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Reliable disease biomarkers characterizing and identifying electrohypersensitivity and multiple chemical sensitivity as two etiopathogenic aspects of a unique pathological disorder

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Abstract: Much of the controversy over the causes of electrohypersensitivity (EHS) and multiple chemical sensitivity (MCS) lies in the absence of both recognized clinical criteria and objective biomarkers for widely accepted diagnosis. Since 2009, we have prospectively investigated, clinically and biologically, 1216 consecutive EHS and/or MCS-self reporting cases, in an attempt to answer both questions. We report here our preliminary data, based on 727 evaluable of 839 enrolled cases: 521 (71.6%) were diagnosed with EHS, 52 (7.2%) with MCS, and 154 (21.2%) with both EHS and MCS. Two out of three patients with EHS and/or MCS were female; mean age (years) was 47. As inflammation appears to be a key process resulting from electromagnetic field (EMF) and/or chemical effects on tissues, and histamine release is potentially a major mediator of inflammation, we systematically measured histamine in the blood of patients. Near 40% had a increase in histaminemia (especially when both conditions were present), indicating a chronic inflammatory response can be detected in these patients. Oxidative stress is part of inflammation and is a key contributor to damage and response. Nitrotyrosin, a marker of both peroxynitrite (ONOO°-) production and opening of the blood-brain barrier (BBB), was increased in 28% the cases. Protein S100B, another marker of BBB opening was increased in 15%. Circulating autoantibodies against O-myelin were detected in 23%, indicating EHS and MCS may be associated with autoimmune response. Confirming animal experiments showing the increase of Hsp27 and/or Hsp70 chaperone proteins under the influence of EMF, we found increased Hsp27 and/or Hsp70 in 33% of the patients. As most patients reported chronic insomnia and fatigue, we determined the 24 h urine 6-hydroxymelatonin sulfate (6-OHMS)/creatinin ratio and found it was decreased (<0.8) in all investigated cases. Finally, considering the self-reported symptoms of EHS and MCS, we serially measured the brain blood flow (BBF) in the temporal lobes of each case with pulsed cerebral ultrasound computed tomosphygmography. Both disorders were associated with hypoperfusion in the capsulothalamic area, suggesting that the inflammatory process involve the limbic system and the thalamus. Our data strongly suggest that EHS and MCS can be objectively characterized and routinely diagnosed by commercially available simple tests. Both disorders appear to involve inflammation-related hyper-histaminemia, oxidative stress, autoimmune response, capsulothalamic hypoperfusion and BBB opening, and a deficit in melatonin metabolic availability; suggesting a risk of chronic neurodegenerative disease. Finally the common co-occurrence of EHS and MCS strongly suggests a common pathological mechanism.

Keywords: biomarkers; cerebral hypoperfusion; electrohypersensitivity; limbic system; multiple chemical sensitivity.

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Introduction

In 1962, Randolph first described clinically (1) what is today commonly called multiple chemical sensitivity (MCS) (2); a human pathological disorder that has been identified and defined in 1999 during an international consensus meeting on the basis of the six following criteria: "1. The symptoms are reproducible with [repeated chemical] exposure; 2. The condition is chronic; 3. Low levels of exposure [lower than previously or commonly tolerated] result in manifestations

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of the syndrome; 4. The symptoms improve or resolve when the inciting agents are removed; 5. Responses occur to multiple chemically unrelated substances; 6. [Added in 1999]: Symptoms involve multiple organ systems" (3). Although the precise worldwide prevalence of MCS remains unclear, it is expected that due to the vastly increased number of the various chemical products that have been put on the market during the last few decades, MCS is becoming an increasing prevalent pathological disorder (4).

The recent rise of wireless telecommunication worldwide also confronts scientists with the question of whether anthropogenic electromagnetic fields (EMFs) such as emitted by cell phones, wireless internet, and high voltage power lines, can cause adverse health effects as it is the case for chemicals. In 1991 Rea first described what he called electromagnetic field sensitivity (5). Six years later, Bergqvist et al., in a report prepared by a European group of experts for the European Commission coined the term electrohypersensitivity (EHS) to encompass in a unique concept the clinical conditions in which EHS self-reporting patients complain of symptoms they attribute to EMF exposure (6). Since 1998, Santini et al. in France, reported symptoms experienced by users of digital cellular phones and the health risk of people living near cellular phone base stations (7, 8).

In 2004, because of the increasing worldwide prevalence of EHS, the World Health Organization (WHO) organized an international scientific workshop in Prague (Czech Republic) in order to define and characterize EHS. Although not acknowledging EHS as being caused by EMF exposure, the Prague working group defined EHS as "a phenomenon where individuals experience adverse health effects while using or being in the vicinity of devices emanating electric, magnetic, or electromagnetic fields … whatever its cause, EHS is a real and sometimes a debilitating problem for the affected persons" (9). However, following this meeting, WHO proposed to use the alternative term "idiopathic environmental intolerance (IEI) attributed to electromagnetic fields" (IEI – EMF), indicating there is no proven causality between the occurrence of IEI – EMF (formerly EHS) and EMF exposure (9).

In view of the poor knowledge of pathogenesis and etiology of EHS and MCS, most mainstream medical, sanitary and societal bodies maintain there is not sufficient scientific proof to support the concept that clinical symptoms experienced by EHS and/or MCS self-reporting patients are really caused by EMF and/or chemical exposure, respectively. This is particularly the case for EHS patients, for whom in comparison to sham controls, the reproduction of clinical symptoms in the presence of EMFs have globally failed to demonstrate a causal link, in blind or double-blind studies (10).

Moreover, the lack of recognized disease biomarkers objectively characterizing EHS and MCS has resulted in clinical symptoms being dismissed as psychogenic; and/or EHS and MCS are conflated with psychosomatic or psychiatric diseases, and not recognized as true organic disorders caused by the environment (11–16). This is particularly the case for radiofrequency EMF, for which some scientists believe that EHS is an uncertain and confusing concept (17); whereas some others, on the basis of their own clinical experience agree that excessive exposure may cause EHS (5, 18, 19).

Here, we present our own experiences based on the preliminary analysis of a series of 1216 consecutive investigated cases of self-claimed EHS and/or MCS, in the framework of an ongoing prospective clinical study aiming at identifying and characterizing EHS and MCS both clinically and biologically; through the use of biomarkers detected and measured in the peripheral blood and the urine of patients. Our data clearly shows that EHS and MCS should be recognized as genuine somatic pathological entities; that patients with EHS and/or MCS are non-psychosomatic nor psychiatric patients; and probably that EHS and MCS are two etiopathogenic aspects of a single pathological disorder.

Search for reliable disease biomarkers

The identification and measurement of reliable biomarkers is a crucial step for identifying and characterizing diseases. This is *a fortiori* the case for any new pathological entity or clinical syndrome such as MCS, EHS or other environmental intolerance syndrome. However, to our knowledge, such an approach has proven inconclusive for MCS (20) and EHS (21).

We thus searched for characteristic biomarkers and selected a battery of biological tests which could be routinely used clinically in environmental medicine practice for taking care of EHS and/or MCS self-reporting patients.

In addition, due to the reported clinical symptoms, we systematically measured the brain blood flow (BBF) in both cerebral hemispheres of these patients by using echo-doppler of the middle cerebral artery (22) and measured centimeter by centimeter brain pulsatility by using pulsed ultrasound-based cerebral computerized tomosphygmography, which allows centimetric resolution pulsed ultrasound recording of cerebral pulsatility (23–25), to localize more precisely the BBF in the different areas of the two temporal lobes. Our working hypothesis was that under

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the influence of environmental factors such as EMFs and/or chemicals, some neuro-inflammation and oxidative stress might occur in the brain, with blood-brain barrier (BBB) disruption as a consequence.

We thus routinely measured the inflammation-associated high-sensitivity C reactive protein (hs-CRP) in the peripheral blood; and levels of vitamin D2-D3, as it has been suggested that low levels of its metabolite, the secosteroid 25 hydroxy-vitamin D (25-D) could be a consequence rather than a cause of inflammatory and/or autoimmune processes (26), and that vitamin D deficiency is associated with abnormal development and functioning of the central nervous system (CNS) (27, 28). Since it has been shown that upon brain injury, degeneration or infection, the inflammatory response may trigger degranulation of mast cells, leading to a massive release of histamine in the blood (29), we systematically measured the levels of histamine in the peripheral blood. In addition, as the best known mast cell degranulation mechanism involve crosslinking of the high affinity surface IgE receptor (30), we also measured total IgE levels in the peripheral blood. It is well known that histamine is a potent mediator of inflammation and is able to increase BBB permeability through oxidative and/or nitrosative stress (31, 32). So we looked for possible oxidative and/or nitrosative stress-related biomarkers of BBB disruption; and identified nitrotyrosine (NTT), because it results from the toxic effects of peroxynitrite ($ONOO^{\circ -}$) on proteins (33–36). Such a BBB opening marker has also been shown for the calcium-binding protein S100B, produced and released predominantly by peri-vascular astrocytes (37–40). During the inflammatory process, it is well known that cells produce excessive amount of superoxide (O_2°) and nitric oxide (NO°), and that although NO° is a weak free radical resulting from the action of nitric oxide synthase, its excessive intracellular production is associated with cytotoxic properties because of the formation of extremely reactive nitrogen species such as peroxynitrite. The biochemical reaction in the form of $O_2^{\circ}+NO^{\circ}=>ONOO^{\circ -}$ may thus explain why NTT (which results from oxidative and nitrosative stresses) is associated with BBB disruption (32, 41). Dosage of free NTT and protein-combined NTT as well as protein S100B in the peripheral blood of EHS and/or MCS patients was thus an important element of the battery of biological tests we used.

We also considered that non thermal radiofrequency often is a repetitive stress leading *inter alia* to continuous heat shock protein (HSP) over-expression and release in exposed tissues, particularly in the brain (42–46). HSPs are a family of highly conserved proteins with chaperone functions acting to maintain the structural conformation of cellular proteins. Their over-expression under stress conditions which promotes an inflammatory response is well known (47–49). We thus speculated that the major inducible stress protein HSP70, which has been shown to oppose to neuronal apoptosis (50, 51) and to BBB disruption (51, 52), so eliciting some neuroprotection could be involved as it could be also the case for HSP27 (53, 54). However, under chronic EMF exposure it was reported that, as compared to controls, intracellular HSP70 levels may decline (55). We thus systematically measured HSP70 and HSP27 levels in the peripheral blood of EHS and/or MCS patients in order to try to determine whether these chaperone proteins are a marker of EMF and/or chemicals chronic exposure; as it has been shown for non-thermal EMF exposure in experimental studies (42–46).

Moreover, during oxidative and nitrosative stress proteins may be extensively modified and denatured and so acquire new epitopes which can explain their loss of specificity and biological activity, hence the synthesis of autoantibodies (56, 57). This is the case for EMF exposure which has been shown to alter DNA replication and mitosis and form abnormal proteins (42, 58, 59) and so to produce electro-oxidation-related IgE autoantibodies (60). We consequently hypothesized that under the influence of environmental EMFs and/or chemicals, CNS proteins such as O-myelin may be so denatured that they acquire autoantigenic properties. Consequently we thus systematically searched for and measured autoantibodies against O-myelin in the blood of patients.

Finally, since some effects of EMF exposure have been reported to be mediated by the pineal hormone, melatonin (61), and given the fact that in our series many patients had sleep disturbance, we also systematically measured melatonin metabolism in these patients. However, as measurement of endogenous melatonin in urine is not useful because of its low unmetabolized levels (62), we measured levels of its metabolite 6-hydroxymelatonin sulfate (6-OHMS) and creatinine in 24 h urine, to determine the 6-OHMS/creatinine ratio. Note that since creatinine is excreted in a relatively constant amount in each patients, we used this ratio to reduce the variability of 6-OHMS measurement attributed to urine dilution.

The test battery for identifying and characterizing EHS and MCS is summarized in Table 1. Technical information about the methods we used for carrying out all biological tests and the BBF analysis are summarized as follows:

For the biomarker study, all patients were investigated by using commercially available biochemical tests and values for each patient were compared to the normal reference values obtained from the commercial companies. Sensitivity, specificity and reproducibility of these tests were thus those defined by these companies. Each

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Table 1: Disease biomarkers investigated in self-reporting EHS and/or MCS patients with their normal values.

