Ibitoye, R., Kemp, K. C., Rice, C. M., Hares, K. M., Scolding, N. J., & Wilkins, A. (2016). Oxidative stress-related biomarkers in multiple sclerosis: a review. *Biomarkers in Medicine*, *10*(8), 889-902. https://doi.org/10.2217/bmm-2016-0097

Abstract

Objective: To provide an up to date review of oxidative stress biomarkers in multiple sclerosis and thus identify candidate molecules with greatest promise as biomarkers of diagnosis, disease activity or prognosis.

Method: A semi-systematic literature search using PubMed and other databases.

Results: Nitric oxide metabolites, superoxide dismutase, catalase, glutathione reductase, inducible nitric oxide synthase, protein carbonyl, 3-nitrotyrosine, isoprostanes, malondialdehyde and products of DNA oxidation have been identified across multiple studies as having promise as diagnostic, therapeutic or prognostic markers in MS.

Conclusion: Heterogeneity of study design, particularly patient selection, limits comparability across studies. Further cohort studies are needed, and we would recommend promising markers be incorporated into future clinical trials to prospectively validate their potential.

Keywords

Biomarkers
Multiple Sclerosis
Oxidative Stress
Reactive oxygen species
Diagnosis
Disease activity
Disability progression
Therapeutic monitoring

Body of Article

Introduction

Despite major advances in understanding multiple sclerosis (MS) pathophysiology and therapeutics, there remains a need for better biomarkers of disease activity, therapeutic response and prognosis. Clinical disability scores such as the Expanded Disability Scale Score (EDSS), lack sensitivity and their use in trials for progressive MS are limited by the lengths of time required to detect meaningful disability progression. Other scores [1] developed to address these shortcomings have their own limitations. Magnetic resonance imaging (MRI) markers of disability progression such as brain atrophy attempt to improve on this but have a poor predictive value [2]. There remains a need for sensitive markers of disease activity, closely related to the underlying neuropathology and predictive of disability progression.

Genomic [3], metabolomic [4] and proteomic [5] approaches to MS biomarkers hold promise. Profiles of putative markers are compared between groups to identify a profile of diagnostic or prognostic value. These techniques generate large data sets requiring tailored multivariate statistical analysis. Individual markers of high predictive value are seldom identified, and the physiological implications are frequently unclear. MicroRNAs, small non-coding RNAs which regulate post-transcriptional gene expression, are an emerging group of micromolecules with potential as biomarkers in MS [6].

Studies of specific metabolic pathways identified to be relevant to MS pathogenesis provide another approach to biomarker identification. For example, the kynurenine pathway, the major degradative pathway for tryptophan, has been shown to regulate immune function. Studies show kynureinine acid, a neuroprotective metabolite, is increased in the CSF of MS patients following relapses [7] .

Oxidative damage to DNA, protein and lipids, is a major feature of MS neuropathology in both relapsing-remitting and progressive disease [8]. Reactive oxygen species (ROS) (Figure 1), the mediators of oxidative damage, are released as part of the respiratory burst of activated neutrophils, monocytes and microglia and result in oligodendrocyte injury and axonal damage. Immune system activation usually occurs in response to pathogens, but in MS, auto-reactive T-cells initiate and maintain a maladaptive response to central nervous system (CNS) targets. Major enzymes in ROS

detoxification are superoxide dismutases (SOD) and peroxidases (including catalase) as illustrated in Figure 2.

Regulation of antioxidant defences is a key determinant of the cytosolic redox environment. Nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) is a principal transcriptional regulator of genes with antioxidant effect [9]. Other transcription factors with antioxidant effect such as peroxisome proliferator-activated receptor (PPAR)-gamma coactivator-1α (PGC-1α) have also been implicated in MS pathogenesis [10]. The Nrf2 pathway has been implicated as the target of dimethyl fumarate, a recently licenced disease modifying therapy in relapsing-remitting MS (RRMS) [11, 12].

There is evidence from other inflammatory disorders, such as rheumatoid arthritis and systemic lupus erythematosus, that oxidative stress biomolecules can be surrogates of disease activity. In rheumatoid arthritis, plasma thioredoxins show particular promise as they prospectively track disease activity and correlate with C-reactive protein, a marker of disease activity [13].

The biomarker challenge in MS is in many ways more significant, as the substrate of disease – the CNS is less accessible, and definite clinical outcomes are reached only after years of disease. Identifying sensitive markers which prospectively track disease activity or predict outcomes would thus transform clinical management.

We review and summarise the evidence on major oxidative stress biochemical markers in MS. We consider specifically whether the evidence supports a diagnostic, disease activity monitoring, therapeutic monitoring or prognostic role for these molecules. We focus on studies measuring markers in accessible tissue fluids such as peripheral blood and cerebrospinal fluid (CSF). We review enzymatic antioxidants and do not consider non-enzymatic antioxidants in this review. We highlight the most promising oxidative stress biomarkers and implications for future research.

Methods

We searched PubMed (http://www.ncbi.nlm.nih.gov/pubmed) in December 2015 using the following search terms: ‘multiple sclerosis’ AND [carbonyl, catalase, glutathione peroxidase, glutathione reductase, hydrogen peroxide, iNOS, malondialdehyde, myeloperoxidase, nitrates, nitric oxide, nitric oxide synthase, nitrites, Nrf2, PGC, PPAR, reactive oxygen species, superoxide, superoxide dismutase, nitrotyrosine]. RI reviewed the titles and abstracts of articles with regard to

our remit and selected studies for inclusion (Table 1 and Appendix). Additional relevant publications were identified from the references of selected articles and from focused searches of other databases such as Google Scholar (https://scholar.google.co.uk/).

One major problem when reviewing biomarker studies was the disparity of terminology used to describe various types or phases of MS. In general, we have included studies on patients with clinically isolated syndromes (CIS) / RRMS who fulfil revised McDonald criteria [14] and studies on patients with progressive MS who fulfil criteria established by Lublin and Reingold [15], or relevant antecedent criteria. In addition, there was variation in how authors defined disease activity and relapses with the potential to confound results. We have reflected on these disparities, where relevant, throughout our discussion.

Most articles reported single centre cross-sectional studies with significant heterogeneity in the choice of control group, patient group, biological sample, assay method and statistical method. We considered in general terms the quality of the evidence and implications for future research. Unless otherwise stated, results reached statistical significance.

CSF and peripheral blood were commonly sampled in the search for biomarkers of disease, reflecting the systemic autoimmune basis of MS, and the biological compartmentalisation of the CNS.

Reactive Oxygen Species

ROS are free radical or non-radical species which oxidise biological molecules. We include nitrogen-based oxidants in this definition, sometimes referred to as ‘reactive nitrogen species’. Most ROS have a very short half-life and are highly reactive, so direct quantification in biological tissues is difficult.

Nitric oxide

NO is mainly produced through a nitric oxide synthase (NOS) catalysed reaction of L-arginine and oxygen (Figure 1) and acts in autocrine and paracrine signalling pathways. Three isoenzymes exist in humans and of these inducible NOS (iNOS) is expressed in response to immune activation and is the major producer of NO. Subsequent oxidation produces nitrate and nitrite, more stable end-products which reflect NO production.

