The memory lymphocyte immunostimulation assay in immune system disorders: Is useful or useless?

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Abstract:

AIM: The aim of the study was to evaluate the clinical relevance, sensitivity and specificity of in vitro blood test, Memory Lymphocyte ImmunoStimulation Assay (MELISA®), in genetically predisposed patients that suffer by autoimmune/inflammatory syndrome induced by adjuvants, after HPV-vaccination and that could have a high metal hypersensitivity.

MATERIALS AND METHODS: Sixteen girls (aged 12–24 years) that developed long-lasting and invalidating somatoform symptoms occurring within 20 days postvaccination are included in this descriptive study. The hypersensitivity to five metals (aluminum, nickel, mercury, methyl mercury, and thimerosal) was measured by MELISA® test.

RESULTS: Seven girls showed negativity to all the five metals tested. The findings showed metal hypersensitivity only in nine patients: Toxicity to aluminum (two girls), reactivity to nickel (seven girls), followed by mercury (seven girls).

CONCLUSION: The MELISA® assay is neither sensitive nor specific in detecting metal hypersensitivity and associated chronic diseases, including autoimmune pathologies.

Key words:
Hypersensitivity, Memory Lymphocyte ImmunoStimulation Assay, metal, test

Introduction

Several studies show that metals, including mercury, aluminum, nickel, methyl mercury thiosalicylate, thimerosal (used with aluminum as vaccine adjuvant) can be a risk factor for the development of various autoimmune pathologies, including autoimmune thyroiditis,¹,² multiple sclerosis,³ kidney disease,⁴ and myalgia.⁵,⁶ These metals act as immunosuppressants (cytostatically), or as immunoadjuvants (through nonspecific activation of the immune response),⁷,⁸ resulting in cytokine release and abnormalities of the hypothalamus-pituitary-adrenal axis, and causing changes in the brain, fatigue, and severe psychological symptoms such as as are seen in chronic fatigue syndrome, fibromyalgia, and autoimmune thyroiditis.⁹ However, the metal hypersensitivity has been found most common in genetically predisposed individuals.¹⁰ The enzymatic processes blocked by metals also result in chronic formation of metal-protein compounds (human leukocyte antigen [HLA] antigens or antigen-presenting macrophages) that the T-lymphocytes do not recognize, resulting in autoimmune reactions. The metals bind to SH-groups on proteins which can then be recognized as “foreign” and attacked by T-lymphocytes.¹¹

However, the interaction of T-lymphocytes with a metal determines the basis of the so-called Memory Lymphocyte ImmunoStimulation Assay (MELISA®), which detects the proliferation of memory lymphocytes (T-lymphocytes that had

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contact with a sensitizing allergen) after exposure to metals in vitro.\[^{12-14}\] We examined the findings of MELISA® Test in genetically predisposed patients that developed autoimmune/inflammatory syndrome induced by adjuvants (ASIA syndrome) after HPV vaccination.

**Materials and Methods**

Sixteen young girls, aged 12–24 years who developed clinical manifestations (such as asthenia, severe pain, skin rashes, sinus tachycardia, amenorrhea, optic neuritis, headache, and sleep disturbances) and elevated titers of autoantibodies (e.g., Anti-EBV, ANA, HLA) after HPV-vaccination, already referred to our “Second Opinion Medical Network for the evaluation of ASIA syndrome,” participated in this descriptive design[^15][Table 1 and 2].

The selected patients were informed, through an individual interview, and informed consent previously approved by the Local Institutional Review Board under the Helsinki Declaration.

The blood sample of each girl was collected into six vacutainer tubes, containing sodium citrate, and sent to licensed Laboratory (InVitaLab Medizindiagnostik, Neuss, Germany).

The choice of five metals for testing (aluminum, mercury, nickel, methylmercury, and thimerosal) was based on informations derived from possible exposure to adjuvant stimuli that may occur through HPV-vaccine precoated with metal solutions in 2–3 concentrations; then, the plates were incubated for 5 days at 37°C with 5% CO₂.

Three negative controls (only lymphocytes in 10% medium) and one positive control (lymphocytes in 10% medium plus pokeweed mitogen) were included in each test. After 5 days, 600 µl of cell suspension from each well was transferred to a new 24-well plate (second monocyte depletion) and the cells incubated for 4 h.[^16]

The subsequent cell proliferation is measured by the incorporation of radioactive isotope 3H-thymidine in metal cultures. An increase in thymidine uptake could point to the presence of hypersensitivity to the metal tested. These findings are expressed as a stimulation index, calculated as the thymidine uptake in treated cultures divided by the mean isotope uptake in untreated control cultures [Table 3].