Biomarker	Normal range
High-sensitivity C reactive protein (hs-CRP)	≤3 mg/L
Vitamin D2-D3	≥30 ng/mL
Histamine	≤10 nmol/L
IgE	≤100 UI/mL
Protein S100B	≤0.105 μg/L
Nitrotyrosine (NTT)	≥0.6 μg/L and ≤0.9 μg/mL
Heat shock protein 70 (HSP70)	≤5 ng/mL
Heat shock protein 27 (HSP27)	≤5 ng/mL
Anti-O-myelin autoantibodies	Negative
Hydroxy-melatonin sulfate (6-OHMS)	≥5 ng/L and ≤40 ng/L
6-OHMS/creatinine	≥0.8 and ≤8

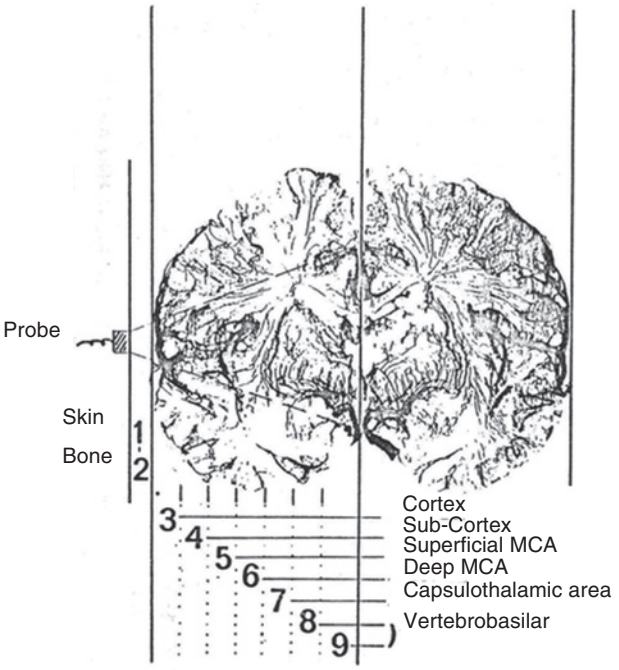

Figure 1: Pulsometric index (PI) obtained by a computerized ultrasonic cerebral tomosphygmography (UCTS) in the different area of temporal lobes.
Data are expressed as mean pulsometric index (PI). PI varies between territories studied: 3+4 correspond to cortico sub-cortical area; 3+4+5, to the superficial area of the middle cerebal artery (MCA); 5+6+7, to the deep area of the MCA; 7, to the capsulothalamic area; 3+4+5+6+7, to the complete area depending of the MCA; 8+9, to the vertebrobasilar area; 3+4+5+6+7+8+9, to the complete temporal lobe.

assay was performed according to the manufacturer's method. Hs-CRP and 25-OH vitamin D were measured by using an automated immunoassay [Architect Ci 4100 (Abbott Laboratories, Abbott Park, Chicago, IL, USA)]; for Histamine measurement we used an ELISA specific test; for protein S100B, a quantitative automated chemiluminescent immunoassays [Liason S100 (DiaSorin Deutschland GmbH, Dietzenbach, Germany)]; for NTT, a competitive ELISA test (Cell Biolabs Inc., San Diego, CA, USA); for anti-O-myelin antibody detection, a Western Blot qualitative analysis (IMMCO Diagnostics, Buffalo, NY, USA); for HSP 27 and HSP 70, specific high sensitivity enzymatic immunoassays (Stressgen Biotechnologies Corporation, San Diego, CA, USA); and for 5-hydroxy-melatonin-sulfate, a urine ELISA test (IBL International GmbH, Hamburg, Germany).

In addition, to these biochemical tests we used a non-invasive ultrasonic cerebral tomosphygmography method that we specifically set-up to investigate the blood flow in the patient temporal lobes and determined for each patient a pulsometric index (PI) that we measured centimeter by centimeter from the cortex to the diencephalic medial area (see Figure 1). This index varies between the territories studied. In this study, Pi determination for each cerebral territory in 727 EHS and/or MCS patients was compared to a retrospective series of 141 normal subjects which allowed to establish the normal median reference values of PI (see Figure 2). Finally since our study is still ongoing we did not reported any statistical analysis. This will follow in specific further papers.

Search for clinical diagnosis criteria

In 2009, at the time we initiated this prospective cohort study, we were aware there was no available recognized biological markers for defining objectively EHS and MCS; this led us to use clinical criteria as inclusion criteria. For MCS, as already above mentioned, we used the six criteria that had been reported in the 1999 international workshop (3) and for EHS, we used similar criteria. However, as in an unpublished feasibility study we showed that many EHS patients when they are in the vicinity of chemicals may present with olfactory abnormalities consisting in subjective odor disruption; we systematically added a seventh clinical criteria to the six ones already defined during the 1999 consensus meeting on MCS, in order to further characterize clinically MCS and distinguish it from EHS. Accordingly patients with MCS, unlike EHS patients, were characterized not only by the simple odor intolerance, but more specifically by symptoms of mucous inflammation in the nose, the oropharynx and/or the laryngo-tracheo-bronchus tract; manifesting clinically as rhinitis, oropharyngeal dysesthesia or laryngitis and/or bronchospasms, respectively.

To further avoid any confounding pathology, all patients of the present prospective series have been

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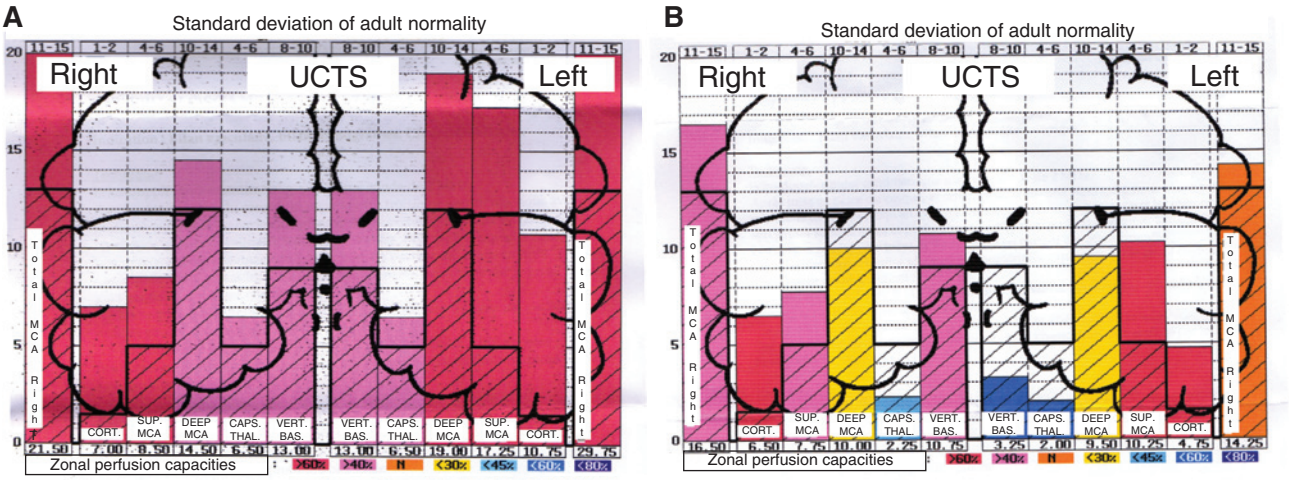

Figure 2: Example of diagrams obtained by using UCTS exploring the global centimetric ultrasound pulsatility in the two temporal lobes of a normal subject (A) and in a EHS self-reporting patient (B).
Measurements are expressed in Pulsometric index (PI). Note that in A and B mean values of PI in each explored area recorded is from the cortex to the internal part of each temporal lobe; so on the left part of the two diagrams A and B for the right lobe from the left to the right; and on the right part of these diagrams for the left lobe from the right to the left. Note also that in A (normal subject) all values are over the normal median values whereas in B (EHS-self reporting patients) values in the capsulothalamic areas (the fifth and the second column for the right and left temporal lobe, respectively) are under the normal median values.

interviewed face to face at length during their medical consultation and questioned systematically about their past medical history and the type and conditions of occurrence of their clinical symptoms, thanks to the use of a validated pre-established questionnaire. In addition, all patients have been carefully physically examined. Also, before inclusion, all patients were systematically investigated by usual routine blood tests and medical imaging including Brain MRI and/or scanner and carotid echodoppler in order to eliminate any known unrelated CNS pathology.

Finally, based on the above clinical finding for both EHS and MCS patients we used the following inclusion criteria:

1. Absence of known pathology accounting for the observed clinical symptoms;
2. Reproducibility of symptom occurrence under the influence of EMFs and/or multiple chemicals whatever their incriminated source;
3. Regression or disappearance of symptoms in the case of EMF and/or multiple chemical avoidance;
4. Chronic evolution;
5. Symptoms such as headache, superficial and/or deep sensibility abnormalities, skin lesions, sympathetic-nerve dysfunction, reduced cognitive ability including loss of immediate memory and attention and/or concentration deficiencies, insomnia, chronic fatigue and depressive tendency, all main clinical symptoms reported as non-specific symptoms in the scientific literature (13, 19), but which when grouped together may evoke clinically the diagnosis of EHS (data not shown);
6. No serious pre-existing pathology such as atherosclerosis, diabetes, cancer; and/or neurodegenerative or psychiatric diseases which have been associated with EHS and/or MCS in the past or at the inclusion time but would render difficult the interpretation of clinical symptoms and biomarker data (see Section "EHS/MCS as a possible sentinel pathological disorder"); and finally
7. For each patient written informed consent.

Study of this large cohort of patients was not a case-control study neither a randomized study so there was no specific control group.

As depicted in Table 2, on a total of 1216 investigated consecutive cases, 839 are presently analyzed of whom 727 are evaluable, 521 with EHS (71.7%), 52 with MCS (7.1%) and 154 with both EHS and MCS (21.2%), regardless of whether MCS occurred before or after EHS. Only 29 patients, i.e. 3% claimed to suffer from EHS and/or MCS but did not meet the inclusion criteria. In fact most of these patients claimed to be electrohypersensitive. Although many of them were associated with a putative neurologic or psychiatric disorder, EHS could not be clearly established. Also excluded were patients with EHS and/or MCS who were in addition, diagnosed as suffering from heavy pathology evidenced after inclusion, or who were lost to follow-up, or for whom results of the biological investigation were not available at the time of analysis.

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Table 2: Summary of the present ongoing prospective clinic-biological study of EHS and/or MCS-self reporting patients.

Patients groups	Total	EHS	MCS	EHS/MCS
Total investigated	1216			
Total presently analyzed	839			
Neither EHS nor MCS	29			
Not evaluable	83			
Evaluable	727	521	52	154
Sex ratio	495 W/232 M	344 W/177 M	34 W/18 M	117 W/37 M
	68%/32%	66%/34%	65%/35%	76%/24%
Mean age	47.9±12.4	48.2±12.9	48.5±10.3	46.7±11.2
Median age [range][a]	47 [16–83]	48 [16–83]	47 [31–70]	46 [22–76]

[a]The range of values is indicated in square brackets, e.g. [minimum-maximum].

Demographic panorama

This large cohort of investigated patients originated from many different European countries, and from other countries worldwide such as the US, Canada, Australia, Russia, China, Middle East and Africa. This allows some estimation the demographic picture of so called EHS and/or MCS patients. The demographic data are depicted in Table 2 and Figure 3.

A noteworthy finding which was observed in many countries is that women appear to be much more susceptible to EHS and/or MCS than men, since in our series two thirds are female, with no difference between EHS and MCS rates. Note however, that the female predominance appears to be more pronounced for patients with both EHS and MCS, where three out of four are female (Table 2).

In this series, median age is about 47 years and does not differ according to EHS, MCS and EHS/MCS diagnosis. As indicated in Figure 3, all age categories are represented and mainly include young and old adults, but it appears that adolescents may be also associated with EHS. This may be due to their excessive use of wireless technology (essentially mobile phones and other devices) at this age. In fact, outside of the present series, we have observed that infants and children could also be suffering from EHS.

Analysis of biochemical markers

Biomarker results are indicated in Tables 3–5 and in Figure 4.

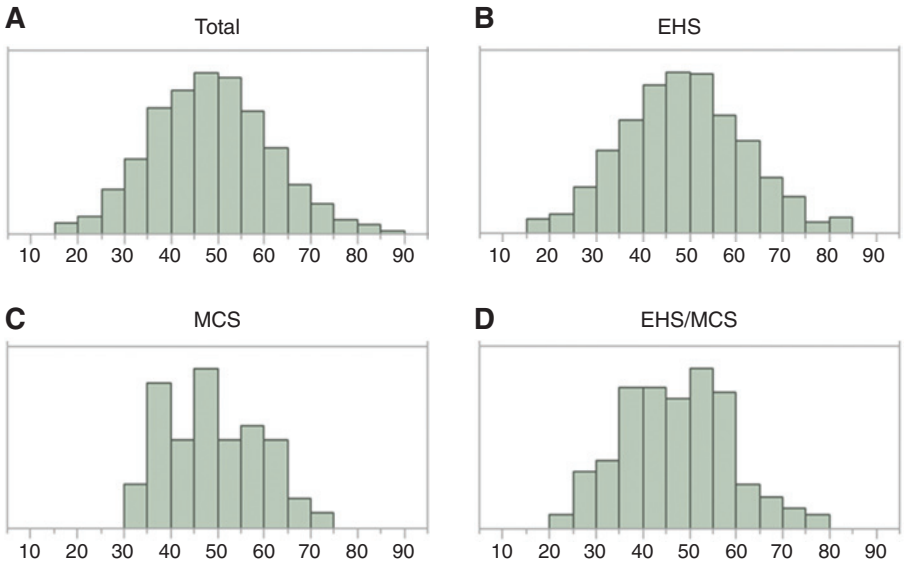

Figure 3: Age categories according to the total number of evaluable patients (A) and to the three EHS (B), MCS (C) and EHS/MCS (D) analyzed groups of patients.