Peripheral blood

Cross-sectional studies have measured nitrate (NO_3^-) and nitrite (NO_2^-), often as a total value (tNO_x) in plasma or serum. Most studies reported significantly higher mean tNO_x in RRMS compared to control groups, in acute relapse and in remission [16-23]. Most noteworthy is Tavazzi et al.'s study [16] of a large cohort of 113 RRMS patients in whom serum tNO_x levels were significantly higher ($p<0.001$) in RRMS patients than in health controls. Three studies described no significant difference but were heterogeneous as to the MS disease phenotype, and none considered patients following an acute relapse [24-26]. A single study [27] found the opposite: significantly lower mean tNO_x in RRMS patients compared to control groups; no significant study characteristics were identified to explain this disparity. tNO_x has been considered across RRMS and SPMS groups with mixed results – one study reported significantly higher mean tNO_x in RRMS than SPMS [22] whereas others reported no significant difference [16, 17, 23].

Cross-sectional studies considering tNO_x in relation to EDSS [16, 20] or disease duration [20] reported no significant relationship. A longitudinal study found higher tNO_x significantly correlated with lower relapse rates over an 18 month period, supporting a prognostic role [22].

Stępień et al. [28] reported the effect of 3 years of interferon (IFN)-β1a and β1b treatment on serum nitrite. They found a significant reduction in mean serum nitrite levels ($p<0.0001$), along with a reduction in relapse rates with treatment. In the absence of a control group, it is not possible to directly ascribe these changes to a treatment effect, but serum nitrite may be a marker of disease activity.

CSF

Most studies of CSF reported significantly higher mean CSF tNO_x in active RRMS [19, 29-31], mixed MS populations [32-40] and PPMS [25] compared to controls, supporting a role for tNO_x as a diagnostic marker. In one study, mean CSF tNO_x was significantly higher in RRMS than SPMS groups [33], supporting a role for tNOx as a marker of inflammatory disease activity. Two studies described no significant difference in CSF tNO_x between MS and control groups but neither offered details on the disease activity of their patient populations [24, 26]; case-mix differences may account for the disparity in the outcomes of these studies. Overall, there is good evidence for tNOx as a diagnostic marker in MS, particularly in acute MS relapse.

The potential of tNO_x as a biomarker of disease activity has been explored in cross-sectional and longitudinal studies. Studies have reported significant association between high tNO_x and the number of gadolinium-enhancing lesions [19], as well as the volume of gadolinium-enhancing lesions [32] (51 MS patients, 14 healthy controls, p=0.01). The longitudinal arm of the latter study [32] found higher baseline CSF tNO_x in patients with EDSS disability progression when compared to stable disability (p=0.02). Cross-sectional studies reported higher CSF tNO_x in patients with acute relapsing disease, compared to those with stable remitting disease [37, 38], and higher mean CSF nitrite in RRMS than progressive MS (PMS) groups [33]. One study reported higher tNO_x immediately following relapse when compared to remission [41]. Another reported a negative correlation between post-relapse tNO_x and the EDSS decrease at 8 weeks [29], however changes in EDSS over short periods of time should be interpreted with caution. Studies using heterogeneous methods however, failed to identify a significant relationship between tNO_x and disease subtype [35], disease activity [34] or disability [36].

Other

A single study of urinary tNOx [42] reported elevated tNOx/creatinine quotients in patients with demyelinating disease compared to healthy controls ($p<0.0001$), and in patients with clinically isolated syndrome (CIS) or RRMS when compared to PMS (p=0.006).

Summary

The evidence strongly supports raised serum, plasma and CSF tNO_x, as diagnostic markers in multiple sclerosis, especially in patients with acute relapsing disease. Despite the aforementioned limitations, Stępień et al.'s work [28] suggests serum nitrite is a marker of disease activity.

CSF tNO_x may be a surrogate for inflammatory disease activity, and may differentiate disease phenotypes. A single study suggests urinary tNO_x is similarly elevated in MS.

Transcriptional Regulators of Reactive Oxygen Species

Nrf2 is constitutively bound to cytoplasmic Kelch-like erythroid cell-derived protein with Cap'n'Collar homology-associated protein 1 (KEAP-1) which serves to prevent its nuclear translocation, and targets Nrf2 for ubiquitin-proteasome degradation. The transcriptional targets of Nrf2 are genes with the antioxidant response element (ARE) promoter, or homologous polymorphisms. These

include catalase, glutathione peroxidases (GPx), glutathione reductase (GRx), glutathione S-transferase and SOD isoenzymes.

A single study measured peripheral blood mononuclear cell (PBMC) Nrf2 as part of a 14 month longitudinal study of 22 patients on natalizumab, an alpha4 integrin receptor blocker. In association with clinical improvement, Nrf2 protein expression was elevated in the cytoplasmic and nuclear fractions of PBMCs [43]. The interpretation of these findings is challenged by the absence of a control group, but the evidence suggests a role for Nrf2 in therapeutic monitoring.

PGC1-α expression is reduced in normal appearing MS grey matter and this reduction is associated with neuronal loss [10]. A single study reported an increase in PPARγ in MS CSF compared to controls [44].

Summary

Despite in vitro and animal model evidence for these transcriptional regulators of antioxidant defence as relevant to disease, few published studies have explored them in human subjects with MS. The discovery of an Nrf2-dependent mechanism of action for dimethyl fumarate reinforces the importance of work on this area.

Reactive Oxygen Species Enzymes

Phagocyte myeloperoxidase (MPO) catalyses the production of hypochlorous acid (a potent oxidant) from hydrogen peroxide (H_2O_2). Enzymatic detoxification of superoxide is through the actions of SODs and peroxidases (Figure 2), and for nitric oxide is through caeruloplasmin-catalysed oxidation to nitrite. No studies were identified measuring NADPH-oxidase in tissue fluids as a biomarker in MS.

Myeloperoxidase

Three studies considered mean peripheral blood MPO expression by diagnostic group with mixed results. Minohara et al. [45], in a cohort of 86 Japanese MS patients reported significantly higher serum MPO expression when compared to healthy controls. Tasset et al. [46] also found mean MPO expression in peripheral leukocytes higher in RRMS than in control groups though this did not reach statistical significance. Mostert et al. [18] measured MPO activity in peripheral leukocytes and found

reduced mean activity in a mixed group of MS patients compared to controls. Though discrepant with the findings of studies of MPO expression, no single study reported on both MPO expression and activity. Further work is needed to clarify whether MPO is a useful biomarker in MS. No studies of myeloperoxidase in MS patient CSF were identified.

Superoxide dismutases

In humans there are three SOD isoenzymes: dimeric copper or zinc co-ordinated SOD1 (Cu/ZnSOD) in the cytoplasm, tetrameric manganese-coordinated SOD2 (MnSOD) in mitochondria and tetrameric extracellular copper or zinc co-ordinated SOD3.

SODs are implicated in MS pathogenesis, as part of a physiological response to oxidative stress and may be therapeutic targets. An EAE mouse model study, for example, found suppressing SOD activity increased tissue injury, and over-expressing SOD reduced injury with reduced disability [47]. An *in vitro* study of human mesenchymal stem cell mechanisms of neuronal protection identified SOD3 expression as necessary for the neuroprotective effect [48].

Blood

Studies considering SOD activity in erythrocyte lysates reported significantly higher mean activity in RRMS compared to control groups [49, 50], in keeping with a diagnostic role. The strongest evidence for this arises from Ljubisavljevic et al.'s cross-sectional study [50] of 50 CIS and 57 RRMS patients. Not only did they find higher SOD activity in RRMS than controls ($p=0.009$), they also reported higher SOD activity in their CIS group when compared to RRMS ($p<0.05$). In further analysis they identified CIS and RRMS patients with lower EDSS had lower SOD activity, and CIS patients with more gadolinium enhancing MR lesions had lower SOD activity.

