Study on lymphocyte proliferation in nickel sensitive patients*

*Estudo da proliferação linfocitária em pacientes sensibilizados ao níquel**

Ana Paula Galli Sanchez[1] Celina Wakisaka Maruta[2] Maria Notomi Sato[3]
Ricardo Luiz Ribeiro[4] Carolina de Almeida Zomignan[5] Ricardo Spina Nunes[6]
Vitor Manoel Silva dos Reis[7]

Abstract: BACKGROUND: The diagnosis of nickel sensitivity is made by epicutaneous patch testing.
OBJECTIVE: To develop a more sensitive and specific test.
PATIENTS AND METHODS: Nineteen patients with positive patch test reactions to nickel and 25 controls were submitted to lymphocyte proliferation test. Mononuclear cells were isolated from peripheral venous blood and cultivated in triplicate in culture plates ($2x10^5$ cells/well) with: culture medium only, nickel sulfate (156.25; 78.13; 19.53; 9.77 and 2.44μM) and optimal concentrations of Candida albicans antigen as well as pokeweed, phytohemagglutinin A and anti-CD3 antibody (OKT3) mitogens. Tritiated thymidine was added to plates, radioactivity incorporated by cells was measured and the results expressed by the stimulation index (SI).
RESULTS: The lymphocyte proliferative response was higher in cases than in controls in all nickel concentrations tested. Considering positive test reactions when SI $\geq$ 3, none of the controls and 16 (84.21%) cases were positive in at least one of five concentrations used. The proliferative responses to Candida albicans and mitogens were similar in cases and controls, demonstrating normal cellular immunity in both groups.
CONCLUSION: The lymphocyte proliferation test is useful in diagnosis of nickel sensitivity.
Keywords: Lymphocyte activation; Dermatitis, contact; Nickel.

Resumo: *FUNDAMENTO: O diagnóstico da alergia ao níquel é estabelecido com a realização do teste de contato.*
OBJETIVO: Desenvolver um método diagnóstico mais sensível e específico.
CASUÍSTICAS E MÉTODOS: Dezenove pacientes com teste de contato positivo para o níquel e 25 controles foram submetidos ao teste da proliferação linfocitária. As células mononucleadas foram isoladas do sangue venoso periférico e cultivadas em triplicatas, em placas de cultura ($2x10^5$ células/orifício) com: meio de cultura apenas; sulfato de níquel (156,25; 78,13; 19,53; 9,77 e 2,44μM) e concentrações ideais do antígeno Candida albicans e dos mitógenos pokeweed, fito-hemaglutinina A e anticorpo anti-CD3 (OKT3). Timidina tritiada foi adicionada às placas, a radioatividade incorporada pelas células medida e os resultados expressos pelo índice de estimulação (IE).
RESULTADOS: A resposta proliferativa dos linfócitos dos casos foi superior à dos controles em todas as concentrações de níquel testadas. Considerando teste positivo para níquel quando IE $\geq$ 3, nenhum dos controles e 16 (84,21%) dos casos apresentaram teste positivo em pelo menos uma das cinco concentrações usadas. As respostas à Candida albicans e aos mitógenos foram semelhantes nos casos e controles, demonstrando a integridade da imunidade celular em ambos os grupos.
CONCLUSÃO: O teste da proliferação linfocitária mostra-se útil no diagnóstico da alergia ao níquel.
Palavras-chave: Ativação linfocítica; Dermatite de contato; Níquel.

Received on January 05, 2005.
Approved by the Consultive Council and accepted for publication on March 03, 2005.
* Study conducted in the Dermatology Outpatients Clinic, Teaching Hospital, Medical School, Universidade de Sao Paulo (HC-FMUSP) and in the Laboratory of Investigation in Dermatology and Immunodeficiencies (LIM 56) of the FMUSP (SP).

[1] M.Sc., Assistant physician of the Dermatology Service of the Complexo Hospitalar Padre Bento de Guarulhos (SP).
[2] Ph.D., Lecturer of the Department of Dermatology of the FMUSP (SP).
[3] Ph.D., Lecturer of the Department of Dermatology of the FMUSP and Researcher of LIM-56, Biomedical scientist
[4] M.Sc., Pediatrician, Lecturer of the Department of Public Health of the Medical School of Santo Amaro (SP).
[5] Technician of Laboratory of Investigation in Dermatology and Immunodeficiencies (LIM 56) of the FMUSP (SP).
[6] Biologist of the Department of Dermatology of HC-FMUSP (SP).
[7] Ph.D., Lecturer of the Department of Dermatology of HC-FMUSP (SP).