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Table 3: High-sensitivity C reactive protein (hs-CRP), immunoglobulin E (IgE), vitamin D2-D3 and histamine in the peripheral blood of EHS and/or MCS self-reporting patients.

Patients groups	EHS	MCS	EHS/MCS
n	521	52	154
hs-CRP	78 (14.97%)	3 (13.46%)	22 (14.28%)
>3 mg/L	[3.27–51.91]	[3.5–10]	[3.27–21.61]
Vitamine D	33 (6.33%)	5 (9.62%)	16 (10.39%)
<10 ng/mL	[4.81–9.70]	[4.80–8.00]	[7.10–9.90]
Vitamine D	300 (57.58%)	25 (48.07%)	92 (59.74%)
≥10 ng/mL and <30 ng/mL	[10.40–29.70]	[10.70–27.90]	[15.00–28.60]
Histamine	182/491 (37%)	18/44 (36.7%)	59/142 (41.5%)
>10 nmol/L	[10.08–360.00]	[10.80–90.00]	[10.10–1797.50]
IgE	115 (22.07%)	8 (15.38%)	38 (24.68%)
>100 UI/mL	[101–1387.60]	[131.10–294.87]	[103.30–1200.00]

Note that for each biomarker the range of values is indicated in square brackets, e.g. [minimum-maximum].

Table 4: Protein S100B and nitrotyrosin (NTT) in the peripheral blood of EHS and/or MCS self-reporting patients.

Patients groups	EHS	MCS	EHS/MCS
n	521	52	154
S100B	73/495 (14.7%)	6/51 (19.7%)	28/142 (10.7%)
>0.105 μg/L	[0.105–2.090]	[0.110–0.500]	[0.110–0.470]
NTT	77/259 (29.7%)	6/29 (26%)	22/76 (28.9%)
>0.9 μg/mL	[0.92–8.20]	[1.10–3.10]	[0.91–3.10]
Increased S100B and/or NTT	133/250 (53.2%)	12/22 (54.5%)	46/73 (63%)
Increased histamine, S100B and/or NTT	220/327 (71.8%)	27/36 (75%)	91/125 (79.1%)

Note that for each marker the range of values is indicated in square brackets, e.g. [minimum-maximum].

Table 5: HSP70 and HSP27 chaperone proteins and anti-O-myelin autoantibodies in the peripheral blood of EHS and/or MCS self-reporting patients.

Patients groups	EHS	MCS	EHS/MCS
n	521	52	154
Hsp 70	91/486 (18.7%)	4/52 (7.7%)	36/142 (7.6%)
>5 ng/mL	[5.90–11.20]	[7.10–7.70]	[5.20–32.20]
Hsp 27	123/476 (25.8%)	6/52 (11.5%)	42/132 (11.5%)
>5 ng/mL	[5.20–11.20]	[5.90–9.20]	[5.10–25.00]
Hsp70 and/or Hsp27	162/487 (33.3%)	9/52 (25%)	56/142 (39.4%)
Anti-O-myelin autoantibodies	109/477 (28.8%)	8/47 (17%)	33/140 (23.4%)

Note that for each marker the range of values is indicated in square brackets, e.g. [minimum–maximum].

High-sensitivity C reactive protein (hs-CRP)

An increase in hs-CRP levels was found globally in 107 patients (14.7% of the cases), and more precisely in 78 patients (15%), seven patients (13.5%) and 22 patients (14.3%), respectively in the three EHS, MCS, EHS/MCS individualized groups (Table 3); suggesting that in such cases, patients were associated with some type of systemic inflammation. We thus systematically looked for unrelated causes of inflammation and/or infection in these patients, but with the exception of three cases, we did not found any. Furthermore, since hs-CRP is considered as a biomarker of age-related cognitive decline or dementia, and more particularly of Alzheimer's disease (63, 64), we systematically searched for Alzheimer's disease in these patients. In two cases, Alzheimer's disease was discovered after inclusion and considered as possibly the results of excessive past EMF exposure (see Section "EHS/MCS as

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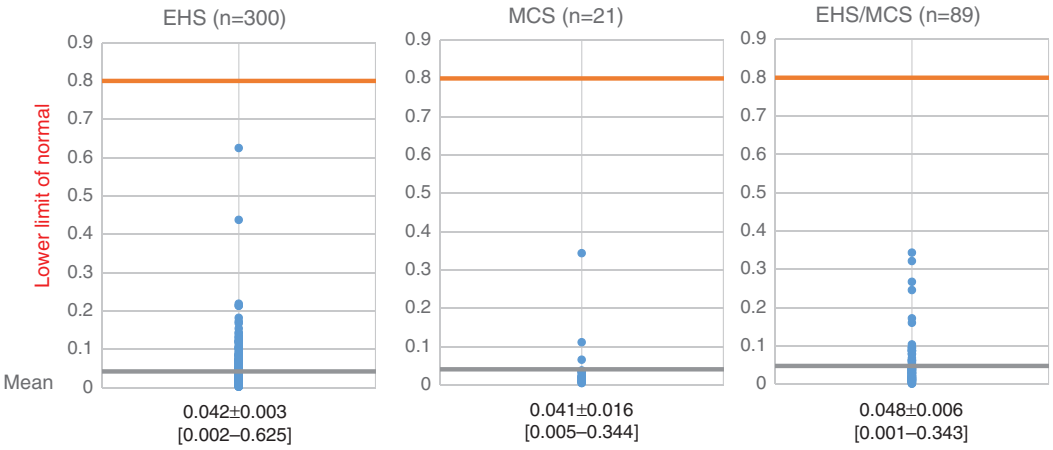

Figure 4: 24 H urine 6-OHMS/creatinine ratio in EHS and/or MCS self-reporting patients.

a possible sentinel pathological disorder"). But, because chronologically, Alzheimer's disease appeared to follow the initial occurrence of EHS, we considered that for these two patients, Alzheimer's disease might have been the consequence of EHS rather than simply associated with it. Nevertheless, these two cases were categorized as non-evaluable cases in the present analysis.

Vitamin D2–D3

As indicated in Table 3, a profound decrease in the levels of the secosteroid 25-D is found globally in 184 patients (25.3% of the cases), and in 121 patients (23.2%), 12 patients (23.1%) and 51 patients (33.1%) in the three groups, respectively. As already discussed (see Section "Search for reliable disease biomarkers"), these data agree with the concept that decrease in vitamin D2–D3 levels appear to be a consequence rather than a cause of inflammation and so need to be therapeutically normalized.

Histamine

An important finding in our study is the discovery that histamine in the peripheral blood is increased in nearly 40% of the patients and that this increase does not differ between the three groups investigated (Table 3). This finding suggests that histamine is not only a natural clinical biomarker of EHS and MCS, but also may play a crucial role in the pathogenesis of both clinical entities, since it has been shown to be not only a neurotransmitter produced and released by the CNS, but also an inflammatory mediator produced and released by mast cells in many inflammatory processes including neuro-inflammation (see Section "Pathophysiological relevance").

IgE

Levels of circulating IgE were found to be increased in 22%, 15.4% and 24.7% of the three EHS, MCS and MCS/EHS groups, respectively. Since histamine release from mast cells involve the high affinity IgE mast cell surface receptor and IgE (30, 65), we searched for a correlation between histamine and IgE levels in the peripheral blood of the patients. As it will be further discussed, it seemed not to be the case (see Section "Pathophysiological relevance").

Protein S100B

Levels of circulating protein S100B have been found to be globally increased in 107 patients (15.5%), with no differences between the three groups (Table 4). As we will discussed (see Section "Some insight into etiopathogeny") this finding confirms previously reported data showing the glia-derived S100B protein is a biomarker of hypoperfusion-associated brain damage or dysfunction (39, 40, 66–68), and more particularly of neurodegenerative diseases such as Alzheimer's disease (69) and amyotrophic lateral sclerosis (70); but differs from the negative results obtained in non EHS healthy subjects for whom protein S100B levels has been shown to be normal within the 2 h following GSM mobile phone use (71–73).

Nitrotyrosin

Likewise, increased NTT blood levels have been detected globally in 105 patients (29%), with no difference between the three groups. Moreover, as indicated in Table 4, it appears that increased levels of protein S100B and/or NTT can be detected in approximately 55%–60% of the cases.

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Since, as previously indicated, protein S100B and NTT could be potential markers of BBB disruption, we consider that such disruption could be evidenced clinically in over 50% of the patients, whatever their EHS and/or MCS clinical presentation.

HSP70 and HSP27

As indicated in Table 5, depending of the group considered, increased levels of the HSP70 and HSP27 chaperone proteins were detected in the peripheral blood in about 7%–19% and about 11%–26% of patients, respectively. Collectively, 25%–40% of the patients were found to be associated with increased levels of HSP70 and/or HSP27, without difference between the 3 so far individualized groups, meaning that HSP70 and HSP27 are circulating biomarkers not only of EMF chronic exposure as it is the case in animal experimental studies (42–46) but also of chemical chronic exposure. HSP70 and HSP27 seem to be more frequent in EHS patients than in MCS patients.

Autoantibodies against O-myelin

As indicated in Table 5, autoantibodies against O-myelin have been detected globally in 17% to nearly 29% of the patients studied with no difference between the three groups, suggesting that in these patients EHS and/or MCS were associated with some type of autoimmune response. Here too, it is more frequent in EHS than in MCS.

Melatonin

6-OHMS and creatinine were measured in the 24 h urine of a number of patients. As indicated in Figure 4, all investigated patients had a decrease in the 6-OHMS/creatinine ratio; suggesting that these patients have decreased antioxidant defenses (74, 75), and so may be at risk of chronic diseases (see Sections "Pathophysiological relevance" and "EHS/MCS as a possible sentinel pathological disorder"). Moreover, this decrease might explain why such patients present sleep disturbance.

Clinical forms of EHS and/or MCS without detectable biomarkers

Increase in hs-CRP and vitamin D2–D3 blood levels are non-specific biological parameters. On the other hand, although none of our biomarkers are per se specific (see Section "Some insight into etiopathogeny") the increased serum level of histamine, protein S100B and NTT in the peripheral blood seems more characteristic of EHS and MCS, because of their pathophysiological relevance. However, as indicated in Table 4, increased levels of histamine, protein S100B and/or NTT were found in only 70%–80% of the patients, meaning that in 20%–30% of the cases in our series, EHS and MCS could not be objectively characterized by these biomarkers. However, in such patients in addition, to the clinical picture the objective diagnosis of EHS and/or MCS could still be made based on the abnormal recording of brain pulsed ultrasound computed tomosphygmography.

Pathophysiological relevance

In our study we have shown that EHS and MCS both are associated with the same biological abnormalities. This strongly suggests that both pathological entities share a unique common pathophysiological mechanism.

Since histamine was found to be increased in the peripheral blood of nearly 40% of the patients, this molecule appears to be a key pathogenic mediator, whatever the environmental stressors. Indeed, the fact that histamine levels were not found to be increased in all patients doesn't mean that patients for whom there is no histamine blood level increase have no local histamine production and release in their tissues or at other times. Moreover, we will outline below that histamine is not just a neuro-inflammation mediator. Histamine plays a critical pathophysiological role as a neurotransmitter in the brain. For example neuronal histamine has been shown to be involved in the sleep cycle, motor activity, synaptic plasticity and memory (76–79): all types of neurologic and/or psychologic altered functions or symptoms that we have observed clinically in EHS and/or MCS bearing patients (data not shown). In addition, histamine release from sympathetic nerves can be experimentally induced by nerve stimulation (80) and it has been shown that H1 receptor may play a major role in the regulation of sympathetic nerve activity (81). This may explain why EHS and/or MCS patients may present clinically with some transitory sympathetic-related symptoms such as tachycardia, tachyarrhythmia and/or arterial pressure instability (data not shown) when exposed to EMF and/or chemical stressors (82). Moreover, following ischemic-hypoxic damage, histamine release from nerve endings has been found to be enhanced, possibly contributing to some neuroprotection (83).