Longitudinal studies have explored how mean SOD activity changes during treatment interventions. Mitosek-Szewczyk et al. [51] considered changes in corticosteroid-treated acute relapse and showed mean erythrocyte SOD activity was significantly lower in RRMS immediately following relapse than in control groups ($p<0.05$), increased following IV methylprednisolone treatment and remained significantly higher during remission than in control groups. Tasset et al. [43] measured mean erythrocyte SOD activity before and after 14 months of natalizumab treatment; they found no significant difference, despite post-treatment reductions in carbonylated protein and oxidised guanosine, end products of oxidative stress. Damiano et al. [52] measured PBMC SOD1 protein and mRNA expression before and after 3 months of IFN-β1b treatment, confirming significantly lower

mean baseline SOD1 expression in MS compared to control groups ($p<0.001$), with significantly increased expression following treatment. These studies suggest SOD activity varies during relapse, and SOD expression may be a marker of IFN-β1b treatment response. Adamczyk-Sowa et al. [53] reported on a complex study of MS patients receiving melatonin supplements, with mixed results; IFN and glatiramer acetate, but not mitoxantrone were associated with increased serum SOD activity ($p<0.05$). An earlier article [54] showed elevated CSF and serum MnSOD but reduced CSF Cu/Zn SOD in RRMS compared to controls, and a treatment effect for mitoxantrone.

Acar et al. [55] and Ljubisavljevic et al. [56] reported mean SOD activity in serum/plasma and confirmed this was significantly higher in RRMS compared to control groups. Inarrea et al. [57] measured platelet SOD1 and SOD2 activity in MS and found no significant difference between groups.

CSF

Two studies of CSF SOD expression and activity were identified suggesting a diagnostic and prognostic role for this marker. Ljubisavljevic et al. [56] measured CSF and plasma SOD activity in CIS, RRMS and control groups. They found reduced CSF SOD activity in CIS ($p<0.05$) and RRMS patients ($p<0.05$) compared to controls. In contrast, plasma SOD activity was increased in CIS ($p<0.05$) and RRMS ($p<0.05$) compared to controls. They identified a negative correlation between SOD activity and EDSS suggesting SOD activity may be a surrogate for disability outcomes. Damiano et al. [52] also found significantly reduced mean SOD1 expression in RRMS compared to control groups.

Summary

Peripheral blood studies of SOD activity strongly and consistently support a role as a diagnostic marker in MS. More work is needed to determine whether SOD has potential as a surrogate for disease activity or disability outcomes.

The finding of reduced CSF SOD activity in MS in contrast to increased activity in peripheral blood defies a straightforward explanation. This highlights a need for caution in extrapolating findings between biological tissues and fluids, and the need for further work to understand the changes in SOD isoenzyme expression in MS.

Catalase

Catalase is a ubiquitously expressed peroxisomal enzyme and is a major intracellular peroxidase. Expression is high in active demyelinating plaques and in MS grey matter astroglia [58]. In support of a protective role, a mouse optic neuritis model showed overexpression of catalase reduced optic nerve injury [59].

Blood

Few studies have looked at peripheral blood catalase activity in MS, with equivocal results. Jensen & Clausen [60] measured catalase activity in lymphocyte and granulocyte lysates. They found lower activity in MS patient granulocytes compared to controls ($p<0.05$), but no difference within lymphocytes. Others looked at catalase activity in erythrocyte lysates [61] and found no difference between MS and control groups. None of these studies detailed disease activity in their MS populations making comparisons difficult.

Ljubisavljevic et al.'s [56] aforementioned study also measured plasma catalase activity in CIS, RRMS and control groups. They reported CSF and plasma catalase activity to be increased in CIS ($p<0.05$) and RRMS patients ($p<0.05$) compared to controls. In support of a role in monitoring disease activity, they found patients with lower EDSS had higher CSF and plasma catalase activity.

CSF

A number of studies across mixed MS populations considered CSF catalase activity and confirmed increased catalase activity in MS cohorts compared to controls [54, 56, 62], supporting a diagnostic role.

Summary

There is evidence from a single study for plasma catalase activity as a diagnostic biomarker in MS. Plasma catalase activity may also be a marker of disease activity. The evidence in other blood fractions was disparate and likely confounded by variations in case-mix. In CSF, results were more consistent, with good evidence in support of catalase activity as a diagnostic marker in MS.

Glutathione Peroxidase

Glutathione peroxidases (GPx) are selenoproteins which catalyse the reduction of peroxides through the oxidation of glutathione. Isoenzymes are preferentially expressed in specific tissues and have differing substrate affinity. GPx1 is the most abundant intracellular isoenzyme and GPx3 predominates in plasma.

Glutathione peroxidases are implicated in MS pathogenesis and for example GPx1 is upregulated in MS plaques. EAE rat model studies showed elevation of GPx3 in CSF compared to controls [63].

Blood

Peripheral GPx activity in erythrocyte, granulocyte, lymphocyte and plasma fractions has been considered with mixed results. Within erythrocytes, most authors reported significantly lower mean GPx activity in MS compared to control groups [46, 64-67]; though the MS populations were mixed and many were in remission. Zachara et al. [68] in a study of MS patients with an acute relapse however found significantly higher mean erythrocyte GPx activity in their MS group compared to controls ($p<0.01$).

Studies of lymphocytes reported mixed results. In a study of MS patients, most of whom where in remission, GPx levels were significantly lower than in controls [69] ($p<0.05$), and in another, in which half the patients were sampled after an acute relapse, there was no significant difference [70] between MS and control subjects. Case-mix differences may thus explain this discrepancy. Jensen et al. [69] also looked at granulocyte GPx activity, finding a significantly lower activity in their MS cohort compared to controls ($p<0.05$).

One study examining serum GPx [71] found significantly increased mean GPx in RRMS patients compared to controls ($p<0.05$). Another study, of 101 RRMS patients [72] found the opposite, though 65% of participants were on disease modifying treatment.

CSF

Two studies explored CSF GPx activity in MS and control groups with inconsistent results. Calabrese et al. [73] compared MS patients to healthy volunteers and found significantly lower mean GPx activity in MS. Kaplan et al. [74] compared MS patients to dementia and stroke cohorts, reporting no significant differences.

Summary

GPx activity studies have been performed across a range of biological tissues and patient populations with mixed results. Methodological differences alone are unlikely to account for these disparities. More work is needed to clarify whether GPx has potential as a diagnostic marker in MS.

Glutathione Reductase

Glutathione reductase (GRx) is an important enzymatic regulator of intracellular oxidative stress. By catalysing the reduction of glutathione disulphide to glutathione, the latter antioxidant is regenerated.

Blood

We identified one study of GRx activity in blood [69] which compared MS patients to healthy controls. No significant differences were seen in lymphocyte and granulocyte lysates, though a trend to lower activity in MS was described. Interestingly, a significant correlation between GPx and GRx activity in controls was noted, which was absent in MS patients.

CSF

A study of CSF GRx in 24 MS patients and controls [73] found significantly higher mean GRx activity in MS compared to control groups; GPx activity was found to be reduced.

Summary

There is a paucity of studies of GRx in MS and, at present, the evidence does not endorse a clear role for GRx as a biomarker in MS. A single study supports CSF GRx as a diagnostic marker in MS.

Inducible Nitric Oxide Synthase

The relationship between inducible nitric oxide synthase (iNOS) and the pathogenesis of MS is complex, likely reflecting its dual role in signalling and tissue injury. The case for a pathogenic role is built by studies showing iNOS is expressed in acute MS plaques [75]. This is supported by correlations in animal models between iNOS burden and disease activity [76]. The evidence against a

purely pathogenic role arises from EAE studies showing iNOS inhibition results in increased disease severity [77].

