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Observational studies: why are they so important?

Estudos observacionais: por que são tão importantes?

Alessandro Wasum Mariani[I], Paulo Manuel Pêgo-Fernandes[II]

Instituto do Coração (InCor), Hospital das Clínicas (HC), Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil

[I]MD. Thoracic Surgeon, Instituto do Coração (InCor), Hospital das Clínicas (HC), Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

[II]MD, PhD. Associate Professor, Discipline of Thoracic Surgery, Instituto do Coração (InCor), Hospital das Clínicas (HC), Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

Randomized clinical trials (RCTs) are known to be the gold standard for medical research, and this study design is preferred for investigating the efficacy of new interventions. Many authors and editors believe that the RCT design always surpasses other research designs. However, this is not an unquestionable truth.

The main advantage of RCTs is that they provide better control over possible bias through randomization and blinding. In other words, the great strength of an RCT is its high internal validity. On the other hand, rigid design control could reduce the ability to generalize the results.[1] Another issue relating to RCTs is the fact that recruitment, randomization and blinding are not always possible because of technical issues (e.g. surgical procedures) or ethical issues (e.g. in a hypothetical trial to prove the association between smoking and lung cancer, it would be unethical to randomly assign a group to smoke).

But what should be done when RCTs are unfeasible or unethical? The answer could be a well-designed observational study. This term describes a range of study designs that includes cross-sectional, case-control, prospective and retrospective cohort studies. The main characteristic of observational studies is that any intervention is not determined by the protocol, but by clinical practice.[2]

Cross-sectional studies are used to estimate prevalence. They are relatively quick and cheap, and can be used to study multiple outcomes. However, they do not differentiate between cause and effect or within the sequence of events. They are useful for identifying associations that can then be more rigorously studied using a cohort study or randomized controlled study. The most important problem with this type of study is in differentiating between cause and effect from a simple association.[3]

Case-control studies are considered to be simple to organize and useful for hypothesis generation. They retrospectively compare two groups in order to identify predictors of an outcome. They allow calculation of odds ratios. The disadvantages are that they can only evaluate one outcome and that the presence of bias is usually high and difficult to assess. The major difficulty could lie in determining an adequate control group.[3]

Cohort studies are excellent for estimating the incidence and natural history of a condition. They may be prospective or retrospective and sometimes two cohorts are compared. They analyze predictors (risk factors) that enable relative risk calculation. Since they measure events in temporal sequence, they can distinguish causes from effects. Retrospective cohorts are considered to be cheaper and quicker, but may have fragile results, particularly if the database is inadequate. Prospective cohorts are more accurate and, with a good protocol (with adequate sample size and follow-up) can have results that are as reliable as those of RCTs.[3]

The main problem in observational studies is the presence of confounders and selection bias (which are prevented in RCTs through randomization and blinding). A confounder can be defined as any factor that is related not only to the intervention (e.g. treatment) but also to the outcome and could affect both.[4] One good example is age: in a study on the relationship between smoking (exposure) and lung cancer (outcome), age could be implicated as a factor that would increase the incidence of the outcome. Thus, if one of the groups (smokers or non-smokers) has an older population, the increase in lung cancer could be influenced by age (as a confounder), and not by the exposure studied.

However, advanced statistical tools may enable good and reliable control over many confounders. Some tools like propensity scores and sensitivity analysis, when correctly performed, could drastically reduce the bias caused by the lack of randomization.[5]

Some authors have studied the results from RCTs, compared with similar observational studies. Concato et al. published an evaluation of meta-analyses that compared outcomes between RCTs and observational studies and reached the following conclusion: "The results from well-designed observational studies (with either a cohort or a case-control design) do not systematically overestimate the magnitude of the effects of treatment, as compared with those in randomized, controlled trials on the same topic".[6] In other words, if the observational study has good methodological quality, the results are quite similar.

Many investigators have pointed out that the main strength of observational studies is their greater proximity to "real life situations", since RCTs have stricter inclusion criteria and rigid protocols that may not reflect clinical practice. By definition, observational studies have greater heterogeneity of medical interventions and patient populations that are closer to clinical practice.[2] Other advantages of observational studies are that they are usually cheaper than RCTs and can be used to investigate rare outcomes and to detect unusual side effects, and that some designs are easily and quickly performed.

Observational studies also are important for creating new hypotheses, proving the external validity of RCTs already performed, establishing the sample size for an RCT and evaluating which patient subsets really benefit from each alternative intervention of effective alternative therapies.[1] In this way, it can be said that observational studies can be complementary to RCTs.

Although the evidence level of observational studies appears to be lower than that of RCTs, it is clear that this kind of investigation is crucial for elucidating many scientific questions. Not only authors but also editors around the world are giving more attention to these studies. The take-home message is that the study question and the quality of the methodology applied to answer it are much more important than the study design. In this context, observational studies may be the best way to answer the many medical questions in situations in which the classical RCT approach does not apply.

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Address for correspondence:
Alessandro Wasum Mariani
Rua Treze de Maio, 1.217 — apto 31
Bela Vista — São Paulo (SP) — Brasil
CEP 01327-001
E-mail: alessandro_mariani@hotmail.com

Knowledge, attitudes and practices regarding the Pap test among women in northeastern Brazil

Conhecimentos, atitudes e práticas sobre o exame de Papanicolaou em mulheres do nordeste brasileiro

Carla Lorenna Ferreira de Albuquerque[I], Marla da Paschoa Costa[I], Felipe Moreira Nunes[I], Roberto Wagner Junior Freire de Freitas[II], Paulo Roberto Medeiros de Azevedo[III], José Veríssimo Fernandes[IV], Juciane Vaz Rego[II], Humberto Medeiros Barreto[II]

Universidade Federal do Piauí (UFPI), Campus Amílcar Ferreira Sobral, Piauí, Brazil

[I]Nursing Undergraduate, Department of Health Sciences, Universidade Federal do Piauí (UFPI), Floriano, Piauí, Brazil.

[II]MSc. Professor, Department of Health Sciences, Universidade Federal do Piauí (UFPI), Floriano, Piauí, Brazil.

[III]PhD. Professor, Department of Statistics, Universidade Federal do Rio Grande do Norte (UFRN), Natal, Rio Grande do Norte, Brazil.

[IV]PhD. Professor, Department of Parasitology and Microbiology, Universidade Federal do Rio Grande do Norte (UFRN), Natal, Rio Grande do Norte, Brazil.

KEY WORDS:
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PALAVRAS-CHAVE:
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Saúde da mulher.
Fatores socioeconômicos.

ABSTRACT

CONTEXT AND OBJECTIVE: The Papanicolaou (Pap) test has been shown to be effective in preventing cervical cancer. However, both the national and international literature shows that Pap testing has not reached the level of coverage desired. The objective of this study was to assess women's knowledge, attitudes and practices regarding the Pap test and to investigate whether there are any associations between these three factors and the women's sociodemographic characteristics.

DESIGN AND SETTING: Cross-sectional descriptive study conducted in Floriano, Piauí.

METHODS: The study was conducted among 493 women between November 2009 and December 2010. A questionnaire with precoded questions was used, and the responses were analyzed in terms of appropriateness in relation to the Pap test.

RESULTS: The degrees of adequacy of knowledge, attitudes and practices regarding the Pap test were 36.7%, 67.2% and 69.6%, respectively. Among the main barriers against testing, absence of symptoms and a sense of embarrassment were the most notable.

CONCLUSIONS: Women who visit doctors periodically had the most appropriate practices regarding the Pap test, but their knowledge of the procedure was poor. This suggests that these women were not receiving adequate information about the benefits of periodic testing.

RESUMO

CONTEXTO E OBJETIVO: O exame de Papanicolaou já mostrou efetividade na prevenção de câncer do colo do útero. A literatura nacional e internacional tem mostrado que o exame de Papanicolaou não tem alcançado o índice de cobertura desejado. O objetivo deste estudo foi avaliar os conhecimentos, atitudes e práticas entre mulheres em relação ao exame de Papanicolaou e verificar se existe associação entre esses comportamentos e as características sociodemográficas.

TIPO DE ESTUDO E LOCAL: Estudo descritivo e transversal realizado em Floriano, Piauí.

MÉTODOS: O estudo foi conduzido com 493 mulheres no período de novembro de 2009 a dezembro de 2010. Utilizou-se um questionário com perguntas precodificadas, cujas respostas foram analisadas quanto à adequação dos comportamentos em relação ao exame.

RESULTADOS: Os graus de adequação dos conhecimentos, atitudes e práticas em relação ao exame foram de 36,7%, 67,2% e 69,6%, respectivamente. Dentre as principais barreiras para a sua realização, destacaram-se a ausência de sintomas e a vergonha.

CONCLUSÃO: As mulheres que realizam consultas periodicamente apresentam prática mais adequada, porém com baixa adequação de conhecimentos frente ao procedimento, sugerindo que não estejam recebendo as informações adequadas sobre os benefícios da realização periódica do exame de Papanicolaou.

INTRODUCTION

Cervical cancer is the second biggest cause of cancer-related deaths among women worldwide, but the incidence is higher in developing countries.[1,2] In Brazil, this disease remains the third most common malignant neoplasm after non-melanoma skin cancer and breast cancer.[3,4] In 2012, the approximate incidence rate of cervical cancer per 100,000 women was 17.49 for Brazil, 17.96 for northeastern Brazil and 22.58 for the state of Piauí.[3]

The etiology of cervical cancer is directly related to persistent infection of the uterine cervix with human papillomavirus (HPV) genotypes that have a high oncogenic potential.[5,6] HPV infection is considered to be a necessary but insufficient cause of development of neoplasms or their precursor lesions, because viral deoxyribonucleic acid is present in 99.7% of cervical cancer cases.[7-9]

Importantly, this malignant neoplasm is one of many cancers with great potential for prevention and cure. The progression of cervical cancer is relatively slow, passing through various stages of precancerous intraepithelial lesions before advancing to its invasive form.[10] This characteristic of the disease, combined with the relative ease of diagnosis, has allowed physicians to detect this cancer during its earliest stages, when treatment results in a high cure rate.[10,11] Moreover, the infectious nature of cervical cancer's etiological agent has made implementation of preventive measures possible, including active immunization against HPV genotypes with higher oncogenic potential.[7,11,12]

The Papanicolaou (Pap) test is a simple method for detecting morphological changes in the uterine cervix from desquamated epithelial cells. Because the test is quick, painless, broadly applicable, easy to perform, performable in outpatient clinics and inexpensive, it has been considered to be the best method for cervical cancer screening.[10,13,14] However, an estimated 40% of Brazilian women have never been tested.[15] Low compliance arises for many reasons, including difficulties in accessing healthcare services, emotional discomfort for some women, embarrassment, social taboos, socioeconomic conditions and poor understanding of the benefits of testing for preventing cervical cancer.[16] These barriers have hindered achievement of the desired level of test coverage. Information concerning test coverage and the factors associated with test noncompliance among women in northeastern Brazil is still scarce.

OBJECTIVE

The purpose of the present study was to assess the knowledge, attitudes and practices of women in the city of Floriano, Piauí, regarding the Pap test, and to determine whether there was any association between the appropriateness of these three factors and the sociodemographic characteristics and other variables of this population.

METHODS

A cross-sectional descriptive study was conducted in the city of Floriano, Piauí, from November 2009 to December 2010, through home visits and interviews using a standardized questionnaire. The study included 493 women between 15 and 69 years of age (mean: 35.4 years) residing in both the urban and the rural areas of the municipality.

The urban center of the municipality is located 240 km from the state capital and the total estimated population of the municipality is 57,690 inhabitants, of whom 49,970 live in urban areas, 7,720 live in rural areas and 30,381 are female. The primary economic activities include agriculture, raising livestock, extracting natural resources and trade. Most of the population is poor and depends on the public health system, which consists of one 97-bed hospital, 25 primary healthcare units and 24 teams within the Family Health Program.

A sample size calculation was used to determine the number of women to be interviewed. This calculation used a statistical method based on the demographics of the municipality's female population and taking into consideration both rural and urban areas. The variable considered for defining the sample calculation was p equals the proportion of women between 20 and 59 years of age within the population of women aged over 15 years. In this case, the calculation of sample size was given by:

$$n = \frac{Np(1 - p)}{ND + p\,(1 - p)}$$

where n equals the sample size, N equals the total number of women aged over 15 years in the city and D equals $\varepsilon^2/4$, such that ε is a boundary error estimation of p, which satisfies $P(|\,p - \hat{p}| < \varepsilon) = 0.95$, in which $\hat{p}$ is an estimate for p. According to a survey conducted by the Municipal Health Department, N = 23,318 women and a preliminary estimate for p was given by $\hat{p}$= 0.72. By taking $\varepsilon = 0.04$ and inserting these values to determine n in the formula above, it was found that n = 493 women.

Three interviewers were trained to administer the survey and collect data. The research project and its objectives were explained to potential participants and assurances were given that confidential information would be safeguarded. These potential participants were then asked whether they would like to participate in the study. Those who voluntarily agreed to participate then signed an informed consent form and answered the questions on the questionnaire. The questions, which were designed to evaluate the three factors, consisted of direct questions in which the participants were asked to state their age, place of residence, education level, marital status, ethnicity, religion, family income, number of visits to a doctor during the previous year, sexual activity, use of contraceptives and parity. Only the women who voluntarily decided to participate in the study gave responses to the questionnaire and therefore there were no sample losses. The questions were asked by the interviewer orally without inducing responses.

To analyze the data we adopted the following definitions:

- **Inadequate knowledge:** a situation in which the women claimed that they had heard of the test but did not know the reason why the test was performed.

- **Inappropriate attitude:** a situation in which the women considered testing unnecessary or had no opinion about receiving the test.
- **Inadequate practice:** a situation in which the women claimed to have been tested more than three years ago, to have been tested only once in their lifetime or to have never been tested.

The data collected were independently digitized twice and were stored in an Excel database. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 16.0. To determine whether there was any association between variables, the chi-squared test was used, and a P-value < 0.05 was considered significant.

This research was submitted for approval to the Ethics Committee for Human Research of Universidade Federal do Piauí and was approved under the protocol number 0156.0.045.000-09.

RESULTS

Analysis of the data collected from the questionnaire allowed a profile to be created for the study participants. All of the women in the study were between 15 and 69 years of age (mean: 35.4 ± 14.3 years), and most were between 20 and 45 years of age. Most of the women lived in the urban areas of the municipality, possessed only elementary school education, possessed a monthly family income at or below the minimum wage level, professed Catholicism as their religion, engaged in an active sex life, were married or living in a stable relationship with their partner and had one to three children.

In terms of the degree of knowledge about the test, 94.5% of the women interviewed had heard of the procedure, but only 36.7% had adequate knowledge of the test. The doctor was cited as the primary source of information about the test by 44.2% of the participants, while friends or relatives were cited by 19.5% of the participants (**Table 1**).

Table 1. Sources of information and adequacy of knowledge relating to the Pap test in the municipality of Floriano, Piauí, Brazil, 2010 (n = 493)

Variable	n	%
Aware of the test		
Yes	466	94.5
No	27	5.5
Purpose of the test		
To prevent cancer	74	15.0
To prevent cervical cancer	107	21.7
Other answers	312	63.3
Knowledge of the test		
Adequate	181	36.7
Inadequate	312	63.3
Who had given information on the test		
Medical doctor	218	44.2
Friend or family	96	19.5
School	44	8.9
Radio/TV	41	8.3
Healthcare unit staff	34	6.9
Community health workers	33	6.6

All of the women interviewed considered the test necessary, but only 67.1% had an appropriate attitude regarding the procedure, through expressing conscious recognition of its advantages and benefits and correctly indicating the reasons for periodically undergoing the test. Among those who had an appropriate attitude, 46.7% justified the need for the test as a means of preventing cervical cancer, and 20.5% justified the test as a means of preventing cancer but without specifying the type of cancer prevented (**Table 2**).

With regard to whether the participants had ever undergone the test, 75.9% of them reported undergoing the procedure at some point, while 24.1% reported never having undergone the test. Among those who said that they had undergone the procedure, 69.6% said that they had been tested at a frequency of at least once every three years, thus demonstrating an appropriate level of test practice, considering that this frequency is within the acceptable limits recommended by the Brazilian Ministry of Health. The primary barriers against adequate frequency of testing were reported to be absence of symptoms (39.5%) and being too embarrassed (26.9%) to undergo the procedure (**Table 3**).

Table 2. Attitudes towards and reasons for undergoing the Pap test reported by women in the municipality of Floriano, Piauí, Brazil, 2010 (n = 493)

Variable	n	%
The need for testing		
Necessary	493	100.0
Unnecessary	0	0.0
Attitude		
Appropriate	331	67.1
Inappropriate	162	32.9
Reason for testing		
To prevent sexually transmitted diseases	113	22.9
To prevent cancer	101	20.5
To prevent uterine cervical cancer	230	46.7
No answer	49	9.9

Table 3. Test practices, adequacy of test practices and barriers against Pap testing reported by women in the municipality of Floriano, Piauí, Brazil, 2010 (n = 493)

Variable	n	%
Type of test practice		
Had been tested at some time in the past	374	75.9
Had never been tested	119	24.1
Frequency of testing		
Had been tested at least once every three years	343	69.6
Had been tested at intervals greater than three years	31	6.3
Had never been tested	119	24.1
Test practice		
Adequate	343	69.6
Inadequate	150	30.4
Barriers against test practice		
No symptoms	47	39.5
Feelings of embarrassment	32	26.9
Fear of pain	5	4.2
Inability to schedule the test	10	8.4
Long distance to healthcare unit	10	8.4
Lack of time	3	2.5
Inability to miss work	1	0.8
Other reasons	11	9.2

Statistically significant associations between knowledge of the test and age, social class, education level and family income level were observed (**Table 4**). The appropriateness of the women's attitude towards the test was associated with age, schooling level, marital status, sexual activity, frequency of consultations with doctors and parity. The adequacy of test practice presented associations with age, sexual activity, numbers of consultations with doctors, contraception and parity.

DISCUSSION

Among the 493 women included in our study, most were between 20 and 45 years of age, nonwhite, Catholic, less formally educated, limited to lower family incomes, sexually active, multiparous and married or living in a stable relationship with a partner. This profile is easily explained given that these women lived in the northeastern region of the country, a region historically associated with high levels of social inequality and poverty. It is not uncommon, therefore, even in the twenty-first century, to find young women with very little formal education and no training who are housewives with children.

Our results showed that 75.9% of the participants claimed to have had a Pap test at least once in their lifetime, a rate lower than that reported for women in the cities of São Paulo (86.0%)[17] and

Table 4. Assessment of adequacy of knowledge, attitudes and practices relating to the Pap test, according to sociodemographic and reproductive characteristics, among women in the municipality of Floriano, Piauí, Brazil, 2010 (n = 493)

Variable	Total	Adequate knowledge			Appropriate attitudes			Adequate test practices		
		n	%	P	n	%	P	n	%	P
Age (years)										
< 20	64	11	17.2		24	37.5		17	26.6	
20-45	303	115	37.9	0.001	212	70.0	0.000	229	75.7	0.000
> 45	126	55	43.7		95	75.4		97	77.0	
Stratum										
Rural area	46	13	28.3		26	56.5		29	63.0	
Lower class	330	114	34.5	0.037	219	66.4	0.101	229	69.4	0.601
Middle class	117	54	46.5		86	73.5		85	72.6	
Schooling										
Incomplete elementary school	180	56	31.1		119	66.1		124	68.9	
Complete elementary school	173	58	33.5	0.009	105	60.7	0.014	113	65.3	0.233
Complete middle school	115	53	46.1		85	73.9		86	74.8	
High school/college	25	14	56.0		22	88.0		20	80.0	
Marital status										
Single	170	43	25.3		90	52.9		79	46.5	
Married	269	114	42.4	0.001	200	74.3	0.000	217	80.7	0.000
Others	54	24	44.4		41	75.9		47	87.0	
Race										
White	94	44	46.8	0.024	65	69.1	0.645	65	69.1	0.764
Nonwhite	399	137	34.3		266	66.6		278	69.7	
Family income (monthly minimum wage)										
Up to 1	349	115	32.9		228	65.3		233	66.8	
2 to 4	126	56	44.4	0.017	90	71.4	0.411	95	75.4	0.085
≥ 5	18	10	55.5		13	72.2		15	83.3	
Visits to a doctor during preceding year										
No	198	56	28.3	0.002	121	61.1	0.019	92	46.5	0.000
Yes	295	125	42.4		210	71.2		251	85.1	
Sexually active										
No	58	13	22.4	0.016	26	44.8	0.000	11	19.0	0.000
Yes	435	168	38.6		305	70.1		332	76.3	
Contraception use										
No	257	88	34.2	0.235	164	63.8	0.101	162	63.0	0.001
Yes	236	93	39.4		167	70.8		181	76.7	
Parity										
None	115	26	22.6		60	52.1		51	44.3	
1 to 3	260	111	42.7	0.002	185	71.2	0.001	207	79.6	0.000
4 to 6	77	31	40.2		58	75.3		53	68.8	
7 or more	41	13	31.7		28	68.3		32	78.0	
Total	**493**	**181**	**36.7**		**331**	**67.1**		**343**	**69.6**	

São Luís do Maranhão (82.4%)[18] and in the municipality of São José de Mipibu, in the state of Rio Grande do Norte (85.0%).[19] A significant proportion of the study participants (24.5%) admitted to never having had the test. Our rate was higher than the rates observed in the three studies mentioned above, where the percentages of women who had never undergone the test were 13.9%, 17.6% and 15.0%, respectively. A similarly low rate of 11.2% was reported in Campinas, state of São Paulo.[16]

However, the proportion of women with adequate test practice was 69.6%, thus representing a coverage rate for the test that was slightly higher than that reported for women in Brazil as a whole (66.0%)[20] and for women in São José de Mipibu (64.4%).[19] Our rate was also very similar to those found in two studies conducted in Pelotas, Rio Grande do Sul,[4,21] in which rates of 68.8% and 68.9%, respectively, were reported. However, our rate was below the coverage rates reported for women in São Paulo (77.3%)[17] and São Leopoldo, Rio Grande do Sul (85.5%).[22] The adequacy of test practice demonstrated by our study participants was not influenced by the level of education. This is different from the results obtained in other studies conducted in this country,[16,19,21] in which an association was found between the adequacy of this factor and the level of education.

Regarding knowledge, 36.7% of participants demonstrated adequate knowledge of the test, but our rate was lower than that of women in São José de Mipibu (46.1%).[19] We observed that adequate knowledge about the Pap test was found among higher proportions of women who were over the age of 20, ethnically white, married or living in a stable relationship with their partner, more highly educated and sexually active; and who possessed higher family income, and reported medical visits in the year preceding the survey and had children.

For women with a higher level of formal education and greater purchasing power, these results could be explained, at least in part, by the greater access to information about the health benefits of the test that these women had and, possibly, the greater opportunity for them to receive the test. However, the higher rates of adequate knowledge about the test observed among women who were sexually active, who visited a doctor more often and who had children may have been because they sought medical advice more often, either to obtain information regarding contraceptive methods or to receive prenatal care.

With regard to attitudes towards the test, 67.1% of the women interviewed had appropriate attitudes, such that they were able to mention the advantages and benefits of the procedure for their health and to correctly state the reason for periodically undergoing the test. This rate was similar to those reported for women in São José de Mipibu (63.3%)[19] and South Africa (60.6%),[23] but it was lower than the rate reported for Argentinean women (80.5%)[24] and higher than the rate observed for women in the Brazilian state of Rio Grande do Sul (45.6%).[25]

Among the women who had an appropriate attitude towards the test, 46.7% considered it necessary to undergo the test periodically to prevent cervical cancer specifically, while 20.5% considered it necessary for preventing cancer, without specifying the type of cancer. A significant proportion of the women studied (9.9%) considered the test necessary but did not know what the benefits of the test were for women's health. This finding suggests that when they learned about the test, the women participating in this study were not well informed regarding the importance of the procedure as a screening method for early diagnosis and treatment of lesions before those lesions progressed to a more malignant form of cancer. Better knowledge of this testing resource is essential for preventing cervical cancer.

The proportion of the women with an appropriate attitude towards the test was significantly higher among those who were already sexually active and married or living in a stable relationship, and among those who consulted a gynecologist during the year prior to the survey. The rates of appropriate attitudes increased proportionally with age, level of education and number of children. These findings were probably due to the greater degree of awareness that these women had about the advantages and benefits of periodic testing, in addition to the more readily available access to information and healthcare services that these women had. Other studies have also reported an association between appropriate attitudes and formal education.[13,25] A study conducted in Campinas,[16] for example, found an association between appropriate attitudes towards the test and formal education and family income.

In comparing our results regarding test practices with the results available in the literature, we saw that the rate of adequate test practice shown by the women in our study was similar to those described for women in Santo Angelo, Rio Grande do Sul[25] and São José de Mipibu,[19] but higher than the rates reported for women in South Africa[23] and Argentina.[24] The primary reasons given by women for not undergoing the test included absence of symptoms and being too embarrassed to undergo the test. These barriers were similar to those reported in other studies conducted in Brazil[16,19,25] and Argentina.[24]

CONCLUSION

Clearly, health professionals including physicians and nurses play an important role in shaping the knowledge, attitudes and practices of women with regard to the Pap test. Success in this is reflected in the level of adherence to periodic testing among women and, consequently, the coverage rate for the test. Nevertheless, it appears that the forms of communication and/or methodology used by these professionals to inform women about the benefits and advantages of periodic testing may not be sufficiently clear or adequate. However, we cannot rule out the possibility that the time devoted to each consultation and the number of consultations received by

these women may not have been sufficient to clarify all matters of concern, or that the women participating in this study had failed to make adequate use of the healthcare services provided. These potential problems require further attention from the municipality's healthcare managers, who need to commit to providing correct information about the test and its advantages and benefits for women's health, in order to improve adherence among the female population, thus meeting the coverage recommendations of the Brazilian Ministry of Health.

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Address for correspondence:
Humberto Medeiros Barreto
BR 343, KM 3,5
Meladão — Floriano (PI)
CEP 64800-000
Tel. (+55 89) 3522-0136
Cel. (+ 55 89) 9928-2467
E-mail: hmbarreto@ufpi.edu.br

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Patent blue and air as an alternative for resection of nonpalpable breast lesions: a case series

Azul patente e ar como uma alternativa para a ressecção de lesões não palpáveis de mama: série de casos

Sabas Carlos Vieira[I], Viviane Carvalho Alves[II], Tayná Cristinne Barros de Oliveira[II], Jacira Oliveira Ibiapina[II], Emmyle Cristyne Alves Soares[II], Marcus Luciano Lopes de Paiva Crisanto[II]

Universidade Federal do Piauí (UFPI), Teresina, Piauí, Brazil

[I]PhD. Adjunct Professor of Oncology, Department of General Practice, Universidade Federal do Piauí, Teresina, Brazil.
[II]Medical Student, Universidade Federal do Piauí, Teresina, Brazil.

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Mamografia.
Detecção precoce de câncer.

ABSTRACT

CONTEXT AND OBJECTIVE: Use of mammography for breast cancer screening has resulted in a significantly increased number of patients with nonpalpable radiological findings that need histopathological study for better management. The present study evaluated an alternative to excision of nonpalpable breast lesions, using injection of patent blue (CAS 3536-49-0) dye and air.

DESIGN AND SETTING: Cohort study of 64 consecutive patients at a private clinic in the city of Teresina (Piauí), between January 2009 and December 2010.

METHODS: The patients had received mammographic diagnoses of nonpalpable breast lesions classified as BI-RADS 3, 4 and 5, with indication of histopathological study. They underwent stereotaxy and/or ultrasound-guided injection of patent blue, for marking and subsequent excision of the lesion.

RESULTS: The patients' mean age was 47.7 years. Nodes accounted for 53.1% of the breast abnormalities; microcalcifications, 37.5%; and complex cysts, 9.4%. In 89.1% of cases, the lesions were BI-RADS 4; 7.8% were BI-RADS 5 and 3.1% were BI-RADS 3. The histopathological findings were benign in 70.3% of the cases; atypical hyperplasia, 9.4%; and malignant, 20.3%. Among the malignant cases, 53.8% were carcinoma *in situ* and 46.2%, invasive carcinoma. The percentage of malignancy was 0% in BI-RADS 3 lesions; 14.3% in BI-RADS 4 and 100% in BI-RADS 5. In the cases of malignancy, the margins were clear in 92.3%. Reoperation to widen the margins was required in one patient.

CONCLUSION: Excision of nonpalpable breast lesions marked with patent blue and air was possible in all cases.

RESUMO

CONTEXTO E OBJETIVOS: A utilização da mamografia no rastreamento para o câncer de mama tem apresentado significativo aumento do número de pacientes com achados radiológicos impalpáveis que, para melhor orientação terapêutica, necessitam de estudo histopatológico. O presente estudo avaliou uma alternativa à ressecção de lesões impalpáveis de mama utilizando injeção de corante azul patente e ar.

TIPO DE ESTUDO E LOCAL: Coorte de 64 casos consecutivos de uma clínica particular da cidade Teresina (PI), no período de janeiro de 2009 a dezembro de 2010.

MÉTODOS: As pacientes receberam o diagnóstico mamográfico de lesões impalpáveis de mama classes BI-RADS 3, 4 e 5 com indicação para estudo histopatológico. Elas foram submetidas à injeção de azul patente, orientada por estereotaxia e/ou ultrassonografia para marcação e posterior ressecção da lesão.

RESULTADOS: A média de idade das pacientes foi 47,7 anos. Os nódulos representaram 53,1% das anormalidades; as microcalcificações, 37,5% e os cistos complexos, 9,4%. Em 89,1% dos casos, as lesões eram BI-RADS 4; 7,8%, BI-RADS 5 e 3,1%, BI-RADS 3. Os achados histopatológicos foram benignos em 70,3% dos casos; com hiperplasia atípica em 9,4% e malignos em 20,3%. Dos casos de malignidade, 53,8% eram carcinoma *in situ* e 46,2%, carcinoma invasor. O percentual de câncer foi 0% nas lesões BI-RADS 3;14,3% nas BI-RADS 4 e 100% nas BI-RADS 5. Nos casos de neoplasia maligna, as margens estavam livres em 92,3%, sendo necessária reoperação para ampliação de margens em uma paciente.

CONCLUSÃO: A ressecção de lesões impalpáveis de mama marcadas com azul patente e ar foi possível em todos os casos.

INTRODUCTION

The dissemination of breast cancer screening associated with better imaging techniques has resulted in increased incidence of nonpalpable breast lesions, as classified according to the Breast Imaging Reporting and Data System (BI-RADS), published by the American College of Radiology (ACR) and recommended by the Brazilian College of Radiology (CBR).[1]

The initial approach is to perform core biopsy or complete excision of the lesion by means of mammotomy. In cases of inconclusive biopsy or in the presence of carcinoma, surgery is indicated. In nonpalpable lesions, surgical biopsy should be preceded by preoperative ultrasound-guided or mammography-guided marking, (freehand, biplanar or stereotactic).[2] The exact preoperative site of the lesion is a determining factor for whether high rates of total resection are achieved, thereby decreasing the need for re-excision.

Currently, the most widely used methods involve techniques with radioactive material (radioguided occult lesion localization, ROLL), metal wire, activated charcoal or dyes, such as patent blue,[3] methylene blue[4] and indocyanine green.[5] Nowadays, the majority of breast surgery services use ROLL as the standard procedure. In a literature review conducted by the authors of the present study, only nine studies used dye for localization of nonpalpable breast lesions.[3-11] Among these studies, two used a dye in association with ROLL[6,7] and one used a dye in association with a metal wire.[8] Of these nine, only two studies were Brazilian.[3,10] The aim of the present study was to evaluate the resection of nonpalpable breast lesions stained with patent blue dye and air.

METHODS

This was a retrospective cohort study. The medical files of 64 patients seen at a private breast disorder clinic in the city of Teresina (PI) between January 2009 and December 2010 were analyzed after gaining approval from the Ethics Committee of Universidade Federal do Piauí. These patients had received imaging diagnoses of nonpalpable breast lesions classified as BI-RADS 3, 4 and 5 and an indication for histopathological study. The imaging diagnosis was performed by means of mammography and/or breast ultrasonography.

On the day of the surgery, the lesions were marked with patent blue dye as close as possible to the time scheduled for the surgical procedure. The interval between dye injection and the surgical procedure ranged from 21 to 320 minutes, with a mean time of 156 minutes.

Initially, 2% lidocaine chloride was infiltrated at the puncture site where the dye would be injected. Subsequently, 0.2 ml of patent blue dye and 0.4 ml of air were injected by means of a syringe that was stereotaxy-guided and ultrasound-guided or only ultrasound-guided. The purpose of dye injection was to facilitate lesion localization by the surgeon, using ultrasound. After the end of the procedure, ultrasonography was performed to mark the skin location with the shortest tissue route for excision of the lesion. This site was used to guide surgical incision (**Figure 1**).

The surgical procedure was performed under local anesthesia and sedation. Antibiotic prophylaxis was not administered. A perpendicular incision was made in the subcutaneous tissue and breast parenchyma until the area marked in blue had been located. The node marked in blue was then excised (**Figure 2**) and subjected to histopathological analysis. Specimens with microcalcifications were previously radiographed in order to confirm that

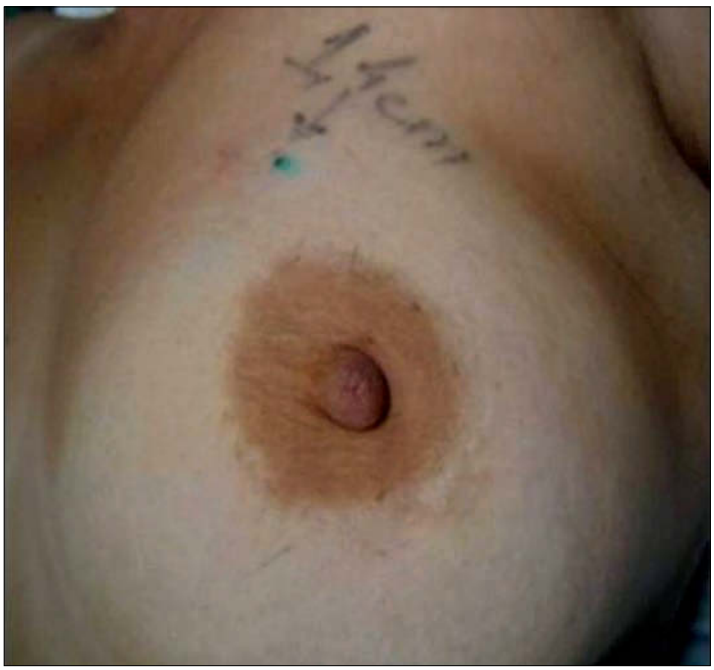

Figure 1. Marking of puncture site with the shortest route.