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However, histamine is also a unique molecule which fulfils all criteria that have been historically established for defining an inflammatory mediator (84). Histamine is mainly produced and stored in perivascular tissue resident mast cells and circulating basophils, and released in inflammatory tissues through established mechanisms predominantly involving cell surface receptors. Regarding histamine release from skin mast cells, the best known degranulation mechanism involves IgE and the high affinity IgE cell surface receptor (30).

In our study, we found elevated levels of circulating IgE in about 20% of the patients, whatever the EHS and/or MCS group considered. However, in such cases, we didn't find any positive correlation between the levels of circulating histamine and the levels of circulating IgE nor the presence of skin lesions. This suggests that skin lesions and circulating histamine level increase in EHS and/or MCS patients are not related to an allergic process.

Also it has been shown that advanced glycation end products (AGEs) can activate mast cells through RAGE, the receptor of AGEs, and may contribute to initiating a vicious circle involving increased AGE formation and ROS production, hence increased low-grade chronic inflammation (85). Similar biological effects may also be obtained with protein S100B which has been shown to engage RAGE in macrophage/microglia and endothelial cells; and so depending of its extracellular concentration, to contribute either to chronic inflammation via NFκB activation or to anti-apoptotic effects and trophic protection in the course of pathological conditions such as brain insult or diabetes (86). Since AGEs have been shown to be involved in diabetes mellitus (87) although all included patients had no diabetes type II at inclusion time, we systematically search for a possible occurrence of diabetes type II in EHS and/or MCS patients during the follow up of this study, but with the exception of two cases, all patients were free from diabetes.

Predominantly found at host/environment interfaces such as skin, respiratory and gastrointestinal tracts (88) and closely associated with blood vessels, mast cells play a crucial sentinel role in host defense (89). Consequently, more precise investigations remain to be done in EHS and/or MCS patients to determine what mast cell-associated tissue histamine release come from.

However, since brain mast cells have been shown to be critical regulators of the pathogenesis of CNS diseases including stroke, traumatic injury and neurodegenerative diseases (83, 90) (see also Section "EHS/MCS as a possible sentinel pathological disorder") we systematically looked for brain pathologic alterations in EHS and /or MCS patients. Routine cerebral MRI and/or scanner as well as carotid echography were critically considered to be normal in all evaluable cases. We thus measured the BBF-related pulsatility in the patient hemispheres by using echodoppler of the middle cerebral artery, and found that resistance index and systolic and diastolic velocity indexes were associated with cerebral hypoperfusion in one or the two hemisphere in 50.5% of the cases, whatever the patient group considered (data not shown). More precisely, by using pulsed ultrasound computed tomosphygmography, we found that in comparison to normal subjects, cerebral pulsatility in EHS and /or MCS patients was decreased or even completely abolished in one or the two temporal lobes (Figure 2), suggesting that BBF might be specifically decreased or abolished in this brain area. We found that this abnormality, although being not specific, was so frequently observed in these patients that it may represent a typical brain alteration similar to that found in Alzheimer's disease and other neurodegenerative diseases (see Section "EHS/MCS as a possible sentinel pathological disorder"). This finding therefore, strongly suggests that brain could be the main target of environmental EMFs and/or chemicals in EHS and/or MCS patients, and that both cerebral hypoperfusion and subsequent histamine release whatever its neuronal or mast cell origin could be main contributing factors to BBB disruption. Furthermore, we found that cerebral blood pulsatility was quasi-constantly decreased in the capsulothalamic area of the temporal lobes, which includes the *limbic system* and the *thalamus*, and so correspond to particularly vulnerable areas to environmental stressors in the brain.

Confirming this capsulothalamic hypothesis, it has been shown that experimentally-induced brain ischemia-hypoxia can increase BBB permeability (91–94) and that hippocampal pathology arising after chronic hypoperfusion can give rise to cognitive impairment and more particularly memory deficit (95), a pathophysiological mechanism that supports both the key role of cerebral hypoperfusion/hypoxia in neurodegenerative diseases such as Alzheimer's disease (96) and our clinical observation of frequent cognitive defects in EHS and/or MCS patients. How cerebral hypoperfusion/hypoxia may arise from the neuro-inflammation process remains however, unclear. Cerebral blood flow restriction and consequently impaired oxygen supply may occur due to local oedematous swelling, artery and/or capillary vasoconstriction and/or increased BBB permeability induced by histamine or other neuro-inflammation mediators (97, 98). While hypoxia itself rather than ischemia can induce histamine release (99). In addition, less efficient oxygen utilization due to mitochondrial uncoupling may be associated with impaired oxygen supply (100). As a consequence of hypoxia and impairment of mitochondrial functioning, reduced sensorial excitability, hence transitory loss of

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motor, sensory and cognitive function may occur during EHS and/or MCS processes; but this loss of function may progress to permanence and universality in the case of chronic neurodegenerative diseases (97, 101).

Under the influence of environmental stressors, not only mast cells (102, 103), but also microglia cells and astrocytes (31, 104–106) play a crucial role in BBB disruption. Indeed the resident CNS tissue macrophages glial cells such as microglia cells and astrocytes, and the resident CNS mast cells are probably the first cells to respond to any neuro-inflammatory stimuli. In addition, it has been shown that tachykinin peptides such as substance P, can trigger microglial activation and subsequent release of proinflammatory molecules, thereby contributing in addition, to mast cells to the development of microglia-mediated inflammation and BBB break down (107–109). It is indeed well known that under the influence of neuro-inflammatory stressors, such as EMF and particularly during mobile device (GSM) prolonged exposure, microglia cells can migrate to the site of injury, proliferate and recruit astrocytes (110), what is commonly called gliosis – a first cellular neuro-inflammation response which produces and releases NO°, ROS and inflammatory mediators (105, 111). Moreover, astrocytes express histamine receptors (112) which after activation can trigger release of cytokines, which are themselves able to induce histamine release through mast cell degranulation in positive feedback loop (113). Finally, our finding of both cerebral hypoperfusion and histamine release, supports previous data according to which BBB disruption is obtained more efficiently when these two factors are combined (91).

At a molecular level it has been evidenced that histamine and other neuro-inflammation mediators induce oxidative and nitrosative stress and so change the molecular composition and functional state of the BBB endothelial tight junctions, hence increasing permeability of the BBB (32, 104, 114, 115). As a consequence of this process circulating inflammatory cells may thus transmigrate into the CNS and so amplify the neuro-inflammation response (116, 117). Note that such oxidative/nitrosative stress-induced BBB disruption has not only been evidenced as a consequence of chronic cerebral hypoperfusion (118) but also proved to occur under the influence of EMF exposure at non thermal as well as thermal levels in several animal studies (104, 119–122).

Melatonin suppression as a consequence of EMF exposure has been experimentally evidenced both in animals and humans (123–125). We found that 6-OHMS 24 h-urine excretion was decreased in all the investigated cases, whatever the EHS and/or MCS patient group considered. Although this finding suggests that melatonin production might have been decreased in these patients, EMF exposure have been reported to be incapable of altering melatonin synthesis and secretion (126). So an alternative plausible explanation is that decrease in 6-OHMS excretion may reflect decreased melatonin metabolic availability, due to an increased uptake and utilization of melatonin as a free radical scavenger (127, 128). Such reduction in melatonin bioavailability may thus contribute to decrease host defence mechanisms and may account for the fact that patients submitted to prolonged and intensive EMF exposure may be at risk of neurodegenerative diseases and cancer (129), particularly of breast cancer (130) (see Section "EHS/MCS as a possible sentinel pathological disorder").

The development of the oxidative/nitrosative stress-related autoimmune response may also contribute to weaken the protective effect of the chaperone proteins HSP70 and HSP27 (131) as has been evidenced for example in stroke patients (132). Indeed the role of histamine in modulating the immune system (133), the disturbance of the immune system by EMFs (134) and the progressive increase in oxidative and nitrosative stress as long as chronic exposure to EMFs and/or chemicals persists may explain why the physiological defence mechanisms of these patients may finally collapse.

On the basis of our data we therefore, propose the following pathophysiological model of co-MCS/EHS exposure: 1) Under the influence of EMFs and/or chemicals a cerebral hypoperfusion/hypoxia-related neuro-inflammation may occur; 2) Due to the release of histamine and other mediators BBB disruption and permeability increase may be induced through resulting oxidative and/or nitrosative stress; 3) Circulating inflammatory cells could then enter the brain to initiate a vicious circle which may considerably amplify the neuro-inflammation process; and finally 4) Because of oxidative and nitrosative stress and subsequent decreased melatonin bioavailability and autoimmune response, physiological defence mechanisms are weakened making EHS and/or MCS patients potentially at risk of chronic neurodegenerative diseases and cancer.

Part of this model has been proposed separately for histamine release from mast cells in EHS (135) and for the NO/NOOH- nitrosative stress cycle in MCS (136). Our proposed EHS/MCS common pathogenic model is summarized in Figure 5.

Some insight into etiopathogeny

Certainly this study does not prove a causal link between EMFs and EHS, or between chemicals and MCS, but it does strengthen the evidence for such a possibility. To our

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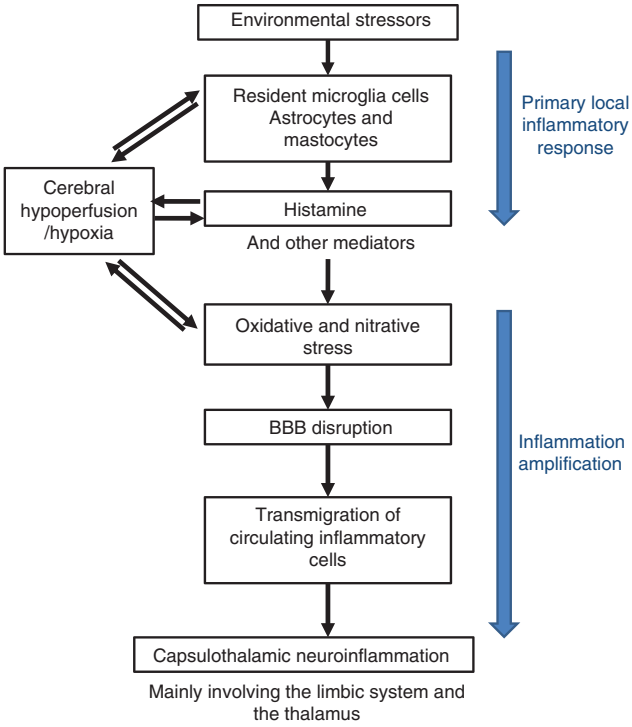

Figure 5: Proposed hypothetic EHS/MCS common pathogenic model based on EHS/MCS induced-neuroinflammation, cerebral hypoperfusion, histamine release, oxidative/nitrosative stress and BBB disruption.

knowledge this is the first time that EHS and/or MCS have been objectively characterized by the use of several different types of biomarkers and in a large prospective series of patients. This finding should avoid the frequent erroneous interpretation that EHS and/or MCS patients are psychosomatic patients (11–14, 17, 137) and so strongly suggests that EHS and MCS are genuine somatic pathological entities. Furthermore, our study revealed that with the exception of the two cases of Alzheimer's disease which were detected soon after inclusion, and several other cases of neurodegenerative diseases which were diagnosed during the follow-up (these cases have been considered as non-evaluable cases) (see Section "EHS/MCS as a possible sentinel pathological disorder"), all EHS and/or MCS patients had no detectable psychiatric disease.

As previously mentioned we should however, note that none of the biomarkers so far identified in our study are specific of EHS and/or MCS. This is the case for histamine which is known to be increased in the serum of patients with typical migraine (138–140) and/or allergy (30) and for HSP70 and HSP27 which has been shown to be increased in several neurodegenerative diseases (141, 142); and for protein S100B which acts normally as a physiological intracellular regulator and extracellular signal and so has been shown to be expressed and released not only by damaged CNS cells such as glial cells and neurons, but also by different non CNS cells such as chondrocytes, adipocytes, melanocytes, myofibers and other non CNS cells (67, 86, 143). This explains why the detection of increased levels of protein S100B in the serum of patients does not mean they are necessarily EHS and/or MCS patients. Other pathological disorders such as neurodegenerative diseases, psychiatric diseases such as bipolar disorder or cancer (70, 144, 145) may be indeed also concerned by such S100B protein levels. Likewise NTT is not only a general marker of inflammation but also more particularly a marker of atherosclerosis (146). Increased levels of NTT are thus also non-specific. As already indicated (see Section "Search for reliable disease biomarkers") we therefore, paid attention for excluding from our series all cases associated with neuropsychiatric diseases and/or other serious pathologies such as atherosclerosis and type 2 diabetes in order to eliminate any confounding factors.