INTRODUCTION

Nickel is the major cause of allergic contact dermatitis (ACD) in Brazil and in the industrialized world.[1,2] According to a multicenter study, 25.1% of Brazilian population investigated presented sensitization to nickel.[1] Nickel ACD predominates in females, young and non-atopic patients.[3,4]

The diagnosis of nickel ACD is based on the history and clinical picture of patients, and corroborated by patch test (PT) with 5% nickel sulfate in petrolatum.[1]

Although the PT technique is well defined, the result depends on correct preparation, application and reading of the test. Patients cannot rinse the application site and have to go to hospital three times to perform the test. PT requires previous clinical control of the dermatosis, it may produce exacerbation of the dermatitis and should not be used in patients on systemic steroids. Use of topical corticosteroids in the test site in the past 15 days and recent sun exposure on the back are also contraindications of the test.[1]

Moreover, applying nickel in petrolatum to the skin may result in follicular lesions and pustules like irritating reactions, as well as doubtful and false-negative results.[4,5]

Another diagnostic method mentioned in the literature but with varied and controversial results is the lymphocyte proliferation test - LPT.[6-24] Peripheral blood lymphocytes of patients are placed in contact with nickel in LPT. If the patient is sensitive to nickel, the circulating memory lymphocytes are activated, synthesize DNA and split. Cell proliferation is measured by incorporation of thymidine radiolabeled by activated cells.

In the first reports on LPT in nickel-sensitive individuals the authors used nickel chloride and acetate.[6,7,9] Lymphocyte proliferative response was unspecific in cases and normal in controls, that is, nickel acted as a mitogen, stimulating the lymphocytes of sensitive patients as well as of non-sensitive controls.[6,9]

Later, most studies were performed with nickel sulfate,[8,10,11,13-23] but there is no standardization in the literature about cell and nickel concentration used. False-positive results occur very often.[10,14,15,17-23]

In order to better interpret the results already published, the authors performed LPT with nickel sulfate in five concentrations, using samples of heparinized peripheral venous blood of 44 individuals.

PATIENTS AND METHODS

Cases - 1Nineteen female patients, aged 15-71 years (mean 38.42 years; median 37 years), with history of nickel sensitization and/or lesions suggesting nickel-related contact eczema or dyshidrosis. After clinical control of the dermatosis, the diagnosis of nickel ACD was confirmed by PT1. In case of a positive reading for nickel sulfate after 96 hours, a 15-ml peripheral venous blood collection was scheduled for LPT.

Controls - Twenty-five individuals (7 males and 18 females), aged 18-50 years (mean 30.84 years; median 26 years), with no history of nickel sensitization and negative PT for nickel sulfate. After PT, blood collection was scheduled for LPT.

On the first appointment, the patients of both groups were informed about the objective of the study and signed an informed consent. The exclusion criterion adopted was use of systemic corticosteroids up to three weeks before PT.

TC - It was carried out with the standard Brazilian series comprising 30 substances, which were applied in aluminum chambers (Finn Chamber®, Epitest Ltd, Finland) in the standardized concentrations and quantities and attached to the healthy back skin. Patients were oriented to not rinse the site and come back to hospital 48 hours later, when the material was removed and the first reading was performed. Forty-eight hours later patients came back for the second reading. The test preparation, application and both readings of all patients were performed by a single examiner. The reading criteria adopted were established by the International Contact Dermatitis Research Group:[25] negative reaction (-): no reaction; doubtful reaction (+?): poorly defined mild erythema; weak positive reaction (+): defined erythema and papule; strong positive reaction (++): erythema, papule and vesicle; very strong positive reaction (+++): erythema, papule, coalescent vesicles forming bullae. The positive reactions in 48 and 96 hours were considered positive for sensitization, as well as those negative in 48 hours and positive in 96 hours. We deemed negative reactions for sensitization both negative reactions in 48 and 96 hours and those positive in 48 hours and negative in 96 hours.

LPT - The test was conducted at the LIM 56 of FMUSP. Working in a laminar flow, the mononucleated cells were isolated from blood of patients by density gradient, using a Ficoll-Hypaque solution.[26] After washing (centrifugation in saline solution), the cells were placed in a test tube with the culture medium (RPMI 1640). Next, one sample was taken to automatic cell count in the equipment Cell Dyn 1400. The cultures were performed with $2x10^6$ mononucleated cells/ml, in triplicate, in a 96-well