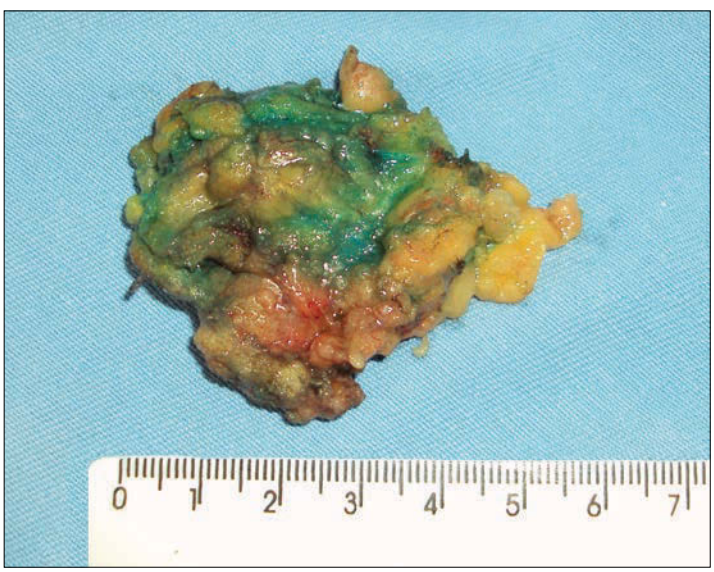

Figure 2. Excised blue node.

complete excision of the lesion had been achieved. Hemostasis was checked. The incision was subsequently closed using a fine skin suture and compression dressing was applied. No drainage tube was used.

RESULTS

The patients' mean age was 47.7 ± 7.5 years. In 89.1% of the cases, the lesions were classified as BI-RADS 4, while 3.1% were BI-RADS 3 and 7.8% were BI-RADS 5 (**Table 1**). Nodes accounted for 53.1% of the abnormalities, microcalcifications for 37.5% and complex cysts for 9.4% (**Table 2**).

Twenty-five patients underwent biopsy prior to lesion excision. Of these, 7 cases were malignant, 14 were benign, 2 consisted of atypical hyperplasia and 2 were inconclusive. We considered that the lesions classified as BI-RADS 4C or 5, in which biopsies did not reveal malignancy due to possible sampling error, were inconclusive. Out of the 14 lesions previously classified as benign, 12 (87.5%) were benign, one (6.25%) showed atypical hyperplasia and one (6.25%) had a final result of malignancy after excision. Neither of the two cases of atypical hyperplasia was confirmed as such: one (50%) was revealed to be malignant, while the other (50%) proved to be benign. All seven cases previously diagnosed as malignant was confirmed as such. The two cases with previously inconclusive biopsies proved to be benign after excision (**Figure 1**).

According to the pathological analyses on the excised specimens, 70.3% of the lesions were benign and 9.4% were atypical hyperplasia. Among the benign lesions, fibroadenoma predominated (35.6%). In 20.3% of the cases, the final histopathological diagnosis was breast cancer. Of these, 10.9% were carcinoma *in situ* and 9.4% were invasive carcinoma (**Table 3**). All of these cases (both the carcinoma in situ and invasive carcinoma cases) consisted of ductal carcinoma. One patient was re-excised to widen the margins of excision. None of the BI-RADS 3 lesions were malignant. Among the BI-RADS 4 lesions, the percentage of cancer was 14.3% and among the BI-RADS 5 lesions, 100% (**Table 4**).

Five patients with a malignant biopsy prior to resection underwent sentinel lymph node biopsy. None of these cases exhibited any compromised tissue.

Table 1. Patients according to BI-RADS classification

BI-RADS	Number of patients	%
3	2	3.1
4	57	89.1
5	5	7.8
Total	**64**	**100.0**

BI-RADS = Breast Imaging Reporting and Data System.

Table 2. Types of lesions according to radiological characteristics

Type of lesion	Number of patients	%
Complex cyst	6	9.4
Node	34	53.1
Microcalcification	24	37.5
Total	**64**	**100.0**

Table 3. Patients according to histopathological study on resected lesions

Histopathological finding	Number of patients	%
Benign	45	70.3
Atypical hyperplasia	6	9.4
Carcinoma *in situ*	7	10.9
Invasive carcinoma	6	9.4
Total	**64**	**100.0**

Table 4. Percentage of benign and malignant lesions, according to BI-RADS classification

BI-RADS	% benign	% malignant	Total
3	100.0	0	100.0
4	85.7	14.3	100.0
5	0	100.0	100.0

BI-RADS = Breast Imaging Reporting and Data System.

DISCUSSION

The development of mammographic techniques, as well as increased awareness about and access to breast cancer treatment has enabled earlier diagnosis and has led to higher incidence of nonpalpable breast lesions. Lesion excision is indicated when there is a previous diagnosis of malignancy (BI-RADS 6), presence of a suspicious lesion (BI-RADS 4) or a lesion highly suspected of malignancy (BI-RADS 5), or presence of lesions that are probably benign (BI-RADS 3) with an indication for surgical biopsy.[10]

In the present study, the majority (89.1%) of the patients belonged to BI-RADS category 4 (**Table 1**). Two BI-RADS 3 lesions were excised: one because there was a history of breast cancer in a first-degree relative and another because there was growth of a palpable and a nonpalpable node. In this case, despite the benign characteristics seen on imaging examinations, the decision to excise the lesion was made in conjunction with the patient. Several methods have been developed for surgical localization of nonpalpable breast lesions, such as metal wire, ROLL and dyes.

Metal wire-guided excisional biopsy is a safe and accurate method that has been widely adopted. However, displacement, folding or breakage of localization wires may occur. When the wire is sectioned during surgery, part may remain in the breast parenchyma, which may have the implication of legal actions brought by the patient. If the procedure is not started in the incision made at the skin puncture site for placement of the metal

wire, it may be difficult to locate its tip. If an incision is made at the puncture site, the procedure will be more invasive and traumatic. Furthermore, the wire is relatively expensive.[4]

ROLL was proposed as the best alternative, compared with the standard method of wire-guided excision of nonpalpable breast lesions. A small dose (3.7 MBq) of albumin labeled with ^{99m}Tc is injected by means of ultrasound or stereotactic imaging. During surgery, the lesion is located using a gamma ray probe.[6] The main disadvantage of this technique consists of difficulty in establishing the depth of the breast parenchymal lesion, since the probe cannot distinguish between the depths of the lesion, which may give rise to wider excision of the breast segment than desired.[12] This difficulty was found to be overcome when a dye is used in association with ROLL for marking nonpalpable lesions.[7] In that study, 157 patients with nonpalpable lesions were evaluated. Among these, marking was performed using a metal wire in 78 cases and ROLL associated with methylene blue in 79 cases. Surgery and ROLL combined with methylene blue was performed in a shorter time and achieved a clearer surgical margin and a smaller specimen size. In addition, there was a lower rate of re-excision and the size of the skin incision was smaller than with wire-guided excision.[7] However, with regard to marking, use of dye alone is sufficient to completely excise the lesion, as demonstrated in our current study.

Another disadvantage of using ROLL is that an additional professional is required in the team (the nuclear physician), thus raising the cost of the procedure, since it includes performing breast scintigraphy. According to research carried out by the present authors in private clinics in the city of Teresina (capital of the state of Piauí), ROLL surpasses the costs of excision with dyes by about 75%.

The effectiveness of dye used alone was also demonstrated in another study, in which 57 lesions of 51 patients were marked with 0.4-0.7 ml of methylene blue, with ultrasound guiding, 20 to 180 minutes before the surgical procedure.[9] All the lesions were successfully excised. Adequate localization was possible in the cases of 56 lesions. In a single patient, the lesion was not found because the dye had been absorbed before the beginning of the procedure. In that case, the lesion was excised using intraoperative ultrasound. The authors attributed this failure to the interval of 100 minutes between dye injection and the beginning of surgery, and suggested that the surgery should be started as soon as possible after dye injection. The procedure involving dye injection was reported as painful by 5.3% of the patients, tolerably painful by 28% and painless or mildly uncomfortable by 66.7%. No allergic reactions were observed.[9]

In a study by Prudêncio et al., patent blue dye was used in preoperative marking of 285 patients with nonpalpable breast lesions. In 153 patients, the marking was guided by ultrasound and, in 132 patients, mammography was used. Marking was performed between 30 and 180 minutes before the surgical procedure. The marked area was identified during the intraoperative period and was successfully excised in all cases (100%).[10]

The main advantage of marking with a dye is that it enables lesion removal under direct viewing of the blue area. In the past, after dye injection, the route of the puncture site was marked during needle removal. The incision was made along the marked track. If the puncture site was situated far from the lesion, wider incisions and trauma were inevitable, thereby compromising the cosmetic results from the procedure.[4] A small amount of air between the plunger and the dye is currently used, which facilitates lesion localization by means of ultrasound, thus marking the site closest to the lesion to be excised. The puncture path is not impregnated with dye, which gives rise to less tissue trauma. The excision is only made when the marked blue area is viewed. Thus, there is a smaller area of excised breast parenchyma, which improves the cosmetic results.[3] One initial concern with this method was the dissemination of dye, which could make the technique difficult if surgery was not performed promptly after injection.

One important advantage of patent blue is its capacity to diffuse to adjacent tissues. Its capacity to diffuse is intermediate, in comparison with methylene blue, which diffuses greatly, and charcoal, which does not diffuse. Since it does not require the use of nuclear medicine and special metal wires, the cost of the procedure is also lower. As shown in this case series, the time that elapsed between dye injection and performing the surgical procedure was 163 minutes on average, ranging from 21 to 320 minutes. In all cases, the dye was restricted to the area of nonpalpable lesion, thus not compromising the excision. In patients with invasive carcinoma and carcinoma *in situ*, the margins were not clear in only one patient, who needed to be re-excised to widen the margins of excision.

In order to introduce an alternative technique and determine its applicability for surgical removal of nonpalpable breast lesions, Aydogan et al. performed occult lesion localization guided with indocyanine green dye, in a case report. Lesion localization was performed in two patients before surgery under ultrasonographic control by injecting indocyanine green into the lesion and its subcutaneous tissue projection. During surgery, the site of the skin incision and the resection margins were identified by observing the area of indocyanine-derived fluorescence under the guidance of a near-infrared-sensitive camera. In both cases, the breast lesion was correctly localized, and the area of fluorescence corresponded well to the site of the lesions. On histopathological examination, the surgical margins were found to be clear. The authors concluded that indocyanine green is an excellent dye for localization of nonpalpable breast lesions.[5]

Another argument against the use of dyes is the possibility of allergic events. In the present case series, there was no occurrence of allergic reaction. In the literature, the incidence of allergic phenomena seen through using patent blue dye has been 0.06 to 2.7%, with a mean value of 0.71%,[13] particularly in surgery for investigation of sentinel lymph nodes in which a higher volume is used, usually 2 to 4 ml. When nonpalpable lesions are marked, only 0.2 ml is used. One precautionary measure is to avoid performing this procedure in patients with a significant history of allergy, such as severe hives and angioedema.

CONCLUSION

Excision of nonpalpable breast lesions marked with patent blue dye and air was possible in all lesions. In the cases of malignancy, there were clear margins in 92.3%, while re-excision to widen the margins of excision was required in one patient.

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Address for correspondence:
Tayná Cristinne Barros de Oliveira
Rua Desembargador Pires de Castro, 2.575
Aeroporto — Teresina (PI) — Brasil
CEP 64002-490
Tel. (+ 55 86) 3225-3626/(+ 55 86) 9929-1773
E-mail: taynacristinne@hotmail.com

Follow-up of women with atypical squamous cells cannot exclude high-grade squamous intraepithelial lesions (ASC-H)

Acompanhamento de mulheres com células escamosas atípicas não pode excluir lesão intraepitelial de alto grau (ASC-H)

Fanny López-Alegría[I], Dino Soares De Lorenzi[II], Orlando Poblete Quezada[III]

Primary Healthcare Clinics, Santiago, Chile

[I]PhD. Professor, Department of Nursing, School of Nursing, Universidad Andres Bello, Santiago, Chile.

[II]MD. Professor, Department of Obstetrics and Gynecology, Universidade de Caxias do Sul, Rio Grande do Sul, Brazil.

[III]Medical Technologist. Cytology Laboratory, Complejo Asistencial Barros Luco, Santiago, Chile.

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Seguimentos.

ABSTRACT

CONTEXT AND OBJECTIVE: The concept that the presence of atypical squamous cells cannot exclude high-grade squamous intraepithelial lesions (ASC-H) was introduced in the 2001 Bethesda System of cervical cytology classification. This nomenclature defines cervical cancer precursor lesions. The objective of this study was to investigate the colpocytological-histological results from a three-year follow-up conducted on a cohort of women with reports of ASC-H who were attended during 2005-2006 at clinics of the Southern Metropolitan Healthcare Service of Santiago, Chile.

DESIGN AND SETTING: Prospective cohort study at primary healthcare clinics in Santiago, Chile.

METHODS: Colpocytological-histological follow-up was conducted over a three-year period on 92 women with cytological reports of ASC-H who were attended at primary healthcare clinics during 2005-2006.

RESULTS: At the end of the follow-up period, high-grade lesions were evaluated and the following outcomes were observed: seven women presented invasive cancer (7.6%), 49 presented high-grade lesions (53.3%), 26 presented low-grade lesions (28.2%) and 10 presented normal results (10.9%). The "Conditional Probabilities Tree Diagram" was used to show the results from tests and the times of lesion detection. It demonstrated that, after a first report of ASC-H, clinical management needed to be interventionist.

CONCLUSION: The follow-up on our cohort of women showed that the majority of uncertain ASC-H diagnoses (82.6%) had abnormal colposcopic results and that during the follow-up using ASC-H smears, two out of every three women developed high-grade lesions.

RESUMO

CONTEXTO E OBJETIVO: O conceito de que à presença de células escamosas atípicas não se pode excluir lesão intraepitelial de alto grau (ASC-H) foi introduzido pelo Sistema de Bethesda 2001, na classificação de citologia cervical. Esta nomenclatura define lesões precursoras do câncer cervical. O objetivo deste estudo foi investigar os resultados colpo-cito-histológicos de três anos de acompanhamento realizado em uma coorte de mulheres com relatórios de ASC-H que receberam atendimento no período 2005-2006 em clínicas do Serviço Metropolitano de Saúde Sul de Santiago, Chile.

TIPO DE ESTUDO E LOCAL: Estudo de coorte prospectivo em unidades básicas de saúde de Santiago, Chile.

MÉTODOS: Foi conduzido um acompanhamento colpo-cito-histológico por um período de três anos em 92 mulheres com laudos citológicos de ASC-H, que receberam atendimento nas unidades básicas de saúde de 2005-2006.

RESULTADOS: No final do período de acompanhamento, as lesões de alto grau foram avaliadas e os resultados foram observados: sete mulheres apresentaram câncer invasivo (7,6%), 49 apresentaram lesões de alto grau (53,3%), 26 apresentaram lesões de baixo grau (28,2%) e 10 apresentaram resultados normais (10,9%). O "Diagrama de Árvore Condicional de Probabilidades" foi utilizado para mostrar os resultados dos testes e o período de detecção das lesões, demonstrando que, depois de um primeiro relatório de ASC-H, o manejo clínico deve ser intervencionista.

CONCLUSÃO: O acompanhamento de nossa coorte de mulheres mostra que a maioria dos diagnósticos incertos de ASC-H (82,6%) tiveram resultado colposcópico anormal e, durante o acompanhamento de esses esfregaços ASC-H, duas de cada três mulheres desenvolvem lesões de alto grau.

INTRODUCTION

The concept that the presence of atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H) was introduced by the 2001 Bethesda System of cervical cytology classification. This system addresses the need to define a cytological category between atypical squamous cells of undetermined significance (ASCUS) and high-grade squamous intraepithelial lesion (HSIL).[1] The definition of this nomenclature resulted from the ASCUS/LSIL Triage Study (ALTS) carried out between 1996 and 2000, in which interpretations of ASCUS were subcategorized as "equivocal LSIL" (ASCUS-L) and "equivocal HSIL" (ASCUS-H). Through a consensus among expert pathologists, it was concluded that ASCUS-H represented a cytological category that differed from ASCUS-L and HSIL.[2] ASCUS-H has been seen to present a risk association of 27.2% with cervical cancer precursor lesions, which is higher than the risk association for the ASCUS-L category of 11.4%, but lower than the risk association for HSIL of 44.8%.[2,3]

Subsequent studies, such as a study by Sherman et al., detected high-grade lesions (HSIL) in 41% of the women with initial ASC-H smears.[4] Supporting previous findings, Patton obtained a high positive predictive value for lesions of cervical intraepithelial neoplasia (CIN II/III), derived from the ASC-H diagnosis category in a general population of women.[5]

Bonvicino et al. used computed data from two medical centers in San Antonio, Texas, United States, in which 260 women diagnosed with ASC-H underwent cytological-histological follow-up, and found that 25.4% had a high-grade lesion at the end of the follow-up period.[6] Using the same methodology in Brazil, Cytryn et al. found high prevalence of high-grade lesions among a total of 57 ASC-H cases.[7] Thus, the abovementioned follow-up studies demonstrated that there are connections between ASC-H smears and high-grade lesions.[4,6,7]

In Chile, the 2001 Bethesda System was adopted in 2005 via Ordinance Number B232 1771 issued by the Department of Non-Communicable Diseases, within the Division of Disease Prevention and Control. This ordinance standardized the use of the nomenclature that had started to be used in previous years.[8] In 2006, the National Program for Research and Control of Cervical Cancer collected 779,068 Pap smears nationwide, from which 14,608 (1.9%) were found to be atypical using the new nomenclature.[9]

Although other studies such as Gaete et al.[10] and Yazigi et al.[11] were conducted in Chile, they were limited because they did not discriminate the type of atypia given that they were conducted using cytological reports that were produced before the 2001 Bethesda System was incorporated in Chile. The scarce scientific evidence regarding the ASC-H category among the female population of Chile motivated the present study.

OBJECTIVE

The objective of this study was to investigate the cytological-histological results from a three-year follow-up conducted on a cohort of women with reports of ASC-H who were attended during 2005-2006 at clinics within the Southern Metropolitan Healthcare Service in Santiago, Chile.

METHODS

The present research comprises a prospective cohort study on women who were followed up over a three-year period. During 2005-2006, 88,438 cervical cytological smears were collected by midwives using Ayre spatulas and cytobrushes in primary healthcare clinics, in the southern area of Santiago, Chile.

These smear screenings were fixed and sent to Barros Luco Hospital Laboratory (central hospital). In this laboratory, the cytological smears were stained by means of the conventional Pap smear technique and were classified using the national nomenclature system, which is equivalent to the 2001 Bethesda system.[8] These tests were processed by a team of cytotechnologists with an average of 20 years of experience. Subsequently, these records were registered in the "Cytological Expert Diagnosis Archive System" at the Chilean Ministry of Health. This provided the cytological-histological database that we searched to obtain data for the present research study.[9]

The ASC-H cases were selected (n = 106) and the following selection criteria were applied: the women needed to be without uterine pathological conditions, without prior cervical procedures and with normal Pap smear results for the last three years. These criteria resulted in a cohort of 92 women who were followed up colpocytologically-histologically for a three-year period or until their cases were resolved.

The clinical management of these women included gynecological examinations, cytological tests (Pap smears), colposcopic examinations and biopsies. HPV testing was not performed due to cost constraints. All of the follow-up tests were performed by professionals at the central hospital's Cervical Pathology Clinic.

The variables studied included the following: the women's age at the time of ASC-H identification, the number and type of cytological, colposcopic and histological results and the length of follow-up (months) among the women with ASC-H cytology, which ended with diagnostic confirmation, defined as diagnosis of a more severe lesion (CIN II+) or negative confirmation.

To define the cytological variable (Papanicolaou), the 2001 Bethesda nomenclature was utilized. The results were classified as follows: negative for intraepithelial lesion or malignancy (Neg); atypical squamous cells of undetermined significance (ASCUS); atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesion (ASC-H); low-grade squamous

intraepithelial lesion (LSIL); high-grade squamous intraepithelial lesion (HSIL); or squamous cell carcinoma.

To define the histological variable (biopsy), cervical intraepithelial neoplasia (CIN) grades were used and divided as follows: cervical intraepithelial neoplasia of low grade (CIN I); cervical intraepithelial neoplasia of moderate grade (CIN II); cervical intraepithelial neoplasia of high grade (CIN III); carcinoma *in situ* (CIS); or invasive carcinoma.

For the colposcopic variable, a standard protocol was used, which included conventional visual examination, application of 5% acetic acid and identification of the squamocolumnar junction. This variable was defined as follows: negative (-) colposcopy, when colposcopic findings did not show severe lesions or the need for a biopsy; or positive (+) colposcopy, when colposcopic findings showed lesions of severity that required biopsy.

The data collected were analyzed electronically using Microsoft Excel (version 2007) and the "Conditional Probabilities Tree Diagram," which shows the number, type, outcome and time interval between patient tests.

RESULTS

Out of the 88,438 cervical exfoliative smears collected, 752 (0.85%) were atypical Pap smears. The latter contained atypical squamous cells, which were divided into atypical squamous cells of undetermined significance (ASC-US) (619 cytological tests, 0.69%) and atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesion (ASC-H) (106 cytological tests, 0.11%) (**Table 1**).

After applying the selection criteria to the group of women with ASC-H Pap smears, the cohort consisted of 92 women, with a mean age of 38.2 years and an age range from 19 to 71 years.

The follow-up results from this cohort were illustrated using the "Conditional Probabilities Tree Diagram" (**Figure 1**), which begins with the ASC-H Pap smear ("phase 0") and illustrates the tests performed on the women and their outcomes over time. Squares signify a biopsy and circles indicate a Pap smear.

It needs to be borne in mind that the ASC-H cytological finding is defined as a suspected case (positive Pap smear) in the cervical cancer program. These 92 women who were attended at primary healthcare clinics were managed in accordance with the central hospital's "management algorithm for specialist or cervical pathology clinic treatment from the time of the first atypical Pap", in order to make the diagnosis and/or proceed with treatment.

During the first contact with this clinic, the patient's history was taken, a gynecological examination was made and colposcopy was performed. Colposcopy is considered to be the first procedure for diagnostic confirmation in the management algorithm. As shown in "Phase 1", 76 cases (82.6%) showed abnormal colposcopic findings and required biopsy for histological analysis, while in 16 cases (17.4%), the initial colposcopic findings were normal and were followed up by a Pap test.

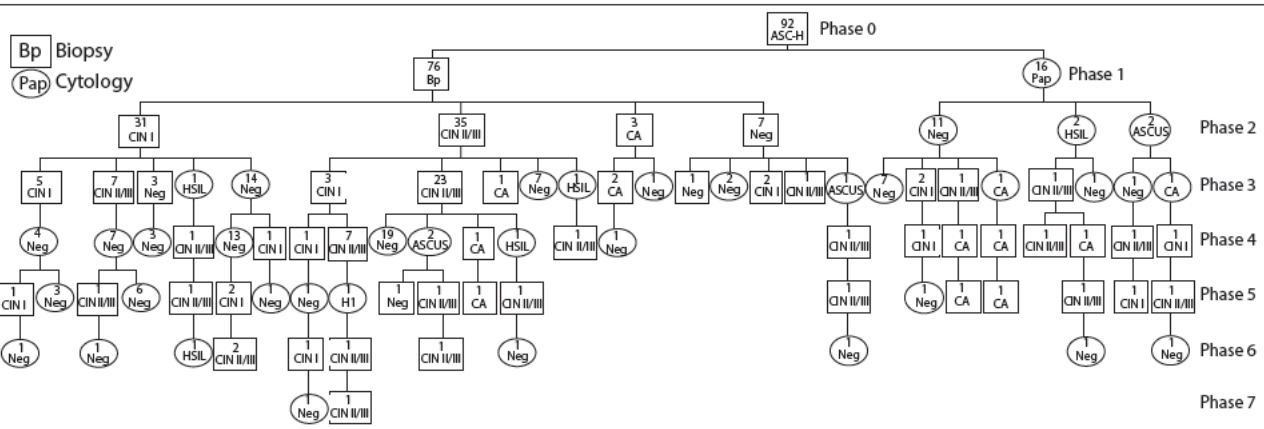
The average length of time taken for the patients to complete these medical procedures was 3.15 months, with a minimum of two days and a maximum of 19.6 months.

Subsequently, the cytological-histological results collected in the previous stage were known as "Phase 2". For the group that started with a histological study, the results were distributed as follows: 31 biopsies showed CIN I; 35 showed CIN II/III; three showed carcinoma; and seven were negative. For the group that started with a Pap smear, three of these tests indicated HSIL, 11 were negative for neoplastic cells and two indicated ASC-US. Given these results, and in accordance with the derivation algorithm, a reassessment was performed and the previous procedures were repeated. Pap and colposcopy were obligatory, while the histological examination was made according to the colposcopic findings.

In "Phase 3" and as a result of the repeated examinations, 12 women were found (through biopsy) to have CIN I; 34 women were found to have CIN II/III; three women were found to have carcinoma and four women were negative. In the cases with Pap smears, 38 tests were performed and most of them (33) showed normal outcomes. These results mostly corresponded to patients

Table 1. Exfoliative cervical cytological examinations from primary healthcare clinics in Santiago, Chile, 2005 - 2006

Cytological finding	Result	Number	%
	Satisfactory	77,411	87.53
Negative cytological findings	Less than optimal	4,923	5.56
	Inadequate	4,145	4.68
Atypical			
Atypical squamous cells of undetermined significance (ASCUS)		619	0.69
Atypical squamous cells cannot exclude high-grade SIL (ASC-H)		106	0.11
Atypical glandular cells of undetermined significance (AGC-US)		25	0.02
Atypical glandular cells suggestive of adenocarcinoma *in situ*		2	0.00
	Low-grade Pap	503	0.56
Positive cytological findings	High-grade Pap	638	0.72
	Invasive cancer	66	0.07
Total		**88,438**	**99.94**



Neg = negative; ASCUS = atypical squamous cells of undetermined significance; LSIL = low grade squamous intraepithelial lesion; HSIL = high grade squamous intrapithelial lesion; CIN I = mild dysplasia; CIN II/III = moderate and severe dysplasia; CA = carcinoma.

Figure 1. Follow-up of women with high-grade squamous intraepithelial lesions (ASC-H) in Chile.

who had been treated by means of surgical conization consequent to the diagnostic-treatment biopsies that were performed prior to gathering material for Pap smears. Following conization, and in accordance with the Chilean Ministry of Health's guidelines, two Pap smears must be performed, with an interval of six months, before the patient returns to the primary healthcare clinic.

In "Phase 4", on the one hand, the outcomes from the earlier 14 biopsies are shown: four were classified as carcinoma, six as high-risk lesions and four as low-risk lesions. On the other hand, cytological outcomes are shown, and these mostly related to patients according to the process of epidemiological monitoring.

In "Phase 5," there were also results with high-grade lesions from a total of 20 women. In "Phases 6 and 7", the cases were predominantly monitored by means of Pap smears and there were four patients with CIN II/III and one with CIN I who was diagnosed via biopsy.

In this diagnostic search process, 166 oncotic cytological tests were performed via conventional Pap smears, with an average of 1.8 examinations per woman and a minimum of zero and maximum of five cytological tests per woman. With regard to histological examinations, the total numbers of uterine cervix biopsies guided by colposcopy was 158, with an average of 1.7 per woman, and a minimum of zero and maximum of four. The total number of cytological-histological examinations that patients required to obtain a definitive diagnosis was as follows:

- 1 biopsy = 27 women (29.3%);
- 2 biopsies = 46 women (50%);
- 3 biopsies = 8 women (8.7%);
- 4 biopsies = 3 women (3.3%);
- 2 Papanicolaou smears = 8 women (8.7%);

The average length of time from the ASC-H Pap smear to the final diagnosis of the most severe lesion was 5.29 months in the biopsy group and 12.26 months in the Pap smear group.

In evaluating this three-year cytological-histological follow-up period, the 92 women were found to have the following lesion distribution:

- 7 women had invasive carcinoma (7.6%);
- 49 women had high-grade lesions (53.3%);
- 26 women had low-grade lesions (28.2%);
- 10 women had normal cytological outcomes (10.9%).

DISCUSSION

ASC-H is a type of atypical smear characterized by follow-up that includes a range of clinical procedures to reach a definitive diagnosis. Knowledge of the prevalence of women carrying ASC-H is essential for estimating the proportion of women at risk of developing a high-grade lesion.

One of the first studies aimed at investigating the prevalence of this new cytological classification category was carried out by the College of American Pathologists in the United States in 2002-2003: this was a nationwide survey and it revealed that ASC-H represented approximately 0.2% of cytological interpretations.[12] These first studies indicated that using this category was relatively congruent with the frequency provided by the 2001 Bethesda System.[1] Thus, studies including this new category were started worldwide, thereby providing ranges of ASC-H prevalence. This prevalence ranged from 0.22% out of a total of 27,367 Pap smears performed in a screening program in India, up 8.8% out of a total of 12,188 Pap smears in South Africa.[13,14]

In Latin America, and specifically in Brazil, Yamamoto et al. found ASC-H smear prevalences of 0.23% in 2007 and 0.54% in 2008, out of a total of 56,179 smears collected through the Cervical Cancer Research and Control Program.[15] Within this line of research, our study results, which demonstrated ASC-H prevalence of 0.11%, or 106 Pap smears out of a total of 88,438 between 2005 and 2006, are congruent with the established 2001 Bethesda System (**Table 2**).[5,13-24]

Table 2. Prevalence of atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesions (ASC-H) in population screening studies

Reference	Place and period	Total Pap tests	Number of ASC-H cases	Prevalence of ASC-H %
Louro et al.[18]	Birmingham, USA January 2000 to December 2001	43,840	368	0.84
Selvaggi[19]	Wisconsin, USA March to September, 2002	9,214	25	0.27
Duncan and Jacob[22]	Tennessee, USA October 2002 to March 2004	60,390	414	0.69
Elsheikh et al.[20]	Indiana, USA July 2001 to July 2003	129,911	7,698	0.24
Barreth et al.[17]	Alberta, Canada January to December 2002	241,841	727	0.30
Saad et al.[16]	Pittsburgh, USA January 2003 to June 2004	152,495	800	0.52
Gupta et al.[13]	Noida, India January 2001 to December 2004	27,367	60	0.22
Patton et al.[5]	Tennessee, USA March 2003 to December 2006	150,702	591	0.39
Okonda et al.[14]	Swaziland, Southern Africa June 2004 to May 2006	12,188	1,072	8.8
Alsharif et al.[21]	Minnesota, USA July 2004 to December 2007	235,645	1,219	0.52
Yamamoto et al.[15]	São Paulo, Brazil 2007	30,869	72	0.23
Rekhi et al.[23]	Mumbai, Parel, India January 2005 to December 2008	9,190	29	0.31
Giorgi Rossi et al.[24]	Italy December 2007 to September 2008	3,410	10	0.29
Present study	Santiago, Chile 2005 to 2006	88,438	106	0.11

Concerning the percentage of ASC-H among the total volume of atypical squamous cells (ASC), the 2001 Bethesda System indicates that between 5 and 10% (approximately) should be expected.[1,3] In our study, the ASC-H prevalence (106) represented 14% of the total atypia (752 women). The national survey conducted by the College of American Pathologists (2002-2003) found that 5% of the squamous atypias corresponded to ASC-H.[12] In Brazil, Yamamoto et al. obtained 2,622 atypical Pap smears in 2007-2008 and 210 (0.8%) of these were ASC-H smears.[15]

For the clinical management of patients with ASC-H smears, the American Society for Colposcopy and Cervical Pathology (ASCCP) published an algorithm in relation to clinical management of these patients in order to unify the criteria.[25] In Chile, and taking this algorithm into consideration, the Chilean Ministry of Health implemented its "Clinical Guidance for Cervical Cancer" (2005 and update 2010), which includes flow charts for clinical decision-making, such as the "management algorithm for specialists or Cervical Pathology Clinics (CPC) from the time of the first atypical Pap smear according to the 2001 Bethesda System" and the "algorithm of diagnostic confirmation". In the first algorithm, it is established that women with atypical smears must be managed at a cervical pathology clinic.[26,27] The follow-up for our population study was started at a cervical pathology clinic. The population's demographic profile comprised young women (average age: 38.2 years), which was similar to the populations of several other studies, which had averages ranging from 29 to 38.1 years. It is noteworthy that these outcomes corresponded to women in the screened population (**Table 3**).[6,13,17,18,28]

Table 3. Average age of women with atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesions (ASC-H) in population screening studies

Reference	Average age
Louro et al.[18]	36.8
Barreth et al.[17]	29.0
Gupta et al.[13]	38.1
Bonvicino et al.[6]	35.6
McHale et al.[28]	32.8
Present study	38.2

Table 4. Outcomes from histology follow-up of women with smears showing atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesions (ASC-H)

Reference	Women with ASC-H with histological follow-up	Women with CIN II or higher
Selvaggi[19]	22	15 women (68%)
Louro et al.[18]	Pap test preparation types with ASC-H: 190 conventional smear 28 liquid-based preparation (ThinPrep)	Prevalence of CIN II+ according to Pap test preparation type: Liquid-based preparations = 9 (45%) Conventional smears = 70 (46.1%) There was no statistically significant difference in the incidence of CIN II or higher on subsequent biopsies after interpretation of ASC-H based on the preparation type
Duncan and Jacob[22]	99	40 women (40.4%)
Barreth et al.[17]	Follow-up on 517 women: Histological = 454 Cytological alone = 63	Prevalence of CIN II+ or HSIL according to examination type: Histological = 363 (79.9%) Cytological alone = 11 (17.5%)
Saad et al.[16]	Cytological and histological follow-up on women: 127 perimenopausal 90 postmenopausal	Prevalence of CIN II+ or HSIL according to menopausal period: perimenopausal = 28 (22.0%) postmenopausal = 5 (6%)
Elsheikh et al.[20]	Comparative: Women with ASCUS = 410 Women with ASC-H = 110	Prevalence of CIN II+ according to epithelial cell abnormalities: ASCUS = 35 women (8.5%) ASC-H = 49 women (44.6%)
Srodon et al.[3]	Histological follow-up on HPV (+) women ASCUS = 266 ASC-H = 45	Prevalence of CIN II+ in HPV(+) patients: ASCUS = 27 (10.2%) ASC-H = 18 (40.0%)
McHale et al.[28]	Histological follow-up on 229 women	Prevalence of CIN II/III according to time: At the initial colposcopy, 23 of the patients (10%) had histological evidence of CIN II/III. The cumulative risk of CIN II/III was 12.2% at 12 months
Gupta et al.[13]	Colposcopic-histological follow-up: ASCUS = 218 women ASC-H = 52	Prevalence of CIN II/III or higher according to epithelial cell abnormalities: ASCUS = 7 (3.2%) ASC-H = 16 (30.8%)
Bonvicino et al.[6]	Histological follow-up on 122 women	Number of follow-up cervical biopsies for a definitive diagnosis of CIN II+ First = 35 (72%) Second = 7 (14%) Third = 4 (8%) Fourth = 3 (6%) Total: 49 women (40%) with CIN II+
Mokhtar et al.[29]	Histological follow-up on 123 women	Prevalence ratio of CIN II/III according to age group > 40 years = 65.1% < 40 years = 47.5%
Patton et al.[5]	Histological follow-up on 195 women Postmenopausal = 89 women Pregnant = 44 women Postpartum= 27 women Contraceptive use = 35 women	Prevalence of CIN II+: Postmenopausal: 20 women (22.5%) Pregnant: 35 women (79.6%) Postpartum 18 women (66.7%) Contraceptive use: 21 (60%) The diagnosis of ASC-H in postmenopausal Pap smears has a low predictive value in stark contrast to the pregnant, postpartum, and contraceptive-use categories
Alsharif et al.[21]	Histological follow-up on 691 women	Prevalence of CIN II+ according to cytologic categories: LSIL = 370 (16.1%) LSIL-H = 112 (33.1%) HSIL = 468 (69.0%) ASC-H = 182 (26.3%)
Cytryn et al.[7]	Histological follow-up on 57 women	Prevalence of CIN II/III according to age group of women : < 50 years = 10 (22.2%) 50 years or older = 1 (8.3%) Total: 11 women (19.3%) biopsy with CIN II+
Present study	92	Prevalence of CIN II+: 56 women (60.9%)

CIN = cervical intraepithelial neoplasia; LSIL = low grade squamous intraepithelial lesion; HSIL = high grade squamous intrapithelial lesion.