Unlike the reported negative result of histamine increase in MCS patients (147), we found increased histamine levels globally in about 40% of MCS, EHS or MCS/EHS patients. Since it has been shown that increased histamine levels may in fact appear only when MCS patients are submitted to environmental stressors such as volatile organic compounds (VOC) (147), we thus wonder whether the 60% of patients in our series who were not associated with detectable increased histamine levels may in fact be patients who were not exposed to environmental EMF and/or chemical stressors just before histamine measurement. Such interpretation may also involve the fact that in our series we detected increased S100B protein levels in only 15% of the patients, since the increased levels of protein S100B following brain injury are fleeting (39, 40, 66–68).

However, since EHS and MCS share similar biological abnormalities and so may share a common pathophysiological mechanism (see Section "Analysis of biochemical markers"), these two so far clinically individualized entities may represent two etiopathogenic aspects of a unique common pathological disorder. Arguments in support are the following: 1. EHS and MCS are associated with a similar symptomatic clinical picture; 2. Both entities share identical biological abnormalities including histamine release, oxidative and nitrosative stress, and BBB opening; 3. Both entities are characterized by a similar BBF decrease, and this cerebral hypoperfusion take place in the majority of cases predominantly in the same areas, i.e. mostly in the temporal lobes, more precisely in the capsulothalamic area; 4. either EHS or MCS occur first; 5. Using the same therapeutic protocol, similar positive clinical results can be obtained in both cases (data not shown).

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Because EHS and MCS were historically identified clinically and distinguished from each other on the basis of individual potentially environmental stressors, some confusion has emerged. That is, unlike EHS and/or MCS which are still considered as subjective entities because of a lack of etiological substratum, many other internationally recognized diseases were medically characterized before discovery of their etiopathological mechanisms. In fact, the acknowledgment of EHS and MCS as resulting from environmental causes oppose to powerful socioeconomic interests and may explain why they are still not recognized as genuine pathological disorders by national or international bodies and health institutions (137).

Moreover, it is well known that diseases are multifactorial and this may explain why current research failed to attribute a causal origin to EHS and/or MCS. Case-control epidemiologic studies and provocation studies, globally have failed to demonstrate a causal link between EMF and EHS (13, 137), as it may also be the case for chemicals and MCS. These negative results however, do not exclude the possibility of a causal link, as observational studies are difficult to conduct and objective inclusion/exclusion criteria and endpoint evaluation criteria were not clearly defined because of a lack of objective reliable biomarkers. Moreover, if we accept the concept that EHS and/or MCS are part of a common multifactorial disease, clearly those findings may also have been biased by multiple related or unrelated confounding exposure factors and so may have been associated with a reduction of signal-to-noise ratio, thereby obscuring evidence of a possible causal link. Moreover, black box epidemiology and provocation studies focus on risk factors without satisfactory understanding pathogenesis.

There are in fact several arguments for a causal role of EMFs and/or multiple chemicals in the genesis of the so far individualized EHS/MCS pathological disorder: 1. Self-reporting occurrence of clinical symptoms depending on electromagnetic and/or chemical sources, 2. Efficient removing or lessening of clinical symptoms in EHS patients and/or MCS patients in case of avoidance of EMFs and/or chemicals, respectively (19); 3. Appearance of biological abnormalities (positive detection of biomarkers) when patients are exposed to electromagnetic and/or chemical sources, and regression or disappearance of these biological abnormalities (normalization of biomarkers) when patients are withdrawn from electromagnetic and/or chemical sources, a finding that confirm objectively self-reporting patient symptoms (data not shown); 4. a possible common underlying pathophysiological mechanism involving oxidative and/or nitrosative stress-associated neuro-inflammation and BBB opening (see Sections "Demographic panorama" and "Analysis of biochemical markers"); and finally 5. Identical or similar biological abnormalities detected in humans as compared to those evidenced experimentally in animals submitted to EMF and/or chemicals exposure. Although our data account for clinical symptoms and biological abnormalities associated with an intolerance syndrome and highlight its pathogenesis, they do not account for susceptibility and more particularly, hypersensitivity which in addition, to intolerance both characterize EHS and MCS. Virtually all diseases result from the interaction of genes and the environment, hence the concept of genetic susceptibility via constitutive genes which can further the pathogenic role of environmental stressors (148). Theoretically such susceptibility could explain why some subjects are particularly suffering from EHS and/or MCS and not others. A genetic predisposition including gene variants of drug-metabolizing enzymes has been reported for MCS (149–151) but this has not been confirmed (152, 153), suggesting that to define MCS biologically, redox state and cytokine profiling should be considered instead (153).

Our data reveal that women are more susceptible than men to EHS or MCS and this susceptibility concerns both EHS and MCS (see Section "Search for clinical diagnosis criteria"). This suggests some still undetermined sex-related genetic susceptibility. To our knowledge there is no reported study on genetic predisposition in EHS patients. As magnetosomes are detectable in the human brain and meninges (pia mater and dura mater) (154), and because some EMF-related biological effects are achieved through magneto-reception (155), we speculated that some type of innate genetic predisposition to EHS might result from the presence of a high number of magnetosomes in the brain and meninges of susceptible patients. This may reveal to be true particularly for non-thermal EMFs (156). Other hypothesis may include acquired susceptibility through epigenetic mechanisms related to EHS and/or MCS prolonged exposure and some biological synergistic potential between EMF exposure and low dose organic or inorganic chemical contamination (157, 158). This may be particularly the case for heavy metals which, as for EMF, have been shown to release proinflammatory cytokines (159, 160).

It is worthy of note that metallic dental alloys are associated with release of heavy metals such as mercury, lead and cadmium into oral cavity (161, 162) and so may contribute to EHS (158). It has been shown that EMFs such as GSM frequencies emitted from mobile phone may induce or accelerate the mercury vapor release from dental amalgam (163) and consequently may contribute not only to EHS but also to MCS (164).

An intriguing unknown pathophysiological mechanism referred to as sensitivity-related illness (SRI) (4) or

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as toxicant-induced loss of tolerance (TILT) (165) has been put forward in order to account for the fact that patients with EHS and/or MCS cannot tolerate weak intensity of EMFs and/or low concentration of chemicals. We define acquisition of such a hypersensitivity state with two criteria: 1. Decrease in the tolerance threshold for EMFs or chemicals; and 2. Extension of this decreased tolerance threshold to the whole electromagnetic spectrum or to multiple structurally unrelated chemicals, as disease progress. Although our data may suggest a role of the limbic system and the thalamus, to our knowledge no clear pathophysiological explanation of this intriguing brain-related hypersensitivity condition has yet been given.

EHS/MCS as a possible sentinel pathological disorder

The BBB protects the brain against potentially harmful toxic chemicals which may have contaminated the blood and thereby is currently regarded as a physiological structure that plays a crucial role in maintaining brain homeostasis (166–169). However, the BBB cannot protect the brain against EMFs (170). This may explain why EMFs are probably a major stressor associated with BBB disruption and brain inflammation, and why oxidative stress and more particularly oxidative/nitrosative stress-induced BBB breakdown may be causally involved in neurodegenerative diseases (171, 172), such as Alzheimer's disease (AD) (173–176), Parkinson's disease (PD) (177), multiple sclerosis (MS) (178), Huntington's disease and amyotrophic lateral sclerosis (70) and even possibly psychiatric diseases such as schizophrenia, autism and bipolar disorder (179–182).

Since the first reports on EMF exposure-related BBB disruption (119, 183) conflicting data have emerged (122) leading to search for new tests for evidencing BBB disruption in EHS and/or MCS patients. BBB permeability imaging (184) in addition, to search of peripheral biomarkers could be helpful. Using protein S100B and NTT as biomarkers our data tend to show that BBB opening could be detected in 55%–60% of patients; but this result does not mean the remaining cases could not have been associated with BBB opening we were unable to detect.

There is indeed compelling evidence that chronic neuro-inflammation is a long lasting and potentially self-perpetuating process including an initially long-standing release of inflammatory mediators, leading to increased oxidative and nitrosative stress. This process may thus persist long after the initial environmental trigger and consequently can contribute to neurodegeneration through free radical attack on neural cells (185). This is particularly the case in AD and PD for which toxicity of free radicals have been demonstrated to contribute to protein and DNA injury, inflammation, tissue damage and subsequent neuronal degeneration and apoptosis (175, 176, 183, 185–187).

We have shown that patients with EHS and/or MCS often have cerebral hypoperfusion and histamine release, two factors that in addition, to the production of autoantibodies have been evidenced to occur in AD (173, 174) and PD (188–192); hence contributing to neuro-inflammation and BBB dysfunction. Moreover, several studies have shown that prolonged occupational exposures to low or extremely low frequency EMFs are associated with AD (193–196) and such a link has recently been confirmed in a meta-analysis based on more than twenty epidemiological studies (197). Although it has been shown in a single study that long term high frequency EMF exposure could protect against and even reverse cognitive impairment in mice bearing a so called animal equivalent of AD (198), there is currently no scientific reason to believe that in humans prolonged radiofrequency EMF exposure as it is the case with excessive cell phone and/or mobile phone use will be not also causally related to AD occurrence (199). Moreover, it has been shown that neurodegenerative diseases are in fact multifactorial and that, as it has been hypothesized, ferrimagnetic metals in food chain may contribute to initiate these neurodegenerative diseases under the influence of EMF exposure (200).

Typically AD starts with mild memory deficits, primarily affecting short term memory and gradually progresses to loss of retrospective memory and dementia. An important finding in our still ongoing study is that most of EHS and/or MCS patients had decreased cognitive ability manifested by loss of immediate memory and attention and concentration deficiency (see Sections "Search for reliable disease biomarkers" and "Analysis of biochemical markers"). Since EHS and/or MCS pathogenesis appears to be associated with brain pathophysiological abnormalities similar to that occurring in neurodegenerative disorders, a question is whether EHS and/or MCS are either a pre-neurodegenerative state or an unrelated pathological disorder whose environmental causal origin might however, be similar to that of neurodegenerative diseases. Nevertheless, whichever these two possible etiopathologic alternatives, EHS and/or MCS might be considered as some type of environmental sentinel pathological disorder.

It is worthy of note that in our series, in addition, to the two cases of AD, which were diagnosed a few months after inclusion, another case of AD and two cases of PD were discovered in association with EHS during the patient follow up. Moreover, at inclusion time we excluded two cases of AD, two cases of PD, three cases of multiple sclerosis,

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and one case of Huntington disease, which were found to be associated with EHS. In addition, we excluded seven EHS or EHS/MCS cases because they were associated with previous or simultaneous carcinoma: breast carcinoma (3 cases), brain tumor (2 cases) and lymphoma (1 case). We also excluded three MCS cases because they were associated with lymphoma (1 case) and thyroid endocrinopathy (2 cases).

Certainly long term longitudinal analysis and replication of this ongoing prospective study will be necessary to establish whether EHS and/or MCS could be related to neurodegenerative disease and/or cancer, and thus may announce or reflect occurrence of these pathologies.

The growing worldwide health problem

Whatever the causal origin of EHS and/or MCS, there is compelling evidence that EHS and/or MCS self-reporting patients constitute an unsolved, large and growing health problem worldwide.

As far as EHS is concerned, about 1%–10% of the investigated population, e.g. 5% in Switzerland (13), 5% in Ireland, 9% in Sweden, 9% in Germany and 11% in England are presently estimated to be EHS self-reporting persons (201). Given the seven billion persons worldwide using cordless and/or mobile phone it is expected these percentages may increase in the 50 next years. However, because at the time these estimations were made there was no objective criteria for identifying EHS (21), these data require confirmation by more objective investigations.

By using the battery of biomarkers we have investigated in this study it now seems possible to objectively characterize and identify EHS and MCS. Although termed "idiopathic", IEI has been defined as abnormal responses possibly triggered by exposure to organic chemicals and/or metals. It is believed that in addition, to MCS several pathological disorders such as fibromyalgia and chronic fatigue syndrome, because they may share a similar environment-related intolerance condition, could be part of IEI. We have shown multiple lines of evidence that EHS and MCS share a similar pathogenesis and so might be the same pathological disorder whatever their putative causal stressors. This strongly reinforces the concept that both EHS and MCS must be part of the so called IEI syndrome.

Since the WHO publication in 1993 on EMFs (202), much progress have been made in the identification and understanding of EMF effects on the organism, while EHS has still not been clearly characterized and acknowledged by WHO.

Present research vainly focus on the causal role of EMFs and chemicals as possible triggers of EHS and MCS, respectively and not enough on the actually unmet health care needs at a socioeconomic and public health setting for persons with environmental sensitivity (203), as it is particularly the case for EHS and/or MCS persons.

We therefore, strongly propose that whatever their proofs for their causal origins, EHS and MCS should clearly be added to the next version of the WHO international classification of diseases (ICD) on the basis on their clinical and pathological description; as has been the case for many other diseases.

