Article published online: 2020-02-19

Original Article

Access this article online

Quick Response Code:

Website: www.jlponline.org

DOI: 10.4103/JLP.JLP_137_16

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

Maria Vadalà[1,2], Carmen Laurino[1,2], Beniamino Palmieri[2]

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 asthenia, severe pain, sleep disturbances, gastrointestinal, and neurological problems 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

[1]Department of General Surgery and Surgical Specialties, University of Modena and Reggio Emilia Medical School, Surgical Clinic, [2]Network of the Second Opinion, Modena, Italy

Address for correspondence:
Dr. Maria Vadalà, Department of General Surgery and Surgical Specialties, University of Modena and Reggio Emilia Medical School, Surgical Clinic, Modena, Italy.
Network of the Second Opinion, Modena, Italy.
E-mail: mary.vadala@gmail.com

Submission: 07-10-2016
Accepted: 04-07-2017

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Vadalà M, Laurino C, Palmieri B. The memory lymphocyte immunostimulation assay in immune system disorders: Is useful or useless?. J Lab Physicians 2017;9:223-6.

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] [Tables 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 administration.

The lymphocytes were isolated from blood sample and subsequently cultured in medium containing 20% autologous inactivated human serum and incubated with 5% CO_2 atmosphere for 30 min at 37°C in cell culture flasks for partial depletion of monocytes. After incubation, cells were counted, diluted with medium plus 10% serum in a concentration of 1×10^6 lymphocytes/ml and successively were cultured in 48-well tissue plates precoated with metal solutions in 2–3 concentrations; then, the plates were incubated for 5 days at 37°C with 5% CO_2.

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].

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

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].

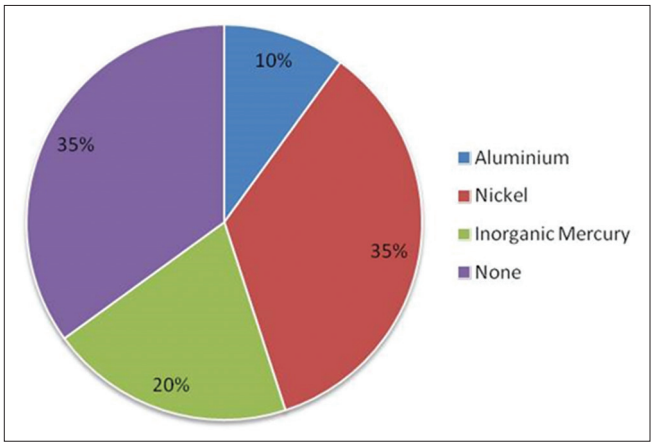

Figure 1: Metal hypersensitivity to Memory Lymphocyte ImmunoStimulation Assay® test

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) Autoantibodies (anti-VCA IgM, anti-EBNA IgG)	Fibromyalgia, 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 Type 1
#5	Gardasil® (1 dose)	Recurrent syncope, headache, muscle weakness, severe stomach pain, pins and needles	Autoantibodies (anti-VCA IgG, anti-EBNA IgG)	-
#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 Type 1
#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	-	-
#13	Gardasil® (3 doses)	Arthralgia, sleep disturbances, headache, abdominal pain	Autoantibodies (anti-TG and anti-TPO, 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	-	Sideropenic anemia, idiopathic thrombocytopenia
#16	Cervarix® (1 dose)	Fever, CFS, sleep disturbances, dizziness, concentration problems	Autoantibodies (anti-VCA IgG and IgM)	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 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 ($HgCl_2$ ≤ 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 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, orthodontics	Inorganic Hg	6.6 (++)
			Nickel	2.4 (++)
#6	14	Vaccine	Negative	-
#7	15	Vaccine	Negative	-
#8	17	Vaccine, nickel allergy, environment	Nickel	2.4 (+)
			Inorganic Hg	2.9 (+)
#9	15	Vaccine, environment, orthodontics	Inorganic Hg	2.4 (+)
#10	13	Vaccine, environment	Nickel	9 (+++)
			Inorganic Hg	5.9 (+++)
#11	15	Vaccine	Nickel	4.4 (+)
#12	17	Vaccine, orthodontics	Nickel	3.8 (++)
			Inorganic Hg	4.5 (+)
#13	15	Vaccine, orthopedics	Aluminum	0.3 (toxic)
			Nickel	5.7 (++)
#14	15	Vaccine, environment	Negative	-
#15	20	Vaccine	Negative	-
#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 girls, respectively).