In the present study, women with ASC-H atypia underwent on average 1.8 cytological tests per woman (0 to 5 Pap smears) and half of them (46 women) underwent a minimum of two biopsies, in order to reach the definite CIN II+ diagnosis. It is possible to compare these outcomes with Bonvicino's data, in which among 260 ASC-H smears, there was an average cytological-histological follow-up of 1.35 Pap smears per woman, with a range of 1 to 4, and the biopsy follow-up consisted of an average of 0.64 biopsies per woman. In other words, the majority of the women (72%) underwent only one biopsy for a diagnosis of CIN II+ to be reached.[6]

The average length of follow-up in the present study was 5.29 months, to reach the most severe diagnosis (CIN II+), using only the biopsy. For confirmation of this cytological-histological diagnosis, a period of 12.26 months was needed, during which two normal cytological examinations were needed, with an interval of six months between them. These results are contrary to those of Bonvicino, in which the CIN II/III diagnosis required 18.5 months.[6]

In our study, 60.9% (56 women) had a definitive high-grade diagnosis (CIN II+) that was verified by biopsy. Comparing this ratio with the literature, we found that it was intermediate between the studies by Saad et al.[16] and Barreth et al.,[17] which found that 6% and 79.9% of the women had high-grade lesions, respectively. **Table 4** shows 14 studies in which different variables were analyzed. For instance, Louro et al.[18] made a comparison between conventional collection and liquid-based smears, and found that in spite of being different techniques for cytological sample collection, they resulted in similar definitive diagnoses of CIN II.[18] This is consistent with the results obtained from the majority of the studies observed, which demonstrated CIN II+ prevalences of more than 40%, according to the biopsy results.[3,5,6,17-24,29]

Our results confirmed that at the time of the first ASC-H report, immediate intervention with colposcopy was necessary, followed by a biopsy. This validates the medical management of ASC-H that is required by the "management algorithm from the time of the first atypical Pap," which is included in the Chilean Ministry of Health's Clinical Guidelines for Cervical Cancer.

CONCLUSIONS

The follow-up of our cohort of women showed the following:

- The majority of the uncertain ASC-H diagnoses (82.6%) also had abnormal colposcopic results.
- During the follow-up on ASC-H smears, two out of every three women developed high-grade lesions (CIN II+), which were detected after an average of 3.15 months.
- Detection of high-grade lesions in a female population through screening reflects the importance of public health programs within the National Cervical Cancer Research and Control Program in Chile.

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Address for correspondence:
Fanny López Alegría
Capullo, 2245
Providencia — Santiago — Chile
CEP 7510-196
Tel. (56-2) 84729215
E-mail: fanny.lopez@usach.cl

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Relationship between mental health and spiritual wellbeing among hemodialysis patients: a correlation study

Relação entre saúde mental e bem-estar espiritual em pacientes de hemodiálise: um estudo correlacional

Beatriz Bertolaccini Martínez[I], Rodrigo Pereira Custódio[II]

Universidade do Vale do Sapucaí (Univás), Pouso Alegre, Minas Gerais, Brazil

[I]MD, MSc, PhD. Professor, Department of Medicine, Universidade do Vale do Sapucaí (Univás), Pouso Alegre, Minas Gerais, Brazil.

[II]Nursing Student. Universidade do Vale do Sapucaí (Univás), Pouso Alegre, Minas Gerais, Brazil.

KEY WORDS:
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PALAVRAS-CHAVE:
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ABSTRACT

CONTEXT AND OBJECTIVE: The stress of living with a terminal disease has a negative impact on the mental health of hemodialysis (HD) patients. Spirituality is a potential coping mechanism for stressful experiences. Studies on the relationship between spirituality and mental health among HD patients are scarce. The purpose of this study was to evaluate the relationship between mental health and spiritual well-being among HD patients.

DESIGN AND SETTING: Cross-sectional observational study on hemodialysis patients at a single center in Brazil, between January and December 2011.

METHODS: Mental health was assessed using the General Health Questionnaire and spiritual wellbeing was assessed using the Spiritual Wellbeing Scale; 150 HD patients participated in the study.

RESULTS: A significant correlation was found between mental health and spiritual wellbeing (P = 0.001). Spiritual wellbeing was the strongest predictor of mental health, psychological distress, sleep disturbance and psychosomatic complaints.

CONCLUSION: Poor mental health was associated with lower spiritual wellbeing. This has important implications for delivery of palliative care to HD patients.

RESUMO

CONTEXTO E OBJETIVO: O estresse de viver com uma doença terminal tem impacto negativo sobre a saúde mental de pacientes em hemodiálise. A espiritualidade é um mecanismo de enfrentamento em potencial para experiências estressantes. Estudos sobre a relação entre espiritualidade e saúde mental de pacientes em hemodiálise são escassos. O objetivo deste estudo foi avaliar a relação entre saúde mental e bem-estar espiritual dos pacientes em hemodiálise.

TIPO DE ESTUDO E LOCAL: Estudo observacional e transversal de pacientes em tratamento de hemodiálise de centro único no Brasil, no período de janeiro a dezembro de 2011.

MÉTODOS: A saúde mental foi avaliada pelo Questionário Geral de Saúde e o bem-estar espiritual foi avaliado usando a Escala de Bem-Estar Espiritual. Participaram do estudo 150 pacientes em hemodiálise.

RESULTADOS: Foi encontrada correlação significante entre a saúde mental e o bem-estar espiritual (P = 0,001). Bem-estar espiritual foi o mais forte preditor de saúde mental, sofrimento psíquico, distúrbios do sono e queixas psicossomáticas.

CONCLUSÃO: A saúde mental deficiente associou-se com menor bem-estar espiritual. Isso tem implicações importantes para a prestação de cuidados paliativos para pacientes em hemodiálise.

INTRODUCTION

Psychiatric disorders are common among hemodialysis (HD) patients and are associated with increased morbidity and mortality, and reduced quality of life.[1] Spirituality is an important factor in the quality of life of HD patients.[2] According to Koenig et al.,[3] spirituality is a personal quest to understand aspects of life, its meaning and the relationship with the sacred, which may or may not involve religious practices or formation of religious groups. Spirituality is a potential resource in relation to mental health and is a coping mechanism for stressful experiences.[4]

The relationship between spirituality and health is a relevant factor to be assessed among HD patients. However, there are few studies in the literature correlating spirituality and mental health in this population.

OBJECTIVE

This study was undertaken to evaluate the relationship between mental health and spiritual wellbeing among HD patients.

METHODS

This cross-sectional correlation study was approved by the Research Ethics Committee of Universidade do Vale do Sapucaí (Univás), Brazil, and was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments. Written informed consent was obtained from all patients prior to their inclusion in the study and anonymity was assured.

One hundred and sixty-eight HD patients from a single medical center in Brazil were considered for the study, but were excluded if they had hearing impairment (three subjects), were younger than 18 years of age (three subjects), had been on hemodialysis for less than 12 months (nine subjects) or declined to participate in the study (two subjects). The remaining 151 patients were approached and asked to participate in the study. Of these, 150 agreed to participate and one subject declined, resulting in 150 patient completing the study. The recruitment period was from January to December 2011.

Sociodemographic, economic and clinical data were obtained from all participants.

Mental health was assessed using the validated Brazilian Portuguese version of the General Health Questionnaire (GHQ).[5,6] The GHQ measures mental health and consists of 60 items assessing the presence or absence of current non-psychotic symptoms and common mental disorders. The items are grouped into five subscales: psychological stress, death ideation, performance anxiety, sleep disturbance, and psychosomatic complaints. The GHQ also yields a total score, in which higher scores indicate lower mental health status.

The validated Brazilian Portuguese version of the Spiritual Wellbeing Scale (SWBS) was used to assess spiritual wellbeing.[7,8] The SWBS contains 20 items, of which 10 assess religious wellbeing and 10 assess existential wellbeing. The total score, which is obtained by adding the scores for the two subscales, is a measurement of spiritual wellbeing.

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) 18.0 (SPSS Inc., Chicago, IL, USA). The results were expressed as means ± standard deviations, medians and frequencies. Pearson's correlation coefficient (r) was used for bivariate analysis. Stepwise multiple logistic regression analysis was used to evaluate the correlation of mental health with other variables when $P < 0.25$ in the bivariate analysis. The significance level was set at 5% ($P < 0.05$).

RESULTS

The sociodemographic, economic and clinical characteristics of the participants are listed in **Table 1**.

The correlations of GHQ scores with the other variables are shown in **Table 2**. We found that high total GHQ scores (poor mental health) were associated with lower household income ($P = 0.02$); psychological stress was associated with younger age ($P = 0.02$) and with the female gender ($P = 0.03$); death ideation, with alcohol drinking ($P = 0.03$); high performance anxiety, with shorter length of time on HD ($P = 0.005$); and sleep disturbance, with longer length of time on HD ($P = 0.01$).

Poor mental health and the presence of psychological stress, sleep disturbance and psychosomatic complaints were associated with lower existential and spiritual wellbeing ($P < 0.05$). The multiple logistic regression results showed that spiritual wellbeing was the strongest predictor of mental health ($P = 0.003$), psychological stress ($P = 0.006$), sleep disturbance ($P = 0.002$) and psychosomatic complaints ($P = 0.0003$), as shown in **Table 3**.

Table 1. Characteristics of the hemodialysis patients (n = 150)

Variables	n (%)
Age, years - mean ± SD (range)	56.9 ± 13.4 (20-90)
Male	93 (62)
< 4 years of education	81 (54)
Household income < US$ 300	33 (22)
Urban resident	118 (79)
Unemployed or retired	142 (95)
Alcohol drinking	63 (42)
Length of time on HD, months - median (range)	36 (12-216)
Etiology of chronic kidney disease	
Hypertension	60 (40)
Diabetes *mellitus*	47 (31)
Chronic glomerulonephritis	21 (14)
Polycystic kidney disease	11 (7)
Other	11 (7)

SD = standard deviation; HD = hemodialysis.

Table 2. Correlation of General Health Questionnaire (GHQ) scores with Spiritual Wellbeing Scale (SWBS) scores and patient characteristics

Variables	GHQ											
	Psychological stress		Death ideation		Performance anxiety		Sleep disturbance		Psychosomatic complaints		Total score	
	r	P	R	P	r	P	r	P	r	P	r	P
Age	-0.19	0.02	0.01	0.87	0.03	0.63	0.02	0.78	-0.03	0.65	-0.04	0.62
Male	-0.18	0.03	-0.05	0.47	0.12	0.13	-0.05	0.46	-0.13	0.10	-0.10	0.22
Income	-0.14	0.07	-0.04	0.59	-0.05	0.49	-0.09	0.25	-0.12	0.12	-0.19	0.02
Time on HD	0.09	0.24	0.13	0.09	-0.23	0.005	0.20	0.01	-0.02	0.78	0.001	0.99
Drinker	0.12	0.13	0.18	0.03	-0.11	0.18	0.15	0.08	0.07	0.37	0.13	0.12
SWBS												
Religious WB	-0.09	0.25	0.08	0.35	-0.04	0.6	-0.04	0.62	-0.1	0.08	-0.13	0.11
Existential WB	-0.2	0.01	0.02	0.8	-0.08	0.33	-0.26	0.001	-0.3	0.001	-0.27	0.001
Spiritual WB	-0.23	0.005	-0.01	0.91	-0.09	0.27	-0.26	0.001	-0.3	0.0001	-0.31	0.001

HD = hemodialysis; WB = wellbeing: household income, alcohol drinking and existential and spiritual wellbeing.

Table 3. Logistic regression analysis on General Health Questionnaire (GHQ) scores, Spiritual Wellbeing Scale (SWBS) scores and patient characteristics

GHQ	Variables	Adjusted R2	F	P
Psychological stress	Spiritual wellbeing	0.0511	7.9772	0.006
	I	0.146	3.4844	0.002
Death ideation	Alcohol drinking	0.0319	4.8735	0.03
	II	0.0505	3.907	0.02
Performance anxiety	Length of time on HD	0.0465	7.219	0.008
	II	0.0606	4.743	0.01
Sleep disturbance	Spiritual wellbeing	0.0685	10.88	0.002
	III	0.10	5.404	0.002
Psychosomatic complaints	Spiritual wellbeing	0.0937	15.304	0.0003
	IV	0.01284	4.242	0.002
Total score	Spiritual wellbeing	0.0967	15.848	0.0003
	V	0.01247	5.165	0.0009

I = adjusted according to age, sex, household income, length of time on hemodialysis, alcohol drinking, existential wellbeing and spiritual wellbeing; II = adjusted according to length of time on hemodialysis and alcohol drinking; III = adjusted according to alcohol drinking, existential wellbeing and spiritual wellbeing; IV = adjusted according to sex, household income and existential, religious and spiritual wellbeing; V = adjusted according to household income, alcohol drinking and existential and spiritual wellbeing.

DISCUSSION

Recent advances in HD technology have increased the life expectancy of HD patients, but their quality of life has not changed appreciably. HD patients still experience a number of adverse situations relating to health, survival, limitations in activities of daily living, losses and biopsychosocial changes.[9] These stressful situations result in psychiatric symptoms, especially depression and anxiety. Our results showed that there was an association between poor mental health and lower household income. This is consistent with the findings of studies conducted in the general population, indicating that financial stress may be an important source of distress in people's lives and may be associated with minor psychiatric disorders.[10,11]

The highest levels of psychological stress were observed among younger and female patients. Patients' life histories influence their present situation; thus, life experiences may trigger self-defense mechanisms against mental health disorders. In women, high levels of psychological stress may be attributed to factors including marriage, raising children, work and hormonal changes.[12-18]

Performance anxiety was associated with shorter length of time on HD. According to Diniz et al.,[19] the initial phase of HD results in psychological distress due to the required use of life-support equipment, which limits patient autonomy. Some studies have reported that patients undergoing HD develop strategies over time for coping with the disease and treatment, thus resulting in less impact on their mental health.[20,21]

The strongest predictor of death ideation was alcohol abuse in our study. Studies have reported that alcohol abuse is associated with increased risk of suicidal behavior.[22,23]

The results revealed that poor mental health was associated with lower spiritual wellbeing, and that psychological stress, sleep

disturbance and psychosomatic complaints were associated with lower existential and spiritual wellbeing. Spiritual wellbeing was a strong predictor of overall mental health, as well as psychological stress, sleep disturbance and psychosomatic complaints. This is in agreement with the findings of other studies, thus suggesting that spiritual wellbeing is a protective factor against minor psychiatric disorders.[8,24]

The higher the scores for spiritual wellbeing and, especially, for existential well-being are, the higher the likelihood of better mental health is. There are different ways of coping with disease and treatment. Suffering is a personal experience, but it is still possible to extract lessons from suffering and to rethink values, thereby giving life a new meaning.[25]

One limitation of this study is that the association between mental health and comorbidities, such as diabetes mellitus, bone disease, neurological deficits and cardiovascular diseases, was not investigated.

Further multicenter and prospective studies are necessary to better understand the relationship between spiritual wellbeing and mental health among HD patients.

CONCLUSION

In conclusion, our results revealed that spiritual wellbeing was negatively related to and the strongest predictor of psychological stress, sleep disturbance, psychosomatic complaints and mental health.

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Address for correspondence:
Beatriz Bertolaccini Martínez
Av. Alfredo Custódio de Paula, 320
Centro — Pouso Alegre (MG) — Brasil
CEP 37550-000
Tel. (+ 55 35) 3449-8772
E-mail: beatrizz@uai.com.br

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Cold ischemia or topical-ECMO for lung preservation: a randomized experimental study

Preservação pulmonar por isquemia fria ou ECMO-tópico: um estudo aleatório experimental

Alessandro Wasum Mariani[I], Israel Lopes Medeiros[II], Paulo Manuel Pêgo-Fernandes[III], Flavio Guimarães Fernandes[IV], Fernando Do Vale Unterpertinguer[IV], Lucas Matos Fernandes[V], Paulo Francisco Cardoso[VI], Mauro Canzian[VII], Fabio Biscegli Jatene[VIII]

Department of Experimental Surgery, Instituto do Coração (InCor), Hospital das Clínicas (HC), Faculdade de Medicina da Universidade de São Paulo (FMUSP), and Laboratório de Pesquisa em Cirurgia Torácica, Faculdade de Medicina da Universidade de São Paulo (LIM 61), São Paulo, Brazil

[I]MD, PhD. Attending Physician, Department of Thoracic and Cardiovascular Surgery, Instituto do Coração (InCor), Hospital das Clínicas (HC), Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

[II]MD, PhD. Attending Physician, Department of Thoracic Surgery, Hospital de Messejana, Fortaleza, Brazil.

[III]MD, PhD. Full Professor of Thoracic Surgery, Instituto do Coração (InCor), Hospital das Clínicas (HC), Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

[IV]Medical Student, Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

[V]MD. Attending Physician, Department of Thoracic and Cardiovascular Surgery, Instituto do Coração (InCor), Hospital das Clínicas (HC), Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

[VI]MD, PhD. Professor, Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

[VII]MD, PhD. Attending Physician, Department of Pathology, Instituto do Coração (InCor), Hospital das Clínicas (HC), Faculdade de Medicina de Universidade de São Paulo (FMUSP), São Paulo, Brazil.

[VIII]MD, PhD. Full Professor of Cardiovascular Surgery, Head of Thoracic and Cardiovascular Surgery Departament, Instituto do Coração (InCor), Hospital das Clínicas (HC), Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

KEY WORDS:
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PALAVRAS-CHAVE:
Preservação de órgãos.
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ABSTRACT

CONTEXT AND OBJECTIVE: Lung preservation remains a challenging issue for lung transplantation groups. Along with the development of *ex vivo* lung perfusion, a new preservation method known as topical-ECMO (extracorporal membrane oxygenation) has been proposed. The present study compared topical-ECMO with cold ischemia (CI) for lung preservation in an *ex vivo* experimental model.

DESIGN AND SETTING: Randomized experimental study, conducted at a public medical school.

METHOD: Fourteen human lungs were retrieved from seven brain-dead donors that were considered unsuitable for transplantation. The lung bloc was divided and each lung was randomized to be preserved by means of topical-ECMO or CI (4-7 °C) for eight hours. These lungs were then reconnected to an *ex vivo* perfusion system for functional evaluation. Lung biopsies were obtained at three times. The functional variables assessed were oxygenation capacity (OC) and pulmonary artery pressure (PAP); and the histological variables were lung injury score (LIS) and apoptotic cell count (ACC).

RESULTS: The mean OC was 468 mmHg (± 81.6) in the topical-ECMO group and 455.8 (± 54) for CI (P = 0.758). The median PAP was 140 mmHg (120-160) in the topical-ECMO group and 140 mmHg (140-150) for CI (P = 0.285). The mean LIS was 35.57 (± 4.5) in the topical-ECMO group and 33.86 (± 6.1) for CI (P = 0.367). The ACC was 25.00 (± 9.34) in the topical-ECMO group and 24.86 (± 10.374) for CI (P = 0.803).

CONCLUSIONS: The present study showed that topical-ECMO was not superior to cold ischemia for up to eight hours of lung preservation.

RESUMO

CONTEXTO E OBJETIVO: A preservação pulmonar permanece um desafio para os grupos transplantadores. Com o desenvolvimento da perfusão pulmonar *ex vivo*, foi proposto um novo método de preservação chamado de ECMO-tópico (oxigenação de membrana extracorpórea). O presente estudo compara ECMO-tópico com isquemia fria (IF) para preservação pulmonar em um modelo experimental *ex vivo*.

TIPO DE ESTUDO E LOCAL: Estudo experimental randomizado, conduzido em uma faculdade de medicina pública.

MÉTODO: Quatorze pulmões humanos foram retirados de sete doadores de morte cerebral considerados não aptos a transplante. O bloco pulmonar foi dividido e cada um foi aleatorizado para preservação por ECMO-tópico ou IF (4-7 °C) durante oito horas. Esses pulmões foram então re-conectados a um sistema de perfusão *ex vivo* para avaliação funcional. Biópsias pulmonares foram obtidas em três tempos. As variáveis funcionais avaliadas foram: capacidade de oxigenação (CO) e pressão de artéria pulmonar (PAP). As variáveis histológicas estudadas foram escore de lesão pulmonar (ELP) e contagem de células apoptóticas (CCA).

RESULTADOS: A média da CO foi de 468 mmHg (± 81.6) no grupo ECMO-tópico e 455.8 (± 54) no grupo IF (P = 0,758); a PAP média foi de 140 mmHg (120-160) para ECMO-tópico e 140 mmHg (140-150) para IF (P = 0,285); o ELP médio foi 35,57 (± 4,5) no ECMO-tópico e 33,86 (± 6,1) no IF (P = 0,367). A CCA foi 25,00 (± 9,34) no grupo ECMO-tópico e 24,86 (± 10,374) no IF (P = 0,803).

CONCLUSÕES: O presente estudo demonstrou que o ECMO-tópico não é superior a IF para oito horas de preservação pulmonar.

INTRODUCTION

Lung transplantation has been established as a treatment option for end-stage lung disease.[1] However, the low tolerance of lungs to ischemia is notorious and can lead to graft dysfunction that impacts on the recipient's outcome.[2] Despite the variety of preservation techniques proposed, such as topical cooling,[3] autoperfusion with extracorporeal circulation[4] and donor core cooling,[5] pulmonary artery flush perfusion with cold preservation solution has endured the test of time and has remained the most frequently used technique because of its practicality and effectiveness.[6] Extracellular preservation solutions such as Perfadex (VitroLife AB, Gothenburg, Sweden) and Celsior (Sang Stat, Lyon, France) have been used frequently for lung preservation.[7]

Concomitantly to the introduction of *ex vivo* lung perfusion, a method of preservation named topical-ECMO (extracorporeal membrane oxygenation) has been proposed by Steen et al. It has been tested experimentally[8] and used clinically.[9,10] This method was designed to preserve the lungs after *ex vivo* assessment and consists of immersion of the lung in a semi-inflated state in Steen solution diluted in Perfadex inside the *ex vivo* box where the lungs were placed after reconditioning. Although topical-ECMO has been proposed and used clinically, the method has not been compared with cold ischemia after single-flush perfusion for preservation so far.

OBJECTIVE

The aim of this study was to evaluate whether topical-ECMO can provide better preservation quality than shown by regular cold ischemia, as methods for lung preservation.

METHODS

This was a randomized experimental study approved by our university hospital's ethics committee (CAPPESQ 0212/08). Between December 2009 and August 2010, lungs retrieved from brain-dead donors that were refused for transplantation based on current clinical criteria were used in this study. The organs were included in the protocol if all efforts to recover the lungs failed. Written consent was obtained from family members of the donors to permit the use of the organs in this study. The lungs were perfused through the pulmonary artery with cold Perfadex (Vitrolife, Gothenburg, Sweden) and harvested in the usual fashion at the time of multiorgan retrieval. The organs were immersed in cold Perfadex, stored in a cooler and transported to our laboratory.

Upon arrival, the lung block was dissected out, and the left and right lungs were separated by sectioning the left atrium, main pulmonary artery and the tracheal carina (**Figure 1**). Separation of the lungs allowed each lung to be subjected to a different forms of preservation. The lungs were then randomly assigned for topical-ECMO or cold ischemia (CI). The randomization was done by means of numbered envelopes containing computer-generated random sequences of numbers.

Topical-ECMO was carried out by means of complete lung immersion in solution (Steen Solution; VitroLife AB, Gothenburg, Sweden) within a containment box (VitroLife AB, Gothenburg, Sweden) (**Figure 2**). The solution was continuously circulated through this box by means of a centrifugal pump (Braile Biomedica, São José do Rio Preto, Brazil) with a flow of 4 l/min and through a membrane oxygenator (Braile Biomedica, São José do Rio Preto, Brazil) receiving oxygen at 5 l/min in order to achieve oxygenation. The average temperature was maintained by means of a heat exchanger with a target of 8 °C. The lung temperature was monitored continuously using a thermometer placed inside the pulmonary vein.

CI lungs were stored in a bag containing Perfadex solution and were immersed in a second plastic bag containing saline solution at 4 °C. The bags were then stored in a refrigerator at a temperature of 4-7 °C.

After eight hours of preservation, the left and right lungs were reconnected in parallel, by means of two Y-shaped cannulae: one in the trachea and the other in the pulmonary artery. The pulmonary veins remained separated. This reconnection technique made it possible for the topical-ECMO and CI lungs to undergo reperfusion and ventilation in the *ex vivo* perfusion system simultaneously, with the same reperfusion solution and exactly the same ventilation parameters. Since the pulmonary veins remained independent, it enabled sampling for blood gas analysis separately (**Figure 3**).

The system used during the reperfusion phase consisted of the XVIVO box (VitroLife AB, Gothenburg, Sweden), attached to the centrifugal pump, heat exchanger, membrane oxygenator and a venous reservoir (Braile Biomedica, São José do Rio Preto, Brazil), as we previously described.[11] The Y cannula for the pulmonary arteries had two separate probes connected to pressure transducers that allowed continuous and independent monitoring of pulmonary artery pressure. The right and left lung pulmonary vein effluents were collected directly into the XVIVO box, where they were mixed and drained into the venous reservoir by gravity. We elected to keep the atria open in order to facilitate assembly of the system, by eliminating the need for special atrial cannulae. This is feasible during short-term perfusion, whereas in cases requiring long-term perfusion, a closed system is preferred so as to avoid pulmonary edema.[12] The system was filled with 1,500 ml of Steen Solution (VitroLife AB, Gothenburg, Sweden), and we chose to use a bloodless cellular solution. The pH was adjusted between 7.35-7.45 by adding trometamol (Addex-THAM; Fresenius-Kabi AB, Uppsala, Sweden). A maximum perfusion flow of 40% of the estimated cardiac output (CO) was utilized, and this was calculated

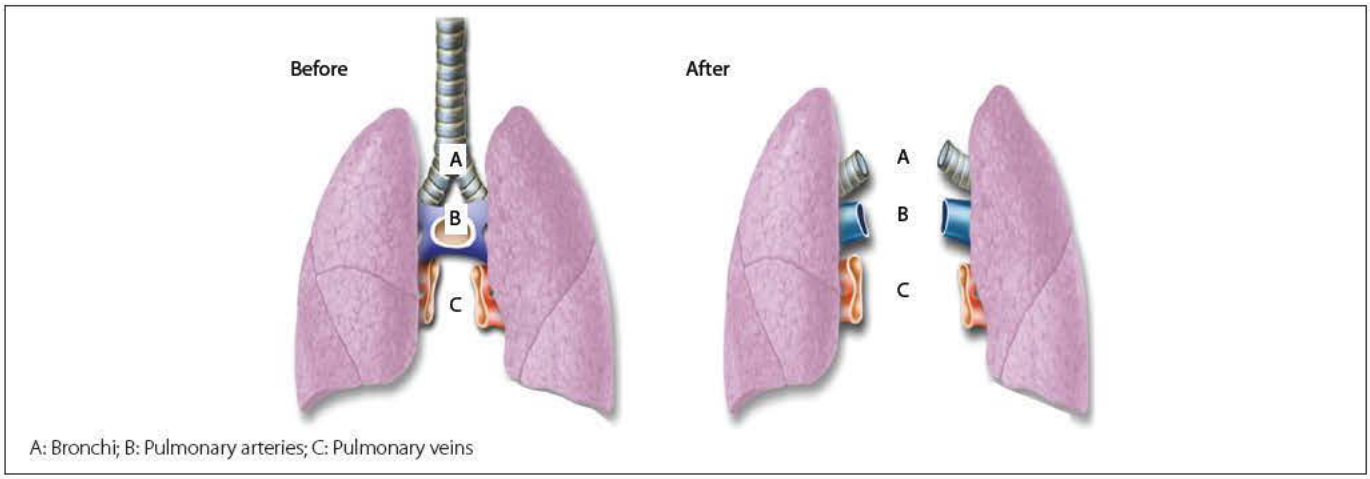


Figure 1. Preparation of the double-lung block before (left) and after (right) separation of the grafts.

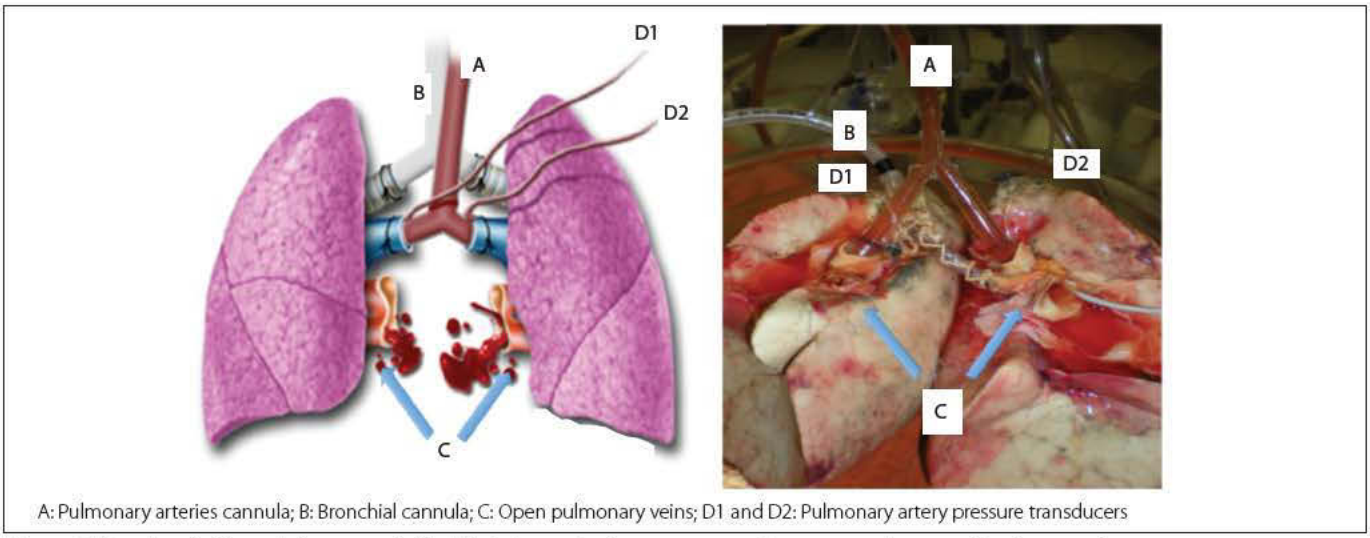
Figure 2. Containment box for topical-ECMO, with lung immersed in Steen Solution.

using a formula based on the size of the donor (CO = 3 * body surface area, assuming target cardiac index = 3). This flow was sufficient to assess the lungs in the system and low enough to avoid pulmonary edema formation.