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References

1. Randolph TG. Human ecology and susceptibility to the chemical environment, ed. Springfield, IL: Charles C Thomas. 1962:148pp.
2. Nethercott JR, Davidoff LL, Curbow B, Abbey H. Multiple chemical sensitivities syndrome: toward a working case definition. Arch Environ Health 1993;48(1):19–26.
3. Multiple chemical sensitivity (MCS): a consensus. Arch Environ Health 1999;54(3):147–9.
4. Genuis SJ. Sensitivity-related illness: the escalating pandemic of allergy, food intolerance and chemical sensitivity. Sci Total Environ 2010;408(24):6047–61.
5. Rea WJ, Pan Y, Fenyves EJ, Sujisawa I, Suyama H, et al. Electromagnetic field sensitivity. J Bioelectricity 1991;10(1–2):241–56.
6. Bergqvist U, Vogel E, Editors. Possible health implications of subjective symptoms and electromagnetic fields. A report prepared by a European group of experts for the European Commission, DGV. Arbete och Hälsa, 1997:19. Swedish National Institute for Working Life, Stockholm, Sweden. Available at: http://www2.niwl.se/forlag/en/.
7. Santini R, Seigne M, Bonhomme-Faivre L, Bouffet S, Defrasme E, et al. Symptoms experienced by users of digital cellular phones: a study of a French engineering school. Electromagn Biol Med 2002;21(1):81–8.

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8. Santini R, Santini P, Le Ruz P, Danze JM, Seigne M. Survey study of people living in the vicinity of cellular phone base. Electromagn Biol Med 2003;22(1):41–9.
9. Hansson Mild K, Repacholi M, Van Deventer E, Ravazzani P, editors. 2006. In: Proceedings, International Workshop on EMF Hypersensitivity, Prague, Czech Republic, October 25–27, 2004. Milan: World Health Organization. Working group report, 15–26. Available at: http://www.who.int/peh-emf/publications/reports/EHS_Proceedings_June2006.pdf.
10. Rubin GJ, Nieto-Hernandez R, Wessely S. Idiopathic environmental intolerance attributed to electromagnetic fields (formerly'electromagnetic hypersensitivity'): an updated systematic review of provocation studies. Bioelectromagnetics 2010;31(1):1–11.
11. Bornschein S, Förstl H, Zilker T. Idiopathic environmental intolerances (formerly multiple chemical sensitivity) psychiatric perspectives. J Intern Med 2001;250(4):309–21.
12. Röösli M. Radiofrequency electromagnetic field exposure and non-specific symptoms of ill health: a systematic review. Environ Res 2008;107(2):277–87.
13. Röösli M, Mohler E, Frei P. Sense and sensibility in the context of radiofrequency electromagnetic field exposure. C R physique 2010;11:576–84.
14. Baliatsas C, Van Kamp I, Bolte J, Schipper M, Yzermans J, et al. Non-specific physical symptoms and electromagnetic field exposure in the general population: can we get more specific? A systematic review. Environ Int 2012;41:15–28.
15. Genuis SJ, Lipp CT. Electromagnetic hypersensitivity: fact or fiction? Sci Total Environ 2012;414:103–12.
16. Köteles F, Szemerszky R, Gubányi M, Körmendi J, Szekrényesi C, et al. Idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF) and electrosensibility (ES) – are they connected? Int J Hyg Environ Health 2013;216(3): 362–70.
17. Marc-Vergnes JP. Electromagnetic hypersensitivity: the opinion of an observer neurologist. C R Physique 2010;11:564–75.
18. Carpenter DO. Excessive exposure to radiofrequency electromagnetic fields may cause the development of electrohypersensitivity. Altern Ther Health Med 2014;20(6):40–2.
19. Hagström M, Auranen J, Ekman R. Electromagnetic hypersensitive Finns: symptoms, perceived sources and treatments, a questionnaire study. Pathophysiology 2013;20(2):117–22.
20. De Luca C, Raskovic D, Pacifico V, Thai JC, Korkina L. The search for reliable biomarkers of disease in multiple chemical sensitivity and other environmental intolerances. Int J Environ Res Public Health 2011;8(7):2770–97.
21. Baliatsas C, Van Kamp I, Lebret E, Rubin GJ. Idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF): a systematic review of identifying criteria. BMC Public Health 2012;12:643.
22. Jorgensen LG. Transcranial Doppler ultrasound for cerebral perfusion. Acta Physiol Scand Suppl 1995;625:1–44.
23. Texier JJ, Grunitsky E, Lepetit JM, Lajoix M, Cognard J, et al. Variation in the functional circulatory value measured by ultrasonic cerebral tomosphygmography during the administration of general intravenous anesthesia. Agressologie 1986;27(6):487–94.
24. Parini M, Lepetit JM, Dumas M, Tapie P, Lemoine J. Ultrasonic cerebral tomosphygmography. Application in 143 healthy subjects. Agressologie 1984;25(5):585–9.
25. Lajoix M, Bechonnet G, Lepetit JM. Ultrasonic cerebral tomosphygmography and cerebral perfusion pressure. Agressologie 1983;24(9):425–7.
26. Albert PJ, Proal AD, Marshall TG. Vitamin D: the alternative hypothesis. Autoimmun Rev 2009;8(8):639–44.
27. Tuohimaa P, Keisala T, Minasyan A, Cachat J, Kalueff A. Vitamin D, nervous system and aging. Psychoneuroendocrinology 2009;34(Suppl 1):S278–86.
28. Eyles DW, Feron F, Cui X, Kesby JP, Harms LH, et al. Developmental vitamin D deficiency causes abnormal brain development. Psychoneuroendocrinology 2009;34(Suppl 1):S247–57.
29. Rocha SM, Pires J, Esteves M, Graça B, Bernardino L. Histamine: a new immunomodulatory player in the neuron-glia crosstalk. Front Cell Neurosci 2014;8:120.
30. Greaves MW, Sabroe RA. Histamine: the quintessential mediator. J Dermatol 1996;23(11):735–40.
31. Abbott NJ. Inflammatory mediators and modulation of blood-brain barrier permeability. Cell Mol Neurobiol 2000;20(2):131–47.
32. Mayhan WG. Role of nitric oxide in histamine-induced increases in permeability of the blood-brain barrier. Brain Res 1996;743(1–2):70–6.
33. Tan KH, Harrington S, Purcell WM, Hurst RD. Peroxynitrite mediates nitric oxide-induced blood-brain barrier damage. Neurochem Res 2004;29(3):579–87.
34. Phares TW, Fabis MJ, Brimer CM, Kean RB, Hooper DC. A peroxynitrite-dependent pathway is responsible for blood-brain barrier permeability changes during a central nervous system inflammatory response: TNF-alpha is neither necessary nor sufficient. J Immunol 2007;178(11):7334–43.
35. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev 2007;87(1):315–424.
36. Yang S, Chen Y, Deng X, Jiang W, Li B, et al. Hemoglobin-induced nitric oxide synthase overexpression and nitric oxide production contribute to blood-brain barrier disruption in the rat. J Mol Neurosci 2013;51(2):352–63.
37. Kanner AA, Marchi N, Fazio V, Mayberg MR, Koltz MT, et al. Serum S100beta: a noninvasive marker of blood-brain barrier function and brain lesions. Cancer 2003;97(11):2806–13.
38. Kapural M, Krizanac-Bengez Lj, Barnett G, Perl J, Masaryk T, et al. Serum S-100beta as a possible marker of blood-brain barrier disruption. Brain Res 2002;940(1–2):102–4.
39. Marchi N, Cavaglia M, Fazio V, Bhudia S, Hallene K, et al. Peripheral markers of blood-brain barrier damage. Clin Chim Acta 2004;342(1–2):1–12.
40. Koh SX, Lee JK. S100B as a marker for brain damage and blood-brain barrier disruption following exercise. Sports Med 2014;44(3):369–85.
41. Gunaydin H, Houk KN. Mechanisms of peroxynitrite-mediated nitration of tyrosine. Chem Res Toxicol 2009;22(5):894–8.
42. de Pomerai D, Daniells C, David H, Allan J, Duce I, et al. Non-thermal heat-shock response to microwaves. Nature 2000;405(6785):417–8.
43. French PW, Penny R, Laurence JA, McKenzie DR. Mobile phones, heat shock proteins and cancer. Differentiation 2001;67(4–5):93–7.
44. Blank M, Goodman R. Electromagnetic fields stress living cells. Pathophysiology 2009;16(2–3):71–8.
45. Yang XS, He GL, Hao YT, Xiao Y, Chen CH, et al. Exposure to 2.45 GHz electromagnetic fields elicits an HSP-related stress response in rat hippocampus. Brain Res Bull 2012;88(4):371–8.

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46. Kesari KK, Meena R, Nirala J, Kumar J, Verma HN. Effect of 3G cell phone exposure with computer controlled 2-D stepper motor on non-thermal activation of the hsp27/p38MAPK stress pathway in rat brain. Cell Biochem Biophys 2014;68(2):347–58.
47. Berberian PA, Myers W, Tytell M, Challa V, Bond MG. munohistochemical localization of heat shock protein-70 in normal-appearing and atherosclerotic specimens of human arteries. Am J Pathol 1990;136(1):71–80.
48. Georgopoulos C, Welch WJ. Role of the major heat shock proteins as molecular chaperones. Annu Rev Cell Biol 1993;9:601–34.
49. Hartl FU. Molecular chaperones in cellular protein folding. Nature 1996;381(6583):571–9.
50. Sabirzhanov B, Stoica BA, Hanscom M, Piao CS, Faden AI. Over-expression of HSP70 attenuates caspase-dependent and caspase-independent pathways and inhibits neuronal apoptosis. J Neurochem 2012;123(4):542–54.
51. Yenari MA, Liu J, Zheng Z, Vexler ZS, Lee JE, et al. Antiapoptotic and anti-inflammatory mechanisms of heat-shock protein protection. Ann NY Acad Sci 2005;1053:74–83.
52. Kelly S, Yenari MA. Neuroprotection: heat shock proteins. Curr Med Res Opin 2002;18(Suppl 2):s55–60.
53. Leszczynski D, Joenväärä S, Reivinen J, Kuokka R. Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer- and blood-brain barrier-related effects. Differentiation 2002;70(2–3):120–9.
54. Leak RK, Zhang L, Stetler RA, Weng Z, Li P, et al. HSP27 protects the blood-brain barrier against ischemia-induced loss of integrity. CNS Neurol Disord Drug Targets 2013;12(3):325–37.
55. Di Carlo A, White N, Guo F, Garrett P, Litovitz T. Chronic electromagnetic field exposure decreases HSP70 levels and lowers cytoprotection. J Cell Biochem 2002;84(3):447–54.
56. Ohmori H, Kanayama N. Mechanisms leading to autoantibody production: link between inflammation and autoimmunity. Curr Drug Targets Inflamm Allergy 2003;2(3):232–41.
57. Profumo E, Buttari B, Riganò R. Oxidative stress in cardiovascular inflammation: its involvement in autoimmune responses. Int J Inflam 2011;2011:295705.
58. Lin H, Opler M, Head M, Blank M, Goodman R. Electromagnetic field exposure induces rapid, transitory heat shock factor activation in human cells. J Cell Biochem 1997;66(4):482–88.
59. Tsurita G, Ueno S, Tsuno NH, Nagawa H, Muto T. Effects of exposure to repetitive pulsed magnetic stimulation on cell proliferation and expression of heat shock protein 70 in normal and malignant cells. Biochem Biophys Res Commun 1999;261(3):689–94.
60. Bozic B, Cucnik S, Kveder T, Rozman B. Autoimmune reactions after electro-oxidation of IgG from healthy persons: relevance of electric current and antioxidants. Ann NY Acad Sci 2007;1109:158–66.
61. Burch JB, Reif JS, Yost MG, Keefe TJ, Pitrat CA. Reduced excretion of a melatonin metabolite in workers exposed to 60 Hz magnetic fields. Am J Epidemiol 1999;150(1):27–36.
62. Kovács J, Brodner W, Kirchlechner V, Arif T, Waldhauser F. Measurement of urinary melatonin: a useful tool for monitoring serum melatonin after its oral administration. J Clin Endocrinol Metab 2000;85(2):666–70.
63. Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, et al. Early inflammation and dementia: a 25-year follow-up of the Honolulu-Asia Aging Study. Ann Neurol 2002;52(2):168–74.
64. Dik MG, Jonker C, Hack CE, Smit JH, Comijs HC, et al. Serum inflammatory proteins and cognitive decline in older persons. Neurology 2005;64(8):1371–7.
65. Gazerani P, Pourpak Z, Ahmadiani A, Hemmati A, Kazemnejad A. A correlation between migraine, histamine and immunoglobulin E. Scand J Immunol 2003;57(3):286–90.
66. Stamataki E, Stathopoulos A, Garini E, Kokkoris S, Glynos C, et al. Serum S100B protein is increased and correlates with interleukin 6, hypoperfusion indices, and outcome in patients admitted for surgical control of hemorrhage. Shock 2013;40(4):274–80.
67. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 2001;33(7):637–68.
68. Michetti F, Corvino V, Geloso MC, Lattanzi W, Bernardini C, et al. The S100B protein in biological fluids: more than a lifelong biomarker of brain distress. J Neurochem 2012;120(5):644–59.
69. Sheng JG, Mrak RE, Griffin WS. Glial-neuronal interactions in Alzheimer disease: progressive association of IL-1alpha+ microglia and S100beta+ astrocytes with neurofibrillary tangle stages. J Neuropathol Exp Neurol 1997;56(3):285–90.
70. Migheli A, Cordera S, Bendotti C, Atzori C, Piva R, et al. S-100beta protein is upregulated in astrocytes and motor neurons in the spinal cord of patients with amyotrophic lateral sclerosis. Neurosci Lett 1999;261(1–2):25–28.
71. Söderqvist F, Carlberg M, Hardell L. Biomarkers in volunteers exposed to mobile phone radiation. Toxicol Lett 2015;235(2):140–6.
72. Söderqvist F, Carlberg M, Hansson Mild K, Hardell L. Exposure to an 890-MHz mobile phone-like signal and serum levels of S100B and transthyretin in volunteers. Toxicol Lett 2009;189(1):63–6.
73. Söderqvist F, Carlberg M, Hardell L. Use of wireless telephones and serum S100B levels: a descriptive cross-sectional study among healthy Swedish adults aged 18-65 years. Sci Total Environ 2009;407(2):798–805.
74. Brzezinski A. Melatonin in humans. N Engl J Med 1997;336(3): 186–95.
75. Baydas G, Ozer M, Yasar A, Koz ST, Tuzcu M. Melatonin prevents oxidative stress and inhibits reactive gliosis induced by hyperhomocysteinemia in rats. Biochemistry (Mosc) 2006;71 (Suppl 1):S91–5.
76. Wada H, Inagaki N, Yamatodani A, Watanabe T. Is the histaminergic neuron system a regulatory center for whole-brain activity? Trends Neurosci 1991;14(9):415–8.
77. Onodera K, Yamatodani A, Watanabe T, Wada H. Neuropharmacology of the histaminergic neuron system in the brain and its relationship with behavioral disorders. Prog Neurobiol 1994;42(6):685–702.
78. Haas HL, Sergeeva OA, Selbach O. Histamine in the nervous system. Physiol Rev 2008;88(3):1183–241.
79. Panula P, Nuutinen S. The histaminergic network in the brain: basic organization and role in disease. Nat Rev Neurosci 2013;14(7):472–87.
80. Chen YY, Lv J, Xue XY, He GH, Zhou Y, et al. Effects of sympathetic histamine on vasomotor responses of blood vessels in rabbit ear to electrical stimulation. Neurosci Bull 2010;26(3):219–24.
81. Murakami M, Yoshikawa T, Nakamura T, Ohba T, Matsuzaki Y, et al. Involvement of the histamine H1 receptor in the regulation of sympathetic nerve activity. Biochem Biophys Res Commun 2015;458(3):584–9.