Blood

We identified one study [78] of iNOS in lymphocytes from 15 MS patients, mostly with active relapsing disease, compared to controls which found increased activity and expression in the MS group.

CSF

Calabrese et al. [62] measured CSF iNOS expression and activity in RRMS patients in remission and controls. They found iNOS expression to be restricted to MS patients, and mean NOS activity significantly higher in MS ($p<0.05$), and abolished by iNOS inhibitors. These findings were associated with elevated tNO_x in keeping with a nitrosative stress hypothesis.

Summary

There is a paucity of studies exploring iNOS as a biomarker in MS. The limited evidence supports iNOS as a diagnostic marker for MS in CSF and peripheral blood lymphocytes.

End-products of oxidation

Protein Carbonylation

Carbonylation of proteins is a common end-point of reactions between free radicals and proteins. Loss of function or aggregation may result, with consequent disruption to cellular homeostasis. The modified proteins are targets for ubiquitin-proteasome degradation. Protein carbonylation is part of the milieu of oxidative injury in MS and is present in non-lesional grey and white matter, associated with markers of astrocytosis [79].

Studies in plasma and serum in RRMS patients in remission using the reaction with dinitrophenylhydrazine products have consistently demonstrated increased protein carbonylation in MS compared to control groups [80-82]. One study of protein carbonylation in CSF [83] confirmed higher levels in a mixed MS population compared to controls ($p=0.034$).

Protein Nitrosylation

Peroxynitrous acid, a highly reactive ROS, reacts with tyrosine residues to form nitrotyrosines. Such post-translational modification may alter protein conformation and function with pathogenic effect.

Studies have measured 3-nitrotyrosine (3-NT) in serum, plasma, peripheral leukocytes and CSF. In serum and plasma, authors reported significantly higher mean 3-NT in MS compared to controls [62, 81, 84, 85], including following acute relapse [84]. A single study also reported significantly higher 3-NT in an SPMS group compared to RRMS [81]. Conversely, Seven et al. [27] reported reduced 3-NT immediately following relapse and following corticosteroid treatment ($p<0.001$). Case mix differences may partly explain this discrepancy as the latter is the only study in this section in which all MS subjects had RRMS and were enrolled after an acute relapse.

In a study of response to INF-β1b treatment, serum 3-NT was found to be significantly lower in MS patients compared to controls ($p<0.05$) [86]. Another study reported reduced peripheral leukocyte 3-NT with glatiramer acetate treatment [85] ($p<0.001$) and together these findings suggests a role in monitoring response to therapies.

Summary

There is good evidence for protein carbonylation and nitrosylation as diagnostic biomarkers in MS. In addition, evidence supports a role in monitoring response to therapies.

Lipid peroxidation

Lipid peroxidation is a common consequence of oxidative stress mechanisms leading to disruption of cell membranes and cellular injury. Malondialdehydes (MDA) and isoprostanes are most frequently assayed as measures of lipid peroxidation; of these, isoprostanes may be the more appropriate measure as unlike MDA, isoprostanes are specific to lipid peroxidation [87]. Other end-products of lipid oxidation such as hexanals are less frequently measured; a single study [88] reported the use of hexanals in exhaled air to distinguish MS from controls.

Isoprostanes

Isoprostanes are prostaglandin F-like products of cyclooxygenase-independent peroxidation of fatty acids, such as arachidonic acid. As a group they are considered ideal measures of in vivo lipid peroxidation [87, 89]. 8-iso-15(S)-prostaglandin F2α is most frequently studied.

Blood

Studies of isoprostane levels in plasma have consistently reported increases in MS, including in RRMS and SPMS subtypes when compared to control groups [90, 91]. A single longitudinal study [90] found no evidence to support a prognostic role with regard conversion to MS, EDSS scores, multiple sclerosis functional composite (MSFC) scores or MRI outcomes. A cross-sectional study [92] identified no relationship to gadolinium enhancing lesions on MRI or time since relapse.

CSF

CSF studies have confirmed isoprostane levels are increased in MS compared to controls [93-96]. One study reported a correlation with disability [96], suggesting a role as a prognostic marker. No correlation was however found with MRI measures of disease activity, or time between relapse and CSF sampling.

Malondialdehyde

Malondialdehyde (MDA) is a mutagenic secondary aldehyde formed in the reaction between ROS and polyunsaturated lipids. Other aldehydes are propanal, hexanal and 4-hydroxynonenal. Reaction with thiobarbituric acid to form a fluorescent adduct is the basis for most quantitative assays of this advanced lipoxidation product.

MDA is highly reactive and can form adducts with protein or DNA with deleterious effect. In MS, MDA is present in active inflammatory lesions as well as neurones undergoing axonal degeneration [8], in keeping with pathogenicity. In a rat EAE model, inhibition of MDA production was shown to ameliorate neurological deficit [97], supporting a pathogenic role in MS.

Blood

Studies of plasma or serum MDA have demonstrated higher levels in MS compared to controls, albeit across a mixed population of MS patients [16, 51, 55, 56, 71, 98-101]. One study [102] comparing 77 healthy controls to 87 patients with MS, however, reported no significant difference;

the MS patients were however in clinical remission. Conversely, another study [103] compared 13 MS patients to 15 controls, and found significantly lower MDA in the MS group. One study measured MDA in erythrocyte lysates [50] confirming a significant increase in MS compared to controls.

The relationship between clinical relapse and plasma or serum MDA levels has been explored, and the evidence supports a role in disease activity monitoring. Mitosek-Szewczyk et al. [51] explored serum MDA at relapse, 5 days following the initiation of corticosteroids, in remission, as well as in controls. They confirmed mean MDA levels were significantly higher in RRMS than in remission; and higher in remission compared to controls. MDA was further elevated at relapse and lower at day 5 of corticosteroid treatment. This provides insight into dynamic changes in this marker of oxidative stress during a relapse. Ljubisavljevic et al. [56] in their study of 57 RRMS, 50 CIS and 20 controls considered the relationship between plasma and CSF MDA, and EDSS. They found higher CSF and plasma MDA in patients with higher EDSS scores ($p<0.05$). They also identified a significant correlation between CSF and plasma MDA.

Two studies have looked at the effect of antioxidant supplementation on serum MDA with mixed results [104, 105]. Neither reported a significant change in clinical parameters such as measures of disease activity or disability.

CSF

Studies quantifying CSF MDA consistently reported higher levels in MS than in control groups across mixed MS cohorts [51, 56, 73, 100, 103].

Summary

The evidence shows isoprostanes and MDA are elevated in MS patient plasma, serum and CSF compared to controls in support of a diagnostic biomarker role, and changes may track disease activity.

DNA oxidation

A single study [46] considered the potential of 8-hydroxy-2'-deoxyguanosine (8-OH2dG) as a diagnostic marker for MS. The authors reported elevated plasma 8-OH2dG in RRMS patients compared to controls, in support of DNA oxidation as a diagnostic marker.

Discussion

Biomarker research poses practical challenges with regard to study design such as patient selection, control selection, the choice of assay method, the interval of follow-up in longitudinal studies, outcomes to which the biomarker will be compared, and data interpretation. The heterogeneity of studies included in our review reflects this challenge.

An ideal biomarker study would consider a homogenous population of MS patients compared to truly healthy controls, rather than patients with potentially confounding non-MS neurological illnesses. The time of sampling from clinical relapse would be defined and patients followed up for sufficient time to reach meaningful clinical outcomes, determined by an established measure such as EDSS. Samples would be collected at a specific time of day to minimise the effect of circadian variation, processed soon after collection to avoid degradation and assays would use methods with small co-efficients of variation and good reproducibility. For diagnostic biomarkers, measures of the utility of the test such as positive and negative predictive value, or receiver operator characteristics analysis would place the results in a clinical context.