A steady state was usually reached after 60 minutes of perfusion, and perfusate gases were collected and hemodynamics were recorded at this time. The variables assessed were pulmonary arterial partial oxygen pressure (PvO_2), pulmonary partial oxygen pressure of the effluent from the pulmonary veins (PaO_2) and partial carbon dioxide ($PaCO_2$), pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR) [PVR = PAP/pulmonary artery flow * 80 (dynes/sec/cm^5)].

Lung tissue samples from the middle lobe and ligulae were collected before harvesting, after the topical-ECMO or cold ischemia period and after *ex vivo* lung perfusion (EVLP). The samples were fixed in 10% buffered formalin for 24 hours, embedded in paraffin, sectioned at thicknesses of 5 mm and stained with hematoxylin and eosin (**Figure 4**). For all cases, semiquantitative scoring was performed by an experienced lung pathologist using the following histology parameters: interstitial edema, intra-alveolar edema, arteriolar thickening, vascular thrombosis, intra-alveolar hemorrhage, intra-alveolar fibrin deposition, necrosis, inflammatory cell infiltrate, pleural infiltrate, peribronchiolar inflammatory reaction, organizing pneumonia, peribronchiolar fibrosis, fibroblast foci, peribronchiolar muscular hypertrophy, pleural plaques, interstitial fibrosis, alveolar lining cell hyperplasia, alveolar macrophages, pigmented macrophages, denudated bronchiolar epithelium, vasculitis and emphysema. The severity of these findings was determined using a four-grade scale: absent = 0; minimal = 1; moderate = 2; and intense = 3. The sum of each parameter resulted in the Lung Injury Score (LIS), with values ranging from 0 to 66.[13]

Apoptosis was assessed by means of *in situ* terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate nick end labeling (TUNEL), using the In Situ Cell Death Detection Kit (Roche, Basel, Switzerland). The TUNEL method is based on the enzymatic ability of TdT to catalyze a template-independent addition of deoxyribonucleotide triphosphate to the 3=-OH ends of double- or single-stranded DNA. The sections were deparaffinized and rehydrated. Protein digestion was done by applying proteinase K to the slides for 15 minutes at room temperature, which was followed by four washes in distilled water for two minutes each. Equilibration buffer was applied to the sections, which were then incubated in a humidified chamber for three minutes. Following this, the sections were incubated with TdT in a humidified chamber at 37 °C for 1 hour. Fluorescein-labeled antidigoxigenin antibody was applied to the sections, and they were incubated in a humidified chamber for 30 minutes at



A: Pulmonary arteries cannula; B: Bronchial cannula; C: Open pulmonary veins; D1 and D2: Pulmonary artery pressure transducers

Figure 3. Drawing (left) and photograph (right) of the *ex vivo* lung system with reconnection used in the experiments.

room temperature. The sections were then washed with phosphate-buffered saline.

Immediately after completing the protocol, the slides were viewed using a fluorescence microscope and photographed using a charge-coupled device digital camera with a 590-nm emission filter. To localize apoptotic cells, randomly selected specimens of lung tissue were photographed under the fluorescence optical microscope at high magnification (original magnification 400 x). Apoptotic cells appeared as bright green (**Figure 5**). Counts were obtained from five randomly chosen fields per slide, representing a total area of 0.1 mm^2. An independent, blinded examiner performed the cell counting.

Immunohistochemical analysis was performed using the polyclonal rabbit antibody for CD3 (1:100, Abcam, Cambridge, United Kingdom). The sections were deparaffinized and a 0.3% hydrogen peroxide solution was applied for 35 min to inhibit endogenous peroxidase activity. Antigen retrieval was performed using citrate solution for 45 minutes. The sections were incubated with the primary antibody overnight at 48 °C. The streptavidin-biotin complex (LSAB+; DakoCytomation, Carpinteria, CA, United States) was used as the secondary antibody and 3,3-diaminobenzidine (DAB) (Sigma Chemical Co, St. Louis, MO, United States) was used as the chromogen. The sections were counterstained using Harris hematoxylin. For negative controls, the primary antibody was replaced with phosphate-buffered saline (PBS). The inflammation index was determined semiquantitatively based on the presence of inflammatory cells in lung tissue, using a three-grade scale: minimal inflammation = 1, moderate inflammation = 2 and marked inflammation = 3.

All histological and immunohistochemical parameters were evaluated and measured by a pulmonary pathologist who was blinded to any information regarding the cases.

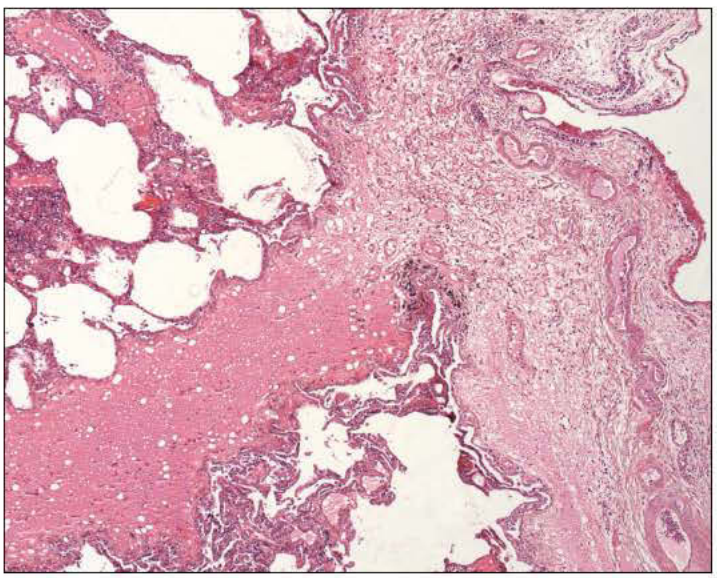


Figure 4. Histological appearance of the lung after *ex vivo* perfusion (original magnification 100 x; hematoxylin-eosin).

Lungs were weighed immediately after harvesting, at the end of the preservation time and after reperfusion. They were also subsequently dried out for 48 hours at 70 °C and weighed again in order to obtain the wet/dry weight ratio.

The normality tests applied were Kolmogorov-Smirnov and Shapiro-Wilk. To compare functional and histological data from before to after the procedure, Student's paired-samples test was performed. The Wilcoxon signed rank test was used for variables that were not normally distributed. Repeated-measurement ANOVA was used to analyze the differences in measurements that evolved over time in pairs of groups. For qualitative variables in 2 x 2 tables, we used the chi-square test, or Fisher's exact test when the expected value was less than five. In tables larger

than 2 x 2, the likelihood function was used because the expected value was less than five in all cases. The results were expressed as the mean and standard error of the mean, or as the median and interquartile range for variables that were not normally distributed. The statistical analyses were performed using the SPSS (Statistical Package for the Social Sciences) 18.0 software (SPSS Inc, Chicago, IL) with a confidence interval of 95% and a significance level of 0.05.

RESULTS

Seven sets of lungs were assessed. The demographics are summarized in **Table 1**. During reperfusion, the oxygenation capacity was estimated from the PaO_2 measured in the effluent perfusate from the pulmonary veins of the lungs. For the lungs preserved by means of topical-ECMO and CI, respectively, the capacities were 468 ± 81.6 mmHg and 455.86 ± 54 mmHg, with no statistically significant differences between the groups (P = 0.758). The mean partial oxygen pressure of the deoxygenated perfusate inflow during EVLP (PvO_2) was 91.94 ± 24.4 mmHg. The mean arterial $PaCO_2$ in lungs preserved by means of topical-ECMO and CI were 17.45 ± 3.6 mmHg and 17.02 ± 3.1 mmHg respectively, with no statistically significant differences between the groups (P = 0.617).

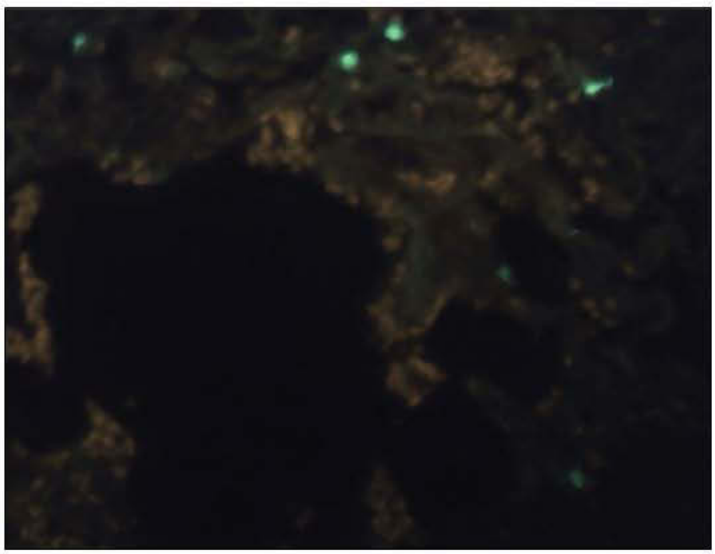


Figure 5. Photomicrograph demonstrating apoptotic cells (green) under a fluorescence optical microscope.

Table 1. Demographics and clinical characteristics of the donors

Donor data	Results
Gender	
Male	4 (57.1%)
Female	3 (42.8%)
Age (years)	53.86 ± 16.7
Time on mechanical ventilation (days)	4.86 ± 3.93
PaO_2 (mmHg)	192.2 ± 92.2
PCO_2 (mmHg)	33.1 ± 9.6
Cause of death	
Hemorrhagic stroke	5 (71.4%)
Head trauma	2 (28.6%)
Reason for rejection for transplantation	
PaO_2 < 300 mmHg	6 (85.7%)
Bilateral contusion	1 (14.3%)
Side of topical-ECMO	
Right	4 (57.1%)
Left	3 (42.9%)

ECMO = extracorporal membrane oxygenation.

Hemodynamics showed that the median PAP in topical-ECMO lungs was 140 mmHg (120-160) and in CI lungs, 140 mmHg (140-150); P = 0.285. The median PVR also showed no statistically significant differences between the groups: topical-ECMO = 459 dynes/sec/cm^5 and CI = 474.50 dynes/sec/cm^5; P = 0.285.

The mean weight changes of the lungs between the groups over time (pre-ischemic, post-ischemic and post-reperfusion times) did not show any significant differences, as depicted in **Figure 6**. The wet-to-dry ratios in the topical-ECMO and CI groups were 2.77 ± 0.93 and 3.21 ± 1.85, respectively, with no statistically significant difference (P = 0.358). The lung histology, studied by means of LIS and the apoptotic cell count (ACC), did not show any significant differences between the groups over time (LIS P = 0.531 and ACC P = 0.803), as shown in **Figures 7** and **8**.

The degree of tissue inflammation was assessed by detecting the presence of inflammatory cells. Infiltration of CD3+ T-lymphocytes into the lung tissue was evaluated at three times and did not significantly differ between the groups, as shown in **Table 2**.

DISCUSSION

Our experiments showed that there were no differences between topical-ECMO and CI in terms of preservation quality, hemodynamic performance and histology of the lungs assessed.

Although topical-ECMO was created and has been used as a method for lung preservation at Lund University in Sweden, it has not been tested for efficacy or safety. In the first paper in which this method appears, 12 pigs were used as non-heartbeat donors and lung function was assessed *ex vivo* after harvesting. After the *ex vivo* evaluations had been completed, the lungs were cooled and preserved using topical-ECMO at a temperature of 12 °C, until transplantation.[8] The next paper described the first human case that received a lung after *ex vivo* evaluation. After perfusion, the left lung was stored using topical-ECMO at 8 °C and remained there for nine hours and 30 minutes, until transplantation.[9]

In 2009, the same group published six human cases of transplantation after *ex vivo* lung perfusion. Once again, after the *ex vivo* perfusion had been completed, the lungs were cooled down

Table 2. Degree of CD3 T-lymphocyte infiltration into lung tissue at three times

Time 1	Topical-ECMO	CI
Mild	85.70%	85.70%
Moderate	14.3%	14.3%
Severe	0	0
		P = 1.000
Time 2	**Topical-ECMO**	**CI**
Mild	71.40%	57.10%
Moderate	14.30%	42.90%
Severe	14.30%	0
		P = 0.280
Time 3	**Topical-ECMO**	**CI**
Mild	71.40%	85.70%
Moderate	14.30%	14.30%
Severe	14.30%	0
		P = 0.478

ECMO = extracorporal membrane oxygenation; CI = cold ischemia.

and stored using topical-ECMO until transplantation. The mean duration of use of topical-ECMO was five hours and 53 minutes for the first implanted lung and 8 hours and 46 minutes for the second lung.[10] Despite the good results in all these papers, it cannot be affirmed whether the use of topical-ECMO interfered with lung preservation or with lung function, either positively or negatively.

Since the time when the topical-ECMO method was designed, this is the first comparative study evaluating it against any other preservation strategy. Our use of rejected human lungs in an experimental study was motivated by the aim of testing the method in a realistic situation that would also be more representative of the clinical lung transplantation scenario. The study was designed such that it would optimize the use of rejected donor lungs and, likewise, would reduce variability by using both lungs from the same donor, i.e. one lung for each of two different preservation techniques.

The difference between the topical-ECMO described by Steen et al. and the one used here was the solution. While the Swedish group[8-10] used a mixture of Steen Solution, Perfadex and a variable quantity of red blood cells in order to reach a hematocrit level of around 5%, pure Steen Solution was used in the present work.

The concept of splitting the double-lung block and sharing both the airway and the inflow perfusion connections had been tested previously in a pilot study in our laboratory and was proven to be feasible without significant functional deterioration of the lungs.[14]

The oxygenation capacity during the *ex vivo* perfusion can be considered to be the most important parameter for functional assessment, since it reflects both gas exchange and graft performance. Previous studies have shown that PaO_2 values correspond more reliably to the quality of lung preservation than do other parameters such as those from pathology or radiology.[15] In the

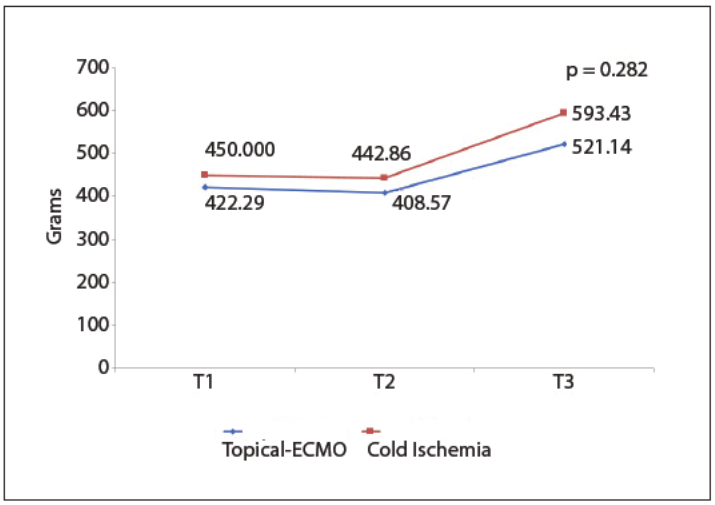


Figure 6. Lung weight gain between groups over time comparing topial ECMO (extracorporal membrane oxygenation) and CI (cold ischemia).

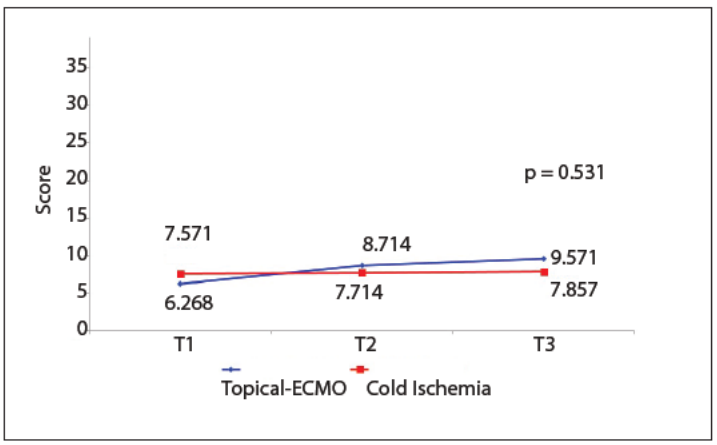


Figure 7. Comparison of lung injury scores between groups over time comparing topical ECMO (extracorporal membrane oxygenation) and CI (cold ischemia).

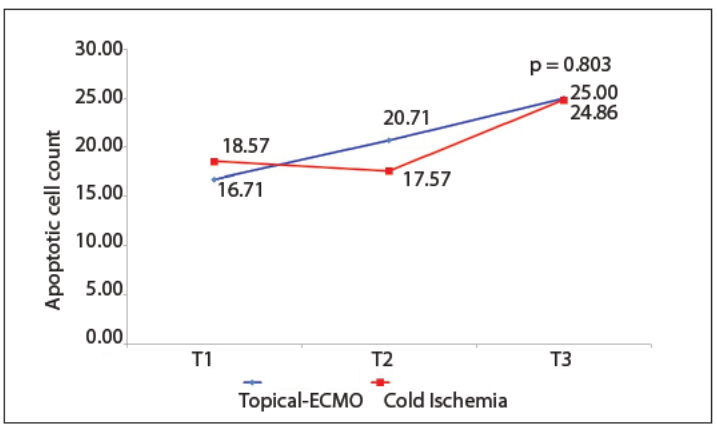


Figure 8. Comparison of apoptotic cell counts between groups over time comparing topical ECMO (extracorporal membrane oxygenation) and CI (cold ischemia).

present study, the absence of significant differences between the groups demonstrates that oxygen capacity and CO_2 clearance were similar in both groups.

The parameter of weight variation has been used experimentally as a reliable measurement of pulmonary edema in the setting of lung preservation. The process of ischemia and reperfusion results in increased vascular permeability and disruption of the alveolar-capillary barrier, which ultimately causes water extravasation. The amount of edema can be therefore considered to be inversely proportional to the quality of preservation.[16] Despite the usefulness of weight variation in determining edema, its accuracy is debatable since it is influenced by other factors such as alveolar hemorrhage, which also renders the method less accurate for detection of milder degrees of edema. In our study, the weight gain after reperfusion was similar in both groups and possibly reflected the degree of edema inherent to the *ex vivo* reperfusion, which may become an adverse factor during longer reperfusions, as described previously.[12] On the other hand, the wet-to-dry ratio has been found to be a more reliable measurement of the amount of water in the specimens at the end of reperfusion.[17] Therefore, the higher the value is, the greater the edema will be. In our study, the wet-to-dry ratio was similar in both groups, thus suggesting that similar amounts of edema were present regardless of the preservation strategy used.

There are two major limitations regarding the histological changes found in the lungs: the degree of the histological changes does not show any linear correlation with the functional outcome; and the presence of pulmonary parenchymal lesions before harvesting, induced by pro-inflammatory agents secondary to brain death, may be great enough to cause profound changes to the pulmonary tissue.[18] Nevertheless, histological evaluation remains a powerful indicator of the quality of lung preservation.[19] The injury score used in this study (ELP) was similar between the groups, both after the period of preservation and after reperfusion. This finding shows that the two preservation methods yielded comparable preservation quality.

ACC performed by means of an immunohistochemical technique (TUNEL) has been studied in human lung transplant cases.[20] Fischer et al. found that there was a significant increase in the number of apoptotic cells two hours after graft reperfusion.[21] These studies demonstrated that there was a significant correlation between the percentage of necrotic cells and deterioration of graft function, as estimated by means of PaO_2 after implantation. In our study, the ACC was equivalent in the two preservation techniques.

Immunohistochemistry was used to stain CD3+ cells. The lymphocytic infiltrate was quantified in order to assess the magnitude of the inflammatory infiltrate, and we found no differences between the groups. This was indicative that the two preservation methods were comparable with regard to inflammation. On the other hand, the shorter period of *ex vivo* reperfusion in our study (eight hours) may have played a role in these findings. Cypel et al. used a similar method for comparing lung preservation by means of an *ex vivo* reperfusion system, with cold ischemia for twelve hours. Their study showed that *ex vivo* reperfusion presented lower levels of lymphocyte infiltrate, thus indicating lower intensity of inflammation and therefore better lung preservation.[22]

The present study has several limitations. The small number of cases and the presence of lung injury prior to harvesting, plus the fact that no lungs were transplanted and reperfused, are among the most prominent of these. Because of the great difficulty in obtaining human lung donors for experimental research (small numbers of cases, non-acceptance by the relatives and logistic difficulties), we had to base our sample on opportunity sampling (with no prior sample calculation). The high variability between cases of lung donors that may exist in such investigations was the reason for developing the "split lung block technique".[14] Our attempt to minimize bias by pairing each set of lungs from the same donor may have mitigated some of the factors, but many aspects remain unclear and will require future studies. The small number of cases makes it impossible for us to extrapolate the results from this study to the general population.

The implications from this study for lung transplantation practice lie in the fact that the topical-ECMO technique, which is more complex and expensive, does not seem to bring any benefits regarding lung preservation. Within the field of research, this work may contribute through providing additional data on the use of *ex vivo* lung perfusion systems, in ischemia-reperfusion studies.

CONCLUSION

The results show that topical-ECMO does not seem to improve lung preservation, compared with cold ischemia, for up to eight hours of preservation.

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Address for correspondence:
Alessandro Wasum Mariani
Rua Treze de Maio, 1.217 - apto 31
São Paulo (SP) — Brasil
CEP 01327-000
Tel. (+55 11) 2661-5248
E-mail: alessandro_mariani@hotmail.com

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Frequencies of interleukin-6, GST and progesterone receptor gene polymorphisms in postmenopausal women with low bone mineral density

Frequência do polimorfismo da interleucina-6, GST, e dos receptores de progesterona em mulheres na pós-menopausa com baixa densidade mineral óssea

Katia Franco Moura[I], Mauro Haidar[II], Claúdio Bonduki[III], Paulo Cezar Feldner Júnior[III], Ismael Silva[II], José Maria Soares Júnior[II], Manoel João Girão[IV]

Department of Gynecology, Universidade Federal de São Paulo – Escola Paulista de Medicina (Unifesp-EPM), São Paulo, Brazil

[I]Postgraduate Student, Department of Gynecology, Universidade Federal de São Paulo – Escola Paulista de Medicina (Unifesp-EPM), São Paulo, Brazil.

[II]MD, PhD. Adjunct Professor, Department of Gynecology, Universidade Federal de São Paulo – Escola Paulista de Medicina (Unifesp-EPM), São Paulo, Brazil.

[III]MD, PhD. Affiliated Professor, Department of Gynecology, Universidade Federal de São Paulo – Escola Paulista de Medicina (Unifesp-EPM), São Paulo, Brazil.

[IV]MD, PhD. Titular Professor, Department of Gynecology, Universidade Federal de São Paulo – Escola Paulista de Medicina (Unifesp-EPM), São Paulo, Brazil.

KEY WORDS:
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PALAVRAS-CHAVE:
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Pós-menopausa.

ABSTRACT

CONTEXT AND OBJECTIVE: Osteoporosis is a skeletal disorder characterized by low bone mineral density (BMD). Studies have shown that some of the genetic components relating to lower BMD may be detected by polymorphisms. Our aim was to evaluate the frequencies of interleukin-6, GST and progesterone receptor gene polymorphisms in postmenopausal women with low BMD.

DESIGN AND SETTING: Cross-sectional study, conducted in a public university in São Paulo, Brazil.

METHODS: We evaluated interleukin-6 (IL-6), progesterone receptor gene (PROGINS) and glutathione S-transferase (GST) polymorphisms in 110 postmenopausal women with no previous use of hormone therapy. Tests were performed using DNA-PCR, from oral scrapings. We used Student's t-test and a logistic regression model for statistical analysis.

RESULTS: Regarding IL-6 polymorphism, 58.2% of the patients were homozygotes (GG) and 41.8% had allele C (heterozygote or mutant homozygote + GC or CC). PROGINS genotype polymorphism was absent in 79% (wild homozygote or P1/P1) and present in 20.9% (heterozygote or P1/P2). Regarding GSTM1 polymorphism, the allele (1/1) was present in 72.7% of the patients and was absent in 27.3%. We found that IL-6 polymorphism had statistically significant correlations with the L2-L4 T-score (P = 0.032) and with BMD (P = 0.005). Women with IL-6 polymorphism were 2.3 times more likely to have a L2-L4 T-score of less than -1, compared with those not presenting this polymorphism.

CONCLUSION: IL-6 gene polymorphism was correlated with low BMD, whereas the PROGINS and GSTM1 polymorphisms did not show any correlation.

RESUMO

CONTEXTO E OBJETIVO: A osteoporose é uma desordem esquelética caracterizada por baixa densidade mineral óssea. Estudos têm demonstrado que alguns componentes genéticos relacionados com a menor densidade mineral óssea podem ser detectados por polimorfismos. Nosso objetivo foi avaliar a presença do polimorfismo de genes em mulheres pós-menopáusicas com baixa densidade mineral óssea.

TIPO DE ESTUDO E LOCAL: Estudo transversal, conduzido em universidade pública em São Paulo, Brasil.

MÉTODOS: Avaliamos os polimorfismos relacionados à interleucina-6 (IL-6), o gene receptor de progesterona (PROGINS) e glutationa S-transferase (GST) em 110 mulheres na pós-menopausa sem terapia hormonal prévia. Os testes foram realizados com DNA-PCR a partir de raspados orais. Foram utilizados teste t de Student e modelo de regressão logística para análise estatística.

RESULTADOS: Em relação ao polimorfismo IL-6, 58,2% dos pacientes eram homozigotos (GG) e 41,8% tinham o alelo C (heterozigoto ou homozigoto mutante + GC ou CC). Nos genótipos do polimorfismo PROGINS, 79% estavam ausentes (homozigoto selvagem ou P1/P1) e 20,9% presentes (heterozigoto ou P1/P2). No polimorfismo do GSTM1, o alelo (1/1) estava presente em 72,7% dos pacientes e ausente em 27,3%. Encontramos significância estatística entre o polimorfismo genético da IL-6 com o T-*score* de L2-L4 (P = 0,032) e a densidade mineral óssea (P = 0,005). As mulheres com polimorfismo da IL-6 tiveram 2,3 vezes mais chance de ter menos de -1 na L2-L4 T-*score*, quando comparadas às não portadoras.

CONCLUSÃO: O polimorfismo do gene da IL-6 está correlacionado com baixa densidade mineral óssea, enquanto os polimorfismos GSTM1 e PROGINS não mostraram correlação.

INTRODUCTION

Osteoporosis is a skeletal disorder characterized by low bone mineral density (BMD) and deterioration of bone microarchitecture, thus predisposing towards a risk of fractures. It is estimated to affect more than 75 million people worldwide. The risk factors among women include: race, lower height, body mass index, low-calcium diet, use of corticosteroids for over six months, smoking and menopausal status.[1]

Earlier studies showed that environmental effects and genetic control influenced bone turnover.[2,3] Back in 1991, genetic inheritance was shown to be 45-85% likely to be a determinant of mineral density.[2] Since then, various genes have been investigated.[3-7] Studies have shown that some of the genetic components relating to lower BMD may be detected by gene polymorphisms such as interleukin-6 (IL-6) polymorphism.[8-11]

IL-6 is a phosphoric acid-containing glycoprotein with 185 amino acids and it is located in chromosome 7p21. It is a multifunctional cytokine produced by mononuclear cells and is regulated by the presence of polymorphisms. In fact, the C-G exchange in nucleotide 174 affects the transcription of this gene by decreasing its expression. IL-6 gene transcriptional activity is also marked in the presence of polymorphism in the promoter region (174 G/C). Presence of the C allele mutant is associated with low IL-6 plasma levels; however, presence of the G wild allele is related to high plasma levels of this cytosine.[12] Some authors have observed increased IL-6 levels in postmenopausal women in response to lower levels of estradiol,[13] and that gene polymorphism influenced bone resorption.[14]

Luo et al.[15] reported that progesterone stimulated osteoblast proliferation and differentiation. This led to increased growth factors in osteoblasts, thus stimulating their proliferation and extracellular matrix synthesis. Progesterone receptor (PR) genes are located in the long arm of chromosome 11 (bands q22-23). Recently, variations in PR genes have been described, such as *PROGINS*. This polymorphism consists of an insertion of the Alu family, with a length of 306 base pairs (bp) in the *G* introns between exons 7 and 8. This event frequently occurs with two other mutations: replacement of a guanine base (G) with thymine (T) in exon 4, thereby exchanging a valine amino acid (Val) for leucine (Leu) in the receptor; and replacement of a cytosine base (C) with thymine (T) in exon 5.[16,17]

Glutathione S-transferases (GSTs) are a family of enzymes that regulate conversion of toxic compounds to hydrophilic metabolites. *GSTs* are responsible for metabolization and peroxidation of estrogens and lipids, and their polymorphism frequency is related to ethnic factors.[18,19] There are three main genes involved in these polymorphisms: GSTM1, GSTT1 and GSTP1. GSTM1 is a gene located in chromosome 1p13.3 and is not expressed by 20% to 50% of individuals.[18] Studies have suggested that genetic alterations such as in *GSTM1* may increase the levels of estrogen and/or catechol estrogens, which might be associated with estrogen-dependent diseases.[20,21]

OBJECTIVE

The aim of our work was to estimate the frequencies of interleukin-6, GST and progesterone receptor gene (PROGINS) polymorphisms in postmenopausal women with low BMD.

METHODS

This was a cross-sectional study, conducted in a public university in São Paulo, Brazil, including 110 patients with no sample size calculation prior to the study initiation.

One hundred and ten women in their first ten years after the menopause (mean age of 52 years) were recruited. All patients provided their informed consent to participate in this study and the Local Ethics Committee approved the related protocol. This study was supported by the Department of Gynecology and there was no external sponsor.

The inclusion criteria were that the women needed to have been postmenopausal for 5 to 10 years and needed to present follicle-stimulating hormone, FSH > 35 mUI/ml and estradiol < 20 pg/ml, and that a bone densitometry scan done before hormone therapy needed to be available.

The exclusion criteria comprised absence of bone densitometry before hormone therapy and no use of corticosteroids. None of the subjects had received any medication known to affect bone metabolism (such as glucocorticoids, thyroxin, anti-epileptics, bisphosphonates, calcitonin or hormone replacement therapy for more than three months).

All the subjects underwent careful physical examination and medical history review, including personal data, such as age, race, age at the menopause and medications currently used. Bone mineral density was evaluated using the Lunar DPX-L equipment (Lunar, Madison, Wisconsin, United States), which, according to the criterion of "z-bottom of form, z-top", described by Kiebzak et al.,[22] achieves a coefficient of variation of 0.62%. The measurements were performed on the lumbar spine. The T-score and the Z-score were calculated. For comparison with polymorphisms, a T-score cutoff at -1 was used to define low bone mineral density.

Samples of oral scrapings were collected using a cytobrush which was rubbed against the oral mucus lining and then placed in tubes containing tris-ethylenediaminetetraacetic acid (EDTA) buffer solution. The cytological samples thus obtained were preserved at -80 °C until subsequent extraction of the genomic deoxyribonucleic acid (DNA).

DNA extraction was performed in accordance with the Amersham-Pharmacia GFX kit protocol for oral cells. The DNA thus obtained was ready for use in the polymerase chain reaction (PCR).

The presence of *IL-6, PROGINS* and *GSTM1* gene polymorphisms was investigated using primers that amplified a small DNA fragment containing the polymorphic site. The primer sequences used for the promoter region of the gene were as follows:

For interleukin *6: 5´ - ATG CCA AGT GCT GAG TCA CTA - 3´ (sense) and 5´ - GGA AAA TCC CAC ATT TGA TA - 3´ (antisense).* For *PROGINS: 5´ - GGC AGA AAG CAA AAT AAA AAG A - 3´ (sense) and 5´ - AAA GTA TTT TCT TGC TAA ATG TC - 3´ (antisense).* For *GSTM: 5´ - GAA CTC CCT GAA AAG CTA AAG C – 3´ (sense)* and *5´ - GTT GGG CTC AAA TAT ACG GTG G – 3´ (antisense).*

Demographic and clinical variables were compared between genotype groups for the *IL6, PROGINS* and *GSTM1* polymorphisms. Statistical analyses were performed using Student's t-test or the Mann-Whitney test. Odds ratios and confidence intervals were derived from binary logistic regression analyses. The significance level was taken to be 0.05. To estimate the risks of the polymorphisms, a logistic regression model was applied to each polymorphism, using a 95% confidence interval.

RESULTS

In our population, 94% of the women were Caucasian. Regarding *IL-6* polymorphism, the composition of the groups was that 64 women were *GG* (homozygotes) and 48 were *GC/CC* (only two patients were CC). In relation to *PROGINS*, there were 87 women with *P1/P1* (wild homozygotes) and 23 with positive *PROGINS* genotypes of the progesterone receptors, *P1/P2* (heterozygote). The *GSTM1* polymorphism analysis showed that in 30 women, the allele was absent (0/0) and, in 80 women, the allele genotype was present (1/1).

Demographic and clinical variables were compared among homozygotes and heterozygotes for the *IL6* genotype. There were significant differences in the L2/L4 T-score (P = 0.03) and BMD (P = 0.05) among the genotype groups (**Table 1**).

To facilitate the odds ratio calculation, the cutoff point for the L2/L4 T-score was adjusted to -1. In this manner, we found that women with polymorphism in one allele, i.e. who were heterozygous for *IL-6*, presented a risk of having a L2/L4 T-score lower than -1.0 that was 2.3 times higher than those without this polymorphism.

No statistical differences were found between the genotype groups for the *PROGINS and GSTM1* polymorphisms regarding clinical variables (**Tables 2 and 3**).

Table 1. Clinical data regarding the *interleukin-6* (IL-6) genotype. Values are given as the mean ± standard deviation (SD)

Variable	n	Genotypes		P
		GG	GC	
		mean (± SD)	mean (± SD)	
Age (years)	110	51.57 (4.35)	52.50 (3.76)	0.24*
Weight (kg)	110	67.18 (12.18)	66.54 (12.84)	0.79*
Height (m)	110	1.53 (0.05)	1.53 (0.06)	0.95*
BMI (kg/m^2)	110	28.44 (5.48)	28.02 (4.64)	0.67*
BMD (g/cm^2)	110	1.13 (0.17)	1.07 (0.15)	0.05*
L_2/L_4 T-score	99	-0.40 (1.44)	-1.04 (1.34)	0.03†

*Student's t; †Mann-Whitney; BMI = body mass index; BMD = bone mineral density.