**Results**

MELISA® test is directly dependent on lymphocyte concentration: the higher the lymphocyte concentration per test, the stronger the reactivity. In this study, the lymphocyte test detected seven patients (42%) who were negative to all the five metals tested and nine patients (53%) who were positive for at least one of the tested metals: toxicity to aluminum (two girls), and reactivity to nickel (four girls), followed to mercury (five girls) [Figure 1]. None of the patients responded to thimerosal and methyl mercury. Some patients had a metal allergy, such as eczema when wearing cheap metal earrings. Other metal exposures, including living in a polluted area (near steelworks), exposure to cigarette smoke were reported by 41% of the patients [Table 4].

![Figure 1: Metal hypersensitivity to Memory Lymphocyte ImmunoStimulation Assay® test](image)

| Table 1: Patients’ characteristics |
|-----------------------------------|
| Number of patients                | 16 |
| Mean age (years)                  | 16.7 |
| Mean weight (kg)                  | 56.3 |
| Mean height (cm)                  | 162.3 |
| HPV vaccine type (n)              |     |
| Gardasil                          | 9 |
| Cervarix                          | 7 |
| Number doses                      |     |
| One dose                          | 4 |
| Two doses                         | 6 |
| Three doses                       | 6 |

HPV = Human papillomavirus
had a low specificity (25%) and therefore was useless for diagnosis of metal hypersensitivity, since a large number of false-positive results could be obtained.\[19\] These false-positive reactions could be due to the use of higher metal concentrations that could result in nonspecific proliferation of the lymphocytes.\[20\] In 1999, the same author tested the validity of the MELISA® test and LTT for the detection of mercury allergy in 62 dental amalgam-bearers (23 amalgam patients, 30 healthy blood donors with amalgam and 9 patients with oral lichen planus adjacent to dental amalgam) and in 10 healthy controls without amalgam (controls).\[21\] Thus, despite the use of low concentration of mercury solution (HgCl₂ ≤ 0.5 µg/mL), a high frequency of positive results was obtained among healthy controls with or without dental amalgam. Consequently, the author concluded that MELISA® cannot be used as an objective marker for mercury allergy in individuals with dental amalgam fillings. Indeed, already in 1998, the

### Table 2: Autoimmune/inflammatory syndrome induced by adjuvants diagnostic criteria in our case series (Shoenfeld et al.)

| Patients | Type HPV vaccine | Major criteria | Minor criteria | Clinical manifestation |
|----------|------------------|----------------|---------------|-----------------------|
| #1       | Cervarix® (3 doses) | Headache, dizziness, synus tachycardia, abdominal pain, CFS, myalgia, arthralgia | Autoantibodies (ANA, anti-cardiolipin IgM) | Fibromyalgia |
| #2       | Gardasil® (2 doses) | Muscle weakness, dry mouth, sleep disturbances, epileptic seizures, CFS | Autoantibodies (anti-EBNA IgG) | - |
| #3       | Gardasil® (2 doses) | Low-grade fever, skin rashes, cognitive impairment | HLA haplotype (HLA-DRB1) | ASIA syndrome |
| #4       | Cervarix® (1 dose) | Headache, muscle weakness, skin rashes, sleep disturbances, memory loss, CFS | Autoantibodies (anti-VCA IgG, anti-EBNA IgG) | Diabetes mellitus |
| #5       | Gardasil® (1 dose) | Recurrent syncope, headache, muscle weakness, severe stomach pain, pins and needles | Autoantibodies (anti-VCA IgG, anti-EBNA IgG) | Type 1 |
| #6       | Cervarix® (1 dose) | Arthralgia, myalgia, concentration problems, memory loss, panic attack symptoms, sleep disturbances, CFS | Autoantibodies (anti-GM1 IgM, anti-Ab IgM, anti-EBNA, anti-VCA) | Pseudoneurological somatoform disorder |
| #7       | Gardasil® (2 doses) | Muscle weakness, dry mouth, CFS, concentration problems, memory loss | Autoantibodies (AbTPO, anti-VCA IgG) | Diabetes mellitus |
| #8       | Gardasil® (3 doses) | Headache, muscle weakness, skin rashes, cognitive impairment, CFS | Autoantibodies (anti-VCA IgG) | Fibromyalgia |
| #9       | Cervarix® (3 doses) | Headache, arthralgia, cognitive impairment, muscle weakness, CFS | Autoantibodies (anti-VCA IgG) | Fibromyalgia |
| #10      | Cervarix® (2 doses) | Arthralgia, muscle weakness, recurrent syncope, asthenia | Autoantibodies (anti-VCA IgG) | Fibromyalgia |
| #11      | Gardasil® (2 doses) | Nausea, asthenia, insomnia, recurrent syncope, abdominal pain | Autoantibodies (anti-VCA IgG, anti-VCA IgM) | Fibromyalgia, Raynaud’s syndrome |
| #12      | Cervarix® (3 doses) | Amenorrhea, abdominal pain, stomach pain | Autoantibodies (anti-TG and anti-TPO, anti-VCA IgG) | - |
| #13      | Gardasil® (3 doses) | Arthralgia, sleep disturbances, headache, abdominal pain | Autoantibodies (anti-VCA IgG) | Autoimmune thyroiditis |
| #14      | Gardasil® (3 doses) | Fever, myalgia, myositis, cognitive impairment, CFS | Autoantibodies (anti-VCA IgG) | Fibromyalgia |
| #15      | Gardasil® (2 doses) | Asthenia, insomnia, concentration problems | Autoantibodies (anti-VCA IgG and anti-EBNA IgG) | Sideropenic anemia, idiopathic thrombocytopenia |
| #16      | Cervarix® (1 dose) | Fever, CFS, sleep disturbances, dizziness, concentration problems | Autoantibodies (anti-VCA IgG and anti-EBNA IgG) | Irritable bowel disease |