The majority of studies reviewed were cross-sectional in nature, the MS populations were mixed and information on the temporal relationship of enrolment to clinical relapse was often not reported. It was nonetheless, apparent for example from the nitric oxide metabolite studies, that results may differ solely as a result of the timing of samples in relation to relapse. Control groups, where present, varied and sometimes included patients with other neurological illnesses deemed to be non-inflammatory. Data on the performance of the assay methods was often not reported and for diagnostic markers, the analysis often did not define predictive values or useful cut-off values.

A number of oxidative stress biomolecules are identified as potential diagnostic markers across CSF and peripheral blood (Table 2). Serum nitrite, CSF and serum SOD, CSF and plasma catalase, serum and peripheral leukocyte 3-NT, CSF isoprostanes, and serum malondialdehyde may also be useful as surrogates of disease activity, disability progression or therapeutic response.

The complex nature of oxidative and anti-oxidant mechanisms in MS coupled with disease heterogeneity makes the interpretation of individual biomarker studies difficult. Studies of oxidative stress regulators and pathways, such as Nrf2, may be more informative, not least as the Nrf2

pathway is implicated as the target of the disease modifying agent dimethyl fumarate. Longitudinal studies are essential to properly evaluate biomarkers as surrogates of disease activity, therapeutic response or outcome.

The challenge in MS of sensitive biomarkers as surrogates of diagnosis, disease activity, disability progression or therapeutic response remains unmet. Oxidative stress biomolecules demonstrate the potential to fulfil this role, but further systematic evaluation in homogeneous populations and using standardised methods is needed. The inclusion of the most promising biomarkers in cohort studies and clinical trials in MS patient populations is essential to achieving this goal.

References

1. Fischer JS, Rudick RA, Cutter GR, Reingold SC. The Multiple Sclerosis Functional Composite Measure (MSFC): an integrated approach to MS clinical outcome assessment. National MS Society Clinical Outcomes Assessment Task Force. *Mult Scler* 5(4), 244-250 (1999).
2. De Stefano N, Giorgio A, Battaglini M *et al*. Assessing brain atrophy rates in a large population of untreated multiple sclerosis subtypes. *Neurology* 74(23), 1868-1876 (2010).
3. Habek M, Borovecki F, Brinar VV. Genomics in multiple sclerosis. *Clin Neurol Neurosurg* 112(7), 621-624 (2010).
4. Bhargava P, Calabresi PA. Metabolomics in multiple sclerosis. *Mult Scler* doi:10.1177/1352458515622827 (2016).
5. Kroksveen AC, Opsahl JA, Guldbrandsen A *et al*. Cerebrospinal fluid proteomics in multiple sclerosis. *Biochim Biophys Acta* 1854(7), 746-756 (2015).
6. Raphael I, Webb J, Stuve O, Haskins W, Forsthuber T. Body fluid biomarkers in multiple sclerosis: how far we have come and how they could affect the clinic now and in the future. *Expert Rev Clin Immunol* 11(1), 69-91 (2015).
7. Lim CK, Brew BJ, Sundaram G, Guillemin GJ. Understanding the roles of the kynurenine pathway in multiple sclerosis progression. *Int J Tryptophan Res* 3 157-167 (2010).
8. Haider L, Fischer MT, Frischer JM *et al*. Oxidative damage in multiple sclerosis lesions. *Brain* 134(Pt 7), 1914-1924 (2011).
9. Ma Q. Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol* 53 401-426 (2013).
10. Witte ME, Nijland PG, Drexhage JA *et al*. Reduced expression of PGC-1alpha partly underlies mitochondrial changes and correlates with neuronal loss in multiple sclerosis cortex. *Acta Neuropathol* 125(2), 231-243 (2013).
11. Linker RA, Lee DH, Ryan S *et al*. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain* 134(Pt 3), 678-692 (2011).

* In vitro and animal model studies showing dimethylfumarate has effect through Nrf2 pathway.

12. Ruggieri S, Tortorella C, Gasperini C. Pharmacology and clinical efficacy of dimethyl fumarate (BG-12) for treatment of relapsing-remitting multiple sclerosis. *Ther Clin Risk Manag* 10 229-239 (2014).

13. Jikimoto T, Nishikubo Y, Koshiba M *et al*. Thioredoxin as a biomarker for oxidative stress in patients with rheumatoid arthritis*. Mol Immunol* 38(10), 765-772 (2002).
14. Polman CH, Reingold SC, Banwell B *et al*. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria*. Ann Neurol* 69(2), 292-302 (2011).
15. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis*. Neurology* 46(4), 907-911 (1996).
16. Tavazzi B, Batocchi AP, Amorini AM *et al*. Serum metabolic profile in multiple sclerosis patients*. Mult Scler Int* 2011 167156 (2011).

* Powered cross-sectional study showing serum nitric oxide metabolites are increased in MS.

17. Ibragic S, Sofic E, Suljic E, Avdagic N, Bajraktarevic A, Tahirovic I. Serum nitric oxide concentrations in patients with multiple sclerosis and patients with epilepsy*. J Neural Transm (Vienna)* 119(1), 7-11 (2012).
18. Mostert JP, Ramsaransing GS, Heersema DJ, Heerings M, Wilczak N, De Keyser J. Serum uric acid levels and leukocyte nitric oxide production in multiple sclerosis patients outside relapses*. J Neurol Sci* 231(1-2), 41-44 (2005).
19. Acar G, Idiman F, Idiman E, Kirkali G, Cakmakci H, Ozakbas S. Nitric oxide as an activity marker in multiple sclerosis*. J Neurol* 250(5), 588-592 (2003).
20. Nazliel B, Taskiran D, Irkec C, Kutay FZ, Pogun S. Serum nitric oxide metabolites in patients with multiple sclerosis*. J Clin Neurosci* 9(5), 530-532 (2002).
21. Sait Keles M, Taysi S, Aksoy H, Sen N, Polat F, Akcay F. The effect of corticosteroids on serum and cerebrospinal fluid nitric oxide levels in multiple sclerosis*. Clin Chem Lab Med* 39(9), 827-829 (2001).
22. Giovannoni G, Miller DH, Losseff NA *et al*. Serum inflammatory markers and clinical/MRI markers of disease progression in multiple sclerosis*. J Neurol* 248(6), 487-495 (2001).
23. Giovannoni G, Heales SJ, Silver NC *et al*. Raised serum nitrate and nitrite levels in patients with multiple sclerosis*. J Neurol Sci* 145(1), 77-81 (1997).
24. Haghikia A, Kayacelebi AA, Beckmann B *et al*. Serum and cerebrospinal fluid concentrations of homoarginine, arginine, asymmetric and symmetric dimethylarginine, nitrite and nitrate in patients with multiple sclerosis and neuromyelitis optica*. Amino Acids* 47(9), 1837-1845 (2015).
25. Peltola J, Ukkonen M, Moilanen E, Elovaara I. Increased nitric oxide products in CSF in primary progressive MS may reflect brain atrophy*. Neurology* 57(5), 895-896 (2001).
26. De Bustos F, Navarro JA, De Andres C *et al*. Cerebrospinal fluid nitrate levels in patients with multiple sclerosis*. Eur Neurol* 41(1), 44-47 (1999).
27. Seven A, Aslan M, Incir S, Altintas A. Evaluation of oxidative and nitrosative stress in relapsing remitting multiple sclerosis: effect of corticosteroid therapy*. Folia Neuropathol* 51(1), 58-64 (2013).
28. Stepien A, Chalimoniuk M, Lubina-Dabrowska N, Chrapusta SJ, Galbo H, Langfort J. Effects of interferon beta-1a and interferon beta-1b monotherapies on selected serum cytokines and nitrite levels in patients with relapsing-remitting multiple sclerosis: a 3-year longitudinal study*. Neuroimmunomodulation* 20(4), 213-222 (2013).
29. Rejdak K, Petzold A, Stelmasiak Z, Giovannoni G. Cerebrospinal fluid brain specific proteins in relation to nitric oxide metabolites during relapse of multiple sclerosis*. Mult Scler* 14(1), 59-66 (2008).
30. Rejdak K, Petzold A, Kocki T *et al*. Astrocytic activation in relation to inflammatory markers during clinical exacerbation of relapsing-remitting multiple sclerosis*. J Neural Transm (Vienna)* 114(8), 1011-1015 (2007).