Table 2. Clinical data regarding *PROGINS*. Values are given as the mean ± standard deviation (SD)

Variable	n	Genotypes		P
		P1/P1	P1/P2	
		mean (± SD)	mean (± SD)	
Age (years)	110	51.78 (3.97)	52.65 (4.69)	0.37*
Weight (kg)	110	67.19 (12.90)	65.86 (10.38)	0.65*
Height (m)	110	1.54 (0.05)	1.53 (0.057)	0.50*
BMI (kg/m^2)	110	28.31 (5.28)	28.11 (4.59)	0.87*
BMD (g/cm^2)	110	1.11 (0.17)	1.09 (0.15)	0.62*
L_2/L_4 T-score	99	-0.60 (1.47)	-0.89 (1.28)	0.47†

*Student's t; †Mann-Whitney; BMI = body mass index; BMD = bone mineral density.

Table 3. Clinical data regarding the *GSTM1* genotype. Values are given as the mean ± standard deviation (SD)

Variable	n	Genotypes		P
		1/1	0/0	
		mean (± SD)	mean (± SD)	
Age (years)	110	51.46 (4.71)	52.15 (3.90)	0.44*
Weight (kg)	110	68.04 (10.47)	66.49 (13.09)	0.56*
Height (m)	110	1.53 (0.05)	1.54 (0.05)	0.59*
BMI (kg/m^2)	110	28.96 (4.23)	28.01 (5.43)	0.38*
BMD (g/cm^2)	110	1.12 (0.16)	1.10 (0.16)	0.58*
L_2/L_4 T-score	99	-0.53 (1.41)	-0.72 (1.45)	0.76†

*Student's t; †Mann-Whitney; BMI = body mass index; BMD = bone mineral density.

DISCUSSION

In our study, we found that *G-C* polymorphism in region 174 of the *IL-6* gene was associated with low bone mineral density. IL-6 is a cytokine with a crucial role in immune, inflammatory, hematopoietic and atherogenic responses and is associated with bone absorption. In bones, IL-6 is synthesized by osteoblasts, monocytes and T-cells, leading to differentiation and activation of osteoclasts. IL-1 and TNF-alpha have a role in activation, whereas estradiol and glucocorticoids suppress transcription of the *IL-6* gene. Thus, the decreased estrogen levels in postmenopausal women may trigger increased *IL-6* expression, thus leading to bone mass loss.[14]

Several variations in alleles have been identified in the *IL-6* promoter region. A common polymorphism, such as *G-C* exchange at position 174, may also interact with estrogen receptors that regulate *IL-6* expression. There is evidence that this polymorphism produces a functional variant in which the allele *174C* results in low stimulation of *IL-6* and also in low concentrations of IL-6 levels, compared with the presence of the *G* allele.[14]

Czerny et al.[23] studied associations between cytokine gene polymorphisms (*IL-1 beta, IL-2* and *IL-6*) and BMD values in postmenopausal women. Their study included 226 postmenopausal women with a diagnosed BMD T-score lower than -2.5 standard deviations (SD) and 224 postmenopausal women with a BMD T-score greater than -2.5 SD. Among the women with T-scores below -2.5 SD, the BMD values were significantly lower in the carriers of the *IL-6 GG* genotype than in those with the *CC* and *GC* genotypes.

In a cohort of 559 postmenopausal Spanish women, two polymorphisms in the *IL-6R* promoter were analyzed in relation to BMD and body mass index. The authors reported that there was a significant association between polymorphisms of the *IL-6R* gene and BMD.[24]

On the other hand, Garnero et al.[25] found that there were no significant associations between genotypes, bone turnover marker polymorphism and bone turnover or BMD in a cohort of healthy French women. They concluded that *IL-6* polymorphism was weakly associated with the peak BMD level and the rate of postmenopausal forearm trabecular bone loss. According to those authors, *IL-6* genotypes accounted only for a small proportion of the variation of both peak BMD and rate of bone loss.

Even though no specific studies have reported polymorphisms of the *PROGINS* and *GSTM1* genes in relation to BMD, we decided to study these polymorphisms. These occurrences can be explained by progesterone action on bone formation and the influence of *GSTM1* on estrogen metabolization.

It has been implied that *GSTs* are important molecules involved in activation of cytoprotection genes[18] and in correlation with the breast because of their ability to metabolize estrogens and lipids through peroxidation.[20] This may also keep osteoporosis from manifesting, due to hyperestrogenism. This change to this gene could influence bone mass.

Some studies have observed that progesterone stimulates proliferation of bone cells. This can be explained by increased insulin growth factor (*IGF*-2) levels, which could potentially stimulate proliferation of osteoblasts.[15] Growth factor beta, together with insulinoid factor, is the most abundant growth factor in the bone, but the effect of progesterone on growth factor beta in osteoblasts is still unknown.[26,27]

In our study, we also evaluated *PROGINS* and *GST* gene polymorphisms regarding age, weight, IMC and BMD variables. We did not find any statistical correlation among them.

We reported that there was a significant association between polymorphisms of the *IL-6* gene and BMD. The clinical importance of these findings may lead to new directions for osteoporosis management, such as biomarkers and molecular targets in therapeutics.

However, in fact, there were several limitations to our study. It is possible that the sample size was inadequate for detecting small differences in the groups, and that the study power was suboptimal. Moreover, the lack of a matched control group consisting of women with normal BMD limits the conclusions of this study. It is important to reproduce this study in other populations in order to achieve better analysis and functional outcomes.

CONCLUSION

This study showed that there was a considerable frequency of polymorphisms of the IL-6 gene in women with low BMD. However, this was not found for the other genes under investigation. Knowledge of osteoporosis-related genetic mechanisms may facilitate prevention and selection of women for therapeutics and prognosis.

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Address for correspondence:
Paulo Cezar Feldner Júnior
Rua dos Otonis, 601
Vila Clementino — São Paulo (SP) — Brasil
CEP: 04025-001
Tel.: (+55 11) 5573-9228
E-mail: pfeldner@alfa.epm.br

Continuous positive airway pressure (CPAP) after lung resection: a randomized clinical trial

Pressão positiva contínua nas vias aéreas (CPAP) após ressecção pulmonar: ensaio clínico randomizado

Lígia dos Santos Roceto[I], Fernanda Diório Masi Galhardo[II], Ivete Alonso Bredda Saad[III], Ivan Felizardo Contrera Toro[IV]

School of Medical Sciences, Universidade Estadual de Campinas (Unicamp), Campinas, São Paulo, Brazil

[I]MSc. Physiotherapist (PT), Intensive Care Unit of Clinical Hospital, School of Medical Sciences, Universidade Estadual de Campinas (Unicamp), Campinas, São Paulo, Brazil.

[II]BSc. Physiotherapist (PT), Intensive Care Unit of Clinical Hospital, School of Medical Sciences, Universidade Estadual de Campinas (Unicamp), Campinas, São Paulo, Brazil.

[III]PhD. Physiotherapist (PT), Pulmonary Rehabilitation Department of Clinical Hospital, School of Medical Sciences, Universidade Estadual de Campinas (Unicamp), Campinas, São Paulo, Brazil.

[IV]MD, PhD. Head and Professor of Thoracic Surgery Department of Clinical Hospital, School of Medical Sciences, Universidade Estadual de Campinas (Unicamp), Campinas, São Paulo, Brazil.

KEY WORDS:
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PALAVRAS-CHAVE:
Pressão positiva contínua nas vias aéreas.
Cirurgia torácica.
Complicações pós-operatórias.
Fisioterapia.
Toracotomia.
Respiração com pressão positiva.

ABSTRACT

CONTEXT AND OBJECTIVE: Noninvasive mechanical ventilation during the postoperative period (PO) following lung resection can restore residual functional capacity, improve oxygenation and spare the inspiratory muscles. The objective of this study was to assess the efficacy of continuous positive airway pressure (CPAP) associated with physiotherapy, compared with physiotherapy alone after lung resection.

DESIGN AND SETTING: Open randomized clinical trial conducted in the clinical hospital of Universidade Estadual de Campinas.

METHOD: Sessions were held in the immediate postoperative period (POi) and on the first and second postoperative days (PO1 and PO2), and the patients were reassessed on the discharge day. CPAP was applied for two hours and the pressure adjustment was set between 7 and 8.5 cmH_2O. The oxygenation index (OI), Borg scale, pain scale and presence of thoracic drains and air losses were evaluated.

RESULTS: There was a significant increase in the OI in the CPAP group in the POi compared to the Chest Physiotherapy (CP) group, P = 0.024. In the CP group the OI was significantly lower on PO1 (P = 0,042), than CPAP group. The air losses were significantly greater in the CPAP group in the POi and on PO1 (P = 0.001, P = 0.028), but there was no significant difference between the groups on PO2 and PO3. There was a statistically significant difference between the groups regarding the Borg scale in the POi (P < 0.001), but there were no statistically significant differences between the groups regarding the pain score.

CONCLUSION: CPAP after lung resection is safe and improves oxygenation, without increasing the air losses through the drains.

CLINICAL TRIAL REGISTRATION: NCT01285648

RESUMO

CONTEXTO E OBJETIVO: A ventilação mecânica não invasiva no período pós-operatório (PO) de ressecção pulmonar pode restaurar a capacidade residual funcional, melhorar a oxigenação e poupar os músculos inspiratórios. O objetivo deste estudo foi avaliar a eficácia da CPAP associada à fisioterapia comparada à fisioterapia unicamente após ressecção pulmonar.

ESTUDO E LOCAL: Ensaio clínico randomizado aberto, realizado no Hospital das Clínicas da Universidade Estadual de Campinas.

MÉTODO: Os atendimentos foram realizados nos PO imediato (POi), primeiro e segundo (PO1, PO2) dias, e a reavaliação na alta hospitalar. A CPAP foi aplicada durante duas horas e o ajuste pressórico estabelecido entre 7 e 8,5 cmH_2O. Foram analisados índice de oxigenação (IO), escala de Borg e de dor, presença e perda aérea dos drenos torácicos.

RESULTADOS: No grupo CPAP ocorreu aumento significativo do IO no POi (P = 0,024), comparado com o grupo fisioterapia respiratória. Houve redução significativa do IO no PO1 (P = 0,042) para o grupo fisioterapia respiratória, comparando-se à CPAP. A perda aérea foi significativamente maior para o grupo CPAP no POi e PO1 (0,001; 0,028), mas nos PO2 e no PO3 não houve diferença significativa entre os grupos. Foi verificada diferença significativa entre os grupos para a escala de Borg no POi (P < 0,001), porém para a escala de dor não foram verificadas diferenças significativas entre os grupos.

CONCLUSÃO: A CPAP após ressecção pulmonar é segura e melhora a oxigenação sem aumentar a perda aérea pelos drenos.

REGISTRO DE ENSAIO CLÍNICO: NCT01285648

INTRODUCTION

Patients in the postoperative period (PO) following lung resection surgery present a high risk of developing pulmonary complications like retention of secretions, atelectasis, pneumonia, prolonged air leaks and respiratory failure, which prolong the duration of mechanical ventilation and hospitalization and contribute towards increasing mortality.[1-3]

Continuous positive airway pressure (CPAP) during the postoperative period, using nasal or full face masks, can restore the residual functional capacity to preoperative levels, improve oxygenation, preserve the inspiratory muscles, restore gas exchange and avoid tracheal intubation due to acute respiratory failure in these patients.[3,4-7] However, it has not yet been established whether use of CPAP during the immediate postoperative period after lung resection is more beneficial than treatment with chest physiotherapy, or whether the positive pressure can increase or worsen the air leaks.

OBJECTIVE

To assess the efficacy of CPAP associated with physiotherapy, compared with physiotherapy alone after lung resection, regarding the following outcomes: oxygenation, dyspnea, pain, duration of stay and air leaks from chest tubes during the postoperative period.

METHODS

Specifications of the study

This was a prospective, non-blinded, randomized, comparative, interventional clinical trial.

Subjects

All the patients who were admitted to the hospital with indications for lung resection were assessed in relation to the eligibility criteria. 60 patients aged 40-75 years of both genders were selected between October 2007 and November 2009. All of them had a medical diagnosis of lung cancer and an indication for lobectomy, bilobectomy or pneumonectomy with posterolateral thoracotomy, and had been admitted to the pulmonology ward of a clinical hospital belonging to a public university. After surgery, the patients were randomized with opaque, sealed envelopes. Both the investigator and the patient knew which group the patient was allocated to. The project was approved by the institution's Research Ethics Committee, under number 388/2007, and all subjects who agreed to participate in the study signed a consent form.

Exclusion criteria

The surgical indication was established by the medical team, and patients with forced expiratory volume in the first second (FEV_1) that was less than 30% of the predicted value, or presented advanced-stage disease with Karnofsky performance status (KPS)[8] 3 or 4, were excluded. Patients who refused to participate in the survey, those who were not aged between 40 and 75 years and those who underwent lung resection with incisions other than posterolateral were excluded. Moreover, patients presenting the following contraindications for use of noninvasive ventilation (NIV) were also excluded: hemodynamic instability that was not responsive to vasoactive drugs; psychomotor agitation or inability to cooperate; evidence of pulmonary thromboembolism and asthma; emergency endotracheal intubation; inability to protect the airway (impaired coughing and swallowing); significant abdominal distension; multiple organ failure affecting more than two organs or systems; or inability to tolerate the nasal mask.

Preoperative evaluation

During the preoperative period (PRE), the subjects who had been selected for the study underwent physiotherapy evaluation that included taking the clinical history, physical examination, pulmonary function tests and arterial blood gas measurement. In addition, during this period, the patients were informed about the surgical procedure, the type of incision, the intubation and sedation procedures and risks, the importance of coughing during the postoperative period and the need for commitment to the required confinement to bed. The patients were also administered chest physiotherapy comprising incentive spirometry and ventilatory patterns.

Surgical procedures

Muscle-sparing thoracotomy with no sectioning of the latissimus dorsi and serratus muscles was performed on all the patients. Epidural anesthesia, as well as regular pain killers, anti-inflammatories and morphine were administered on the first PO. Fissures were treated with cautery dissection and stapling. All the patients received two 38F chest tubes, except in cases of pneumonectomy, which were not drained. The operation was performed by the same surgeon, and the patients were extubated in the operating room.

After surgery, during the immediate postoperative period (POi) and two to four hours after weaning from invasive ventilation and extubation, the patient was again evaluated and was allocated to one of the following two groups:

Chest physiotherapy

Chest physiotherapy (CP) was started with one session in the POi and two sessions on the first and second days after surgery (PO1 and PO2). CP consisted of bronchial hygiene techniques and pulmonary expansion, in addition to exercises, and the patients received oxygen supplementation to maintain pulse

oximetry saturations higher than 90%. The bronchial hygiene techniques used included forced expiration, coughing and vibration.[9] Incentive spirometry and breathing patterns associated with movements of the upper and lower limbs were used to maximize deep diaphragmatic breathing.[10,11] The use of bronchodilators and analgesia complied with the standardization and medical indications of the institution's post-anesthesia intensive care unit (ICU).

CPAP

This group combined chest physiotherapy with NIV via nasal masks for two hours, using the Nellcor/Puritan Bennett GoodKnight 420G CPAP system (United States). The pressure was adjusted according to the patient's tolerance. The starting pressure was between 7 cmH_2O and 8.5 cmH_2O, while the breathing rate was maintained at less than 30 rpm, and the supplemental oxygen was used to maintain pulse oximetry saturation higher than 90%. CPAP was administered from the POi until the second postoperative day, twice a day for a total of five sessions, until reaching 48 to 60 hours after the operation.

Postoperative evaluation and data gathering

From the POi until hospital discharge, the presence of chest tubes and air leaks was recorded.

After pulmonary function tests had been performed preoperatively, and on hospital discharge, the patients were referred to our hospital's pulmonary function laboratory, where spirometry was performed using the MGC pulmonary function analysis system PC-4000-AM, and anthropometric data were gathered. During the testing, the patient remained seated, using a nose clip, and the percentages of forced vital capacity (FVC) and FEV_1, and the ratio between them, were determined. Blood samples for arterial blood gas measurements were collected once a day preoperatively, in the POi and on PO1 and PO2. The measurements were made using the Start New Profile 5 ABL-625 and ABL-700 machines, and the ratio between the partial oxygen pressure (PaO_2) and the inspired oxygen fraction (FiO_2) was calculated as the oxygenation index (OI).

Before beginning physiotherapeutic protocols, the patients were asked to rate their pain from zero to ten according to its intensity (the larger the score, the greater the intensity of pain). In addition, they were also asked about their sensation of dyspnea according to the Borg scale,[12] which also ranged from zero to ten.

Definition of outcomes

The presence of chest tubes and air leaks was verified in the POi and on PO1, PO2 and on the fifth postoperative day or at the time of hospital discharge (PO3). Air leaks were ascertained before beginning the protocol treatment, by watching for one minute to see whether there were any air leaks in the water seal. It was determined whether drain use should continue, or whether the drains should be removed or should be used with wall suction, by applying the institution's medical protocol, through analysis on chest radiographs and on the amount of drained fluid. Thus, it was defined that the drain would be removed when the drainage rate was less than or equal to 200 ml over a 24-hour period.

Statistical analysis

To describe the profile of the sample according to the study variables, frequency tables containing the absolute frequencies (n) and percentages (%) were calculated for the categorical variables, along with descriptive statistics on the continuous variables with means and standard deviations.

All patients admitted to our hospital between October 2007 and November 2009 who conformed to the inclusion criteria were selected for the study. Thus, no sample size calculation was performed and the study subjects constituted a convenience sample. It was found that, considering the OI variable, the number of patients was at least five per group (which would provide a power of 80.0%). The mean sample size therefore provided a power of 87.1%.

To compare the categorical variables between the groups, the chi-square test and Fisher's exact test were used, and for continuous variables compared between pairs of groups at baseline, the Mann-Whitney test was used. To compare measurements between longitudinal groups, we used analysis of variance for repeated measurements (repeated-measure ANOVA), always followed by the Tukey multiple-comparison test for groups. Profile tests with contrasts were used to examine the evolution of the assessments in each group.

Per-protocol (efficacy) analysis was used to assess the outcomes. The variables were transformed into ranks due to lack of normal distribution. The significance level for the statistical tests was 5% ($P < 0.05$).

RESULTS

Forty patients were included in this study: 20 in the CP group, which consisted of 10 males and 10 females; and 20 in the combined CP and CPAP group, which contained 7 females and 13 males. Twenty patients were excluded: 12 underwent nodulectomy; 5 were found to be non-operable during surgery; and there was 1 case each of dependence on mechanical ventilation during the postoperative period; intolerance to NIV; and psychomotor agitation (**Figure 1**). There were 28 cases of lobectomy, 10 of pneumonectomy and 2 of bilobectomy, and the average CPAP pressure used was 7.85 ± 0.4 cmH_2O. **Table 1** shows the analysis on the two groups, with the patients' preoperative characteristics in terms of age, smoking history, body mass index and ventilatory parameters.

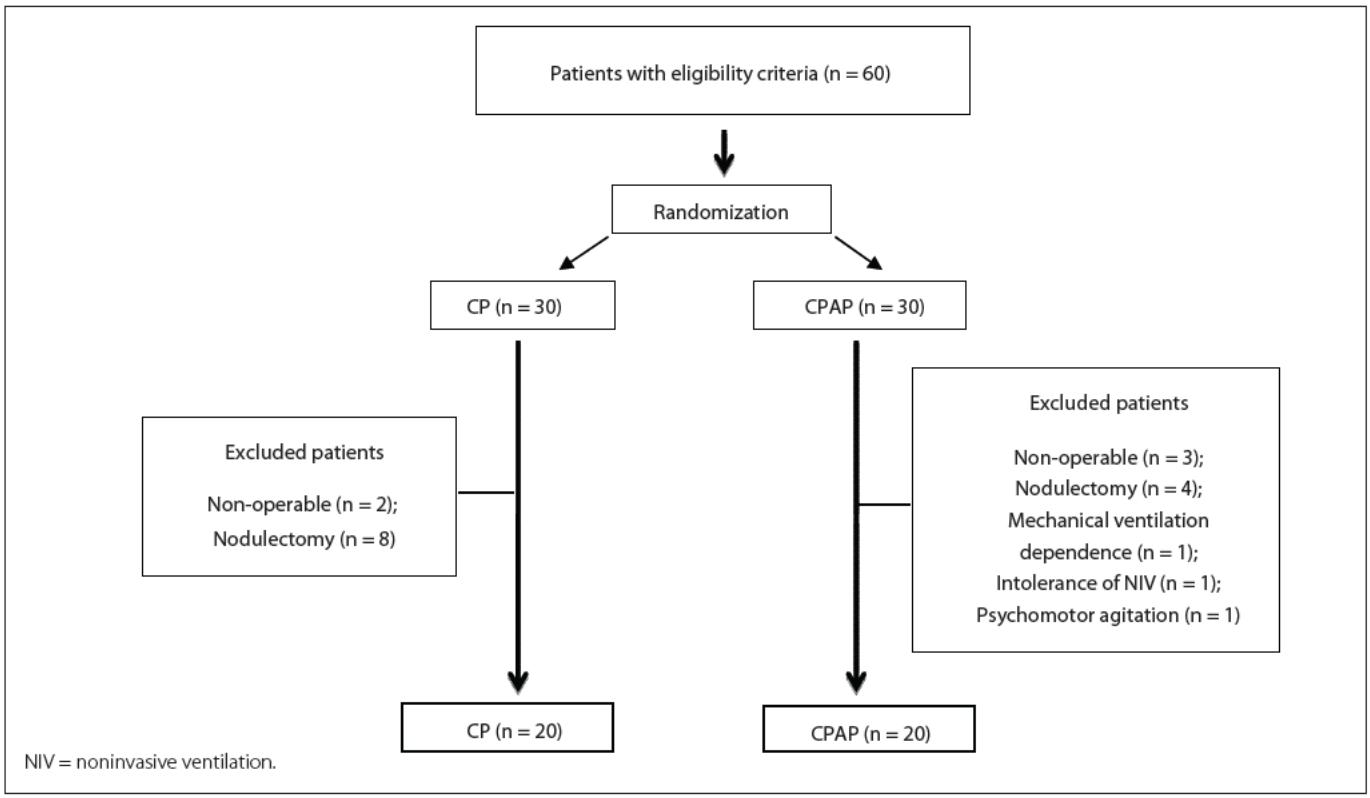


Figure 1. Study flow chart for inclusion and exclusion of patients, for the chest physiotherapy group (CP) and continuous positive airway pressure group (CPAP).

Table 1. Analysis on the preoperative characteristics of the chest physiotherapy group (CP) and continuous positive airway pressure group (CPAP), with regard to age, smoking history, body mass index and ventilatory parameters

	CP (n = 20) Mean ± SD	CPAP (n = 20) Mean ± SD	P-value*
Age (years)	56.05 ± 10.73	60.35 ± 8.93	0.228
Smoking (pack/years)	27.65 ± 27.57	40.95 ± 33.98	0.225
Body mass index (kg/m²)	25.55 ± 4.31	25.12 ± 3.80	0.989
Respiratory rate (breaths/min)	20 ± 3.66	19.80 ± 3.35	0.849
FVC (% pred)	83.80 ± 17.56	78.85 ± 17.88	0.457
FEV_1 (% pred)	80.65 ± 18.37	73.20 ± 20.33	0.208
Duration of surgery (hours)	4.35 ± 1.30	4.20 ± 0.73	0.73
Lobectomy/bilobectomy/ pneumonectomy	13/1/6	15/1/4	0.853

FVC = forced vital capacity; FEV_1 = forced expiratory volume in one second; % pred = percentage of the predicted value. Data are mean ± standard deviation (SD).
*P-values refer to between-group comparisons.

Oxygenation index (OI)

The evolution of OI in the two intervention groups is shown in **Figure 2**, with regard to the preoperative period, immediate postoperative period (POi) and the first and second postoperative days (PO1 and PO2). In the CP group, the OI was significantly lower on PO1 (P = 0.042) than in the CPAP group. The OI in the CPAP group was significantly higher in the POi than the OI in the CP group (P = 0.024).

In a specific analysis on OI with regard to pneumonectomy, there was no significant difference between the groups (P = 0.051; P = 0.0807; P = 0.086), respectively for the times PRE, POi and PO1.

Air leaks

The chest tube drainage was analyzed in relation to the 30 patients who underwent lobectomy or bilobectomy; drainage was not performed after pneumonectomy. There were higher air leaks in the CPAP group in the POi and on PO1, than on the CP group (P = 0.001 and P = 0.028, respectively), but on PO2 and on the fifth postoperative day or at hospital discharge (PO3), there was no significant difference in air leaks between the groups (P = 0.105 and P = 1) (**Figure 3**).

Dyspnea scale

During the preoperative period, 70% of the patients in the CP group did not report dyspnea, whereas 5%, 10% and 15% reported dyspnea when making small, medium and high efforts respectively. In the CPAP group, 60% of the subjects said that they did not have dyspnea, 10% reported dyspnea on making moderate effort and 30% reported dyspnea after great effort, without any statistical difference between the groups (P = 0.704). Analysis on

the dyspnea in the POi and on PO1 showed significant differences between the groups ($P < 0.001$), as can be seen in **Figure 4**.

Pain scale

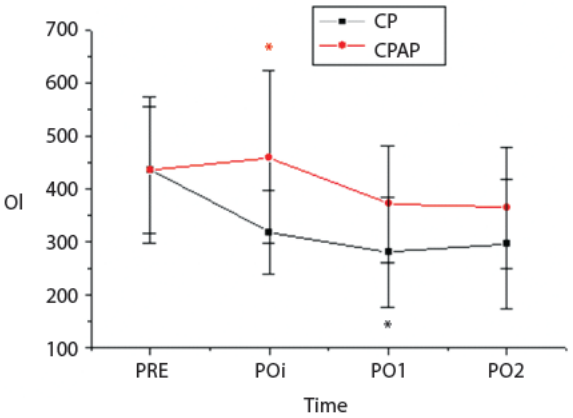
There were no significant differences between the two intervention groups in terms of the analogue pain scale or the presence of epidural catheters for analgesia (ECA) at the three times (**Table 2**).

DISCUSSION

The results presented in this study, including improved OI, no increase in air leaks with positive pressure during the postoperative period and no increases on the pain scale or in relation to dyspnea showed that preventive application of CPAP during the immediate postoperative period after lung resection is safe and is based on the physiological effects of noninvasive ventilation. Likewise, it improved gas exchange, reversed atelectasis and enhanced the distribution of ventilation through recruitment of collapsed areas.[3,6,7,13] These aspects of lung function are directly related to respiratory complications during the postoperative period.

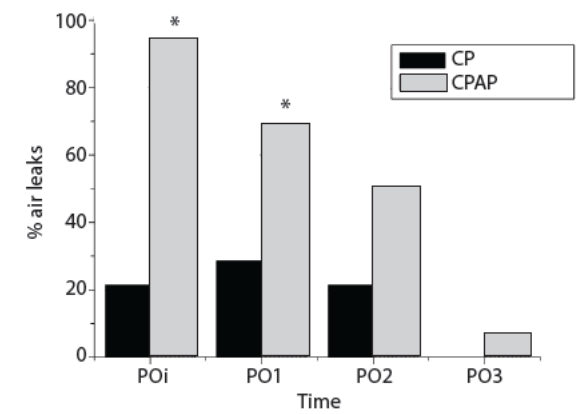
Use of NIV may have provided improved OI in these patients, which was also found in other studies[3,6,7,14] because CPAP ventilation mode generates continuous positive airway pressure during inspiration and expiration that prevents alveolar collapse and atelectasis, maintains residual functional capacity and reduces the burden on the left ventricle through improved cardiac function. The studies by Battisti et al. and Kindgen-Milles et al.[5,15] used CPAP with lower pressures, like in the present study, in patients with non-hypercapnic respiratory failure during the postoperative period after lung resection and thoracoabdominal surgery, respectively, and showed that the OI increased after application of NIV, and that there were fewer pulmonary complications and shorter hospital stays.[13] Moreover, application of CPAP can reduce the respiratory load through an increase in the intrinsic positive end-expiratory pressure (PEEP), which was generated by balancing the load imposed during inspiration.[13,16] The main consequences of high lung volumes during surgery are cell damage caused by over distension and shear forces, and compromised gas exchange, as shown by the oxygenation index.[17]

Pneumonectomy involves greater resection of lung parenchyma and consequently greater impairment of lung function.[18] In the specific OI analysis on pneumonectomy in the present study, it was observed that there was no significant difference between the groups at the times PRE, POi and PO1. It was found that, considering the OI variable to be a primary outcome of the present study, the number of patients was at least five per group (which would provide a power of 80.0%). The mean sample size therefore provided a power of 87.1%, which justified the lack of sample size calculation. Thus, the study subjects constituted a convenience sample.



*P ≤ 0.05. PRE = preoperative period; POi = immediate postoperative period; PO1 = first postoperative day; PO2 = second postoperative day.

Figure 2. Graphical representation of the evolution of the oxygenation index (OI) in the preoperative period and immediate postoperative period, and on the first and second postoperative days, for the chest physiotherapy group (CP) and continuous positive airway pressure group (CPAP).



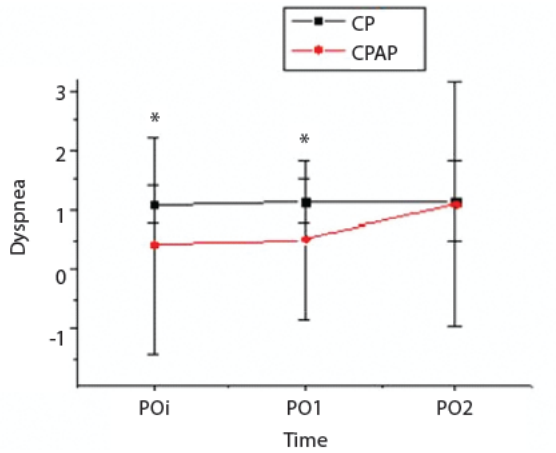
POi = immediate postoperative period; PO1 = first postoperative day; PO2 = second postoperative day; PO3 = fifth postoperative day or at hospital discharge. *P ≤ 0.05.

Figure 3. Percentages of patients with air leaks in the chest physiotherapy (CP) group and continuous positive airway pressure (CPAP) group.

Table 2. Percentages of patients with epidural catheters for analgesia (ECA) and analogue pain scale scores, in the chest physiotherapy (CP) group and the combination CP and continuous positive airway pressure (CPAP) group

	Presence of ECA (%)		P-value	Analogue pain scale		P-value
	CP	CPAP		CP	CPAP	
POi	95	90	1	4.05 ± 3.55	3.20 ± 2.95	0.429
PO1	95	90	1	2.84 ± 2.91	3.39 ± 3.11	0.524
PO2	70	80	0.465	2.22 ± 3.28	2.85 ± 3.05	0.421

POi = immediate postoperative period; PO1 = first postoperative day; PO2 = second postoperative day.



POi = immediate postoperative period; PO1 = first postoperative day; PO2 = second postoperative day.

Figure 4. Graphical representation of the incidence of dyspnea based on the Borg scale in the immediate postoperative period and on the first and second postoperative days, in the chest physiotherapy group (CP) and continuous positive airway pressure group (CPAP).

Comparison of air leaks between two groups showed that a higher percentage of the patients with air leaks were receiving positive pressure. However, this difference was only significant until the first postoperative day. In other words, use of NIV did not cause increased air leaks or fistulas. This was shown in another study by air leak and air drainage times that extended beyond seven days.[19] The study by Lefebvre et al.[14] found that only one of the 89 patients who received NIV after suffering respiratory failure postoperatively subsequent to lung resection presented persistent air leaks, and this result was attributed to application of low pressure, which was used in our study. Other studies have been consistent with our findings, such as the study by Perrin et al.,[3] in which no difference in the duration of chest tube drainage between groups with conventional treatment and NIV was shown, even with use of higher inspiratory pressures during application of bilevel ventilation. Use of positive pressure exerts a force on the suture, increases the spring mechanism and further increases the tendency towards distancing from skin edges.[20] However, other studies have shown that prolonged air leaks after lung resection are related to a number of risk factors, including impairment of lung function, fragility of the lung parenchyma, steroid use, operative protocols for upper lobectomy and presence of pleural adhesions.[19,21,22] In the analysis on air leaks, it could be inferred that the evaluation method was subjective, since no researcher had any device that would numerically quantify air leaks. Instead, they just made observations per minute.

With regard to dyspnea, the results showed that the CP group had higher values on the Borg scale than shown CPAP. However, these findings did not have clinical relevance because the data were correlated with degrees of dyspnea that were defined as very mild or absent. In the analysis on the analogue pain scale, there was no significant difference between the values, but they decreased after the operation and this may have been related to removal of the drains. The study by Lima et al.[23] evaluated the influence of the thoracic drain on the reported pain and found that drain removal resulted in decreased analogue pain scores, which suggests that presence of a chest tube is an important factor associated with pain and functional limitations.

The present study had limitations relating to the public institution where the research was performed, in that there were financial constraints on purchasing and maintaining the equipment that was used in the study. Moreover, mortality rates, infection, reintubation and systemic and local complications during the postoperative period were not analyzed, since hospital length of stay was not verified because of organizational conditions and varied distribution of ICU beds and places for medical specialties. Further investigation on this issue is needed, with larger numbers of patients and blinded evaluators. It is difficult to eliminate bias when a study and its investigators cannot be blinded such that any conscious or unconscious interference in the results from an experiment are avoided. Through other studies in the future, it might be possible to identify the NIV pressure limits, minimum and maximum time of application and long-term outcomes after introduction of NIV during the postoperative period.