Brought to you by | provisional account

82. Havas M. Radiation from wireless technology affects the blood, the heart, and the autonomic nervous system. Rev Environ Health 2013;28(2–3):75–84.
83. Adachi N. Cerebral ischemia and brain histamine. Brain Res Brain Res Rev 2005;50(2):275–86.
84. Dale HH. On some physiological actions of ergot. J Physiol 1906;34(3):163–206.
85. Sick E, Brehin S, André P, Coupin G, Landry Y, et al. Advanced glycation end products (AGEs) activate mast cells. Br J Pharmacol 2010;161(2):442–55.
86. Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, et al. S100B's double life: intracellular regulator and extracellular signal. Biochim Biophys Acta 2009;1793(6):1008–22.
87. Goh SY, Cooper ME. Clinical review: the role of advanced glycation end products in progression and complications of diabetes. J Clin Endocrinol Metab 2008;93(4):1143–52.
88. Padawer J. Quantitative studies with mast cells. Ann NY Acad Sci 1963;103:87–138.
89. Marshall JS. Mast-cell responses to pathogens. Nat Rev Immunol 2004;4(10):787–99.
90. Rinne JO, Anichtchik OV, Eriksson KS, Kaslin J, Tuomisto L, et al. Increased brain histamine levels in Parkinson's disease but not in multiple system atrophy. J Neurochem 2002;81(5): 954–60.
91. Dux E, Temesvári P, Joó F, Adám G, Clementi F, et al. The blood-brain barrier in hypoxia: ultrastructural aspects and adenylate cyclase activity of brain capillaries. Neuroscience 1984;12(3):951–8.
92. Gotoh O, Asano T, Koide T, Takakura K. Ischemic brain edema following occlusion of the middle cerebral artery in the rat. I: the time courses of the brain water, sodium and potassium contents and blood-brain barrier permeability to 125I-albumin. Stroke 1985;16(1):101–9.
93. Hardebo JE, Beley A. Influence of blood pressure on blood-brain barrier function in brain ischemia. Acta Neurol Scand 1984;70(5):356–9.
94. Hatashita S, Hoff JT. Brain edema and cerebrovascular permeability during cerebral ischemia in rats. Stroke 1990;21(4):582–8.
95. Vicente E, Degerone D, Bohn L, Scornavaca F, Pimentel A, et al. Astroglial and cognitive effects of chronic cerebral hypoperfusion in the rat. Brain Res 2009;1251:204–12.
96. Liu H, Zhang J. Cerebral hypoperfusion and cognitive impairment: the pathogenic role of vascular oxidative stress. Int J Neurosci 2012;122(9):494–9.
97. Davies AL, Desai RA, Bloomfield PS, McIntosh PR, Chapple KJ, et al. Neurological deficits caused by tissue hypoxia in neuroinflammatory disease. Ann Neurol 2013;74(6):815–25.
98. Pache M, Kaiser HJ, Akhalbedashvili N, Lienert C, Dubler B, et al. Extraocular blood flow and endothelin-1 plasma levels in patients with multiple sclerosis. Eur Neurol 2003;49(3):164–8.
99. De Ley G, Demeester G, Leusen L. Cerebral histamine in hypoxia. Arch Int Physiol Biochim 1984;94(4):33–5.
100. Yu XX, Barger JL, Boyer BB, Brand MD, Pan G, et al. Impact of endotoxin on UCP homolog mRNA abundance, thermoregulation, and mitochondrial proton leak kinetics. Am J Physiol Endocrinol Metab 2000;279(2):E433–46.
101. Astrup J. Energy-requiring cell functions in the ischemic brain. Their critical supply and possible inhibition in protective therapy. J Neurosurg 1982;56(4):482–97.
102. Ribatti D. The crucial role of mast cells in blood-brain barrier alterations. Exp Cell Res 2015. pii: S0014-4827(15)00193-7.
103. Lindsberg PJ, Strbian D, Karjalainen-Lindsberg ML. Mast cells as early responders in the regulation of acute blood-brain barrier changes after cerebral ischemia and hemorrhage. J Cereb Blood Flow Metab 2010;30(4):689–7.
104. Nordal RA, Wong CS. Molecular targets in radiation-induced blood-brain barrier disruption. Int J Radiat Oncol Biol Phys 2005;62(1):279–87.
105. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 2007;8(1):57–69.
106. Frank-Cannon TC, Alto LT, McAlpine FE, Tansey MG. Does neuroinflammation fan the flame in neurodegenerative diseases? Mol Neurodegener 2009;4:47.
107. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, et al. The role of substance P in inflammatory disease. J Cell Physiol 2004;201(2):167–80.
108. Raslan F, Schwarz T, Meuth SG, Austinat M, Bader M, et al. Inhibition of bradykinin receptor B1 protects mice from focal brain injury by reducing blood-brain barrier leakage and inflammation. J Cereb Blood Flow Metab 2010;30(8):1477–86.
109. Zhu J, Qu C, Lu X, Zhang S. Activation of microglia by histamine and substance P. Cell Physiol Biochem 2014;34(3):768–80.
110. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. Physiol Rev 2011;91(2):461–553.
111. Ammari M, Brillaud E, Gamez C, Lecomte A, Sakly M, et al. Effect of a chronic GSM 900 MHz exposure on glia in the rat brain. Biomed Pharmacother 2008;62(4):273–81.
112. Hösli L, Hösli E, Schneider U, Wiget W. Evidence for the existence of histamine H1- and H2-receptors on astrocytes of cultured rat central nervous system. Neurosci Lett 1984;48(3):287–91.
113. Dong Y, Benveniste EN. Immune function of astrocytes. Glia 2001;36(2):180–90.
114. Majno G, Gilmore V, Leventhal M. On the mechanism of vascular leakage caused by histaminetype mediators. A microscopic study in vivo. Circ Res 1967;21(6):833–47.
115. Mayhan WG. Regulation of blood-brain barrier permeability. Microcirculation 2001;8(2):89–104.
116. Gurney KJ, Estrada EY, Rosenberg GA. Blood-brain barrier disruption by stromelysin-1 facilitates neutrophil infiltration in neuroinflammation. Neurobiol Dis 2006;23(1):87–96.
117. Moretti R, Pansiot J, Bettati D, Strazielle N, Ghersi-Egea JF, et al. Blood-brain barrier dysfunction in disorders of the developing brain. Front Neurosci 2015;9:40.
118. Kasparová S, Brezová V, Valko M, Horecký J, Mlynárik V, et al. Study of the oxidative stress in a rat model of chronic brain hypoperfusion. Neurochem Int 2005;46(8):601–11.
119. Oscar KJ, Hawkins TD. Microwave alteration of the blood-brain barrier system of rats. Brain Res 1977;126(2):281–93.
120. Oscar KJ, Gruenau SP, Folker MT, Rapoport SI. Local cerebral blood flow after microwave exposure. Brain Res 1981;204(1):220–5.
121. Albert EN, Kerns JM. Reversible microwave effects on the blood-brain barrier. Brain Res 1981;230(1–2):153–64.
122. Nittby H, Grafström G, Eberhardt JL, Malmgren L, Brun A, et al. Radiofrequency and extremely low-frequency electromagnetic field effects on the blood-brain barrier. Electromagn Biol Med 2008;27(2):103–26.