31. Yamashita T, Ando Y, Obayashi K, Uchino M, Ando M. Changes in nitrite and nitrate (NO2-/NO3-) levels in cerebrospinal fluid of patients with multiple sclerosis. *J Neurol Sci* 153(1), 32-34 (1997).
32. Rejdak K, Eikelenboom MJ, Petzold A *et al*. CSF nitric oxide metabolites are associated with activity and progression of multiple sclerosis. *Neurology* 63(8), 1439-1445 (2004).
33. Danilov AI, Andersson M, Bavand N, Wiklund NP, Olsson T, Brundin L. Nitric oxide metabolite determinations reveal continuous inflammation in multiple sclerosis. *J Neuroimmunol* 136(1-2), 112-118 (2003).
34. Miljkovic D, Drulovic J, Trajkovic V *et al*. Nitric oxide metabolites and interleukin-6 in cerebrospinal fluid from multiple sclerosis patients. *Eur J Neurol* 9(4), 413-418 (2002).
35. Yuceyar N, Taskiran D, Sagduyu A. Serum and cerebrospinal fluid nitrite and nitrate levels in relapsing-remitting and secondary progressive multiple sclerosis patients. *Clin Neurol Neurosurg* 103(4), 206-211 (2001).
36. Drulovic J, Dujmovic I, Mesaros S *et al*. Raised cerebrospinal fluid nitrite and nitrate levels in patients with multiple sclerosis: no correlation with disease activity. *Mult Scler* 7(1), 19-22 (2001).
37. Svenningsson A, Petersson AS, Andersen O, Hansson GK. Nitric oxide metabolites in CSF of patients with MS are related to clinical disease course. *Neurology* 53(8), 1880-1882 (1999).
38. Brundin L, Morcos E, Olsson T, Wiklund NP, Andersson M. Increased intrathecal nitric oxide formation in multiple sclerosis; cerebrospinal fluid nitrite as activity marker. *Eur J Neurol* 6(5), 585-590 (1999).
39. Giovannoni G. Cerebrospinal fluid and serum nitric oxide metabolites in patients with multiple sclerosis. *Mult Scler* 4(1), 27-30 (1998).
40. Johnson AW, Land JM, Thompson EJ, Bolanos JP, Clark JB, Heales SJ. Evidence for increased nitric oxide production in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 58(1), 107 (1995).
41. Yamashita S, Ando Y. Genotype-phenotype relationship in hereditary amyotrophic lateral sclerosis. *Transl Neurodegener* 4 13 (2015).
42. Giovannoni G, Silver NC, O'riordan J *et al*. Increased urinary nitric oxide metabolites in patients with multiple sclerosis correlates with early and relapsing disease. *Mult Scler* 5(5), 335-341 (1999).

* A study showing urine nitric oxide metabolites may also have potential as a biomarker in MS.

43. Tasset I, Bahamonde C, Aguera E *et al*. Effect of natalizumab on oxidative damage biomarkers in relapsing-remitting multiple sclerosis. *Pharmacol Rep* 65(3), 624-631 (2013).
44. Szalardy L, Zadori D, Tanczos E *et al*. Elevated levels of PPAR-gamma in the cerebrospinal fluid of patients with multiple sclerosis. *Neuroscience Letters* 554 131-134 (2013).
45. Minohara M, Matsuoka T, Li W *et al*. Upregulation of myeloperoxidase in patients with opticospinal multiple sclerosis: positive correlation with disease severity. *J Neuroimmunol* 178(1-2), 156-160 (2006).
46. Tasset I, Aguera E, Sanchez-Lopez F *et al*. Peripheral oxidative stress in relapsing-remitting multiple sclerosis. *Clinical Biochemistry* 45(6), 440-444 (2012).
47. Qi X, Lewin AS, Sun L, Hauswirth WW, Guy J. Suppression of mitochondrial oxidative stress provides long-term neuroprotection in experimental optic neuritis. *Invest Ophthalmol Vis Sci* 48(2), 681-691 (2007).

* Elegant mouse model work showing that transfection of SOD2 to increase SOD2 activity ameliorates allergic encephalomyelitis in a mouse model.

48. Kemp K, Gray E, Mallam E, Scolding N, Wilkins A. Inflammatory cytokine induced regulation of superoxide dismutase 3 expression by human mesenchymal stem cells. *Stem Cell Rev* 6(4), 548-559 (2010).

49. Polidoro G, Di Ilio C, Arduini A, La Rovere G, Federici G. Superoxide dismutase, reduced glutathione and TBA-reactive products in erythrocytes of patients with multiple sclerosis. *Int J Biochem* 16(5), 505-509 (1984).
50. Ljubisavljevic S, Stojanovic I, Cvetkovic T *et al*. Erythrocytes' antioxidative capacity as a potential marker of oxidative stress intensity in neuroinflammation. *J Neurol Sci* 337(1-2), 8-13 (2014).
51. Mitosek-Szewczyk K, Gordon-Krajcer W, Walendzik P, Stelmasiak Z. Free radical peroxidation products in cerebrospinal fluid and serum of patients with multiple sclerosis after glucocorticoid therapy. *Folia Neuropathol* 48(2), 116-122 (2010).
52. Damiano S, Sasso A, De Felice B *et al*. The IFN-beta 1b effect on Cu Zn superoxide dismutase (SOD1) in peripheral mononuclear blood cells of relapsing-remitting multiple sclerosis patients and in neuroblastoma SK-N-BE cells. *Brain Res Bull* 118 1-6 (2015).
53. Adamczyk-Sowa M, Pierzchala K, Sowa P, Polaniak R, Kukla M, Hartel M. Influence of melatonin supplementation on serum antioxidative properties and impact of the quality of life in multiple sclerosis patients. *J Physiol Pharmacol* 65(4), 543-550 (2014).
54. Adamczyk-Sowa M, Sowa P, Pierzchala K, Polaniak R, Labuz-Roszak B. Antioxidative enzymes activity and malondialdehyde concentration during mitoxantrone therapy in multiple sclerosis patients. *J Physiol Pharmacol* 63(6), 683-690 (2012).
55. Acar A, Ugur Cevik M, Evliyaoglu O *et al*. Evaluation of serum oxidant/antioxidant balance in multiple sclerosis. *Acta Neurol Belg* 112(3), 275-280 (2012).
56. Ljubisavljevic S, Stojanovic I, Vojinovic S *et al*. Cerebrospinal fluid and plasma oxidative stress biomarkers in different clinical phenotypes of neuroinflammatory acute attacks. Conceptual accession: from fundamental to clinic. *Cell Mol Neurobiol* 33(6), 767-777 (2013).
57. Inarrea P, Alarcia R, Alava MA *et al*. Mitochondrial complex enzyme activities and cytochrome C expression changes in multiple sclerosis. *Mol Neurobiol* 49(1), 1-9 (2014).
58. Gray E, Kemp K, Hares K *et al*. Increased microglial catalase activity in multiple sclerosis grey matter. *Brain Res* 1559 55-64 (2014).
59. Guy J, Qi X, Hauswirth WW. Adeno-associated viral-mediated catalase expression suppresses optic neuritis in experimental allergic encephalomyelitis. *Proc Natl Acad Sci U S A* 95(23), 13847-13852 (1998).
60. Jensen GE, Clausen J. Glutathione peroxidase and reductase, glucose-6-phosphate dehydrogenase and catalase activities in multiple sclerosis. *J Neurol Sci* 63(1), 45-53 (1984).
61. Kopff M, Zakrzewska I, Czernicki J, Klem J, Strzelczyk M. Red cell superoxide dismutase and catalase activity in multiple sclerosis. *Acta Biochim Pol* 40(1), 154-157 (1993).
62. Calabrese V, Scapagnini G, Ravagna A *et al*. Nitric oxide synthase is present in the cerebrospinal fluid of patients with active multiple sclerosis and is associated with increases in cerebrospinal fluid protein nitrotyrosine and S-nitrosothiols and with changes in glutathione levels. *J Neurosci Res* 70(4), 580-587 (2002).
63. Rosenling T, Stoop MP, Attali A *et al*. Profiling and identification of cerebrospinal fluid proteins in a rat EAE model of multiple sclerosis. *J Proteome Res* 11(4), 2048-2060 (2012).
64. Szeinberg A, Golan R, Ben-Ezzer J, Sarova-Pinhas I, Kindler D. Glutathione peroxidase activity in various types of blood cells in multiple sclerosis. *Acta Neurol Scand* 63(1), 67-75 (1981).
65. Szeinberg A, Golan R, Ben Ezzer J, Sarova-Pinhas I, Sadeh M, Braham J. Decreased erythrocyte glutathione peroxidase activity in multiple sclerosis. *Acta Neurol Scand* 60(5), 265-271 (1979).