There is growing understanding of physiotherapeutic interventions during the postoperative period following lung resection. The recommendations in the literature[4,13,24-26] highlight the importance of using incentive spirometers and applying chest physiotherapy with regard to reducing costs, hospital length of stay and incidence of atelectasis.[24] The present study was able to show that use of NIV in thoracic surgery is safe when applied by trained professionals, and showed the need for further research with larger numbers of patients, in order to determination of other benefits and advantages of NIV during the postoperative period following lung resection.

CONCLUSIONS

Similar to CP, preventive application of CPAP during the postoperative period after lung resection was shown in our study to be a safe technique that was effective in improving oxygenation without increasing air leaks through the thoracic drains. However, further studies with blind assessment that take other relevant outcomes into consideration and include larger numbers of patients are still necessary.

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Address for correspondence:
Lígia Santos Roceto
Rua João Bissoto Filho, 99 — apto 3 — bloco 15
Ortizes — Valinhos (SP) — Brasil
CEP 13275-410
E-mail: ligiasroceto@uol.com.br

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Treatment of children and adolescents with hemangioma using propranolol: preliminary results from a retrospective study

Tratamento de crianças e adolescentes com hemangioma com propranolol: resultados preliminares de um estudo retrospectivo

Juliana Costa Albuquerque[I], Rosane Aline Magalhães[I], Jamille Araújo Félix[I], Maria Vilani Rodrigues Bastos[II], Juvenia Bezerra Fontenele[III], Nádia Mendonça Trompieri[IV], Francisco Helder Cavalcante Felix[V]

Albert Sabin Children's Hospital, Fortaleza, Ceará, Brazil

[I]Pharmacy Student, Universidade Federal do Ceará (UFC), Fortaleza, Ceará, Brazil.
[II]Employee of the Department of Pharmacology and Physiology, Universidade Federal do Ceará (UFC), Fortaleza, Ceará, Brazil.
[III]PhD. Adjunct Professor, Pharmacy Course, Faculty of Pharmacy, Dentistry and Nursing, Universidade Federal do Ceará (UFC), Fortaleza, Ceará, Brazil.
[IV]MD, MSc. Pediatrician in Walter Cantídio University Hospital, Pediatrician and Hemato-oncologist in the Pediatric Cancer Center, Albert Sabin Children's Hospital, Fortaleza, Ceará, Brazil.
[V]MD, MSc. Pediatrician and Hemato-oncologist in Albert Sabin Children's Hospital, Fortaleza, Ceará, Brazil.

KEY WORDS:
Hemangioma, capillary.
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PALAVRAS-CHAVE:
Hemangioma capilar.
Propranolol.
Estudos retrospectivos.
Resultado de tratamento.
Oncologia.

ABSTRACT

CONTEXT AND OBJECTIVE: Hemangiomas are the commonest vascular tumors during childhood. In 2008, the effect of propranolol for treating capillary hemangiomas was demonstrated. Other similar results followed, showing that it rapidly reduces lesion volume. The objective here was to evaluate children and adolescents with hemangiomas that were treated with propranolol.

DESIGN AND SETTING: Retrospective study, conducted in a children's hospital.

METHODS: Patients aged 0-19 years with or without previous treatment, who were treated between January 2009 and December 2010, were included. The response was assessed by comparing the lesion appearance between the start of treatment and the last consultation. We considered partial or complete responses as the response to treatment.

RESULTS: Sixty-nine patients with a median follow-up of 11 months (mean age: 31 months) were included. Of these, 58 patients were recently diagnosed and 11 had had previous treatment. A response (partial or complete) was seen in 60 patients (87%). Among the capillary hemangioma cases, responses were seen in 50 out of 53 (94%), while in other lesion types, it was 10 out of 16 (63%) (P = 0.3; chi-square). Responses in patients less than one year of age were seen in 37 out of 38 (97%), whereas in those over one year of age, in 23 out of 31 (74%) (P = 0.4; chi-square). Side effects were uncommon and mild.

CONCLUSIONS: Propranolol seemed to be effective for treatment of hemangiomas in children and adolescents, and not just in the proliferative stage, with responses in almost all the patients.

RESUMO

CONTEXTO E OBJETIVO: Hemangiomas são os tumores vasculares mais comuns da infância. Em 2008, foi demonstrado o efeito do propranolol no tratamento de hemangiomas capilares. Outros relatos similares seguiram-se, demonstrando seu rápido efeito na redução do volume das lesões. O objetivo foi avaliar crianças e adolescentes com hemangioma tratadas com propranolol.

TIPO DE ESTUDO E LOCAL: Estudo retrospectivo, conduzido em hospital infantil.

MÉTODOS: Foram incluídos pacientes entre 0-19 anos, com ou sem tratamento prévio, tratados entre janeiro de 2009 e dezembro de 2010. A resposta foi avaliada comparando-se o aspecto da lesão entre o início do tratamento e a última consulta. Consideramos resposta parcial ou completa como resposta ao tratamento.

RESULTADOS: Sessenta e nove pacientes foram incluídos, com uma mediana de acompanhamento de 11 meses (idade média: 31 meses). Destes, 58 pacientes eram recém-diagnosticados e 11 tinham tratamento prévio. Resposta (parcial ou completa) foi verificada em 60 pacientes (87%). Entre os hemangiomas capilares, a resposta foi de 50 em 53 (94%), enquanto em outros tipos de lesões, a resposta foi de 10 em 16 (63%) (P = 0,3; teste de qui-quadrado). A resposta em pacientes com até 1 ano de idade foi de 37 em 38 (97%), e naqueles com mais de 1 ano foi de 23 em 31 (74%) (P = 0,4; qui-quadrado). Efeitos colaterais foram incomuns e leves.

CONCLUSÕES: Propranolol parece ser efetivo no tratamento de hemangiomas em crianças e adolescentes, não apenas na fase proliferativa, com resposta em quase todos os pacientes.

INTRODUCTION

Hemangiomas are formed by proliferation of blood vessels and are the commonest vascular tumors during childhood, affecting approximately 3-10% of Caucasian children.[1] They occur more frequent in females (1:1.4-3.0), and in white non-Hispanic children. Their causes are unknown, with the exception of rare genetic syndromes, in which hemangioma is frequent.[2] Histologically, hemangiomas are a heterogeneous group,[3] although the most common type is known simply as infantile or capillary hemangioma.

Hemangiomas display different growth phases over the course of their evolution. Initially, there is a rapidly proliferating phase lasting for up to six months. During this phase, the lesion becomes more erythematous and violacious. Larger, deep hemangiomas may proliferate up to the age of two years. A stationary phase ensues, during which the hemangioma grows in proportion to the child. This is followed by an involutive phase that lasts for up to five years in most cases. Involutive hemangiomas change color to gray. Maximum involution occurs in approximately 50% of children by the age of five years and in 90% of children by the age of nine. Some 20% to 40% of patients keep residual changes of the skin, such as laxity, discoloration, telangiectasia, fibrofatty masses or scarring.[4]

Most hemangiomas are small and never complicated, and they are adequately managed through clinical observation alone. A number of pharmacological interventions have been used for the approximately 10-30% of these patients who present complications. The majority of these treatments have never been properly clinically evaluated, aside from reports on small numbers of cases. The first-line therapy has been oral corticosteroids for many years. Other treatments include topical or intralesional corticosteroids, interferon alpha and vincristine. In the absence of any randomized controlled clinical trials, the dosing, time duration and efficacy of oral corticosteroids are determined empirically at best.[3,4]

In 2008, a letter published in the New England Journal of Medicine first reported use of propranolol for treating childhood hemangiomas.[5] After this initial information, other reports on cases successfully treated with propranolol were published, and the initial article has been cited around 140 times (Google Scholar survey in January 2011). Since January 2009, we have been treating pediatric patients with hemangiomas using off-label oral propranolol in our institution.

OBJECTIVES

To describe the therapeutic effects of propranolol, in a cohort of children and adolescents with hemangioma from a single institution.

METHODS

We planned to evaluate the response of children with hemangiomas to treatment with propranolol. Adverse events reported during the treatment would be recorded. The research project was approved by our institution's Ethics Review Board in 2009. The project is still at the data-gathering phase. This report presents partial preliminary data, according to our database in June 2011.

Parents or guardians received detailed explanations about the treatment and this was started after informed consent had been obtained. A retrospective analysis of the medical records was undertaken, using a semi-structured questionnaire. We included patients ranging in age from 0 to 19 years with a diagnosis of hemangioma, with or without prior treatment. We excluded patients with asthma (as informed by parents).

We started administering treatment with propranolol between January 2009 and December 2010. The response was assessed by comparing the status at the start of treatment and at the last appointment, measuring the two largest diameters of the lesions. Patients with unmeasurable lesions were evaluated by means of a qualitative assessment made by one of the attending physicians (i.e. one of the present authors). The response was classified as stable disease (< 25% variation), partial response (25-95% reduction) or complete response (> 95% reduction). We considered both partial and complete responses to be responses to treatment. Objective measurements were made by means of direct measurement, ultrasound imaging, computed tomography (CT) or magnetic resonance imaging (MRI), depending on the accessibility of the lesion. Deep lesions not measurable by means of ultrasound imaging were followed by means of serial CT or MRI (the number of CT scans was kept to the minimum necessary for response assessment, and was typically two to three scans). The chi-square test was used to compare responses between different groups of patients (infantile hemangiomas versus other types and children less than one year of age versus children older than one year).

RESULTS

We included 69 patients with a mean follow-up of 11 months. The average age at the time of starting the patients' first treatment was 31 months, ranging from one month to 19 years. The median was 8 months. The average age at the time of starting the treatment, for patients with residual lesions or those refractory to prior therapy, was three years, ranging from two months to 16 years. A total of 38 patients started treatment at the age of less than one year, while 31 commenced after completing one year of age. The dose used was 0.5 to 4.0 mg/kg per day, starting with 0.5 mg/kg for all patients in the first week, with weekly increases so as to reach up to 2.0 mg/kg per day. For patients with no initial response over the first 2-3 months, the dose was increased to 4.0 mg/kg per day. The dosing interval was 8 or 12 h.

Fifty-eight patients had not been treated previously, while 11 presented residual lesions or had been refractory to previous therapy.

A response (partial or complete) was seen in 60 patients (87%). Forty-six patients were female and 23 were male (ratio 1:2). The lesions were classified as infantile hemangioma (53), cavernoma (three), syndromic hemangioma (four), congenital lesions or others (nine). Responses were seen in 50 out of 53 cases of infantile hemangioma (94%) and in 10 to 16 cases of the other hemangioma types (63%) (P = 0.3; chi-square). Responses were seen in 37 out of 38 patients who started treatment before reaching the age of one year (97%), whereas the proportion was 23 out of 31 patients who started treatment after reaching one year of age (74%) (P = 0.4; chi-square) (**Figure 1**).

Side effects were uncommon and mild, and the treatment was not discontinued for any child in the series because of side effects. The dose was reduced in some cases, due to side effects. Sixteen patients reported possible side effects. The most common were transitory dyspnea (four patients), cold extremities (two patients), precordial pain (two patients) and slow weight gain (two patients). One patient presented symptomatic hypoglycemia (56 mg/dl) after prolonged fasting. This patient did not have any response to the treatment and it was withdrawn.

Figure 2 illustrates a typical case of unmeasurable infantile hemangioma with complete response to treatment.

Table 1 shows information about the measurable lesions. **Figure 3** shows the change in lesion measurements, from the first to the last objective evaluation. **Figure 4** illustrates the CT scans of a case of non-involutive congenital hemangioma (NICH) that remained stable over the course of the treatment time (i.e. no response).

DISCUSSION

In this preliminary retrospective study, treatment with propranolol was related with a response (consisting of lesion reduction) in most of the children with hemangiomas. It seemed that there was a greater chance of a response in children with infantile hemangiomas who were less than one year of age, in contrast to patients with other types of hemangiomas or who were over one year of age. However, this difference was not statistically significant. The statistical power of the comparison of numbers of responders between those aged less than and more than one year was 80% (data not shown). This indicates that the chance of type II error was small and that there was probably no real difference in the number of responders between children aged less than and more than one year. However, this evaluation did not differentiate between a partial response (defined less strictly in the present study, such that responses that are regarded as minor were also included) and a complete response. One of our goals is to complete the data-gathering for the entire cohort analysis with detailing of the two different outcomes (partial or complete). However, our preliminary results already show that patients who have outgrown the so-called "proliferative phase" of hemangioma development still have a potential for response that should not be underestimated.

The small number of patients with lesions other than infantile hemangiomas does not allow any conclusion about their response potential. However, the statistical power of this comparison was 83%. This heterogeneous group of patients included three patients with cavernous hemangiomas (histologically determined), four syndromic patients with apparently typical infantile hemangiomas (PHACES and Klippel-Trenaunay-Weber syndromes) and nine patients with congenital hemangiomas or late-onset lesions. It is possible that patients with other forms of vascular tumors closely related to infantile hemangiomas may also have a potential for response. It remains to be determined whether this response potential is actually lower than that of patients with infantile hemangiomas.

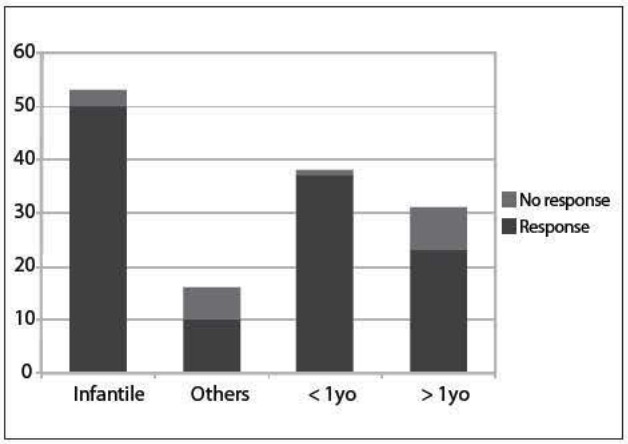

Figure 1. Treatment response in patients with infantile hemangiomas, other types of vascular lesions, patients less than one year of age or over one year of age (number of patients).

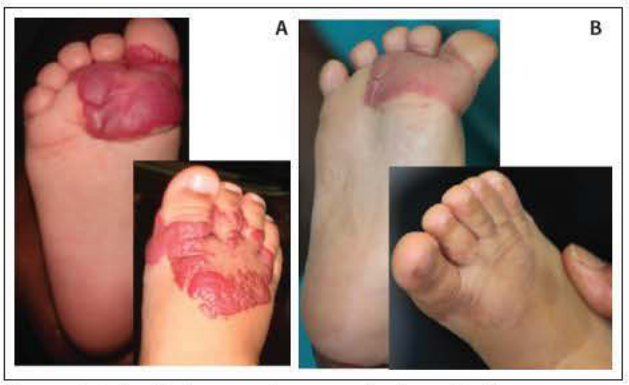

Figure 2. Infantile hemangioma on the feet of a three-month old child A. before treatment, and B. after one year of treatment. Complete remission is apparent. Residual telangiectasia remained.

Table 1. Lesion size in the subgroup of patients with measurable disease

Type	First measurement largest (D)	Smallest (d)	D X d	Second measurement largest (D)	Smallest (d)	D X d	Follow-up	Mode	Age (years)
HI	5	5	25	3	2	6	11	OBS	0.1
HI	6	6	36	4	2	8	6	OBS	0.3
HI	5	5	25	1	1	1	3	OBS	0.5
HI	4.5	4	18	4	4	16	3	OBS	0.5
HI	3.6	2.7	9.72	2	1.6	3.2	3	US	0.6
HI	4.8	4	19.2	4.7	4.6	21.62	3	CT	0.7
HI	3.9	1.9	7.41	2.7	1.4	3.78	15	US	0.8
HI	5.3	3.2	16.96	4.6	4.3	19.78	8	US	0.8
HI	3.5	1.5	5.25	2.5	0.5	1.25	1	OBS	0.8
HI	4	4	16	2.5	2.5	6.25	10	OBS	0.9
HI	1.9	0.9	1.71	0	0	0	18	US	1
HI	5.1	3.9	19.89	5.6	3.5	19.6	7	US	1.6
HI	1.5	1.5	2.25	1	1	1	4	OBS	2.3
HI	3.8	2	7.6	2.4	0.9	2.16	11	US	2.7
HI	2	2	4	3	3	9	27	OBS	3
HI	4.7	1.6	7.52	2.9	1.8	5.22	16	US	5
HI	5.1	3	15.3	3	2	3	3	US	5.8
HI	4	3.5	14	3.5	2.6	9.1	11	CT	11.3
CAV	2.4	2	4.8	1	1	1	17	MRI	8.7
HYGROMA	7	5	35	5.1	4	20.4	7	US	5.4
KTW	18	7	126	16	5	80	7	CT	11.3
LT	7.6	5.2	39.52	8	5.2	41.6	5	US	13.4
LT	5.5	2.4	13.2	6	3	18	10	CT	12.4
NICH	4.1	2.3	9.43	3.6	3.5	12.6	6	US	0.3
NICH	5.1	3.9	19.89	5.6	2.7	15.12	7	US	1.6
NICH	5.6	4.9	27.44	5	2	10	10	MRI	11.5
PHACES	12	7	84	11.3	2.9	32.77	8	MRI	5.5

Types of lesions: capillary hemangioma (HI), cavernous hemangioma (CAV), lymphangioma (HYGROMA), Klippel-Trenaunay-Weber syndrome (KTW), vascular lesions identified in older children (probably malformations - LT), non-involutive congenital hemangioma (NICH), PHACES syndrome (PHACES). First and last measurement are shown, each depicting largest diameter (D), smallest diameter (in fact, the largest perpendicular diameter, d) and the product of the two diameters (estimate of surface area). Follow-up time: from beginning of treatment until last measurement. Mode of examination: direct observation and measurement (OBS), sonography (US), computed tomography (CT), magnetic resonance imaging (MRI).

Infantile hemangiomas have typical presentation and evolution.[2] They express a homogeneous group of immunohistochemical markers, including GLUT1 (glucose transporter 1), which is a surface protein expressed by erythrocytes and the endothelium of infantile hemangiomas.[6] It is possible that propranolol has a specific effect on lesions that express GLUT1, regardless of its presentation and stage of development. Indeed, infantile hemangiomas have been found to express GLUT1 in both the proliferative and regressive phases.[6] In contrast, non-evolutive congenital hemangiomas constitute a group of lesions that are clinically and histologically distinct and do not express this marker.[7] In our series, the patients with congenital hemangiomas (clinical and radiological diagnoses) showed little or no response, unlike most other patients (data not shown). Perhaps the lesions with different presentations or evolution that respond to propranolol are actually GLUT1 positive (+) hemangiomas.

The mechanism of action of propranolol in infantile hemangiomas is still the subject of speculation. Initially, the idea was that this effect could be mediated by binding to beta-adrenergic receptors, leading to reducing of pro-angiogenic factors like

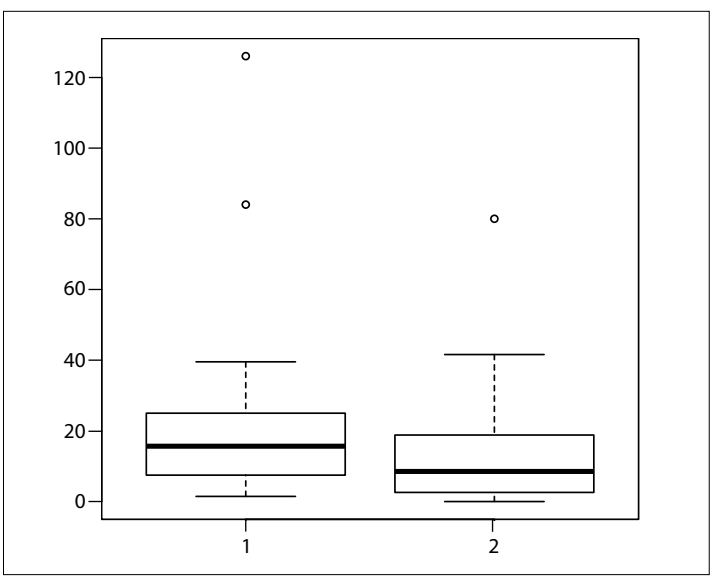

Figure 3. Boxplot of lesion sizes (surface area estimates) at first measurement (1) and last measurement (2). The graph shows the median (band), 25th and 75th quantiles (bottom and top of box), sample minimum and maximum (whiskers) and outliers (dots).

VEGF (vascular endothelial growth factor) and b-FGF (fibroblast growth factor beta).[8] It has already been shown that infantile hemangiomas express adrenergic receptors and are closely related to sympathetic innervation.[9] There has been speculation that, in particular, inhibition of beta-2 adrenergic receptors may lead to vasoconstriction, anti-angiogenesis (via inhibition of VEGF) and induction of apoptosis in hemangiomas.[10] However, no experimental evidence has corroborated these hypotheses. Other possible molecular pathways that are involved in vascular tonus and endothelial proliferation and which could directly or indirectly function as targets of propranolol include: cAMP/PKA, leading to increased VEGF/b-FGF;[11] inhibition of vasodilation through reducing the release of NO mediated by beta-3 receptor ligands;[12] and VEGF production regulated by NF-kB, which relates to the effect of steroids on hemangiomas.[13] Recently, involvement of elements of the renin-angiotensin-aldosterone system has been suggested, via inhibition of the renal renin-angiotensin-aldosterone system by propranolol, thus leading to inhibition of proliferation of endothelial progenitor cells that express receptors for VEGF and the CD34 marker.[14] However, an anecdotal reference to alleged direct binding of propranolol with GLUT1 has no scientific basis.[15]

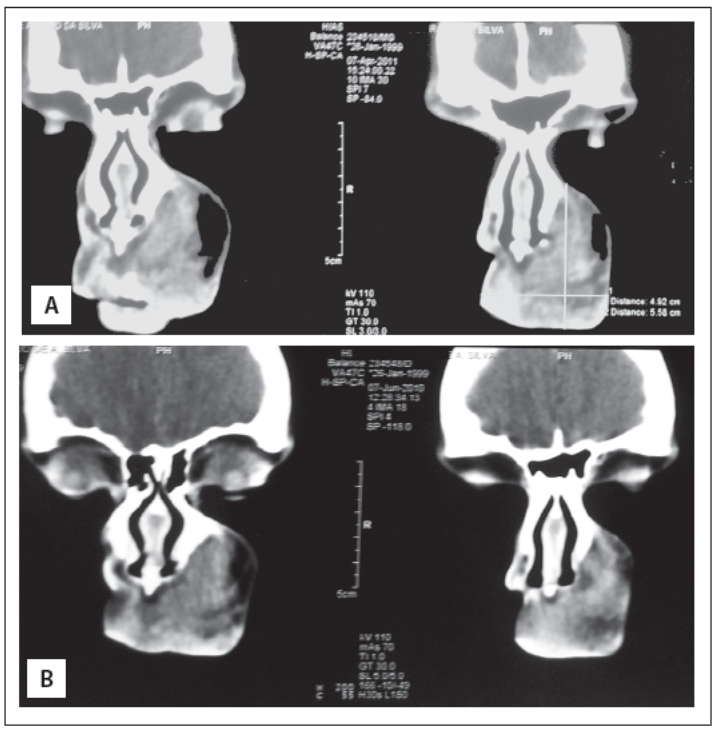

Figure 4. Computed tomography scans on a patient with a congenital facial lesion, contrast enhancing, non-involutive. Imaging and clinical history were used for diagnosing non-involutive congenital hemangioma (NICH). Propranolol treatment did not change the estimated lesion size. Upper panel (A) shows lesion after the treatment, whereas lower panel (B) shows the lesion 10 months earlier.

Regardless of the mechanism of action, it is now indisputable that propranolol has an important effect on infantile hemangiomas, such that it causes their rapid regression.[16] Other groups have also reported similar results,[17,18] in which they showed that the treatment rapidly induced stabilization of lesion proliferation and reduction of the volume of lesions in 100% of the patients. A review of several worldwide series[19] of between one and 58 patients reported that propranolol had been effective in most cases. In 205 pooled cases, there was an "excellent response" in 42 cases, while 69 were classified as "good" or "moderate" or "partial response", 56 had responses that were not quantified and 10 did not respond at all or "deteriorated" or showed "mild recurrence". The rest of the patients' responses were not described. This corresponds to an 82% response rate and a 5% refractoriness or relapse rate. The response in individual series has ranged from 47 to 100%. Doses and administration schedules have varied little: 1-3 mg/kg per day, either with a gradual increase or starting at full dose. The duration of treatment reported has varied considerably, from two to 18 months, which may explain some of the variability of the results. A double-blind, randomized clinical trial of propranolol reported that the treatment was effective in 90% of the 19 children (four months to five years of age), who were treated with 2 mg/kg per day at 8 h intervals. The treatment on the lesions caused them to soften and change color from red to purple within 24 h, stopped their growth in 2-30 days and caused a rapid volume reduction by 4-8 weeks. Thereafter, the reduction of the residual lesions was slower. The trial showed that there was a statistically significant reduction of redness and elevation of infantile hemangiomas.[20] There is no recommended length of treatment, but it has been shown[20] that treating for a minimum of six months and at least until one year of age may prevent recurrences.

These data are comparable with the results from our series. We observed responses in 87% of our 69 patients. Most of the published series included only infants with capillary hemangiomas.[19] The original series included patients aged up to four years, and the only randomized trial completed so far included children of up to five years of age.[17,20] In our series, most of the patients were under one year of age, and 75% were 5 years of age or less, and thus our results are directly comparable with the previously published series. A small number of older children and adolescents (most with lesions other than capillary hemangiomas or residual/refractory hemangiomas) were included in this series. To our knowledge, this is the first report to include patients over five years of age treated with propranolol. Surprisingly, some patients in the older subset did respond to the treatment, thus indicating that propranolol may have some therapeutic utility in these patients.

β-blockers have a well-documented safety and side-effect profile. Over the course of 40 years of clinical use at therapeutic doses in children less than seven years of age, there have been no cases of mortality and no serious cardiovascular events.[20] The safety of propranolol was not addressed in our retrospective series. However, we recorded few side effects, and none requiring treatment discontinuation. Previously published series reported variable incidence of adverse events, ranging from none recorded[20] to two thirds of the patients (a Chinese series that reported diarrhea possibly caused by the oral formulation).[19] Adequate phase I studies on propranolol use among children with hemangiomas may be warranted, in order to ascertain the real adverse event frequency in these patients.

Our report is, as far as we are aware, the largest single-center series so far published in the literature and is one of the few that included syndromic patients with hemangiomas or lesions other from infantile hemangiomas. We were able to reproduce the good results reported by other groups, although we showed that a small number of patients were refractory. These results may have implications for treatments for infantile hemangiomas, given that current practice is to use steroids as first-line therapy in cases of complicated hemangiomas.

Regarding syndromic patients or other types of hemangiomas, and older patients, it is still too early to say for sure whether patients within this heterogeneous group can also benefit from therapy with propranolol. Moreover, it can be hypothesized that propranolol acts specifically on GLUT1-positive lesions, regardless of their clinical presentation. These are interesting directions for future basic and clinical research.

CONCLUSIONS

Propranolol seemed to be effective in treating hemangiomas in children of all ages, and not only in the proliferative stage of the lesions (up to one year of age), with a high response rate. The outcome varied with the type of lesion, and with the age (difference not statistically significant). Infantile hemangiomas in infants under one year of age showed responses in nearly all patients. Randomized clinical trials are necessary to confirm this finding and to assess the safety of the intervention as well.

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Address for correspondence:
Francisco Helder Cavalcante Felix
Hospital Infantil Albert Sabin
Tertuliano Sales 544,
Fortaleza (CE) — Brasil
CEP 60410-794
Tel. (+55 85) 3257-9613
E-mail: heldercfelix@gmail.com

Interaction between pharmaceutical companies and physicians who prescribe antiretroviral drugs for treating AIDS

Interação entre empresas farmacêuticas e médicos que prescrevem antirretrovirais para o tratamento da aids

Mário César Scheffer[I]

Department of Preventive Medicine, Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil

MSc, PhD. Professor, Department of Preventive Medicine, Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, Brazil.

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Ethics, medical.
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Acquired immunodeficiency syndrome.

PALAVRAS-CHAVE:
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Ética médica.
Antirretrovirais.
Síndrome de imunodeficiência adquirida.

ABSTRACT

CONTEXT AND OBJECTIVE: Given that Brazil has a universal public policy for supplying medications to treat HIV and AIDS, the aim here was to describe the forms of relationship between physicians and the pharmaceutical companies that produce antiretrovirals (ARVs).

DESIGN AND SETTING: Cross-sectional epidemiological study conducted in the state of São Paulo.

METHODS: Secondary database linkage was used, with structured interviews conducted by telephone among a sample group of 300 physicians representing 2,361 professionals who care for patients with HIV and AIDS.

RESULTS: Around two thirds (64%) of the physicians prescribing ARVs for HIV and AIDS treatment in the state of São Paulo who were interviewed declared that they had some form of relationship with pharmaceutical companies, of which the most frequent were receipt of publications (54%), visits by sales promoters (51%) and receipt of small-value objects (47%).

CONCLUSIONS: Two forms of relationship between the pharmaceutical industry and physicians who deal with HIV and AIDS can be highlighted: facilitation of professionals' access to continuing education; and antiretroviral drug brand name promotion.

RESUMO

CONTEXTO E OBJETIVO: Diante da existência no Brasil de uma política pública universal de fornecimento de medicamentos para tratamento do HIV e aids, o objetivo é descrever formas de relacionamento entre os médicos e as empresas farmacêuticas produtoras de antirretrovirais (ARVs).

TIPO DE ESTUDO E LOCAL: Estudo epidemiológico de tipo transversal realizado no estado de São Paulo.

MÉTODOS: Foi realizado cruzamento entre bancos de dados secundários e entrevistas estruturadas, por meio telefônico, em amostra de 300 médicos representativa de 2.361 profissionais que assistem pacientes com HIV e aids.

RESULTADOS: Cerca de dois terços (64%) dos médicos entrevistados que prescrevem ARVs para tratamento de HIV e aids no Estado de São Paulo declararam que tiveram alguma relação com empresas farmacêuticas, sendo mais frequentes o recebimento de publicações (54%), de visita de propagandistas (51%) e de objetos de pequeno valor (47%).

CONCLUSÃO: Destacam-se duas formas de relacionamento entre a indústria farmacêutica e os médicos que trabalham com HIV-aids: facilitação do acesso dos profissionais à educação continuada e promoção da marca de medicamentos antirretrovirais.

INTRODUCTION

The AIDS epidemic introduced new elements into the world of drug research and development. It gave rise to unprecedented behavior within the pharmaceutical industry, the fields of medicine and science, the ethics of human research and the organization of services and community mobilization to ensure access to antiretroviral therapy.[1]

In the early 1990s, monotherapy with antiretrovirals (ARVs), followed by combinations of two medications, offered patients modest and ephemeral benefits before the disease evolved. The therapeutic approach to AIDS made unquestionable progress only after the introduction, in 1995, of highly active antiretroviral therapy (HAART), which featured protease inhibitor drugs, thus making ARV combinations more powerful and effective. The role of ARV drugs is to inhibit viral replication, restore the immune system of infected people and reduce occurrences of opportunistic infections and other morbidities.[2]

The pharmaceutical companies responsible for discovering and launching ARVs form one of the most competitive sectors of the global market, dominated by multinational corporations. They are large companies capable of financing and incorporating into their products the main advances that are seen to be possible within the biomedical, biological and chemical sciences.[3]

The main multinationals involved in ARV production in the world have subsidiaries in Brazil. ARVs have been introduced into Brazil in line with the companies' global strategies, local demand and the Ministry of Health's purchasing capacity. The goal of a subsidiary of a foreign pharmaceutical company is to expand the national market of a particular pharmaceutical specialty in order to increase the demand for the drugs developed and produced by the parent company.[4]

Of the 21 ARVs distributed by the Ministry of Health in 2010, 10 were produced by Brazilian pharmaceutical companies in the form of generic drugs. However, foreign companies with patented brands consume most of the public funds earmarked for contracts for purchasing ARVs in Brazil. Approximately 200,000 individuals were undergoing treatment with ARVs within the public healthcare network in Brazil in 2010, and 35,000 new cases of HIV infection are identified per year on average. This is generating a progressive increase in the number of people who will begin to receive ARVs. The Ministry of Health is responsible for recording, purchasing, distributing and preparing clinical guidelines for use of ARVs. These 21 medications, which are in five therapeutic classes, are available through the Brazilian National Health System (Sistema Único de Saúde, SUS), and are dispensed in 677 public pharmacies and healthcare services.[5]

The high consumption of ARVs in Brazil, which are included within a public policy of universal access, means that pharmaceutical companies have set in motion a wide range of promotion strategies and informational and persuasion-related activities, with the objective of encouraging prescription, dispensing and purchasing by the government, and use of their medications.

In this regard, physicians prescribing ARVs, who rely on help from clinical guidelines produced by the government program, but also enjoy professional autonomy at the time of making prescriptions, become the number one target of company marketing.

OBJECTIVES

The present article had the aim of sizing up and describing the main forms of relationship between physicians who prescribe ARVs and the pharmaceutical industry. At the same time, it sought to address the promotion strategies of the companies producing these medications that form part of the Brazilian public policy of universal access to HIV and AIDS treatment.

METHOD

This study used a research database that drew up the profile of physicians who prescribe ARVs in the state of São Paulo,[6] conducted in conjunction with the Department of Preventive Medicine of the University of São Paulo School of Medicine (FMUSP). For the initial study, the population was composed of 2,361 physicians who treated people with HIV in the state of São Paulo and who prescribed antiretroviral drugs between October 2007 and May 2009. The list of prescribing physicians was extracted from the Medication Logistics Control System (SICLOM) of the STD, AIDS and Viral Hepatitis Department of the Ministry of Health. The information about sociodemographic and academic background characteristics was obtained from the physician roster of the Regional Medical Council of the State of São Paulo (Conselho Regional de Medicina do Estado de São Paulo, CREMESP).