flat-bottom plate (Costar® 3596, New York, USA). Each well contained a total volume of 200µl: 100µl of cells in RPMI 1640 enriched with AB human serum 10% ($2x10^5$ cells/well) and 100µl of the antigen or mitogen to be tested, which was diluted in RPMI 1640. A triplicate was prepared for each patient with only 100µl of cells in RPMI 1640 with AB serum and 100µl of RPMI 1640. This triplicate, with no mitogen or antigen, was called baseline. As to nickel, a standard 1M-nickel sulfate solution in distilled water. This solution was diluted in RPMI 1640, and several dilutions of the last solution were made; in that, the final nickel concentrations used were 156.25; 78.13; 19.53; 9.77 and 2.44µM. The cells were cultivated ($2x10^5$ cells/well) for six days, in triplicates, at 37ºC, in a 5% CO_2, environment, in the presence of: 100µl of culture medium only (baseline), 100 µl of each of five nickel sulfate concentrations and 100 µl of ideal concentrations of *Candida albicans* antigen (CMA-5µg/ml) and of pokeweed mitogen (PWM-5µg/ml). The proliferative responses of cells were also separately evaluated in the presence of phytohemagglutinin A mitogens (PHA-2.5µg/ml) and anti-CD3 antibody (OKT3-1/400) in three-day cultures. A baseline triplicate in culture plates was also prepared for each patient. The cultures with CMA and the mitogens mentioned were prepared to assess normal cellular immunity in both groups. An antigen, such as CMA or nickel, induces the proliferation of peripheral lymphocytes of individuals who were previously sensitive to it, whereas a mitogen stimulates lymphocyte proliferation with no specific recognition. Hence, PHA stimulates the polyclonal activation of T-lymphocytes (TL); PWM stimulates both TL and B-lymphocytes; and OKT3 stimulates TL proliferation.[26] Eighteen hours before conclusion of the cultures, tritiated thymidine (3H-thymidine, Amershan Pharmacia Biotech, UK; specific activity 25Ci/mmol) diluted in RPMI 1640 (1:100) was added to each well of the culture plates. After this incubation period, the cultures were aspirated using an automatic cells harvester (Cell-Harvester, Skatron Instruments, UK), and the radioactive material was incorporated to proliferative cells and transferred to glass fiber membranes. These membranes were involved by plastic envelopes, in which scintillation liquid was added. After drying in oven, they were placed in the beta scintillation counter (Betaplate, Wallac, Finland). Based on the results of radioactivity incorporated by the cells and provided by the device as counts per minute (cpm), it was possible to define if there were activation and lymphocyte proliferation or not, The mean cpm of each triplicate was calculated, and the results were expressed by stimulation index (SI). SI was calculated by dividing the mean stimulated triplicate cpm (with one antigen or mitogen) by the mean corresponding baseline triplicate cpm.

Statistical methods - PThe case and control groups were compared by the Mann-Whitney's test[27] regarding the following variables: values of baseline triplicate cpm means, SI values in five nickel sulfate concentrations tested, values of SI in the presence of CMA and mitogens (PHA, OKT3 and PWM). The significance level established was 0.05 or 5% (risk $\alpha \leq 0,05$). The Spearman's correlation coefficient (r_S)[27] was used in the group of cases to correlate the intensity of PT reactions with the intensity of LPT response, as well as to verify if the interval (measured in days) between performing PT and LPT influenced the result of LPT. When the explanation coefficient (r_S^2) fwas greater than 0.80, the correlation between data was considered good. The statistically significant values are marked in the tables with one asterisk (*).

RESULTS

Regarding PT, in the group of cases, one patient presented a weak positive reaction (+), 11 had strong positive reactions (++), and 7 had very strong positive test (+++) for nickel. Nine out of 19 sensitive patients had a positive PT reaction for more than one of the following allergens: thimerosal, ethylenediamine, cobalt chloride or potassium dichromate (Chart 1). In the control group, three patients had a positive PT reaction each for thimerosal, thiuram-mix and carba-mix, respectively (Chart 2).

Tables 1 and 2 show the results of LPT with nickel in the case and control groups. In five nickel concentrations used, the difference in LPT results among cases and controls was statistically significant (Table 3). LPT was considered positive for nickel sulfate when SI was greater than or equal to 3. This value was established considering the mean values (1.25) plus three standard deviations (1.77) of SI values of controls in the presence of 78.13µM nickel, when values greater than the SI were observed in controls.

Sixteen cases (84.21%) had positive LPT in at least one nickel concentration tested. In 15 of these 16 patients, SI was > 3 when in 78.13µM nickel sulfate. Only the lymphocytes of case number 14 did not respond to this concentration and proliferated in the presence of 156.25µM nickel (Table 1). The lymphocyte proliferative response peak of the cases occurred in the presence of 78.13µM nickel, and achieved a SI of 49.87 (Table 1).

Graph 1 illustrates the distribution of SI values of LPT with nickel in both groups, and clearly demon-

