Environmental diseases, oxidative stress and genetic polymorphism

Francesco Cosentino, MD *
Maria Concetta Giuliano, GP #
Fabio Biscachia GP Trainee #

* Gastroenterology and Hepatology Dept - Milton Keynes G.H.- NHS FT UK
# General Practice NHS - Sicily Italy
Enviromental Diseases
Sensitivity Related Illnesses (SRI)

Adverse clinical states elicited by exposure to low dose of enviromental physical, chemical or biological factors mainly xenobiotic chemicals drugs and metals

Multiple Chemical Sensitivity (MCS)

Chronic Fatigue Syndrome (CFS) / Myalgic Encephalomyelitis (ME)

Fibromyalgia (FM)

Electromagnetic Hypersensitivity (EHS)
~ 15-30% of USA population exhibit milder forms of chemical hypersensitivity

~ 3-4% of Americans suffer with severe forms of chemical sensitivity

~ 3% of Canadians have been diagnosed with environmental sensitivities

Comparable data in other countries such as Germany, Sweden, Netherland
Sensitivity Related Illness (SRI)

The marked similarity of symptoms may support common organic etiological biomarkers of disease.

Clinical overlapping of the different syndromes which may represent separated clinical settings sharing some common molecular pathways.
Clinical data prove that functional or genetic defects may cause **CHRONIC OXIDATIVE STRESS** and consequent metabolic and immunologic alterations characteristic for the patients with environmental SRI.

Miller C. S. *J. of Nutritional & Environmental Medicine* 2001, 11, 181-204
Emerging model of disease etiology

- Gene polymorphism phase I/II
- Detoxification enzymes gene
- Free radical antioxidant
- Homeostasis disturbances
- Epigenetic and metabolic factors

Phase I and phase II reactions

**PHASE I**
- Expose or add functional group
- Oxidation
- Reduction
- Hydrolysis

**PHASE II**
- ROS
- Conjugation

**XENOBIOTIC** → PRIMARY PRODUCT → **LIPOPHILIC**

**SECUNDARY PRODUCT** ← EXCRETION ← **HYDROPHILIC**

Expose or add functional group

Oxidation Reduction Hydrolysis

EXCRETION
Nitric oxid - Peroxynitrite at the base of lipid peroxidation and epigenetic alterations

- DNA Damage
- Oxidized Protein
- Lipid Peroxidation

Peroxynitrous acid

ONO\(_{2}^{\cdot}\)

\(\text{H}_2\text{O} + \text{O}_2\)

H\(_2\)O

\(\text{SOD}\)

\(\text{GPx}\)

\(\text{GSH} \rightarrow \text{GSSG}\)

\(\text{GRd}\)

Cellular respiration

Oxidative burst

Environmental factors

Enzyme activity

\(\text{ARG}\)

\(\text{NOS}\)

\(\text{Nitric oxid}\)

\(\text{Peroxynitrite}\)

\(\text{Suoeroxide Anion}\)

\(\text{\(1\)O}_2\)

\(\text{hv}\)

\(\text{DNA Damage}\)

\(\text{Lipid Peroxidation}\)

\(\text{Oxidized Protein}\)
Fig. 1. Peroxynitrite-induced cytotoxic pathways. Nitric oxide and superoxide react to form peroxynitrite which damages cells via various damaging effects such as lipid peroxidation, inactivation of metalloenzymes and other proteins by oxidation and nitration. Peroxynitrite also acts on mitochondria triggering the release of proapoptotic factors such as apoptosis-inducing factor (AIF) and cytochrome c. These factors mediate caspase dependent and independent apoptotic death pathways. Moreover, peroxynitrite-induced DNA breakage activates PARP leading to NAD and ATP depletion and consequently to necrosis.
Sensitivity Related Illnesses (SRI)

**EPIGENETIC FACTORS**

- DNA Methylation
- Histone Modifications
- microRNAs

**Functional Changes in Genoma**

Functional Changes in Genoma occur without a change in nucleotide sequence

Dott. Francesco Cosentino - Dott.ssa Maria Concetta Giuliano - Dott. Fabio Bisicchia
ECIM 2012
Sequelae of toxicant exposure induce loss of Tolerance (TILT) with consequent Allergy, Food Intolerance and Chemical Hypersensitivity.

After primary toxicant individuals become sensitive to low levels of diverse triggers in environment (chemical, inhalant or food antigens).