66. Ljubisavljevic S, Stojanovic I, Cvetkovic T *et al*. Glutathione homeostasis disruption of erythrocytes, but not glutathione peroxidase activity change, is closely accompanied with neurological and radiological scoring of acute CNS inflammation. *Neuroimmunomodulation* 21(1), 13-20 (2014).
67. Shukla VK, Jensen GE, Clausen J. Erythrocyte glutathione perioxidase deficiency in multiple sclerosis. *Acta Neurol Scand* 56(6), 542-550 (1977).
68. Zachara B, Gromadzinska J, Czernicki J, Maciejek Z, Chmielewski H. Red blood cell glutathione peroxidase activity in multiple sclerosis. *Klin Wochenschr* 62(4), 179-182 (1984).
69. Jensen GE, Gissel-Nielsen G, Clausen J. Leucocyte glutathione peroxidase activity and selenium level in multiple sclerosis. *J Neurol Sci* 48(1), 61-67 (1980).
70. Sorensen PS, Hansen JC, Mai J, Nielsen LR, Sorensen T. Lymphocyte glutathione peroxidase activity during exacerbations in multiple sclerosis. *Biol Trace Elem Res* 33 145-150 (1992).
71. Ortiz GG, Macias-Islas MA, Pacheco-Moises FP *et al*. Oxidative stress is increased in serum from Mexican patients with relapsing-remitting multiple sclerosis. *Dis Markers* 26(1), 35-39 (2009).
72. Socha K, Kochanowicz J, Karpinska E *et al*. Dietary habits and selenium, glutathione peroxidase and total antioxidant status in the serum of patients with relapsing-remitting multiple sclerosis. *Nutr J* 13 62 (2014).
73. Calabrese V, Raffaele R, Cosentino E, Rizza V. Changes in cerebrospinal fluid levels of malondialdehyde and glutathione reductase activity in multiple sclerosis. *Int J Clin Pharmacol Res* 14(4), 119-123 (1994).
74. Kaplan E, Bigelow D, Vatassery G, Ansari K. Glutathione peroxidase in human cerebrospinal fluid. *Brain Res* 252(2), 391-393 (1982).
75. Bo L, Dawson TM, Wesselingh S *et al*. Induction of nitric oxide synthase in demyelinating regions of multiple sclerosis brains. *Ann Neurol* 36(5), 778-786 (1994).
76. Okuda Y, Nakatsuji Y, Fujimura H *et al*. Expression of the inducible isoform of nitric oxide synthase in the central nervous system of mice correlates with the severity of actively induced experimental allergic encephalomyelitis. *J Neuroimmunol* 62(1), 103-112 (1995).
77. Aheng C, Ly N, Kelly M *et al*. Deletion of UCP2 in iNOS deficient mice reduces the severity of the disease during experimental autoimmune encephalomyelitis. *PLoS One* 6(8), e22841 (2011).
78. Lopez-Moratalla N, Gonzalez A, Aymerich MS *et al*. Monocyte inducible nitric oxide synthase in multiple sclerosis: regulatory role of nitric oxide. *Nitric Oxide* 1(1), 95-104 (1997).
79. Bizzozero OA, Dejesus G, Callahan K, Pastuszyn A. Elevated protein carbonylation in the brain white matter and gray matter of patients with multiple sclerosis. *J Neurosci Res* 81(5), 687-695 (2005).
80. Oliveira SR, Kallaur AP, Simao AN *et al*. Oxidative stress in multiple sclerosis patients in clinical remission: association with the expanded disability status scale. *J Neurol Sci* 321(1-2), 49-53 (2012).
81. Miller E, Walczak A, Saluk J, Ponczek MB, Majsterek I. Oxidative modification of patient's plasma proteins and its role in pathogenesis of multiple sclerosis. *Clin Biochem* 45(1-2), 26-30 (2012).
82. Sadowska-Bartosz I, Adamczyk-Sowa M, Gajewska A, Bartosz G. Oxidative modification of blood serum proteins in multiple sclerosis after interferon or mitoxantrone treatment. *J Neuroimmunol* 266(1-2), 67-74 (2014).
83. Rommer PS, Greilberger J, Salhofer-Polanyi S, Auff E, Leutmezer F, Herwig R. Elevated levels of carbonyl proteins in cerebrospinal fluid of patients with neurodegenerative diseases. *Tohoku J Exp Med* 234(4), 313-317 (2014).
84. Zabaleta M, Marino R, Borges J *et al*. Activity profile in multiple sclerosis: an integrative approach. A preliminary report. *Mult Scler* 8(4), 343-349 (2002).

85. Iarlori C, Gambi D, Lugaresi A *et al*. Reduction of free radicals in multiple sclerosis: effect of glatiramer acetate (Copaxone). *Mult Scler* 14(6), 739-748 (2008).
86. Stojanovic I, Vojinovic S, Ljubisavljevic S *et al*. INF-beta1b therapy modulates L-arginine and nitric oxide metabolism in patients with relapse remittent multiple sclerosis. *J Neurol Sci* 323(1-2), 187-192 (2012).
87. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. *Am J Clin Nutr* 57(5 Suppl), 715S-724S; discussion 724S-725S (1993).

* A good review of the basic science of measuring lipid peroxidation.