CHART 1: PT date and results of the cases

Nº	Name	Age	Sex	PT	PT result	LPT	Lesion[1]
1	AS	46	F	10/09/01	Nickel sulfate ++/++	11/10/01	-
2	RARS	45	F	10/09/01	Nickel sulfate +++/+++ Thimerosal ++/+++	18/10/01	-
3	ACCS	15	F	16/07/01	Nickel sulfate +++/+++	18/10/01	Eczema on the left cervical region
4	LAP	37	F	01/10/01	Nickel sulfate ++/++ Ethylenediamine +/+	25/10/01	-
5	SMR	33	F	30/07/01	Nickel sulfate ++/++	25/10/01	-
6	IOF	24	F	30/07/01	Nickel sulfate ++/+++	25/10/01	-
7	MTTS	53	F	22/10/01	Nickel sulfate ++/++ Thimerosal +/++	01/11/01	Eczema on nasal dorsum
8	ECM	21	F	07/02/01	Nickel sulfate ++/++ Cobalt chloride ++/++	29/11/01	-
9	MCM	30	F	30/07/01	Nickel sulfate++/++	29/11/01	-
10	DMCV	36	F	26/06/00	Nickel sulfate ++/++ Cobalt chloride ++/+	04/04/02	Eczema on left wrist
11	VA	38	F	02/10/00	Nickel sulfate ++/++	04/04/02	-
12	SFB	30	F	22/04/02	Nickel sulfate +++/+++ Thimerosal ++/++	16/05/02	Disseminated eczematous lesions
13	MLF	52	F	06/05/02	Nickel sulfate +++/+++	16/05/02	-
14	MSO	50	F	03/12/01	Nickel sulfate ++/++	06/06/02	Dyshidrosis on the left hand
15	FMSCR	22	F	15/04/02	Nickel sulfate +/+ Cobalt chloride +/+	20/06/02	-
16	AFS	41	F	06/05/02	Nickel sulfate ++/++	20/06/02	-
17	IQA	53	F	22/04/02	Nickel sulfate +/++ Potassium dichromate ++/+++	02/10/02	-
18	MLT	71	F	10/06/02	Nickel sulfate +++/+++ Cobalt chloride ++/+++	20/06/02	-
19	SMLN	33	F	05/10/98	Nickel sulfate +++/+++	11/09/02	-

Source: Division of Dermatology of HC-FMUSP.
(1) Lesion on the date of LPT

CHART 2: PT date and results of the controls

Nº	Name	Age	Sex	PT result	LPT	Lesion[1]
1	MA	27	M	Thimerosal +/+	11/10/01	–
2	NTV	47	F	–	06/12/01	–
3	RMA	42	F	–	18/10/01	–
4	MM	18	F	Thiuram "mix" +/+	25/10/01	Eczema on hands
5	MHAF	56	F	–	01/11/01	–
6	LFSF	27	M	–	29/01/01	–
7	FAC	47	M	–	04/04/02	–
8	MRV	36	M	Carba "mix" +/+	04/04/02	–
9	ERAN	36	F	–	16/05/02	Eczema on forearms
10	MCCL	50	F	–	16/05/02	–
11	VK	24	F	–	06/06/02	–
12	BMG	30	M	–	20/06/02	–
13	MACGS	24	M	–	20/06/02	–
14	HM	25	F	–	20/06/02	–
15	AAS	26	M	–	11/09/02	–
16	PAVC	26	F	–	11/09/02	–
17	FCP	25	F	–	11/09/02	–
18	CAZ	24	F	–	11/09/02	–
19	VGA	29	F	–	11/09/02	–
20	ESK	26	F	–	11/09/02	–
21	CFC	22	F	–	26/09/02	–
22	SAVF	26	F	–	02/10/02	–
23	LK	26	F	–	02/10/02	–
24	MLM	25	F	–	02/10/02	–
25	PO	27	F	–	02/10/02	–

Source: Division of Dermatology of HC-FMUSP.
(1) Lesion on the date of LPT

TABLE 1: LPT results expressed in SI in five nickel sulfate concentrations tested in cases

	Cases				
Nº	156.25 µM	78.13 µM	19.53 µM	9.77 µM	2.44 µM
1	8.70	22.10	11.34	2.82	1.70
2	4.40	13.59	4.41	6.54	1.05
3	1.55	1.97	1.56	2.30	0.94
4	9.02	31.81	8.11	3.90	1.45
5	2.52	5.88	2.63	1.88	0.99
6	8.42	11.46	3.73	3.46	1.06
7	23.75	19.66	3.27	1.25	1.25
8	19.65	49.87	32.82	23.03	2.29
9	6.12	7.08	3.44	0.74	9.82
10	3.43	3.63	0.77	0.73	0.88
11	0.76	4.56	1.14	1.03	0.74
12	3.69	5.58	2.90	2.43	1.23
13	4.93	7.08	6.26	2.29	1.89
14	4.94	2.36	1.38	1.07	0.93
15	3.64	4.54	0.91	0.76	0.84
16	2.49	1.36	1.14	0.77	0.82
17	0.61	1.10	1.08	0.86	0.84
18	23.71	34.02	10.81	3.28	1.00
19	7.22	23.21	2.62	1.23	0.96
Mean	7.35	13.20	5.28	3.18	1.62
Median	4.93	7.08	2.90	1.88	1.00

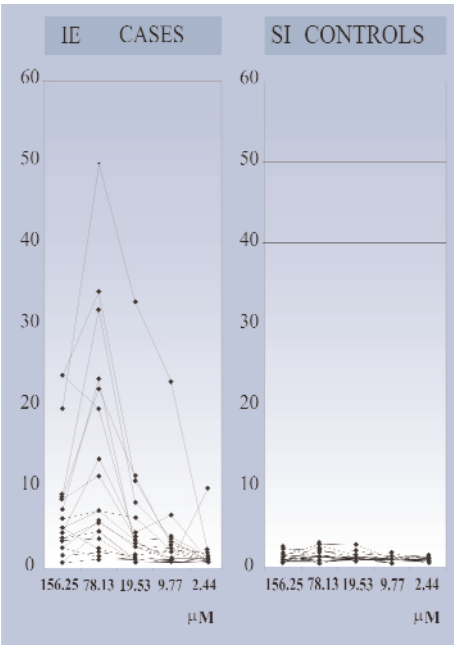
strates that the proliferative lymphocyte responses of the cases were much higher than that of the controls in five concentrations tested.