Cytokine levels in the blood plasma of control subjects \((C, n = 52)\), and patients with diagnosed MCS \((MCS, n = 77)\)

Inter-group significant differences \((P < 0.05\) or \(0.01\)) are reported under each panel:
- MCP-1 (macrophage chemotactic protein)
- PDGF (platelet-derived growth factor)
- VEGF (vascular endothelial growth factor)

Metabolic redox parameters in the blood components

- control subjects (C 52)
- MCS Patients (MCS 133)
- Suspected MCS Patients (S-MCS 93)

GSH (glutathion reduced)
GPX (glutathion peroxidase)
GSSG ((glutathion oxidized)
NO2 (nitric oxide)
CL (luminol-dependent chemiluminescence)
AOA (anti-oxidant activity)


Dott. Francesco Cosentino - Dott.ssa Maria Concetta Giuliano - Dott. Fabio Bisicchia
ECIM 2012
Co-morbidities registered in the MCS group (226) through evaluation of anamnesis.

% of patients affected by each single category of organ pathologies

<table>
<thead>
<tr>
<th>Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastro-intestinal</td>
<td>27.8%</td>
</tr>
<tr>
<td>Thyroideal</td>
<td>24.9%</td>
</tr>
<tr>
<td>Allergies/intollerances</td>
<td>22.7%</td>
</tr>
<tr>
<td>Respiratory</td>
<td>21.6%</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>19.6%</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Multichemical Sensitivity (MCS)

Pesticide and Organic Solvent Action

- Organophosphorus/carbamate pesticides
- Organochlorine pesticides
- Acetylecholine
- Acetylcholinesterase
- Muscarinic activity
- NMDA receptor activity
- GABAa receptors

- Organic solvents
- TRPV1, TRPA1 other TRP receptors
- Sodium channels

- Pesticide and Organic Solvent Action

- Nitric Oxide

- Mercurio
- Solfuro di Idrogeno
- Monossido di Carbonio
Neural Sensitization Cycle

NMDA Receptor

Stimulation of Neurotransmitter Release
(presynaptic cell)

NMDA stimulation (postsynaptic cell)

Increased Nitric Oxide

Increased Peroxynitrite

Retrograde Messenger

ATP (energy) depletion

Martin L. Pall
Prof Emeritus of Biochemistry and Basic Medical Sciences
Washington State University USA
Chemical Intolerance in Primary Care settings
Prevalence Comorbidity and Outcomes

Figure 1. Prevalence of Chemical Intolerance vs Number of Possible Mental Disorders

Note: Possible disorders were major depressive disorder, generalized anxiety disorder, panic disorder, and alcohol abuse disorder.


Dott. Francesco Cosentino - Dott.ssa Maria Concetta Giuliano - Dott. Fabio Bisicchia
ECIM 2012
The search for reliable Biomarkers of Disease in Multiple Chemical Sensitivity and other environmental Intolerances

### Table 1. Genetic Polymorphisms Influencing MCS Susceptibility

<table>
<thead>
<tr>
<th>Gene</th>
<th>Study</th>
<th>Function-chemical metabolism</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1</td>
<td>H,M</td>
<td>Detoxification of organophosphorus toxicants including pesticides</td>
<td></td>
</tr>
<tr>
<td>CYP2D6</td>
<td>M</td>
<td>Hydroxylation of hydrophobic compounds</td>
<td>May be expected to increase activity of strictly hydrophobic solvents on the TRPV1 receptor</td>
</tr>
<tr>
<td>NAT2</td>
<td>M,S</td>
<td>Acetylation</td>
<td>May produce more or less activity, depending on substrate</td>
</tr>
<tr>
<td>GSTM1</td>
<td>S</td>
<td>Provides reduced glutathione for conjugation</td>
<td>Should increase detoxification and excretion</td>
</tr>
<tr>
<td>GSTT1</td>
<td>S</td>
<td>Glutathione conjugation</td>
<td>Should increase detoxification and excretion</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>M&amp;S</td>
<td>Glucuronidation, leading to increased excretion</td>
<td></td>
</tr>
</tbody>
</table>

H=Haley et al, 1999 (11); M=McKeown-Eyssen et al, 2004 (12); S=Schnakenberg et al, 2007 (13); M&S= Müller and Schnakenberg, 2008 (14).


Dott. Francesco Cosentino - Dott.ssa Maria Concetta Giuliano - Dott. Fabio Bisicchia
ECIM 2012
Clinical Audit

MCS Prospective Study

Background

Previous community based studies showed an increasing chemical intolerance and allergy

AIMS

Assess MCS prevalence and comorbidities in a sample of Primary Care Clinic patients

Assess the impact of Genetic and Epigenetic Factors as the cause of MCS

Dott. Francesco Cosentino - Dott.ssa Maria Concetta Giuliano - Dott. Fabio Bisicchia
ECIM 2012
Clinical Audit

MCS Prospective Study

Methods

Patients will be recruited from 10 General Practice Catania Health District Board ASP 3 (Sicily)