88. Ionescu R, Broza Y, Shaltieli H *et al*. Detection of multiple sclerosis from exhaled breath using bilayers of polycyclic aromatic hydrocarbons and single-wall carbon nanotubes. *ACS Chem Neurosci* 2(12), 687-693 (2011).
89. Halliwell B. Lipid peroxidation, antioxidants and cardiovascular disease: how should we move forward? *Cardiovasc Res* 47(3), 410-418 (2000).
90. Teunissen CE, Sombekke M, Van Winsen L *et al*. Increased plasma 8,12-iso-iPF2alpha- VI levels in relapsing multiple sclerosis patients are not predictive of disease progression. *Mult Scler* 18(8), 1092-1098 (2012).
91. Miller E, Mrowicka M, Saluk-Juszczak J, Ireneusz M. The level of isoprostanes as a non-invasive marker for in vivo lipid peroxidation in secondary progressive multiple sclerosis. *Neurochem Res* 36(6), 1012-1016 (2011).
92. Greco A, Minghetti L, Puopolo M *et al*. Cerebrospinal fluid isoprostanes are not related to inflammatory activity in relapsing-remitting multiple sclerosis. *J Neurol Sci* 224(1-2), 23-27 (2004).
93. Mir F, Lee D, Ray H, Sadiq SA. CSF isoprostane levels are a biomarker of oxidative stress in multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm* 1(2), e21 (2014).
94. Sbardella E, Greco A, Stromillo ML *et al*. Isoprostanes in clinically isolated syndrome and early multiple sclerosis as biomarkers of tissue damage and predictors of clinical course. *Mult Scler* 19(4), 411-417 (2013).
95. Mattsson N, Haghighi S, Andersen O *et al*. Elevated cerebrospinal fluid F2-isoprostane levels indicating oxidative stress in healthy siblings of multiple sclerosis patients. *Neurosci Lett* 414(3), 233-236 (2007).
96. Greco A, Minghetti L, Sette G, Fieschi C, Levi G. Cerebrospinal fluid isoprostane shows oxidative stress in patients with multiple sclerosis. *Neurology* 53(8), 1876-1879 (1999).
97. Ljubisavljevic S, Stojanovic I, Pavlovic D, Sokolovic D, Stevanovic I. Aminoguanidine and N-acetyl-cysteine supress oxidative and nitrosative stress in EAE rat brains. *Redox Rep* 16(4), 166-172 (2011).
98. Yousefi B, Ahmadi Y, Ghorbanihaghjo A, Faghfoori Z, Irannejad VS. Serum arsenic and lipid peroxidation levels in patients with multiple sclerosis. *Biol Trace Elem Res* 158(3), 276-279 (2014).
99. Besler HT, Comoglu S, Okcu Z. Serum levels of antioxidant vitamins and lipid peroxidation in multiple sclerosis. *Nutr Neurosci* 5(3), 215-220 (2002).
100. Keles MS, Taysi S, Sen N, Aksoy H, Akcay F. Effect of corticosteroid therapy on serum and CSF malondialdehyde and antioxidant proteins in multiple sclerosis. *Can J Neurol Sci* 28(2), 141-143 (2001).
101. Karg E, Klivenyi P, Nemeth I, Bencsik K, Pinter S, Vecsei L. Nonenzymatic antioxidants of blood in multiple sclerosis. *J Neurol* 246(7), 533-539 (1999).
102. Gironi M, Borgiani B, Mariani E *et al*. Oxidative stress is differentially present in multiple sclerosis courses, early evident, and unrelated to treatment. *J Immunol Res* 2014 961863 (2014).
103. Ghabaee M, Jabedari B, Al EEN, Ghaffarpour M, Asadi F. Serum and cerebrospinal fluid antioxidant activity and lipid peroxidation in Guillain-Barre syndrome and multiple sclerosis patients. *Int J Neurosci* 120(4), 301-304 (2010).
104. Sanoobar M, Eghtesadi S, Azimi A, Khalili M, Jazayeri S, Reza Gohari M. Coenzyme Q10 supplementation reduces oxidative stress and increases antioxidant enzyme

activity in patients with relapsing-remitting multiple sclerosis. *Int J Neurosci* 123(11), 776-782 (2013).
105. Khalili M, Eghtesadi S, Mirshafiey A *et al*. Effect of lipoic acid consumption on oxidative stress among multiple sclerosis patients: a randomized controlled clinical trial. *Nutr Neurosci* 17(1), 16-20 (2014).

Future perspective

Currently, evaluating potential therapies in MS requires lengthy clinical trials where putative immunomodulators are put to the test. After 2 or more years, patients reach meaningful and measurable clinical endpoints, which is unsurprisingly costly and contributes to the slow pace of drug discovery.

Over the next decade, the use of candidate biomarkers in cohort studies and trials will allow reliably biomarkers to be validated as sensitive measures of disease activity and therapeutic response. The process of selecting therapies for clinical trial will thus be foreshortened and these markers will eventually guide clinical management in the age of personalised therapies. The discovery of a specific Nrf2-dependent oxidative-stress mediated mechanism of action for dimethyl fumarate in MS suggests such biomarkers may well emerge from the field of oxidative stress.

Executive summary

Reactive Oxygen Species

- Most are short-lived and difficult to measure directly in tissue fluids.
- Nitric oxide metabolites have been shown to be elevated in MS CSF, plasma and serum, and may have a role in monitoring disease activity.

Transcriptional Regulators of Reactive Oxygen Species

- Nrf2, as a target of effective therapy in MS, is an emerging biomarker of disease activity.

Reactive Oxygen Species Enzymes

- Superoxide dismutase activity has been shown to be decreased in MS CSF but increased in plasma and serum, suggesting a role as a diagnostic marker.
- Catalase activity has been shown to be elevated in MS CSF, plasma and serum, suggesting a role as a diagnostic marker.
- Inducible nitric oxide synthase has been reported elevated in in MS CSF and in MS peripheral blood lymphocytes in keeping with a role as a diagnostic marker.

End-products of Oxidation

- Protein carbonylation and nitrosylation have been shown to be elevated in MS CSF, plasma and serum and may have a role in monitoring treatment response.

- Isoprostanes and MDA have been shown to be elevated in CSF, plasma and serum, and may track disease activity.
- Plasma oxidised DNA has been shown to be elevated in MS and may have a role as a diagnostic marker.

Financial disclosure/Acknowledgements

R. Ibitoye in an Academic Clinical Fellow in Neurology whose post is funded by the National Institute for Health Research, UK.

Figure Captions

Figure 1 - Nicotinamide Adenine Dinucleotide Phosphate (NADPH)-oxidase catalyses the generation of the toxic superoxide (O_2^-) radical anion (right). Coincident transcriptional upregulation of inducible nitric oxide synthase (NOS) increases nitric oxide radical (NO.) production (left). NO. and O_2^- react to form $ONOO^-$ (orange), peroxynitrite, a highly toxic reactive oxygen species.

Figure 2 – Superoxide dismutase catalyses the detoxification of superoxide to hydrogen peroxide which decomposes to water and oxygen, catalysed by peroxidases, in particular, catalase. Glutathione peroxidase (not shown) catalyses the reduction of peroxides through oxidation of reduced glutathione.

Figure 3 - Simplified schematic representing anti-oxidant responses to reactive oxygen species (ROS) via Nrf2 and PGC1α. (ARE: anti-oxidant response element; PPRE: PPAR responsive element)

Table Captions

Table 1 - The review focuses on major oxidative stress markers, closely implicated in MS pathogenesis and reported in the literature.

Table 2 – Oxidative stress biomarkers with diagnostic potential in MS. (-) - not enough evidence; ⇧ - increased; ⇩ - decreased; ⇧/⇩ - inconsistent, mixed or non-significant findings; BOLD – evidence supports a diagnostic role for this marker.