In its second phase, the study gathered the opinions of ARV prescribers, in a selected sample representing the complete group of 2,361 physicians. Drawing on approximation to the binomial distribution for normal distribution, with the aim of estimating the parameter $\pi = 0.50$ (proportion of largest variance) with a sampling error of 0.06 and a confidence interval of 95%, the number of participants required was found to be approximately $n = 300$. The selection was made proportionally to the following strata: volume of patients, length of professional experience and region where the physician lived.

The interviews covered job satisfaction, education, experience, training, working conditions, pay, relationship with patients, opinion about the Brazilian anti-AIDS program, and other points. One of the topics addressed, which is the subject of the present article, was the physicians' relationship with the pharmaceutical companies that produce ARVs.

The geographical limitation of the study to the state of São Paulo was established because this is the state that concentrates the highest number of notified cases of AIDS in Brazil, totaling 207,077 from 1980 to June 2011.[7] This state also contains all of the

Medication Dispensing Units within SUS that are integrated into SICLOM, an information system that makes it possible to obtain data on the prescribing physicians.

RESULTS

Observation of the profile of ARV-prescribing physicians in the state of São Paulo who answered the questionnaire revealed that 48% were male and 52% female; the majority were aged between 24 and 40 years (57%), averaging 39 years; most lived in the metropolitan region of São Paulo (57%), with 52% in the state capital; and 50.6% had completed and 49.4% had not completed their medical residency. Regarding academic background, 30.7% had specialized in infectious diseases; 35.1% had undergone specialized training in other areas and 34.2% did not have any type of specialized training. In this study, specialized training was taken to mean obtainment of a title issued by a medical specialty society, or completion of medicine residency or a master's or doctoral program. Most of the physicians (55%) had up to nine years of experience with HIV and AIDS and, at the time of the survey, 48% were caring for more than 20 patients each. On average, the physicians cared for six patients with HIV and AIDS per day, whereas 75% cared for between one and ten patients on a daily basis. Out of the total number sampled, 11% declared that they were not regular prescribers, since they had seldom prescribed ARVs.

Among the doctors who cared for patients with HIV and AIDS in the state of São Paulo, 64% declared that they had received products, benefits or payments from the pharmaceutical laboratories that produce ARVs (**Figure 1**).

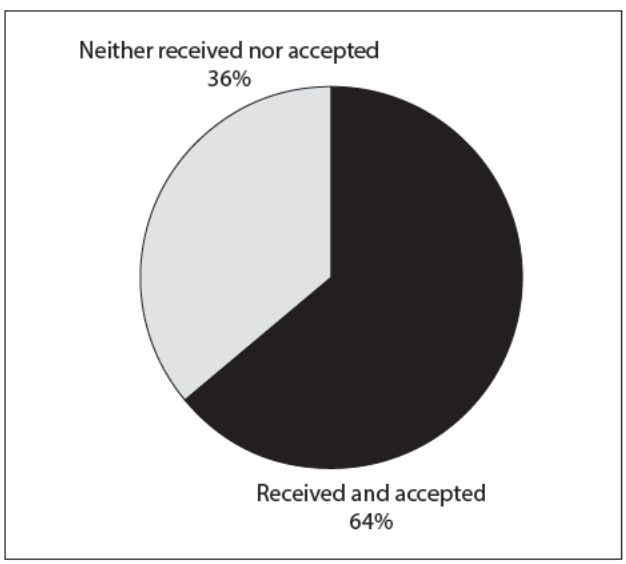

Figure 1. Distribution of physicians who prescribed antiretroviral drugs in the state of São Paulo, according to receipt or non-receipt of benefits from pharmaceutical laboratories, 2010.

The most common benefits received (**Figure 2**) were: informative materials about ARVs (54%); visits by sales promoters and sales representatives (51%); inexpensive objects for the doctor's office (47%); invitations to take part in continuing education courses and events (40%); and scientific journals sponsored by the laboratories (38%).

In smaller proportions, the physicians declared the following benefits: lunches or dinners (27%); trips to national conventions (17%) and international conventions (7%); invitations to take part in or to conduct clinical research (15%); tickets to cultural and leisure events (11%); fees relating to consulting services, lessons, lectures or speaking at conventions (5%); and free ARV drug samples (1%).

With regard to the benefits offered, infectologists (or specialists in infectious diseases) were the physicians who most received small-value objects ($P < 0.001$) and trips to national conventions ($P = 0.021$). This statistical significance was obtained through contingency table analysis using the chi-square test (x^2) and Fisher's exact test, with the significance level of $\alpha < 0.05$.[8] In relation to other benefits, although high numbers were received by the infectologists, statistical significance was not observed (**Annex**).

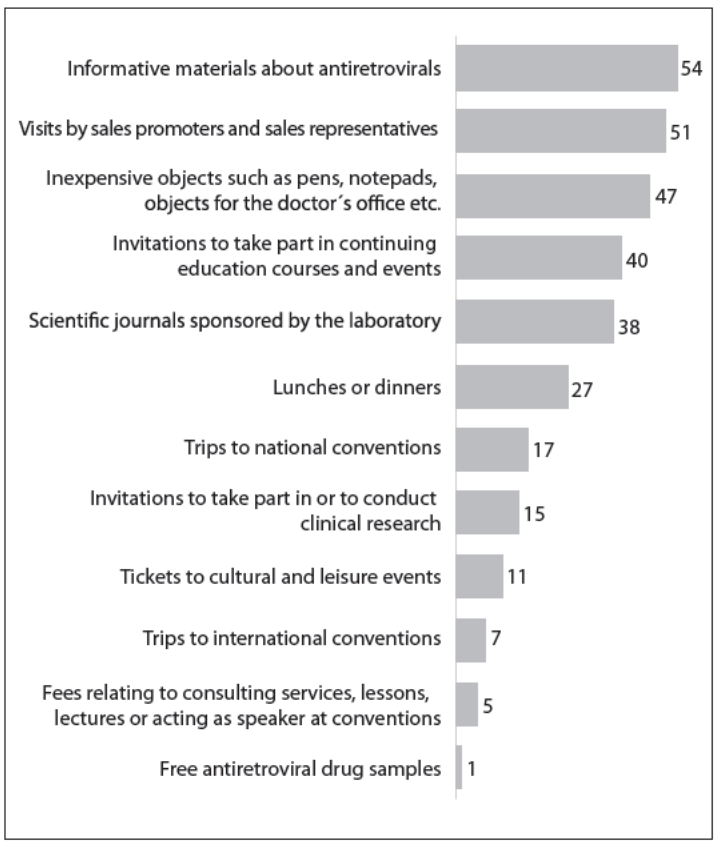

Figure 2. Distribution of physicians who prescribed antiretroviral drugs in the state of São Paulo, according to types of benefits received from pharmaceutical laboratories, 2010.

Asked about the laboratories' level of influence on professional activities, most of the interviewees stated that the pharmaceutical companies' actions had a strong influence (10%), slight influence (50%) or no influence (40%) on their prescribing of antiretrovirals (**Figure 3**).

DISCUSSION

This study shows that one of the strategies of the ARV industry is to invest in training and information for physicians, which includes sponsorship of courses and production of informative material. Another initiative is financial support for physicians' participation in scientific events and congresses, where the pharmaceutical companies take part in scheduling and organizing satellite events, erect stands and develop promotional activities.

However, it is worth emphasizing that this study shows that only a minority of the physicians who were prescribing ARVs (10%) affirmed that the companies exerted considerable influence over them.

Although the percentage of physicians who prescribed ARVs (64%) and had received some benefit from the industry was lower than the level that has been identified in other studies evaluating the relationship between physicians and the industry. In the state of São Paulo, 93% of physicians in general were found to have received some benefit from pharmaceutical, equipment, orthosis and prosthesis companies.[9] In the United States, 94% of physicians were found to have some connection with drug manufacturers.[10]

Some factors may be linked to the relatively low level of interaction between ARV prescribers and the industry. In Brazil, these medications are not traded on the market: they form part of a public program of free distribution through SUS and are included in clinical guidelines that are updated periodically and accepted by the medical community. Another important factor is that some ARVs are already produced in Brazil, in the form of generic drugs, which diminishes the interest in commercial promotion of these medications among national producing laboratories.

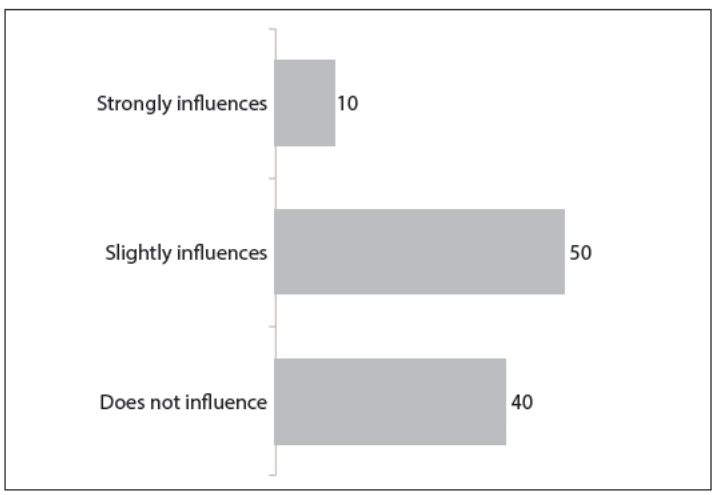

Figure 3. Distribution of physicians who prescribed antiretroviral drugs in the state of São Paulo, according to their opinion regarding the influence of pharmaceutical companies on prescriptions, 2010.

On the other hand, ARV use depends on medical prescription. Several of them compete for the same therapeutic indication and new products of patented brands are constantly being launched, thus causing producing companies to resort to all the available resources to conquer the market.

Several variables have been correlated with the act of medical prescribing: technical capacity, intelligence, skills, common sense, motivation, standards of judgment, knowledge accumulation, clinical experience, time dedicated to refresher courses, level of confidence in the sales promoters of the pharmaceutical companies, specialization level, time since graduation, workplace and coexistence with colleagues.[11]

Medical literature, scientific congresses, information on pharmaceutical companies, professional interactions between physicians, continuing education programs and clinical guidelines have an influence on prescribing of medications. Physicians mainly evoke the scientific literature as the greatest influence on prescriptions, but in practice they can also be influenced by the discourse of the pharmaceutical industry.[12]

The marketing practices of pharmaceutical companies are often subtle and indirect. They consist of amicable pressures and games of influence that can go unnoticed, since they are an integral part of the culture of the drug market. Physicians and the drug industry are bound by mutual necessity.[13] Nonetheless, targeted promotion may possibly create a favorable environment, of sympathy for and receptivity towards the medication.

In Brazil, initiatives targeting improvement of regulations relating to interactions between prescribers and the pharmaceutical industry are still taking shape.

The Medical Ethics Code (updated in 2010) and Resolutions from the Federal Medical Council and the National Health Surveillance Agency may not be sufficient. Likewise, self-regulatory codes of ethics within the current pharmaceutical market, with manuals on advertising and marketing conduct implemented by drug companies, comprise initiatives that may need to be enhanced in order to ensure greater transparency and higher ethical standards in relationships between producers and prescribers.

Furthermore, it can be assumed that there is a considerable imbalance in financial resources between the funds made available by the pharmaceutical industry for promotional information relating to ARVs and the funds from the government and professional associations intended for information addressed to physicians.

The issue of the influence of the industry on prescription choice should form the subject of future studies, considering that much evidence showing that pharmaceutical companies' actions may arouse conflict of interests and may influence physicians' decisions has been presented in the literature.[14]

CONCLUSION

Two activities can be highlighted regarding the forms of relationship between physicians who prescribe ARVs and pharmaceutical companies: continuing education initiatives (brochures, scientific journals, courses and participation in medical conferences) and drug brand name promotion (sales representative visits and brand-orientated gifts and objects). It was not possible to determine the influence of the relationship involved in physicians' choice of ARVs for prescription.

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Address for correspondence:
Mário César Scheffer
Departamento de Medicina Preventiva
Faculdade de Medicina da Universidade de São Paulo
Av. Dr. Arnaldo, 455 — 2º andar
Pacaembu — São Paulo (SP) — Brasil
CEP 01246-903
Tel. (+55 11) 3061-7285
E-mail: mscheffer@uol.com.br

Annex. Distribution of physicians who prescribed antiretroviral drugs in the state of São Paulo, according to products, benefits or payments received and accepted from pharmaceutical laboratories, 2010.

	Total	Operating region		Experience time with HIV/AIDS			Volume of patients			Sex		Age			Speciality			
		Metropolitan region of São Paulo	Inside São Paulo	Up to 3 years	4 to 19 years	20 years or more	Serves up to 20 patients	21 to 120 patients	121 patients or more	Male	Female	Up to 30 years	31 to 50 years	51 years or more	Infectious Diseases	Clinical Medicine	Other specialities	
Received and accepted	**64**	**69**	**58**	**50**	**70**	**81**	**52**	**75**	**91**	**69**	**60**	**54**	**72**	**71**	**97**	**40**	**49**	**P-value**
Informative materials about antiretrovirals	54	60	46	38	60	74	37	68	88	57	51	41	61	64	91	29	34	0.70
Visits by sales promoters and sales representatives	51	54	47	28	61	74	32	65	87	59	43	31	65	61	91	26	30	0.120
Inexpensive objects such as pens, notepads, objects for the doctor's office etc.	47	47	47	31	54	64	31	63	77	50	44	32	62	50	81	26	30	**< 0.001**
Invitations to take part in continuing education courses and events	40	46	32	26	46	55	29	43	66	46	34	29	50	43	66	27	35	0.106
Scientific journals sponsored by the laboratory	38	42	33	22	45	56	23	47	69	43	34	24	48	47	68	18	21	0.985
Lunches or dinners	27	30	23	16	35	33	17	33	47	30	25	18	33	32	46	13	14	0.570
Trips to national conventions	17	15	19	4	23	30	4	20	49	20	14	5	25	24	38	10	2	**0.021**
Invitations to take part in or to conduct clinical research	15	14	16	3	17	35	5	18	34	18	12	3	21	26	29	8	6	0.543
Tickets to cultural and leisure events	11	13	8	4	16	15	7	12	18	13	9	4	16	14	20	2	5	0.141
Trips to international conventions	7	6	9	–	8	20	1	7	23	8	6	0	10	13	17	4	3	0.520
Fees relating to consulting services, lessons, lectures or acting as speaker at conventions	5	4	7	1	5	14	2	6	14	7	4	1	9	8	12	2	1	
Free antiretroviral drug samples	1	–	2	1	1	–	2	–	–	1	1	1	1	–	1	–	3	
Neither received nor accepted	**36**	**31**	**42**	**50**	**30**	**19**	**48**	**25**	**9**	**31**	**40**	**46**	**28**	**29**	**3**	**60**	**51**	
Base	**300**	**177**	**123**	**101**	**135**	**64**	**96**	**101**	**89**	**142**	**158**	**104**	**118**	**78**	**153**	**56**	**46**	

Muir-Torre Syndrome: case report and molecular characterization

Síndrome de Muir-Torre: relato de caso e caracterização molecular

Carolina Alejandra Rios[I], Ricardo Villalón[II], Jorge Muñoz[III], Mónica Acuña[IV], Lucía Cifuentes[V]

Department of Human Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, and Surgical Service, Complejo Asistencial Barros Luco Trudeau, Santiago, Chile

[I]PhD. Scientific Researcher, Genetic Epidemiology Laboratory, Department of Human Genetics, School of Medicine, University of Chile, Santiago, Chile.

[II]MD. Attending Physician, Surgical Service, Complejo Asistencial Barros Luco Trudeau, Santiago, Chile.

[III]BSc. Medical technologist, Pathological Anatomy Service, Clínica Dávila, Santiago, Chile.

[IV]MSc. Associate Professor, Department of Human Genetics, Institute of Biomedical Sciences, School of Medicine, University of Chile, Santiago, Chile.

[V]MD, MSc. Full Professor, Department of Human Genetics, Institute of Biomedical Sciences, School of Medicine, University of Chile, Santiago, Chile.

KEY WORDS:
Muir-Torre syndrome.
Colorectal neoplasms, hereditary nonpolyposis.
Pathology, molecular.
Microsatellite instability.
Mutation.

PALAVRAS-CHAVE:
Síndrome de Muir-Torre.
Neoplasias colorretais hereditárias sem polipose.
Patologia molecular.
Instabilidade de microssatélites.
Mutação.

ABSTRACT

CONTEXT: Muir-Torre syndrome is a rare autosomal dominant genodermatosis caused by mutations in the mismatch repair genes. It is characterized by the presence of sebaceous skin tumors and internal malignancies, affecting mainly the colon, rectum and urogenital tract. Awareness of this syndrome among physicians can lead to early diagnosis of these malignancies and a better prognosis.

CASE REPORT: We report the case of a Chilean patient who, over the course of several years, had multiple skin lesions, endometrial cancer and colon cancer. The syndrome was diagnosed using molecular techniques such as microsatellite instability analysis, immunohistochemistry and DNA sequencing, which allowed us to find the causative mutation.

CONCLUSION: Molecular diagnostics is a highly useful tool, since it allows clinicians to confirm the presence of mutations causing Muir-Torre syndrome. It is complementary to the analysis of the clinical data, such as dermatological presentation, presence of visceral malignancies and family history of colorectal tumors, and it provides important knowledge to help physicians and patients choose between treatment options.

RESUMO

CONTEXTO: A síndrome de Muir-Torre é uma genodermatose autossômica dominante rara causada por mutações nos genes de reparo de incorreções. Caracteriza-se pela presença de tumores sebáceos da pele e doenças malignas internas, afetando principalmente cólon, reto e trato urogenital. A consciência desta síndrome pelos médicos pode levar ao diagnóstico precoce dessas doenças malignas e a um melhor prognóstico.

RELATO DE CASO: Relatamos o caso de uma paciente chilena que, ao longo de vários anos, teve lesões cutâneas múltiplas, câncer de endométrio e câncer de cólon. A síndrome foi diagnosticada com técnicas moleculares, como a análise de instabilidade de microssatélites, imunoistoquímica e sequenciamento de DNA, o que nos permitiu encontrar a mutação causadora.

CONCLUSÃO: Diagnóstico molecular é uma ferramenta muito útil, uma vez que permite que os clínicos confirmem a presença de mutações causadoras de síndrome de Muir-Torre. É complementar para a análise dos dados clínicos, tais como a apresentação dermatológica, a presença de doenças malignas viscerais e história familiar de tumores colorrectais, e fornece conhecimentos importantes para ajudar os médicos e os pacientes a escolher entre opções de tratamento.

INTRODUCTION

Muir-Torre syndrome (OMIM #158320) is a rare autosomal dominant genetic disease. It is characterized by the presence of sebaceous skin tumors and internal malignancies such as colorectal cancer.[1,2] Muir-Torre syndrome is considered to be a phenotypic variant of Lynch syndrome (OMIM #120435), since these conditions have a common molecular etiology.[3,4]

Lynch syndrome accounts for approximately 3% of the incidence of colon cancer worldwide, and can also present with cancer of the urogenital tract, stomach, small bowel, brain and hepatobiliary tract.[5,6] It is caused by germline mutations in genes of the mismatch repair system, mainly *hMSH2* and *hMLH1*. Molecularly, Lynch tumors are characterized by high microsatellite instability and absence of expression of one or more of the proteins that comprise the mismatch repair system.[4-6] These same characteristics are present in Muir-Torre syndrome, but this syndrome differs from Lynch syndrome in that skin lesions are present, such as sebaceomas, sebaceous adenomas and carcinomas, basal cell carcinomas with sebaceous differentiation and seboacanthomas.[7]

Awareness of this syndrome among physicians can help a number of patients and their families, since the proper identification of Muir-Torre syndrome can lead to early diagnosis of visceral malignancies and, hence, better prognosis.

CASE REPORT

A female Chilean patient consulted the Complejo Asistencial Barros Luco Trudeau in Santiago, Chile, for the first time in 1998 due to a cutaneous lesion. She was diagnosed with a sebaceous cyst. Two years later, the lesion recurred and was excised. The histological analysis showed a well-differentiated sebaceous carcinoma. In 2006, the patient (now aged 49 years) presented stage IIIA endometrial cancer with ovarian metastases. She underwent total hysterectomy and bilateral adnexectomy, together with radiotherapy and brachytherapy, at the same hospital.

Over the years, the patient had several sebaceous hyperplasias and carcinomas removed. In 2009, Muir-Torre syndrome was suspected by the medical staff at the hospital and she underwent colonoscopy. The patient did not present gastrointestinal symptoms and the carcinoembryonic antigen level was normal (3.0 μg/ml). However, she had a strong family history of colorectal cancer, with one first-degree relative and two second-degree relatives with the disease.

The colonoscopy detected a lesion in the cecum. The patient underwent right hemicolectomy and received adjuvant chemotherapy (**Figure 1**). The histology showed a well-differentiated adenocarcinoma with mucinous areas (less than 50%), without lymph node involvement.

The genetic testing for Muir-Torre syndrome was performed at the Faculty of Medicine of the Universidad de Chile, Santiago. Paraffin-derived colon tumor tissue was used for all analyses. MLH1 and MSH2 protein expression was studied by means of immunohistochemistry with monoclonal antibodies. Lack of hMSH2 expression was found (**Figure 2**), while hMLH1 was expressed normally (data not shown). Also, DNA from the patient's colon cancer was tested for microsatellite instability, using the markers BAT25, BAT26, D2S123, D5S346 and D17S250.[8] The tumor was MSI-high, since it presented over 30% unstable *loci.* In this analysis, both mononucleotide loci (BAT25 and BAT26) were unstable (**Figure 3**), which is evidence of mismatch repair system malfunctioning.

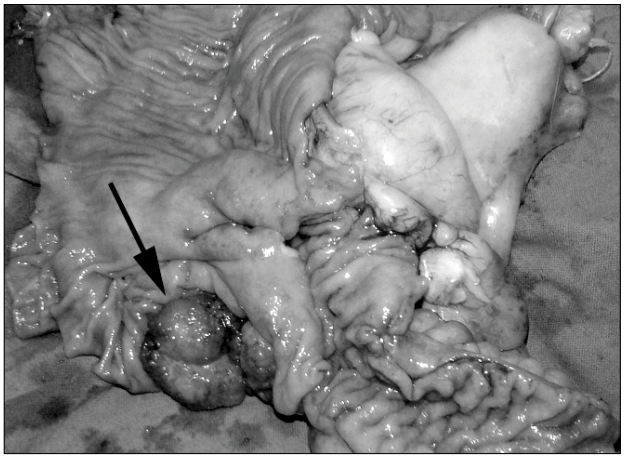

Figure 1. Colon adenocarcinoma.

Following these results, each exon of the *hMSH2* gene was amplified by PCR and sequenced. The subsequent analysis revealed heterozygote single base pair replacement of cytosine (C) by thymine (T) in exon 13 in position 2131. This nucleotide change causes an alteration in the protein sequence, changing an arginine for a stop codon.

DISCUSSION

Muir-Torre syndrome is a rare form of Lynch syndrome and is characterized by tumors of the sebaceous glands or keratoacanthoma and a median age of onset of 50 years.[1,2] This syndrome usually arises within families with colorectal cancer histories, but not necessarily histories of skin tumors. Therefore, a key issue in diagnosing Muir-Torre syndrome is the correct identification of any family history of tumors.[2,3]

Molecularly, Muir-Torre has a common etiology with Lynch syndrome: germline mutations in the mismatch repair genes. However, different studies have shown that Muir-Torre syndrome is preferentially associated with mutations in the *hMSH2* gene,[1-3,7] although mutations in *hMLH1* and *hMSH6* can also be implicated.[9,10] The mutation found in our patient generated a premature stop codon, which produced a truncated MSH2 protein that lacks an important functional domain.[7,11]

This syndrome has also been associated with mutations in the MYH gene, which is inherited in an autosomal recessive

pattern. These mutations are a cause of attenuated familial polyposis, and the Muir-Torre cases associated with them do not show microsatellite instability.[12,13]

Diagnosis of Muir-Torre syndrome is mainly done from the dermatological clinical features and the presence of visceral malignancies or a family history of these.[1,2] A systematic search on this topic showed that this syndrome can go unrecognized if clinicians are unaware of these characteristics (**Table 1**). The role of clinicians in detection and treatment is fundamental. During this search, we found two Brazilian reports of patients with Muir-Torre syndrome.[14,15] Even though the skin lesions presented by these patients were different, they had in common the visceral malignancies and the strong family history, thus highlighting the importance of these clinical characteristics. These two reports did not present any molecular diagnosis, so we were unable to compare them at the molecular level.

Molecular diagnosis has an important role in detecting patients who are carriers of mutations of the mismatch repair system, since such individuals present 80-100% risk of developing cancer.[2,5] Surveillance of these patients and their families is critical, because they can present synchronous or metachronous tumors, especially in the large bowel and the urogenital tract.[16] Frequent colonoscopies are recommended. This situation is likely to require genetic counseling for the patient and the family, in order to help them understand the nature of the syndrome and the relevance of life-long follow-up.[17]

With regard to treatment, in cases with skin lesions, wide local excision with follow-up is standard for detection of possible metastases. Isotretinoin (retinoid) by itself or combined with interferon has been used to prevent some of the cutaneous neoplasms that have been described.[18,19]

Furthermore, use of prophylactic surgery versus endoscopic monitoring has been widely discussed. In patients with colon cancer and proven mutations, total colectomy is recommended, due to the high risk of synchronous and metachronous tumors. However, in patients suspected of having Muir-Torre syndrome, with colon cancer but without a genetic diagnosis,

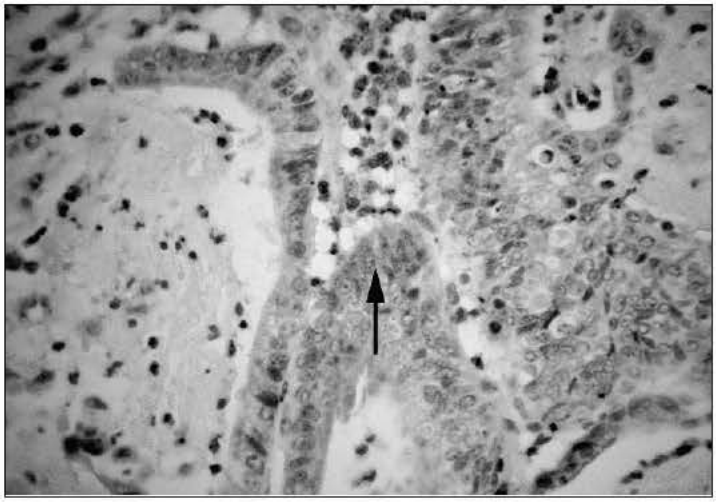

Figure 2. Immunohistochemical staining of colon tissue.

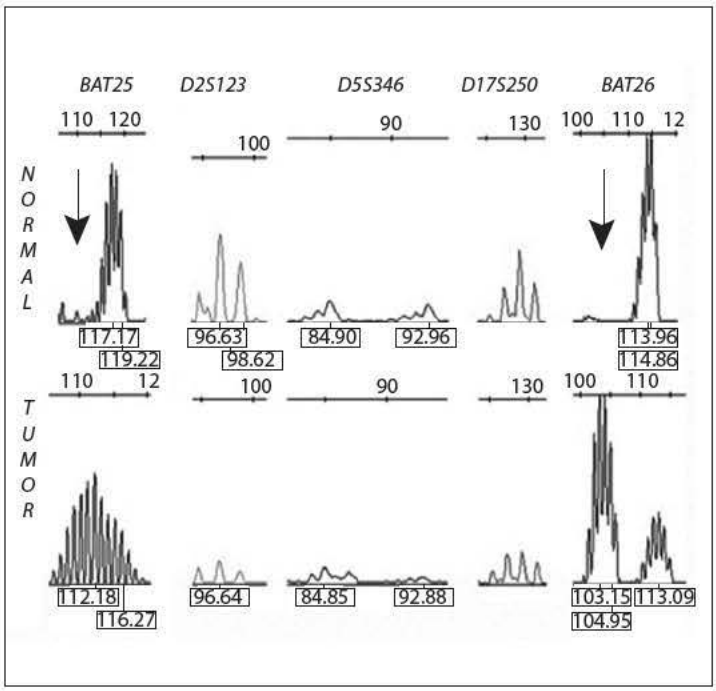

Figure 3. Microsatellite instability analysis.

Table 1. Search strategies regarding the topic of Muir-Torre syndrome. This search was performed on April 8, 2013

Database	Search terms	Results	Relevant findings
Medline (Medical Literature Analysis and Retrieval System Online)	(Muir-Torre syndrome [MeSH terms])	33 case reports	Awareness of clinicians, and particularly dermatologists, on dermatological symptoms is key to diagnosing Muir-Torre syndrome. Molecular testing allows for better diagnosis and surveillance.
Lilacs (Literatura Latino-Americana e do Caribe em Ciências da Saúde)	(Síndrome de Muir-Torre) OR (Muir-Torre syndrome)	3 case reports	The diagnosis can be made with only the clinical characteristics.
Embase (Excerpta Medica Database)	'Muir-Torre syndrome'	204 case reports	Sebaceous adenomas and carcinomas can appear in different parts of face and body. Awareness of the symptoms is vital to diagnosis.

The search was narrowed using filters provided by each database: Article type - case reports (PubMed), Tipo de estudio – informe de casos (Lilacs) and Study type - case report (Embase).

use of prophylactic surgery is controversial.[20] Women with Muir-Torre syndrome are also at higher risk of endometrial and ovarian cancer. In women of childbearing age, total hysterectomy with salpingo-oophorectomy has been discussed as a prophylactic approach, but there is no consensus on this topic.[21]

CONCLUSION

Molecular confirmation of Muir-Torre syndrome and Lynch syndrome is an important tool in clinical diagnosis, because it allows discrimination between non-carriers, who do not have an elevated risk of internal or skin malignancies, and mutation carriers, who need strict surveillance in every organ affected by the syndrome.

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Address for correspondence:
Carolina Alejandra Rios
Programa de Genética Humana, ICBM
Facultad de Medicina, Universidad de Chile
Casilla 70061
Santiago, Chile
Tel.: 56-2-29786016
Email: crios@med.uchile.cl

Intramuscular lipoma of the subscapularis muscle

Lipoma intramuscular no músculo subescapular

Débora Balabram[I], Carla Cristina de Sousa Resende Cabral[II], Omar de Paula Ricardo Filho[III], Cristóvão Pinheiro de Barros[IV]

Hospital Governador Israel Pinheiro (HGIP), Instituto da Previdência dos Servidores do Estado de Minas Gerais (IPSEMG), Belo Horizonte, Minas Gerais, Brazil

[I]MD. Doctoral Student, Department of Anatomical Pathology and Legal Medicine, School of Medicine, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.

[II]MD. Radiologist, Serviço de Radiologia e Ultrassonografia de Minas Gerais (Sermig), Belo Horizonte, Minas Gerais, Brazil.

[III]MD. Pathologist, Laboratory of Anatomical and Diagnostic Pathology, Belo Horizonte, Minas Gerais, Brazil.

[IV]MD. Breast Surgeon, Instituto da Previdência dos Servidores do Estado de Minas Gerais (IPSEMG), Belo Horizonte, Minas Gerais, Brazil.

KEY WORDS:
Lipoma.
Rotator cuff.
Axilla.
Diagnosis, differential.
Magnetic resonance imaging.

PALAVRAS-CHAVE:
Lipoma.
Bainha rotadora.
Axila.
Diagnóstico diferencial.
Imagem por ressonância magnética.

ABSTRACT

CONTEXT: Intramuscular lipomas are benign tumors that infiltrate the muscles.

CASE REPORT: We describe the case of a 58-year-old female patient with an axillary lump. The lump was a lipoma inside the subscapularis muscle. It is important to differentiate these lesions from liposarcomas and from other diseases that may present as axillary lumps. The most accurate imaging method for differentiating benign lipomatous tumors from liposarcomas is magnetic resonance imaging, but surgical removal of these intramuscular lesions to confirm the diagnosis is recommended.

CONCLUSION: Intramuscular lipomas are a rare cause of benign axillary lumps and should be considered in making differential diagnoses on axillary masses.

RESUMO

CONTEXTO: Lipomas intramusculares são tumores benignos que infiltram os músculos.

RELATO DE CASO: Descrevemos o caso de uma paciente de 58 anos com nódulo axilar. O nódulo era um lipoma na intimidade do músculo subescapular. É importante diferenciar essas lesões de lipossarcomas e outras doenças que podem acometer a axila. O método de imagem mais eficaz para diferenciar lesão lipomatosa benigna do lipossarcoma é a ressonância magnética, mas é recomendada a remoção cirúrgica dessas lesões intramusculares para confirmar o diagnóstico.

CONCLUSÃO: Lipomas intramusculares são causas raras de nódulos axilares benignos e devem ser considerados no diagnóstico diferencial dessas lesões.

INTRODUCTION

Intramuscular lipomas are benign tumors that infiltrate the muscles.[1] They are larger than superficial lipomas and are most common in the lower extremities and trunk.[1,2]

We report the case of a 58-year-old patient with a painless axillary lump and discuss possible diagnoses.

CASE REPORT

A 58-year-old woman visited the breast disease clinic of the Public Servants' Social Security Institute of the State of Minas Gerais (Instituto da Previdência dos Servidores do Estado de Minas Gerais, IPSEMG) in November 2010 and reported a lump. On clinical examination, she was found to have a left axillary lump with hard consistency, close to the border of the *latissimus dorsi* muscle. The cytological analysis (using material obtained through an ultrasound-guided procedure) suggested that this was a lipoma. Magnetic resonance imaging (MRI) showed a lesion in the left axilla suggestive of a lipoma inside the subscapularis muscle (**Figure 1**).