Three individuals (numbers 3, 16 and 17) did not present positive LPT in five nickel sulfate concentrations tested (Table 1).

None of the controls presented a positive LPT for nickel (Table 2). The proliferative response peak in controls also occurred in the presence of 78.13µM nickel sulfate, when SI achieved 2.75 (Chart 3 and Table 2).

The responses to PHA, OKT3 and PWM mitogens and to CMA antigen in both groups were similar, demonstrating normal cellular immunity in cases and controls (Tables 4 and 5). There was no statistically significant difference between the mean baseline triplicate cpm of the case and control cultures at three or six days.

By means of the difference in days regarding performance of the two tests and the intensity of PT reactions and LPT response, the correlation between test response intensity *in vivo* and *in vitro* was studied, as well as whether the interval between PT and LPT influenced LPT response intensity. According to the statistical study, there was no correlation between intensity of PT second reading results (+, ++ or +++) and LPT response intensity (expressed as SI) in five nickel concentrations used. In addition, the interval (in days) between PT and LPT did not influence LPT result (SI value) (Table 6).



Graph 1: Comparing the SI values in cases and controls in five nickel sulfate concentrations tested

DISCUSSION

In an attempt to develop a more sensitive and specific laboratory test to diagnose nickel ACD, several studies have been published on LPT with nickel acet,[9,11,14] chloride[6,7,12,24] and sulfate[8,10,11,13-23,28] since 1960.

It was difficult to compare the results of these studies, because the investigators used mononucleated cells in different concentrations and nickel in varied solutions and concentrations (Chart 3). However, these studies enabled isolating specific TL clones recognizing nickel and further studies on immunopathogenesis of nickel ACD. It is worth mentioning that although nickel is the main cause of ACD all over the world, there is still no exact understanding of how this antigen presentation occurs.[29]

According to Gimenez-Camarasa et al.,[13] test sensibility increases when serial dilutions of nickel sulfate are used to perform LPT. Based on this information, the authors performed LPT with five dilutions of the standard nickel sulfate solution.

In this study, 16 (84.21%) out of 19 nickel-sensitive patients presented positive LPT (SI ≥ 3) in at least one of five concentrations tested. Nevertheless, in cases number 3, 16 and 17, the test was negative. For these patients´ lymphocytes, the nickel concentrations used may have been not optimal to induce a proliferative response. A small number of circulating memory TL or an insufficient number of cells isolated from blood and presenting antigen may justify the false-negative results. Another possibility is that memory lymphocytes may concentrate more in the skin of these patients.

Among 16 cases with positive LPT, 15 had a SI ≥ 3 when their lymphocytes were cultivated in the presence of 78.13µM nickel sulfate (Table 1). Only the lymphocytes of case number 14 did respond at this concentration, proliferating in the presence of 156.25µM nickel. The results may be confirmed in the future, by performing LPT in a greater number of cases and controls, and standardization of this technique with two concentrations may be possible, thus making its execution easier.

No false-positive results were observed in the control group, unlike most studies published (Chart 3). The results considered false-positive, may have occurred in individuals already sensitive to it, that is, LPT would identify the presence of sensitization in a threshold still insufficient to produce skin lesions and positive PT reactions.

Based on the LPT results in both groups and using PT as a reference diagnostic method, LPT sensibility with nickel sulfate at five concentrations tested was 84.21%, and its specificity was 100%. LPT speci-

TABLE 2: LPT results expressed in SI in five nickel sulfate concentrations tested in controls

	Controls				
Nº	156.25 µM	78.13 µM	19.53 µM	9.77 µM	2.44 µM
1	1.37	1.21	1.02	1.33	0.97
2	0.53	0.59	0.93	0.76	1.31
3	0.80	1.17	0.70	0.71	0.82
4	0.69	0.74	0.71	0.75	0.90
5	1.40	0.39	1.09	0.44	0.51
6	2.09	2.20	0.58	0.76	0.68
7	0.89	1.01	0.86	0.90	0.84
8	0.94	0.86	0.82	0.93	1.00
9	2.37	1.04	1.07	1.09	1.13
10	0.92	1.07	1.01	1.00	0.95
11	0.72	0.68	0.67	0.51	0.51
12	0.87	1.23	1.12	0.86	1.32
13	1.54	1.96	1.13	1.19	1.02
14	0.80	1.16	1.24	0.97	1.01
15	0.68	0.87	0.85	1.04	0.56
16	1.15	1.70	1.95	1.19	1.41
17	0.73	1.21	0.96	0.82	0.85
18	0.47	1.13	1.00	0.93	0.71
19	0.94	1.30	0.94	0.84	0.79
20	0.71	0.89	0.95	0.87	0.83
21	0.58	1.41	1.05	0.97	0.90
22	0.90	1.35	1.08	0.77	0.67
23	0.41	2.61	1.36	1.60	0.80
24	1.32	2.75	2.71	0.96	0.83
25	1.36	0.67	0.70	1.25	0.39
Mean	1.01	1.25	1.06	0.94	0.87
Median	0.89	1.16	1.00	0.93	0.84