ASIA = Autoimmune/inflammatory syndrome induced by adjuvants, CFS = Chronic fatigue syndrome, VCA = Viral capsid antigen, EBNA = Epstein-Barr nuclear antigen, ANA = Antinuclear antibody, AbTPO = Anticorpi anti-tireoperossidasi, HPV = Human papillomavirus, TG = Thyroglobulin

### Table 3: Values of stimulation index

| Thymidine (SI) | Value     |
|---------------|-----------|
| <0.3          | Toxic     |
| >0.2          | Weak positive (+) |
| >3.0          | Positive (++) |
| >10           | Strong positive (+++) |

SI = Stimulation index

### Discussion

Several studies of Prof. Stejskal (inventor of MELISA® test) reported frequent metal hypersensitivity (e.g., aluminum, nickel, mercury) in patients with chronic fatigue/fibromyalgia by MELISA® assay.\[1,2,17,18\] Nevertheless, in 1997 Cederbrant et al. (coworker of Stejskal) compared the results of cutaneous patch test, conventional lymphocyte transformation test (LTT) and MELISA® test in 34 patients for detection of gold, nickel, and palladium and showed that the MELISA® assay

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Table 4: Description of metal exposure, lymphocyte responses, and stimulation index values for each patient

| Patient code | Age (years) | Reported metal exposure | Positive MELISA | SI value |
|--------------|-------------|-------------------------|----------------|---------|
| #1           | 19          | Vaccine, environment    | Nickel          | 9.2 (++)|
| #2           | 20          | Vaccine                 | Nickel          | 22.7 (+++)|
| #3           | 21          | Vaccine, environment    | Aluminum        | 0.2 (toxic) |
| #4           | 14          | Vaccine, orthodontics   | Negative        | - |
| #5           | 12          | Vaccine, environment,   | Inorganic Hg    | 6.6 (++)|
| #6           | 14          | Vaccine                 | Negative        | - |
| #7           | 15          | Vaccine                 | Negative        | - |
| #8           | 17          | Vaccine, nickel allergy, | Nickel          | 2.4 (+) |
| #9           | 15          | Vaccine, environment,   | Inorganic Hg    | 2.9 (+) |
| #10          | 13          | Vaccine                 | Nickel          | 5.9 (+++)|
| #11          | 15          | Vaccine                 | Nickel          | 4.4 (+) |
| #12          | 17          | Vaccine, orthodontics   | Nickel          | 3.8 (++)|
| #13          | 15          | Vaccine, orthopedics    | Inorganic Hg    | 4.5 (+) |
| #14          | 15          | Vaccine, environment    | Aluminum        | 0.3 (toxic) |
| #15          | 20          | Vaccine                 | Nickel          | 5.7 (++)|
| #16          | 25          | Vaccine                 | Negative        | - |

MELISA = Memory lymphocyte immunostimulation assay, SI = Stimulation index, + = Weak positive, ++ = Positive, +++ = Strong positive

German Contact Allergy Group warned against the use of the MELISA® test for the detection of metal allergy.\(^{[22]}\)

Our findings could confirm the low sensitivity and specificity of the MELISA® test because we observed a high frequency of negative results (seven girls) and reactivity to mercury and nickel in patients that have orthodontics and nickel allergy (three and one girl, respectively).

**Conclusion**

We did not find in the literature evidence-based data supporting the MELISA® test as a reliable, unifying, efficient and meaningful method for detection of metal hypersensitivity and associated diseases. Furthermore, the claim that metal hypersensitivity plays a striking role in immunological, neurological, and metabolic diseases (viz., in the vaccination adverse effects area), does not reach adequate clinical proof of concept and does not justify any chelating therapy to the patients in case of anecdotal positive results.

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**Conflicts of interest**

There are no conflicts of interest.

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