Patients will complete the validated QEESI Quick Environmental Exposure and Sensitivity Inventory
Clinical Audit

MCS Prospective Study

QEESI

Chemical intolerance

Primary Care Evaluation of Mental Disorders

Screen for possible Psychiatric disorders
Criteria for Low, Medium and High scale scores

Table 1. Criteria for low, medium, and high scale scores

<table>
<thead>
<tr>
<th>Scale/Index</th>
<th>Low</th>
<th>Score Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom Severity</td>
<td>0-19</td>
<td>20-39</td>
<td>40-100</td>
</tr>
<tr>
<td>Chemical Intolerance</td>
<td>0-19</td>
<td>20-39</td>
<td>40-100</td>
</tr>
<tr>
<td>Other Intolerance</td>
<td>0-11</td>
<td>12-24</td>
<td>25-100</td>
</tr>
<tr>
<td>Life Impact</td>
<td>0-11</td>
<td>12-23</td>
<td>24-100</td>
</tr>
<tr>
<td>Masking Index</td>
<td>0-3</td>
<td>4-5</td>
<td>6-10</td>
</tr>
</tbody>
</table>

Dr Claudia Miller Dept. Family & Community Medicine Texas University USA
### Clinical Audit

**MCS Prospective Study**

#### LEVEL 1 Criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient case history suggestive of MCS</td>
<td></td>
</tr>
<tr>
<td>Daily activities affected by exposure to chemicals</td>
<td></td>
</tr>
<tr>
<td>Impairment of at least two systems after exposure to chemicals known</td>
<td></td>
</tr>
<tr>
<td>QEESI score $\geq 21$</td>
<td></td>
</tr>
</tbody>
</table>
Clinical Audit
MCS Prospective Study

Level 2 Criteria

Olfactory Assessment
Near Infrared Spectroscopy

Psycological Assessment
MMP1 MMP2
Rorschach Test , Zulliger Test

Neurophysiologic Testing
Clinical Audit
MCS Prospective Study

Level 2 Criteria
Assessment of Oxidative Stress

D-Roms Test
PAB test

Erythrocyte Superoxide Dismutase, Catalase, Glutation peroxidase activity
D-ROMs Test (Reactive Oxygen Metabolites) is a spectrophotometry that determines the concentration of hydroperoxides (ROOH) generated in the cells from the attach of ROS.
**D-ROMs test**

The test is evaluated in a conventional measuring unit called **U CARR** (from the chemist Carratelli, the inventor of the test). Normal values are from 250 to 300 U CARR; above 300 U CARR there is the oxidative stress, but for a thin borderline range (301-320 U CARR).

<table>
<thead>
<tr>
<th>IDROPEROSSIDI (U-CARR)</th>
<th>IDROPEROSSIDI (mg H₂O₂/dl)</th>
<th>Stress Ossidativo (gravità)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 - 320</td>
<td>24,08 - 25,60</td>
<td>Border-line</td>
</tr>
<tr>
<td>321 - 340</td>
<td>25,68 - 27,20</td>
<td>Mild</td>
</tr>
<tr>
<td>341 - 400</td>
<td>27,28 - 32,00</td>
<td>Moderate</td>
</tr>
<tr>
<td>401 - 500</td>
<td>32,08 - 40,00</td>
<td>High</td>
</tr>
<tr>
<td>&gt; 500</td>
<td>&gt; 40,00</td>
<td>Severe</td>
</tr>
</tbody>
</table>

Dott. Francesco Cosentino - Dott.ssa Maria Concetta Giuliano - Dott. Fabio Bisicchia
ECIM 2012
PAB test

Prooxidant-Antioxidant Balance

Rate the effectiveness of the barrier antioxidant which is able to oppose the action of damaging free radicals.

Normal range 1.30 – 1.70 mmol/L
Clinical Audit

MCS Prospective Study

Level 2 Criteria

Autoimmunity Markers
Cytokine Panel
Total IgG IgA IGE
Aga IgA IgG TtG IgA IgG
Iron - Ferritin - Vit B12 - Folate
Homocisteynemi *
MTHFR *
Restriction Diet
Food Allergy Test IgE * – IgG4 *
Patch test *

* Facoltative
Clinical Audit

MCS Prospective Study

Level 3 Criteria

Genetic Polymorphism

PON 1
CYP2D6
NAT2
GSTM1
GSTT1
UGT1A1
The assessment of concentration of hydroperoxides (ROOH) and prooxidant-antioxidant balance by D-ROMs and PAB Test could be a useful and cheap diagnostic device to confirm the suspected diagnosis of SRI in patients with QUEESI score positive.

D-ROMs and PAB test could be a useful diagnostic address in search of Genetic Polymorphism associated with MCS and Epigenetic Factors.