ficity with nickel sulfate was corroborated by performing the test with mononucleated cells of three controls with negative PT reactions for nickel sulfate, but positive reactions for thimerosal, thiuram-mix and carba-mix (Chart 2 and Table 2).

In the case group, the four highest SI values (49.87; 34.02; 31.81 and 23.21) were observed in patients number 8, 18, 4 and 19, respectively, who were sensitive to nickel and to one more allergen (Chart 1 and Table 1). Polysensitization may influence lymphocyte proliferative response intensity, and further studies with a larger number of patients sensitive to nickel and other allergens are required to better understand this observation.

LPT is an assay that depends on strict training to isolate mononucleated cells, as well as to correctly prepare and use mitogens, antigens, culture medium and radioactive materials. Appropriate maintenance and handling of equipment is also necessary. Therefore, LPT performance demands a very elaborated material and human resource infrastructure.

On the other hand, performing LPT in a setting that already has the necessary infrastructure is not expensive. Moreover, patients should come to hospital only once for blood collection. Considering the discomfort PT causes to patients, with restrictions as to bathing and physical activities, as well as time patients spend to have a PT, interrupting their activities three times in one week to conclude the test, the cost of LPT may be advantageous.

While PT requires clinical control of ACD before its performance, LPT may be carried out in patients with lesions. It is a consensus in the literature that this is the main advantage of LPT as compared with PT. Blood sample may be collected and the treatment is initiated soon after, and patients do not run the risk of developing an exacerbation of their skin condition during the tests. In addition, while PT results are expressed in plus, LPT provides results in numerical indices, thus favoring a more objective interpretation.

There are few studies relating intensity of PT reactions and intensity of LPT response. According to Al-Tawil et al.[28] and Everness et al.,[22] there is no

Table 3: Comparing the SI values of LPT with nickel sulfate in five concentrations tested in cases and controls by Mann-Whitney's test

	Concentrations				
Calculations	156.25 µM	78.13 µM	19.53 µM	9.77 µM	2.44 µM
R1	626	640	607	547.5	542
R2	364	350	383	442.5	448
U	39	25	58	117.5	123
U'	436	450	417	357.5	352
µU	237.5	237.5	237.5	237.5	237.5
σU	42.2	42.2	42.2	42.2	42.2
Critical Z (one-tailed)	-1.645	-1.645	-1.645	-1.645	-1.645
Calculated Z	-4.704*	-5.036*	-4.254*	-2.844*	-2.713*
p value	< 10-5*	~0*	< 10^{-5}*	0.0022*	0.0033*

(*) Statistically significant value

Table 4: Comparing the SI values of LPT with pokeweed (PWM), phytohemagglutinin A (PHA) and anti-CD3 (OKT3) mitogens in cases and controls by Mann-Whitney's test

	SI		
Cálculos	PMW	PHA	OKT3
R1	453	481	457
R2	537	509	533
U	212	184	208
U'	263	291	267
µU	237.5	237.5	237.5
σU	42.2	42.2	42.2
Critical Z (one-tailed)	-1.645	-1.645	-1.645
Calculated Z	-0.604	-1.268	-0.699
p value	0.273	0.102	0.242

Table 5: Comparing the SI values of LPT with *Candida albicans* (CMA) antigen in cases and controls by Mann-Whitney's test

	SI
Calculations	CMA
R1	150
R2	228
U	75
U'	95
Critical U unicaudal	51
p value	> 0,05

correlation between the results of PT and LPT in nickel-sensitive patients. However, Von Blomberg-van der Flier et al.,[21] studied a smaller number of sensitive patients and observed a correlation between test responses, that is, patients with strong positive PT reactions presented higher SI.

It is complicated to compare the responses of two tests whose results depend on distinct mechanisms. In PT, the application of nickel on the skin leads to an inflammatory process recruiting lymphocytes able to recognize the metal to the site, whereas in LPT the memory lymphocytes are extracted from the blood. The correlation between *in vivo* and i*n vitro* test results in sensitive patients was also assessed, but no correlation was observed (Table 6).