Brought to you by | provisional account

123. Reiter RJ. Melatonin suppression by static and extremely low frequency electromagnetic fields: relationship to the reported increased incidence of cancer. Rev Environ Health 1994;10 (3–4):171–86.
124. Burch JB, Reif JS, Yost MG, Keefe TJ, Pitrat CA. Nocturnal excretion of a urinary melatonin metabolite among electric utility workers. Scand J Work Environ Health 1998;24(3):183–9.
125. Pfluger DH, Minder CE. Effects of exposure to 16.7 Hz magnetic fields on urinary 6-hydroxymelatonin sulfate excretion of Swiss railway workers. J Pineal Res 1996;21(2):91–100.
126. Reiter RJ. Melatonin in the context of the reported bioeffects of environmental electromagnetic fields. Bioelectroch Bioener 1998;47:135–42.
127. Reiter RJ, Pablos MI, Agapito TT, Guerrero JM. Melatonin in the context of the free radical theory of aging. Ann NY Acad Sci 1996;786:362–78.
128. Reiter R, Tang L, Garcia JJ, Muñoz-Hoyos A. Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sci 1997;60(25):2255–71.
129. The Bioinitiative report 2012. A Rationale for Biologically-based Public Exposure Standards for Electromagnetic Fields (ELF and RF). Available at: www.bioinitiative.org.
130. Girgert R, Hanf V, Emons G, Gründker C. Signal transduction of the melatonin receptor MT1 is disrupted in breast cancer cells by electromagnetic fields. Bioelectromagnetics 2010;31(3):237–45.
131. Hendrick JP, Hartl FU. The role of molecular chaperones in protein folding. FASEB J 1995;9(15):1559–69.
132. Banecka-Majkutewicz Z, Grabowski M, Kadziński L, Papkov A, Węgrzyn A, et al. Increased levels of antibodies against heat shock proteins in stroke patients. Acta Biochim Pol 2014;61(2):379–83.
133. Tanaka S, Ichikawa A. Recent advances in molecular pharmacology of the histamine systems: immune regulatory roles of histamine produced by leukocytes. J Pharmacol Sci 2006;101(1):19–23.
134. Johansson O. Disturbance of the immune system by electromagnetic fields-A potentially underlying cause for cellular damage and tissue repair reduction which could lead to disease and impairment. Pathophysiology 2009;16(2–3):157–77.
135. Gangi S, Johansson O. A theoretical model based upon mast cells and histamine to explain the recently proclaimed sensitivity to electric and/or magnetic fields in humans. Med Hypotheses 2000;54(4):663–71.
136. Pall ML. Post-radiation syndrome as a NO/ONOO- cycle, chronic fatigue syndrome-like disease. Med Hypotheses 2008;71(4):537–41.
137. Levallois P. Hypersensitivity of human subjects to environmental electric and magnetic field exposure: a review of the literature. Environ Health Perspect 2002;110(Suppl 4):613–8.
138. Theoharides TC, Donelan J, Kandere-Grzybowska K, Konstantinidou A. The role of mast cells in migraine pathophysiology. Brain Res Brain Res Rev 2005;49(1):65–76.
139. Ozturk A, Degirmenci Y, Tokmak B, Tokmak A. Frequency of migraine in patients with allergic rhinitis. Pak J Med Sci 2013;29(2):528–31.
140. Alstadhaug KB. Histamine in migraine and brain. Headache 2014;54(2):246–59.
141. Renkawek K, Bosman GJ, de Jong WW. Expression of small heat-shock protein hsp 27 in reactive gliosis in Alzheimer disease and other types of dementia. Acta Neuropathol 1994;87(5):511–9.
142. Renkawek K, Stege GJ, Bosman GJ. Dementia, gliosis and expression of the small heat shock proteins hsp27 and alpha B-crystallin in Parkinson's disease. Neuroreport 1999;10(11):2273–6.
143. Donato R. Intracellular and extracellular roles of S100 proteins. Microsc Res Tech 2003;60(6):540–51.
144. Andreazza AC, Cassini C, Rosa AR, Leite MC, de Almeida LM, et al. Serum S100B and antioxidant enzymes in bipolar patients. J Psychiatr Res 2007;41(6):523–9.
145. Donato R, Cannon BR, Sorci G, Riuzzi F, Hsu K, et al. Functions of S100 proteins. Curr Mol Med 2013;13(1):24–57.
146. Mu H, Wang X, Lin P, Yao Q, Chen C. Nitrotyrosine promotes human aortic smooth muscle cell migration through oxidative stress and ERK1/2 activation. Biochim Biophys Acta 2008;1783(9):1576–84.
147. Kimata H. Effect of exposure to volatile organic compounds on plasma levels of neuropeptides, nerve growth factor and histamine in patients with self-reported multiple chemical sensitivity. Int J Hyg Environ Health 2004;207(2):159–63.
148. Irigaray P, Belpomme D. Basic properties and molecular mechanisms of exogenous chemical carcinogens. Carcinogenesis 2010;31(2):135–48.
149. McKeown-Eyssen G, Baines C, Cole DE, Riley N, Tyndale RF, et al. Case-control study of genotypes in multiple chemical sensitivity: CYP2D6, NAT1, NAT2, PON1, PON2 and MTHFR. Int J Epidemiol 2004;33(5):971–8.
150. Schnakenberg E, Fabig KR, Stanulla M, Strobl N, Lustig M, et al. A cross-sectional study of self-reported chemical-related sensitivity is associated with gene variants of drug-metabolizing enzymes. Environ Health 2007;6:6.
151. Caccamo D, Cesareo E, Mariani S, Raskovic D, Ientile R, et al. Xenobiotic sensor- and metabolism-related gene variants in environmental sensitivity-related illnesses: a survey on the Italian population. Oxid Med Cell Longev 2013;2013:831969.
152. Berg ND, Rasmussen HB, Linneberg A, Brasch-Andersen C, Fenger M, et al. Genetic susceptibility factors for multiple chemical sensitivity revisited. Int J Hyg Environ Health 2010;213(2):131–9.
153. De Luca C, Scordo MG, Cesareo E, Pastore S, Mariani S, et al. Biological definition of multiple chemical sensitivity from redox state and cytokine profiling and not from polymorphisms of xenobiotic-metabolizing enzymes. Toxicol Appl Pharmacol 2010;248(3):285–92.
154. Kirschvink JL, Kobayashi-Kirschvink A, Woodford BJ. Magnetite biomineralization in the human brain. Proc Natl Acad Sci USA 1992;89(16):7683–7.
155. Kirschvink JL, Walker MM, Diebel CE. Magnetite-based magnetoreception. Curr Opin Neurobiol 2001;11(4):462–7.
156. Kirschvink JL. Microwave absorption by magnetite: a possible mechanism for coupling nonthermal levels of radiation to biological systems. Bioelectromagnetics 1996;17(3):187–94.
157. De Luca C, Scordo G, Cesareo E, Raskovic D, Genovesi G, et al. Idiopathic environmental intolerances (IEI): from molecular epidemiology to molecular medicine. Indian J Exp Biol 2010;48(7):625–35.
158. Costa A, Branca V, Minoia C, Pigatto PD, Guzzi G. Heavy metals exposure and electromagnetic hypersensitivity. Sci Total Environ 2010;408(20):4919–20.

Brought to you by | provisional account

159. Burns-Naas LA, Meade BJ, Munson AE. Toxic responses of the immune system. In: Klaassen CD, editor. Casarett and Doull's toxicology: the basic of poisons, 6th ed. New York: McGraw Hill, 2001:419–70.
160. Gardner RM, Nyland JF, Evans SL, Wang SB, Doyle KM, et al. Mercury induces an unopposed inflammatory response in human peripheral blood mononuclear cells in vitro. Environ Health Perspect 2009;117(12):1932–8.
161. Goyer RA, Clarkson TW. Toxic effects of metals. In: Klaassen CD, editor. Casarett and Doull's toxicology: the basic of poisons, 6th ed. New York: McGraw Hill, 2001:822–6.
162. Minoia C, Ronchi A, Pigatto PD, Guzzi G. Blood lead, cadmium, and mercury concentrations in the Korean population. Environ Res 2010;110(5):532.
163. Mortazavi SM, Daiee E, Yazdi A, Khiabani K, Kavousi A, et al. Mercury release from dental amalgam restorations after magnetic resonance imaging and following mobile phone use. Pak J Biol Sci 2008;11(8):1142–6.
164. Störtebecker P. Mercury poisoning from dental amalgam through a direct nose-brain transport. Lancet 1989;1(8648):1207.
165. Miller CS. Toxicant-induced loss of tolerance – an emerging theory of disease? Environ Health Perspect 1997;105(Suppl 2): 445–53.
166. Rubin LL, Staddon JM. The cell biology of the blood-brain barrier. Annu Rev Neurosci 1999;22:11–28.
167. Löscher W, Potschka H. Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. Prog Neurobiol 2005;76(1):22–76.
168. Meairs S, Alonso A. Ultrasound, microbubbles and the blood-brain barrier. Prog Biophys Mol Biol 2007;93(1–3): 354–62.
169. Smith MW, Gumbleton M. Endocytosis at the blood-brain barrier: from basic understanding to drug delivery strategies. J Drug Target 2006;14(4):191–214.
170. Stam R. Electromagnetic fields and the blood-brain barrier. Brain Res Rev 2010;65(1):80–97.
171. Mrak RE, Griffin WS. Glia and their cytokines in progression of neurodegeneration. Neurobiol Aging 2005;26(3):349–54.
172. Griffin WS. Inflammation and neurodegenerative diseases. Am J Clin Nutr 2006;83(2):470S–4S.
173. Erickson MA, Banks WA. Blood-brain barrier dysfunction as a cause and consequence of Alzheimer's disease. J Cereb Blood Flow Metab 2013;33(10):1500–13.
174. Bell RD, Zlokovic BV. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. Acta Neuropathol 2009;118(1):103–13.
175. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, et al. Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21(3):383–421.
176. Sastre M, Klockgether T, Heneka MT. Contribution of inflammatory processes to Alzheimer's disease: molecular mechanisms. Int J Dev Neurosci 2006;24(2–3):167–76.
177. Ionov ID. Self-amplification of nigral degeneration in Parkinson's disease: a hypothesis. Int J Neurosci 2008;118(12):1763–80.
178. Jadidi-Niaragh F, Mirshafiey A. Histamine and histamine receptors in pathogenesis and treatment of multiple sclerosis. Neuropharmacology 2010;59(3):180–9.
179. Anderson G, Berk M, Dodd S, Bechter K, Altamura AC, et al. Immuno-inflammatory, oxidative and nitrosative stress, and neuroprogressive pathways in the etiology, course and treatment of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2013;42:1–4.
180. Ng F, Berk M, Dean O, Bush AI. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int J Neuropsychopharmacol 2008;11(6):851–76.
181. Patel JP, Frey BN. Disruption in the blood-brain barrier: the missing link between brain and body inflammation in bipolar disorder? Neural Plast 2015;2015:708306.
182. Berk M, Kapczinski F, Andreazza AC, Dean OM, Giorlando F, et al. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neurosci Biobehav Rev 2011;35(3):804–17.
183. Merritt JH, Chamness AF, Allen SJ. Studies on blood-brain barrier permeability after microwave-radiation. Radiat Environ Biophys 1978;15(4):367–77.
184. Avsenik J, Bisdas S, Popovic KS. Blood-brain barrier permeability imaging using perfusion computed tomography. Radiol Oncol 2015;49(2):107–14.
185. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 2009;7(1):65–74.
186. Galasko D, Montine TJ. Biomarkers of oxidative damage and inflammation in Alzheimer's disease. Biomark Med 2010;4(1):27–36.
187. Heneka MT, O'Banion MK. Inflammatory processes in Alzheimer's disease. J Neuroimmunol 2007;184(1–2):69–91.
188. Tachibana H, Meyer JS, Kitagawa Y, Tanahashi N, Kandula P, et al. Xenon contrast CT-CBF measurements in parkinsonism and normal aging. J Am Geriatr Soc 1985;33(6):413–21.
189. Abe Y, Kachi T, Kato T, Arahata Y, Yamada T, et al. Occipital hypoperfusion in Parkinson's disease without dementia: correlation to impaired cortical visual processing. J Neurol Neurosurg Psychiatry 2003;74(4):419–22.
190. Kikuchi A, Takeda A, Kimpara T, Nakagawa M, Kawashima R, et al. Hypoperfusion in the supplementary motor area, dorsolateral prefrontal cortex and insular cortex in Parkinson's disease. J Neurol Sci 2001;193(1):29–36.
191. Kasama S, Tachibana H, Kawabata K, Yoshikawa H. Cerebral blood flow in Parkinson's disease, dementia with Lewy bodies, and Alzheimer's disease according to three-dimensional stereotactic surface projection imaging. Dement Geriatr Cogn Disord 2005;19(5–6):266–75.
192. Derejko M, Slawek J, Wieczorek D, Brockhuis B, Dubaniewicz M, et al. Regional cerebral blood flow in Parkinson's disease as an indicator of cognitive impairment. Nucl Med Commun 2006;27(12): 945–51.
193. Sobel E, Davanipour Z, Sulkava R, Erkinjuntti T, Wikstrom J, et al. Occupations with exposure to electromagnetic fields: a possible risk factor for Alzheimer's disease. Am J Epidemiol 1995:142(5):515–24.
194. Sobel E, Dunn M, Davanipour Z, Qian Z, Chui HC. Elevated risk of Alzheimer's disease among workers with likely electromagnetic field exposure. Neurol 1996;47(6):1477–81.
195. Qiu C, Fratiglioni L, Karp A, Winblad B, Bellander T. Occupational exposure to electromagnetic fields and risk of Alzheimer's disease. Epidemiol 2004;15(6): 687–94.

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196. Davanipour Z, Sobel E. Long-term exposure to magnetic fields and the risks of Alzheimer's disease and breast cancer: further biological research. Pathophysiol 2009;16(2–3):149–56.
197. Garcia AM, Sisternas A, Hoyos SP. Occupational exposure to extremely low frequency electric and magnetic fields and Alzheimer disease: a meta-analysis. Int J Epidemiol 2008:37(2):329–40.
198. Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, et al. Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. J Alzheimers Dis 2010;19(1):191–210.
199. Söderqvist F, Hardell L, Carlberg M, Mild KH. Radiofrequency fields, transthyretin, and Alzheimer's disease. J Alzheimers Dis 2010;20(2):599–606.
200. Purdey M. Elevated levels of ferrimagnetic metals in food-chains supporting the Guam cluster of neurodegeneration: do metal nucleated crystal contaminants [corrected] evoke magnetic fields that initiate the progressive pathogenesis of neurodegeneration? Med Hypotheses 2004;63(5):793–809.
201. Hallberg O, Oberfeld G. Letter to the editor: will we all become electrosensitive? Electromagn Biol Med 2006;25(3):189–91.
202. World Health Organisation. Electromagnetic fields (300 Hz–300 GHz) 1993. Available at: http://www.inchem.org/documents/ehc/ehc/ehc137.htm.
203. Gibson PR, Kovach S, Lupfer A. Unmet health care needs for persons with environmental sensitivity. J Multidisc Healthcare 2015;8:59–66.

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