvement of oxygen-free radicals in bile-acid toxicity to isolated rat hepatocytes, Hepatology 17 (5) (1993) 869–881.
[182] R.J. Sokol, et al., Role of oxidant stress in the permeability transition induced in rat hepatic mitochondria by hydrophobic bile acids, Pediatr. Res. 49 (4) (2001) 519–531.
[183] P. Ljubuncic, et al., Effect of deoxycholic acid and ursodeoxycholic acid on lipid peroxidation in cultured macrophages, Gut 39 (3) (1996) 475–478.
[184] Z. Chen, et al., The role of intestinal bacteria and gut-brain Axis in hepatic encephalopathy, Front. Cell. Infect. Microbiol. 10 (2020), 595759.
[185] J. Licinio, M.L. Wong, Pathways and mechanisms for cytokine signaling of the central nervous system, J. Clin. Invest. 100 (12) (1997) 2941–2947.
[186] A.S. Seyan, R.D. Hughes, D.L. Shawcross, Changing face of hepatic encephalopathy: role of inflammation and oxidative stress, World J. Gastroenterol. 16 (27) (2010) 3347–3357.
[187] E. Bourdon, D. Blache, The importance of proteins in defense against oxidation, Antioxidants Redox Signal. 3 (2) (2001) 293–311.
[188] S. Prakash, Role of human serum albumin and oxidative stress in diabetes, J. Appl. Biotechnol. Bioeng. 3 (1) (2017).
[189] M. Sitar, S. Aydin, U. çAkatay, Human Serum Albumin and its Relation with Oxidative Stress, vol. 59, Clinical Laboratory, 2013, 09+10/2013.
[190] J.R. Carvalho, M. Verdelho Machado, New insights about albumin and liver disease, Ann. Hepatol. 17 (4) (2018) 547–560.
[191] S. DeMorrow, Bile acids in hepatic encephalopathy, J. Clin. Exp. Hepatol. 9 (1) (2019) 117–124.
[192] H. Ohkubo, et al., Role of portal and splenic vein shunts and impaired hepatic extraction in the elevated serum bile-acids in liver-cirrhosis, Gastroenterology 86 (3) (1984) 514–520.
[193] R. Jalan, et al., Alterations in the functional capacity of albumin in patients with decompensated cirrhosis is associated with increased mortality, Hepatology 50 (2) (2009) 555–564.