Only two studies published discuss the influence of PT in the result of LPT, in other words, if PT could act as a booster and intensify the lymphocyte proliferative response.[16,22] Their authors demonstrated that performing PT before LPT did not influence the proliferative response of lymphocytes of patients in both groups. In the present study, we analyzed the possibility of the interval between PT and LPT influencing the result of LPT in the case group. There was no correlation of interval (in days) between tests and the results of LPT (in SI). Therefore, shorter intervals between the two tests did not induce more intense responses in *in vitro* tests (Table 6), corroborating the previous findings.

CHART 3: Summary of studies on lymphocyte proliferation test with nickel

Author	Year	Ni[1]	Concentration		Time (days)	FPR[2]
			Nickel (μM)	Cells		
Aspegren et al.	1962	[3]Cl	1; 10; 100; 300 and 1000	1 the 2x10[6]/ml	3	+
Grosfeld et al.	1966	Cl	0.5 and 0.05	[4]NE	4	–
Pappas	1970	[5]Ac	NE	4x10[5]/ml	5	+
MacLeod et al.	1970	[6]S	0.5 and 50	NE	6	–
Forman et al.	1972	S	NE	1x10[6]/ml	4	+
		S	50	1 the 2x10[6]/ml	6	–
Hutchinson et al.	1972					
		Ac	50	1 the 2x10[6]/ml	6	–
Millikan et al.	1973	Cl	2.5; 10; 20 and 30	2.5x10[6]/ml	6	–
Gimenez-Camarasa et al.	1975	S	65; 130; 650; 1300 and 6500	5x10[6]/ml	6	–
		S	3.23; 64.64; 258.57 and 517,13	1.2 the 1.5x10[7]/ml	5	+
Kim et al.	1976					
		Ac	2.83; 56.59; 226.37 and 452.75	1.2 the 1.5x10[7]/ml	5	+
Svejgaard et al.	1978	S	3.6; 9; 18; 36 and 72	2x10[5]/ml	5	+
Veien et al.	1979	S	36 and 72	1x10[5]/wel	6	NE
Silvennoinen-Kassinen	1980	S	8.08 and 40.40	1x10[6]/ml	7	+
Al-Tawil et al.	1981	S	40.40; 80.80; 161.60; 323.21; 646.41 and 1292.82	1x10[5]/wel	1 to 10	+
MacLeod et al.	1982	S	90.5	2x10[6]/ml	6	+
Nordlind	1984	S	7.6; 19; 38 and 76	NE	3 and 6	+
Al-Tawil et al.	1985	S	40.40; 80.80 and 161.60	1x10[5]/wel	5 and 6	[7]NA
von Blomberg et al.	1987	S	7; 14; 40 and 80	1x10[5]/wel	6	+
Everness et al.	1990	S	32.32; 64.64; 96.96 and 129.28	1x10[6]/ml	6 and 7	+
Räsänen et al.	1992	S	10.34; 20.04; 40.72; 80.80 and 161.60	1x10[5]/wel	6	+
Cederbrant et al.	1997	Cl	19.29; 77.15 and 385.77	1x10[6]/ml	5	+

(1) Nickel; (2) False-positive results; (3) Nickel chloride; (4) Not specified; (5) Nickel acetate; (6) Nickel sulfate; (7) Not applicable.

TABLE 6: Spearman's correlation coefficient (r_s) and Spearman's explanation coefficient (r_s^2) calculated to correlate the interval (in days) between PT and LPT with LPT result (SI values) in five nickel sulfate concentrations tested, as well as to correlate the intensity of PT reactions (in plus) with intensity of LPT response (SI values) in five nickel sulfate concentrations tested

	SI-Nickel				
	156.25 μM	78.13 μM	19.53 μM	9.77 μM	2.44 μM
r_s					
Days	-0.10744	0.10114	-0.06582	-0.02766	-0.09120
plus	0.08251	0.09249	-0.00393	0.03236	-0.11322
r_s^2					
Days	0.01154	0.01023	0.00433	0.00077	0.00832
Plus	0.00681	0.00855	0.00002	0.00105	0.01282

CONCLUSION

Based on the data presented, one may conclude that LPT is useful to diagnose nickel ACD, particularly in patients with active lesions. Furthermore, it may be used to isolate nickel-sensitive TL clones, and to carry out studies on cytokines produced by activated cells. Hence, LPT may be useful not only as a diagnostic method, but also to broaden understanding of nickel ACD immunopathogenesis. ❑