Conclusion

We did not find in the literature evidence-based data supporting the MELISA® test as a reliable, unfailing, 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.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Sterzl I, Procházková J, Hrdá P, Bártová J, Matucha P, Stejskal VD. Mercury and nickel allergy: Risk factors in fatigue and autoimmunity. Neuro Endocrinol Lett 1999;20:221-8.
2. Hybenova M, Hrda P, Procházková J, Stejskal V, Sterzl I. The role of environmental factors in autoimmune thyroiditis. Neuro Endocrinol Lett 2010;31:283-9.
3. Siblerud RL, Kienholz E. Evidence that mercury from silver dental fillings may be an etiological factor in multiple sclerosis. Sci Total Environ 1994;142:191-205.
4. Kazantzis G, Schiller KF, Asscher AW, Drew RG. Albuminuria and the nephrotic syndrome following exposure to mercury and its compounds. Q J Med 1962;31:403-18.
5. Stejskal VD, Danersund A, Lindvall A, Hudecek R, Nordman V, Yaqob A, *et al*. Metal-specific lymphocytes: Biomarkers of sensitivity in man. Neuro Endocrinol Lett 1999;20:289-98.
6. Stejskal V, Hudecek R, Stejskal J, Sterzl I. Diagnosis and treatment of metal-induced side-effects. Neuro Endocrinol Lett 2006;27 Suppl 1:7-16.
7. HogenEsch H. Mechanisms of stimulation of the immune response by aluminum adjuvants. Vaccine 2002;20 Suppl 3:S34-9.
8. Havarinasab S, Hultman P. Organic mercury compounds and autoimmunity. Autoimmun Rev 2005;4:270-5.
9. Tomas C, Newton J, Watson S. A review of hypothalamic-pituitary-adrenal axis function in chronic fatigue syndrome. ISRN Neurosci 2013;2013:784520.
10. Loyo E, Jara LJ, López PD, Puig AC. Autoimmunity in connection with a metal implant: A case of autoimmune/autoinflammatory syndrome induced by adjuvants. Auto Immun Highlights 2012;4:33-8.
11. Stejskal J, Stejskal VD. The role of metals in autoimmunity and the link to neuroendocrinology. Neuro Endocrinol Lett 1999;20:351-64.
12. Stejskal VD, Olin RG, Forsbeck M. The lymphocyte transformation test for diagnosis of drug-induced occupational allergy. J Allergy Clin Immunol 1986;77:411-26.
13. Stejskal VD. Allergy to drugs and other chemicals diagnosed by the presence of specific memory cells in human blood. In: Ivanyi P. (eds) Realm of Tolerance. Springer 1989. 213-25
14. Stejskal VD, Forsbeck M, Nilsson R. Lymphocyte transformation test for diagnosis of isothiazolinone allergy in man. J Invest Dermatol 1990;94:798-802.
15. Palmieri B, Poddighe D, Vadalà M, Laurino C, Carnovale C, Clementi E. Erratum to: Severe somatoform and dysautonomic syndromes after HPV vaccination: Case series and review of literature. Immunol Res 2016;65:117-9.
16. Valentine-Thon E, Schiwara HW. Validity of MELISA for metal sensitivity testing. Neuro Endocrinol Lett 2003;24:57-64.
17. Stejskal V. Metals as a common trigger of inflammation resulting in non-specific symptoms: Diagnosis and treatment. Isr Med Assoc J 2014;16:753-8.
18. Stejskal V, Ockert K, Bjørklund G. Metal-induced inflammation triggers fibromyalgia in metal-allergic patients. Neuro Endocrinol Lett 2013;34:559-65.
19. Cederbrant K, Hultman P, Marcusson JA, Tibbling L. *In vitro* lymphocyte proliferation as compared to patch test using gold, palladium and nickel. Int Arch Allergy Immunol 1997;112:212-7.
20. Koene RA. The 'memory lymphocyte immunostimulation assay' (MELISA) is useless for the detection of metal allergy. Ned Tijdschr Geneeskd 2005;149:2090-2.
21. Cederbrant K, Gunnarsson LG, Hultman P, Norda R, Tibbling-Grahn L. *In vitro* lymphoproliferative assays with HgCl2 cannot identify patients with systemic symptoms attributed to dental amalgam. J Dent Res 1999;78:1450-8.
22. Brehler R, Becker D, Merk H. MELISA – *In vitro* test for detection of contact allergy? A comment by the German Contact Allergy Group. Hautarzt 1998;49:418-9.