REFERENCES

1. Grupo Brasileiro de Estudo em Dermatite de Contato (GBEDC). Estudo multicêntrico para elaboração de uma bateria-padrão brasileira de teste de contato. An Bras Dermatol. 2000;75:147-56.
2. Hostýnek JJ. Nickel-induced hypersensitivity: etiology, immune reaction, prevention and therapy. Arch Dermatol Res. 2002;294:249-67.
3. Gawkrodger DJ, Vestey JP, Wong WK, Buxton PK. Contact clinic survey of nickel-sensitive subjects. Contact Dermatitis. 1986;14:165-9.
4. Rietschel RL, Fowler JF. Fisher's contact dermatitis. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2001.
5. Fisher T, Rystedt I. False-positive, follicular and irritant patch test reactions to metal salts. Contact Dermatitis. 1985;12:93-8.
6. Aspegren N, Rorsman H. Short-term culture of leucocytes in nickel hypersensitivity. Acta Derm Venereol. 1962;42:412-7.
7. Grosfeld JCM, Penders AJM, Grood R, Verwilghen L. In vitro investigations of chromium and nickel-hypersensitivity with culture of skin and peripheral lymphocytes. Dermatologica. 1966;132:189-98.
8. Macleod TM, Hutchinson F, Raffle EJ. The uptake of labeled thymidine by leucocytes of nickel sensitive patients. Br J Dermatol. 1970;82:487-92.
9. Pappas A, Orfanos CE, Bertram R. Non-specific lymphocyte transformation in vitro by nickel acetate. J Invest Dermatol. 1970;55:198-200.
10. Forman L, Alexander S. Nickel antibodies. Br J Dermatol. 1972;87:320-6.
11. Hutchinson F, Raffle EJ, Macleod TM. The specificity of lymphocyte transformation in vitro by nickel salts in nickel sensitive subjects. J Invest Dermatol. 1972;58:362-5.
12. Millikan LR, Conway F, Foote JE. In vitro studies of contact hypersensitivity: lymphocyte transformation in nickel sensitivity. J Invest Dermatol. 1973;60:88-90.
13. Gimenez-Camamasa JM, Garcia-Calderon P, Asensio J, Moragas JM. Lymphocyte transformation test in allergic contact nickel dermatitis. Br J Dermatol. 1975;92:9-15.
14. Kim CW, Schöpf E. A comparative study of nickel hypersensitivity by lymphocyte transformation test in atopic and non-atopic dermatitis. Arch Dermatol Res. 1976;257:57-65.
15. Svejgaard E, Morling N, Svejgaard A, Veien NK. Lymphocyte transformation induced by nickel sulphate: an in vitro study of subjects with and without a positive nickel patch test. Acta Derm Venereol (Stockh). 1978;58:245-50.
16. Veien NK, Svejgaard E, Menné T. In vitro lymphocyte transformation to nickel: a study of nickel-sensitive patients before and after epicutaneous and oral challenge with nickel. Acta Derm Venereol (Stockh). 1979;59:447-51.
17. Silvennoinen-Kassinen S. Lymphocyte transformation in nickel allergy: amplification of T-lymphocyte responses to nickel sulphate by macrophages. Scand J Immunol. 1980;12:61-5.
18. Al-Tawil NG, Marcusson JA, Möller E. Lymphocyte transformation test in patients with nickel sensitivity: an aid to diagnosis. Acta Derm Venereol (Stockh). 1981;8:511-5.
19. MacLeod TM, Hutchinson F; Raffle EJ. In vitro studies on blastogenic lymphokine activity in nickel allergy. Acta Derm Venereol (Stockh). 1982;62:249-50.
20. Nordlind, K. Lymphocyte transformation test in diagnosis of nickel allergy. Int Arch Allergy Appl Immunol. 1984;73:151-4.
21. Von Blomberg-van der Flier M, Van der Burg CKH, Pos O, Van de Plassche-Boers EM, Bruynzeel DP, Garotta G et al. In vitro studies in nickel allergy: diagnostic value of dual parameter analysis. J Invest Dermatol. 1987;88:362-8.
22. Everness KM, Gawkrodger DJ, Botham PA, Hunter JAA. The discrimination between nickel-sensitive and non-nickel-sensitive subjects by an in vitro lymphocyte transformation test. Br J Dermatol. 1990;122: 293-8.
23. Räsänen L, Tuomi M. Diagnostic value of lymphocyte proliferation test in nickel contact allergy and provocation in occupation coin dermatitis. Contact Dermatitis. 1992;27:250-4.
24. 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.
25. Wilkinson DS, Fregert S, Magnusson B, Bandmann HJ, Calnan CD, Cronin E, et al. Terminology of contact dermatitis. Acta Derm Venereol (Stockh). 1970;50:287-92.
26. Kurnick JT. Cellular assays. In: Colvi RB, Bhan AK, McCluskey RT, editors. Diagnostic Immunophatology. New York: Raven; 1995. p.751-6.
27. Siegel S, Castellan N J. Non parametric statistics. 2th ed. New York: McGraw-Hill; 1988.
28. Al-Tawil NG, Berggren G, Emtestam L, Fransson J, Jernselius R, Marcusson JA. Correlation between quantitative in vivo and in vitro responses in nickel-allergic patients. Acta Derm Venereol. 1985;65:385-9.
29. Sinigaglia F. The molecular basis of metal racognation by T cells. J Invest Dermatol. 1994;102:398-401.

MAILING ADDRESS:
Ana Paula Galli Sanchez
Rua Cônego Antônio Lessa, 159
03122-060 São Paulo-SP
Tel./Fax: (11) 6606-2800
E-mail: anapgsanchez@ig.com.br