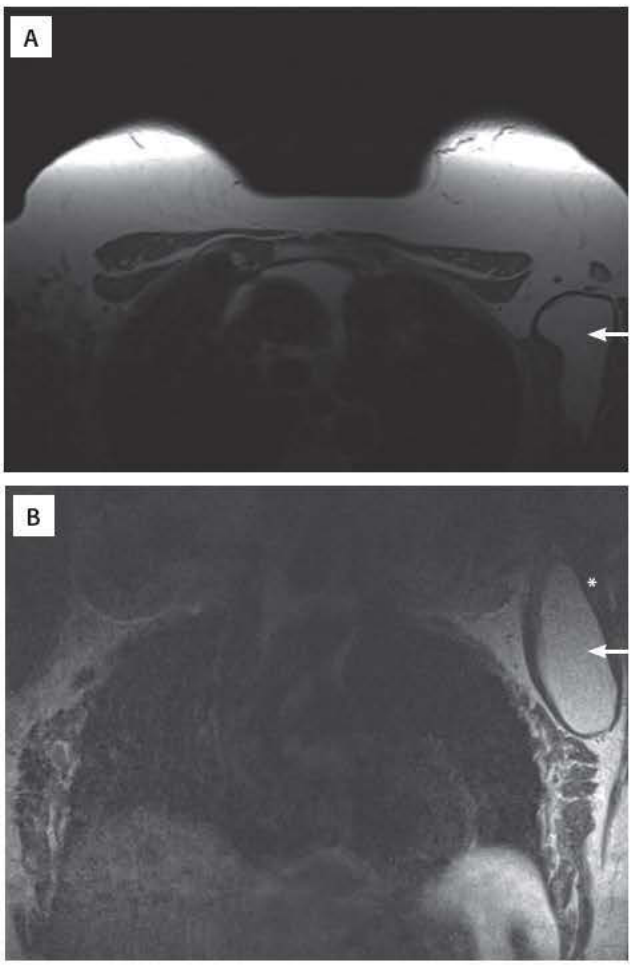

Figure 1. Magnetic resonance imaging. A: T1-weighted axial image; B: T2-weighted coronal image. Arrow, intramuscular lipoma. Asterisk, subscapularis muscle.

In March 2011, the patient underwent surgery to remove the lesion, and the pathological examination confirmed the hypothesis of an intramuscular lipoma (**Figure 2**), measuring nine centimeters.

DISCUSSION

Intramuscular lipomas are an entity comprising slowly growing benign tumors that infiltrate the muscles.[1] They have been called **infiltrating lipomas**. It is important to differentiate them from liposarcomas and, in the axillae, from other axillary diseases (such as lymph node infiltration due to malignant, infectious and immunological diseases).[3-6] Specifically, in this case, a thorough investigation of the breast was carried out to rule out carcinoma. Nowadays, the most accurate imaging examination for differentiating a benign from a malignant lipomatous tumor is magnetic resonance imaging. Infiltration of the muscle bundles, homogenous appearance, lack of peripheral capsule and presence of few fine, regular septa distinguish benign lipomas from liposarcomas.[1,2,7,8] Surgical removal and histological examination should be performed after imaging of the lesion, since neither method is infallible.[1,2,9,10]

We found some case reports in PubMed, Lilacs and Embase, reporting lipomas located in the rotator cuff (**Table 1**), but none of them was located in the subscapularis muscle.[11-14]

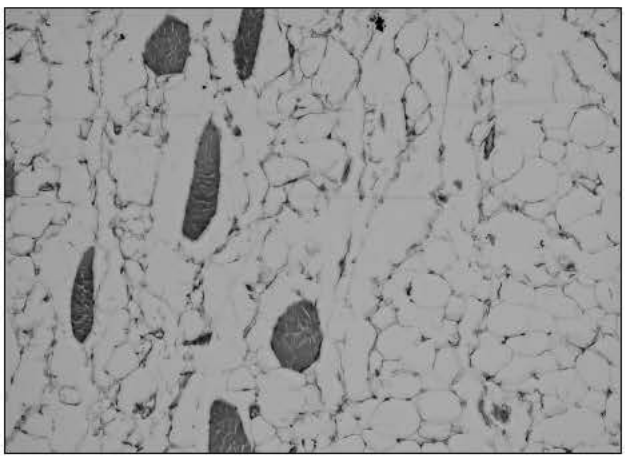

Figure 2. Mature adipocytes with no nuclear abnormalities; muscle fibers within the lipoma.

Table 1. Case reports retrieved from the review of the medical databases. Search date: February 28, 2013

Database	Search strategy	Results
PubMed	"rotator cuff" AND lipoma	3 case reports[11-13]
Lilacs	"rotator cuff" OR "bainha rotadora" OR "manguito de los rotadores" AND lipoma	1 case report[14]
Embase	"rotator cuff" AND lipoma	3 case reports[11-14]

CONCLUSION

Intramuscular lipomas are a rare cause of benign axillary lumps and should be considered in making differential diagnoses on axillary masses.

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Address for correspondence:

Débora Balabram

Rua Maranhão, 774

Santa Efigênia – Belo Horizonte (MG) – Brasil

CEP 30150-330

Tel. (+55 31) 3281-1090

E-mail: debalabra@gmail.com

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Interventions for promoting the initiation of breastfeeding

Lisa Dyson, Felicia M. McCormick, Mary J. Renfrew

The independent commentary was written by Rubens Feferbaum

ABSTRACT

BACKGROUND: Despite the widely documented health advantages of breastfeeding over formula feeding, initiation rates remain relatively low in many high-income countries, particularly among women in lower income groups.

OBJECTIVE: To evaluate the effectiveness of interventions which aim to encourage women to breastfeed in terms of changes in the number of women who start to breastfeed.

METHODS:

Search methods: We searched the Cochrane Pregnancy and Childbirth Group's Trials Register (July 2007), handsearched the Journal of Human Lactation, Health Promotion International and Health Education Quarterly from inception to 15 August 2007, and scanned reference lists of all articles obtained.

Selection criteria: Randomized controlled trials, with or without blinding, of any breastfeeding promotion intervention in any population group except women and infants with a specific health problem.

Data collection and analysis: One review author independently extracted data and assessed trial quality, checked by a second author. We contacted investigators to obtain missing information.

MAIN RESULTS: Main results: Eleven trials were included. Statistical analyses were conducted on data from eight trials (1553 women). Five studies (582 women) on low incomes in the USA with typically low breastfeeding rates showed breastfeeding education had a significant effect on increasing initiation rates compared to standard care (risk ratio (RR) 1.57, 95% confidence interval (CI) 1.15 to 2.15, P = 0.005). Subgroup analyses showed that one-to-one, needs-based, informal repeat education sessions and generic, formal antenatal education sessions are effective in terms of an increase in breastfeeding rates among women on low incomes regardless of ethnicity and feeding intention. Needs-based, informal peer support in the antenatal and postnatal periods was also shown to be effective in one study conducted among Latina women who were considering breastfeeding in the USA (RR 4.02, 95% CI 2.63 to 6.14, P < 0.00001).

AUTHORS' CONCLUSIONS: This review showed that health education and peer support interventions can result in some improvements in the number of women beginning to breastfeed. Findings from these studies suggest that larger increases are likely to result from needs-based, informal repeat education sessions than more generic, formal antenatal sessions. These findings are based only on studies conducted in the USA, among women on low incomes with varied ethnicity and feeding intention, and this raises some questions regarding generalisability to other settings.

This is the abstract of a Cochrane Review published in the Cochrane Database of Systematic Reviews (CDSR) 2005, issue 2, Art. No. CD001688. DOI: 10.1002/14651858.CD001688.pub2 (http://cochrane.bvsalud.org/cochrane/main.php?lib=COC&searchExp=Interventions%20and%20for%20and%20promoting%20and%20the%20and%20initiation%20and%20of%20and%20breastfeeding&lang=pt).

The full text is available from: http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD001688.pub2/pdf.

The abstract is available in the Portuguese, Spanish and Chinese languages from: http://summaries.cochrane.org/pt/CD001688/intervencoes-para-encorajar-as-mulheres-a-iniciar-o-aleitamento-materno.

REFERENCE

1. Dyson L, McCormick F, Renfrew MJ. Interventions for promoting the initiation of breastfeeding. Cochrane Database Syst Rev. 2005;(2):CD001688.

COMMENTS

The prevalence of exclusive breastfeeding until the sixth month of life has increased from 2.5% to 38.6% in Brazil over the last 20 years due to three key factors: NBCAL (the Brazilian rules for marketing of breastmilk substitutes), improvement of the mothers' socioeconomic levels and improvement of their educational profiles.

This interesting review demonstrates that antenatal education for mothers focusing on the benefits of breastfeeding and organization of groups of lactating mothers has a positive influence on improvement of the prevalence of breastfeeding. Thus, prenatal consultations for pregnant women, including with a pediatrician (as recommended by the Brazilian Society of Pediatrics), are effective actions that contribute towards improvement of the prevalence of breastfeeding.

Rubens Feferbaum. Associate Professor of Pediatrics, School of Medicine, Universidade de São Paulo (USP), and Attending Physician in the Neonatal Intensive Care Unit, Children's Institute, Hospital das Clínicas, Universidade de São Paulo (HC/USP), São Paulo, Brazil.

Endovenous ablation (radiofrequency and laser) and foam sclerotherapy versus conventional surgery for great saphenous vein varices

Craig Nesbitt, Ron K. G. Eifell, Peter Coyne, Hassan Badri, Vish Bhattacharya, Gerard Stansby

The independent commentary was written by Marcelo Calil Burihan

ABSTRACT

BACKGROUND: Minimally invasive techniques to treat great saphenous varicose veins include ultrasound-guided foam sclerotherapy (USGFS), radiofrequency ablation (RFA) and endovenous laser therapy (EVLT). Compared with conventional surgery (high ligation and stripping (HL/S)), proposed benefits include fewer complications, quicker return to work, improved quality of life (QoL) scores, reduced need for general anaesthesia and equivalent recurrence rates.

OBJECTIVE: To review available randomized controlled clinical trials (RCT) data comparing USGFS, RFA, EVLT to HL/S for the treatment of great saphenous varicose veins.

METHODS:

Search methods: The Cochrane Peripheral Vascular Diseases (PVD) Group searched their Specialized Register (July 2010) and CENTRAL (The Cochrane Library 2010, Issue 3). In addition the authors performed a search of EMBASE (July 2010). Manufacturers of EVLT, RFA and sclerosant equipment were contacted for trial data.

Selection criteria: All RCTs of EVLT, RFA, USGFS and HL/S were considered for inclusion. Primary outcomes were recurrent varicosities, recanalization, neovascularization, technical procedure failure or need for re-intervention, patient quality of life (QoL) scores and associated complications. Secondary outcomes were type of anaesthetic, procedure duration, hospital stay and cost.

Data collection and analysis: CN, RE, VB, PC, HB and GS independently reviewed, assessed and selected trials which met the inclusion criteria. CN and RE extracted data. The Cochrane Collaboration's tool for assessing risk of bias was used. CN contacted trial authors to clarify details.

MAIN RESULTS: Thirteen reports from five studies with a combined total of 450 patients were included. Rates of recanalization were higher following EVLT compared with HL/S, both early (within four months) (5/149 versus 0/100; odds ratio (OR) 3.83, 95% confidence interval (CI) 0.45 to 32.64) and late recanalization (after four months) (9/118 versus 1/80; OR 2.97; 95% CI 0.52 to 16.98), although these results were not statistically significant. Technical failure rates favoured EVLT over HL/S (1/149 versus 6/100; OR 0.12, 95% CI 0.02 to 0.75). Recurrence following RFA showed no difference when compared with surgery. Recanalization within four months was observed more frequently following RFA compared with HL/S although not statistically significant (4/105 versus 0/88; OR 7.86, 95% CI 0.41 to 151.28); after four months no difference was observed. Neovascularization was observed more frequently following HL/S compared with RFA, but again this was not statistically significant (3/42 versus 8/51; OR 0.39, 95% CI 0.09 to 1.63). Technical failure was observed less frequently following RFA compared with HL/S although this was not statistically significant (2/106 versus 7/96; OR 0.48, 95% CI 0.01 to 34.25). No randomised clinical trials comparing HL/S versus USGFS met our study inclusion criteria. QoL scores and operative complications were not amenable to meta-analysis.

AUTHORS' CONCLUSIONS: Currently available clinical trial evidence suggests RFA and EVLT are at least as effective as surgery in the treatment of great saphenous varicose veins. There are insufficient data to comment on USGFS. Further randomized trials are needed. We should aim to report and analyze results in a congruent manner to facilitate future meta-analysis.

This is the abstract of a Cochrane Review published in the Cochrane Database of Systematic Reviews (CDSR) 2011, issue 5, Art. No. CD005624. DOI: 10.1002/14651858.CD005624.pub2 (http://cochrane.bvsalud.org/cochrane/main.php?lib=COC&searchExp=Endovenous%20and%20ablation%20and%20(radiofrequency%20and%20laser)%20and%20foam%20and%20sclerotherapy%20and%20versus%20and%20conventional%20and%20surgery%20and%20for%20and%20great%20and%20saphenous%20and%20vein%20and%20varices&lang=pt).

The full text is available from: http://dx.doi.org/10.1002/14651858.CD005624.pub2.

The abstract is also available in the Portuguese, French and Spanish languages from: http://summaries.cochrane.org/pt/CD005624/ablacao-endovenosa-por-radiofrequencia-e-laser-e-escleroterapia-com-espuma-versus-cirurgia-convencional-para-o-tratamento-de-varizes.

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COMMENTS

With the advent of new techniques for treating varicose veins, many studies are needed in order to compare the new procedures with the gold-standard treatment, i.e. conventional surgery with removal of either the great or the small saphenous vein and excision of tributaries presenting insufficiency. In this review, many data were flawed or did not lead to a conclusion that would be capable of showing significant details regarding the best technique.

It can be expected that treatments with laser, radiofrequency or foam sclerotherapy may lead to recanalization of the treated veins, since these do not remove the veins but only stop the flow of blood through the lumen. Recurrence of varicose veins within four months suggests that there was an error in marking out the varicose veins before the operation and failure of the planned removal of the saphenous vein or the dilated tributaries. Some technical details of the surgery may differ, such as segmental removal of the great saphenous vein under general anesthesia. This procedure is not customary in many centers, and complete removal of the saphenous vein with intrathecal or regional blockade is preferred. Other extremely necessary data include comparison of the costs of the fiber laser and radiofrequency equipment, costs of procedures and costs of hospitalization when necessary.

Marcelo Calil Burihan. Professor of Vascular Surgery, Hospital Santa Marcelina, São Paulo; Professor of Anatomy, University of Santo Amaro, São Paulo; Professor of Anatomy, Santa Marcelina Medical School, São Paulo; Member of Sociedade Brasileira de Angiologia e de Cirurgia Vascular (SBACV), São Paulo, Brazil; and Member of the Society of Vascular Surgery (SVS), Latin American Chapter.

In the article **"Trends in treatment of anterior cruciate ligament injuries of the knee in the public and private healthcare systems of Brazil"**, published in the São Paulo Medical Journal, volume 131, issue number 4, 2013, the correct name of the third author is Gustavo Gonçalves Arliani and not Arliani Gustavo.

The article should be cited thus: Astur DC, Batista RF, Arliani GG, Cohen M. Trends in treatment of anterior cruciate ligament injuries of the knee in the public and private healthcare systems of Brazil. Sao Paulo Med J. 2013 July 4;131(4):257-63. PubMed PMID: 24141297.

Indexing and scope

The São Paulo Medical Journal/Evidence for Health Care was founded in 1932. Its articles are indexed in Medline, Lilacs, SciELO, Science Citation Index Expanded, Journal Citation Reports/Science Edition (ISI) and EBSCO Publishing.

Published bimonthly by the Associação Paulista de Medicina, the journal accepts articles in the fields of clinical health science (internal medicine, gynecology and obstetrics, mental health, surgery, pediatrics and public health). Articles will be accepted in the form of original articles (clinical trials, cohort, case-control, prevalence, incidence, accuracy and cost-effectiveness studies and systematic reviews with or without meta-analysis), narrative reviews of the literature, case reports, short communications and letters to the editor. Papers with a commercial objective will not be accepted.

The Journal's policy and procedures

After receipt of the article by the Scientific Publications Sector, the authors will be provided with a protocol number. This number serves to maintain good understanding between the authors and the Scientific Publications Sector. Following this, the article will be read by the Editor, who will verify whether it is consonant with the journal's policy and interests, i.e. whether the research or review is within the fields of health or public health.

Next, the Scientific Publications Sector will verify whether the text complies with the journal's Instructions for Authors. If the text is incomplete or if it is not organized as required, the authors will be asked to resubmit their text after resolving such problems. When its format is acceptable, the Scientific Publications Sector will submit the manuscript to closed peer review, in which the reviewers will not sign their verdict and will not know the names of the authors. Each paper will be reviewed by at least three reviewers: one expert in the field, one associate editor (who will evaluate the article from the reader's perspective) and one *ad hoc* editorial advisor (who will assess methodological aspects of the study).

The authors will then receive the reviewers' evaluation and will be asked to resolve all the problems that have been pointed out. Once the Scientific Publications Sector receives the manuscript again, the text will be sent to the scientific editor and the proofreader, who will point out problems with sentence construction, spelling, grammar, bibliographical references and other matters. The authors should then provide all further information and corrections requested and should mark in the text all the points at which modifications have been made, using different colors or electronic text marking systems, so that these modifications are easy to see.

When the text is considered acceptable for publication, and only then, it will enter the queue for publication and the author will receive a letter of acceptance of the article. The Scientific Publications Sector will provide a proof, including any tables and figures, for the authors to approve. No article is published without this last procedure.

Instructions for authors

General guidelines: for all types of articles

Texts must be submitted exclusively through the Internet, using the electronic submission system, which is available at http://www.spmj.hemeroteca.com.br. Submissions sent by e-mail or through the post will not be accepted.

The manuscript must be submitted in English. Nonetheless, it must also include a summary and five key words both in Portuguese and in English. The key words must be selected from the DeCS and MeSH lists only, as explained in detail below (no other key words will be accepted).

Papers submitted must be original and therefore all the authors need to declare that the text has not been and will not be submitted for publication in any other journal. Papers involving human beings (individually or collectively, directly or indirectly, totally or partially, including the management of information and materials) must be accompanied by a copy of the authorization from the Research Ethics Committee of the institution in which the experiment was performed.

All articles submitted must comply with the editorial standards established in the Vancouver Convention (Uniform Requirements for Manuscripts Submitted to Biomedical Journals)[1] and the specific quality guidelines for papers reporting on clinical trials (CONSORT),[2] systematic reviews and meta-analyses (PRISMA),[3,4] observational studies (STROBE)[5,6] and accuracy studies on diagnostic tests (STARD).[7,8]

The style known as the "Vancouver Style" is to be used not only for the format of the references, but also for the whole text. The Editors recommend that authors should familiarize themselves with this style by accessing http://www.icmje.org.

Abbreviations must not be used, even those in common use. Drugs or medications must be referred to using their generic names, avoiding unnecessary mention of commercial or brand names, and should be followed by the dosage and posology. Any product cited in the Methods section, such as diagnostic or therapeutic equipment, tests, reagents, instruments, utensils, prostheses, orthoses and intraoperative devices must be described together with the manufacturer's name and place (city and country) of manufacture in parentheses.

Grants, bursaries and any other financial support for studies must be mentioned separately after the references, in a section named "Acknowledgements", along with any other acknowledgements to individuals or professionals who have helped in producing the study but whose contribution does not constitute authorship (we recommend that the item "Authorship" at http://www.icmje.org should be read to obtain clarifications regarding the criteria for authorship).

For any type of study, all statements in the text that are not results from the study presented for publication in the São Paulo Medical Journal/Evidence for Health Care, but are data from other studies already published elsewhere must be accompanied by citations of the pertinent literature. Thus, statements about the incidence or

prevalence of diseases, costs, frequency of use of certain therapies and epidemiological data in general should be followed by the references for the surveys that generated this information, even if the data come from government institutions or databases, given that these are data from other studies.

Format

First page (cover page)

The first page must contain:

1) the type of paper (original article, review or updating article, short communication or letter to the editor);
2) the title of the paper in English and Portuguese, which must be short but informative;
3) the full name of each author (the editorial policy of the São Paulo Medical Journal is that abbreviations for authors' names must not be used; thus, names should either be sent complete or with middle names omitted, for example: an author whose full name is John Richard Smith can be presented as John Smith or John Richard Smith, but not as John R. Smith; likewise, use Christopher Smith and not Chris Smith, or William Smith and not Bill Smith, and so on)), his/her academic titles (abbreviated in English), in the order obtained (for example: MD for medical doctor, MSc for holders of a master's title, PhD for holders of a doctorate or BSc for bachelor of science, such as in biology), and the positions currently held (for example, Doctoral Student, Attending Physician, Adjunct Professor, Associate Professor, Head of Department, etc.), in the department and institution where he/she works, and the city and country;
4) the place where the work was developed;
5) the complete address (name of street or avenue, building number, city) of the corresponding author, telephone and e-mail that can be published together with the article.

Second page: abstract (English and Portuguese) and key words

The second page must include the title and an abstract (English and Portuguese, maximum of 250 words each),[9] structured in five items:

1) context and objective;
2) design (type of study) and setting (place where the study was developed);
3) methods (described in detail);
4) results; and
5) conclusions.

The abstract (both in English and in Portuguese) should contain five key words. The English terms must be chosen from the Medical Subject Headings (MeSH) list of Index Medicus, which are available on the internet (http://www.ncbi.nlm.nih.gov/sites/entrez?db=mesh).[10] The Portuguese terms must be chosen from the *Descritores em Ciências da Saúde* (DeCS), developed by Bireme, which are available on the internet (http://decs.bvs.br/).[11]

References

The list of references (in the "Vancouver style", as indicated by the International Committee of Medical Journal Editors, ICMJE) should be laid out in the final part of the article, after the conclusions and before the tables and figures. In the text, the references must be numbered according to the order of citation. The citation numbers must be inserted after periods/full stops or commas in sentences (see examples in the preceding section), and must be in superscript form (without using parentheses or square brackets). References cited in the legends of tables and figures must maintain sequence with the references cited in the text.

In the list of references, all the authors must be listed if there are up to and including five authors; if there are six or more, the first three should be cited, followed by the expression "et al." For books, the city of publication and the name of the publishing house are mandatory. For texts published on the internet, the complete uniform resource locator (URL) or address is necessary (not only the main home page of a website or link), so that by copying the complete address into their computer internet browsers, the journal's readers will be taken to the exact document cited, and not to a general website. The following are some examples of the most common types of references:

Article in journal

- Hurt AC, Hardie K, Wilson NJ, et al. Community transmission of oseltamivir-resistant A(H1N1)pdm09 influenza. N Engl J Med. 2011;365(26):2541-2.

Chapter of book

- Miller WI, Achernabb JC, Fluck CE. The adrenal cortex and its disorder. In: Sperling M. Pediatric endocrinology. 3rd ed. Elsevier Health Sciences; 2008. p. 444-511.

Text on the internet

- Centers for Disease Control and Prevention. Children's food environment State Indicator Report, 2011. Available from: http://www.cdc.gov/obesity/downloads/ChildrensFoodEnvironment.pdf. Accessed in 2012 (Mar 7).

Last page

The last page must contain:

1) the date and place of the event at which the paper was presented, if applicable, such as congresses or dissertation or thesis presentations;
2) sources of support in the forms of finance for the project, study bursaries or funding for purchasing equipment or drugs. The protocol number for the funding must be presented;
3) description of any conflicts of interest held by the authors. We recommend that the item "Conflicts of interest" at http://www.icmje.org should be read to obtain clarifications regarding what may or may not be considered to be a conflict of interest;

Figures and tables

Images must have good resolution (minimum of 300 DPI) and be recorded in ".jpg" or ".tif" format. Do not attach images inside Microsoft PowerPoint documents. If photographs are inserted in a Microsoft Word file, the images should also be sent separately. Graphs must

be prepared in Microsoft Excel (do not send them in image formats) and must be accompanied by the tables of data from which they have been generated. The number of illustrations must not exceed the total number of pages minus one.

All figures and tables must contain legends or titles that precisely describe their content and the context or sample from which the information was obtained (i.e. what the results presented are and what the kind of sample or setting was). The legend or title sentence should be short but comprehensible without depending on reading the article.

All the figures and tables should be cited in the text.

São Paulo Medical Journal/Evidence for Health Care is for now published in black-and-white in its printed version. Photographs, photomicrographs, bar and line graphs and any image to be published must be prepared considering that there will be no color differentiation (any color information will be discarded). Shades of gray and printing patterns (dots, stripes and others) should be used instead, with good contrast.

Original articles

Clinical trials, cohort, case-control, prevalence, incidence, accuracy and cost-effectiveness studies, and systematic reviews with or without meta-analysis, are considered to be original articles.

The São Paulo Medical Journal/Evidence for Health Care supports the clinical trial registration policies of the World Health Organization (WHO) and the International Committee of Medical Journal Editors (ICMJE) and recognizes the importance of these initiatives for registration and international dissemination of information on randomized clinical trials, with open access. Thus, from 2008 onwards, manuscripts on clinical trials have been accepted for publication only if they have received an identification number from one of the clinical trial registers that have been validated in accordance with the criteria established by WHO and ICMJE. Authors of randomized clinical trials must thus register their studies before submitting them for publication in the São Paulo Medical Journal/Evidence for Health Care. The addresses for these registers are available from the ICMJE website (http://www.icmje.org). The identification number should be declared at the end of the abstract.

Authors will be required to comply with the guidelines for writing each type of original article, as follows:

1. Observational articles: STROBE Statement;[5,6]
2. Clinical trials: CONSORT Statement;[2]
3. Accuracy studies on diagnostic tests: STARD Statement;[7,8]
4. Systematic reviews of the literature and meta-analyses: PRISMA[4]

The São Paulo Medical Journal takes the view that these guidelines not only aid in writing and organizing the content of articles in a standardized manner, thereby improving their quality and facilitating reading and assessment, but also these guidelines help to avoid situations in which important information on the methodology of studies remains outside of the manuscript.

As a partner institution of the Cochrane Collaboration and the Brazilian Cochrane Center, the *Associação Paulista de Medicina* considers that production of articles in accordance with these guidelines also aids in future production of systematic reviews of the literature and meta-analyses. Thus, articles submitted for publication that are not in accordance with these norms may be returned to their authors for adjustment before the peer review process begins.

Original articles must be structured so as to contain the following parts: Introduction, Objective, Methods, Results, Discussion and Conclusion. The text must not exceed 5,000 words (excluding tables, figures and references), from the introduction to the end of the conclusion, and must include a structured abstract with a maximum of 250 words.[9] "Structured abstract" means that the abstract must contain the following items: Context and objective, Design and setting, Method, Results and Conclusion.

The structure of the document should follow the format laid out below:

1) *Title and abstract*: the study design and/or the way participants were allocated to interventions, for example "randomized" or "retrospective" study, should be mentioned in the title and in the abstract. The abstract should provide a summary of what was done and what was found.

2) *Introduction:* specify the reasons for carrying out the study, describing the present state of knowledge of the topic. Describe the scientific background and "the state of the art". Do not include here any results or conclusions from the study. Use the last paragraph to specify the principal question of the study, and the principal hypothesis tested, if there is one. Do not include discussions about the literature in the introduction; the introduction section should be short.

3) *Objective:* describe briefly what the main objective or question of the study was. Clearly describe the pre-specified hypotheses.

4) *Methods*

4.1) *Type of study:* describe the design of the study and specify, if appropriate, the type of randomization (the way in which draws were conducted), the blinding (how this was ensured), the diagnostic test standards (gold standard or range of normal values) and the time direction (retrospective or prospective). For example: "randomized clinical trial", "double-blind placebo-controlled clinical trial", "cross-sectional accuracy study", "retrospective cohort study", "cross-sectional prevalence study" or "systematic review of clinical trials".

4.2) *Sample, participants or patients*: describe the eligibility criteria for participants (inclusion and exclusion criteria) and the sources and procedures for selection or recruitment. In case-control studies, describe the rationale for distributing the subjects as cases and controls, and the matching criteria. The numbers of patients at the beginning and end of the study (after exclusions) must be made clear. A flow diagram showing the initial recruitment, the exclusions and the

final sample of patients included should be produced and inserted in the article.

4.3) *Setting:* indicate the place where the study was carried out, including the type of healthcare provided (i.e. whether primary or tertiary; and whether in a private or in a public hospital). Avoid stating the name of the institution where the study was developed (for blinding purposes in the peer review). Only the type of institution should be made clear, for example: "public university hospital" or "private clinic".

4.4) *Procedures (*intervention, diagnostic test or exposure): describe the principal characteristics of any intervention, including the method, the timing and the duration of its administration or of data collection. Describe the differences in interventions administered to each group (if the study is controlled). Detail the procedures in such a way that other researchers will be able to repeat them in other localities.

4.5) *Main measurements, variables and outcome*: state what the primary and secondary outcomes analyzed in the study are. Describe the method of measuring the primary result, in the way in which it was planned before data collection. For each variable of interest, detail the assessment methods. If the hypothesis of the study was formulated during or after data collection (and not before), this needs to be declared. Describe the methods used to enhance the quality of measurements (for example, multiple observers, training, etc.) and to avoid bias. Explain how quantitative variables were handled in the analyses.

4.6) *Sample size and statistical analysis*: describe the sample size calculation method, or the study period in the event that patients were consecutively admitted over a period. Readers need to understand why a given number of patients was used. The planned statistical analysis, the statistical tests used and their significance levels, along with any *post hoc* analyses, should be presented in this section. Describe the methods used to control for confounding factors and variables, and explain how missing data and cases lost from the follow-up were dealt with.

4.7) *Randomization*: describe the method used to implement the random allocation sequence (for example, sealed envelopes containing random sequences of numbers or software for generating random numbers). If appropriate, report that the study used "quasi-randomization".[12] In addition, describe who generated the random sequence, who assigned the participants to each group (in the case of controlled trials) and who recruited the participants.

5) Results: describe the main findings. If possible, these should be accompanied by their 95% confidence intervals and the exact level of statistical significance (it is not enough to write "P < 0.05": the exact P value should be supplied). For comparative studies, the confidence interval must be stated for the differences between the groups.

5.1) *Participant flow diagram*: describe the flow of participants through each stage of the study (inclusions and exclusions) and the follow-up period, and the number of participants completing the study (or lost from the follow-up). Use a flow diagram to demonstrate the numbers of patients, from the initial recruitment to the end of the study, and the reasons for exclusions. If there was any "intention-to-treat" analysis, describe it.

5.2) *Deviations*: if there was any deviation from the protocol, away from what was initially planned, describe it and the reasons for it.

5.3) *Adverse events*: describe any side effect, adverse event or complication.

6) Discussion: provide an interpretation of the results, taking into account the study hypotheses and conclusions. Emphasize the new and important factors encountered in the study, which will form part of the conclusion. Do not repeat data presented in the introduction or results in detail. Mention any limitations of the findings that should be noted and any possible implications for future research. Describe any potential bias. Report any relevant findings from other studies: it is important to review the recent literature to seek new evidence that may have been published, which needs to be discussed. State whether the findings can be generalized to populations (i.e. whether the findings have external validity). It is recommended that the last two paragraphs should contain implications for practice and for further research.

7) Conclusions: specify only the conclusions that can be sustained by the results, together with their clinical significance (avoiding excessive generalization). Draw conclusions based on the objectives and hypotheses of the study. The same emphasis should be placed on studies with positive and negative results.

Systematic reviews with or without meta-analyses should comply with the same publication norms established for original articles, and be produced in accordance with PRISMA[4] and the Cochrane Collaboration's systematic review Handbook.[13] The text should not exceed 5,000 words (excluding tables, figures and references)

Short communications, case reports or case series

Short communications and case reports must be limited to 3,000 words (from the introduction to the end of the conclusion). Short communications are reports on the results from ongoing studies or studies that have recently been concluded for which urgent publication is important. They should be structured thus: Introduction, Objective, Methods, Results, Discussion and Conclusion, like in original articles. Individual case reports should contain: Introduction, Case Report, Discussion and Conclusion. Reports on case series constitute

observational studies and these should be structured in accordance with the norms of the STROBE Statement.[5]

Both short communications and case reports must be submitted with abstracts and key words. The abstracts in short communications should be structured with: Context and objective, Design and setting, Methods, Results and Conclusion, like in original articles. The abstracts in case reports and case series should contain: Context, Case Report (with a description of the case and a pertinent discussion) and Conclusion.

The São Paulo Medical Journal/Evidence for Health Care is interested in publishing rare or instructive case reports, accompanied by a systematic search of the literature, in which relevant studies found (based on their level of evidence) are presented and discussed.[14] The results from the systematic search of the main databases — Medline (via PubMed), Embase, Lilacs and Cochrane Library — should be presented in a table with the search strategy for each database and the number of articles obtained.

Narrative reviews

Narrative reviews may be accepted by the São Paulo Medical Journal/Evidence for Health Care and should be structured with: Introduction, Objectives, Methods, Results, Discussion and Conclusions. The abstract must be structured with: Context and objective, Design and setting, Methods, Results and Conclusions, like in original articles. The manuscript must comply with the norms of the Vancouver style[1] and must include a systematic search in the main databases: Medline, Embase, Lilacs and Cochrane Library. The search strategy for each database and the number of articles obtained from each database should be presented in a table. The access route to the electronic databases used should be stated (for example, PubMed, OVID, Elsevier or Bireme). For the search strategies, MeSH terms must be use for Medline, LILACS and Cochrane Library. DeCS terms must be used for LILACS. EMTREE terms must be used for Embase. Also, for LILACS, search strategy must be performed, at the same time, with English (MeSH), Spanish (DeCS) and Portuguese (DeCS) terms. The search strategies must be presented exactly as they were used during the search, including parentheses, quotation marks and Boolean operators (AND, OR, AND NOT).

Letters to the editor

Letters to the editor may address articles published in the São Paulo Medical Journal/Evidence for Health Care publication or may deal with health issues of interest. Case reports must not be submitted as letters. In the category of letters to the editor, the text has a free format, but must not exceed 500 words and five references.

Documents cited

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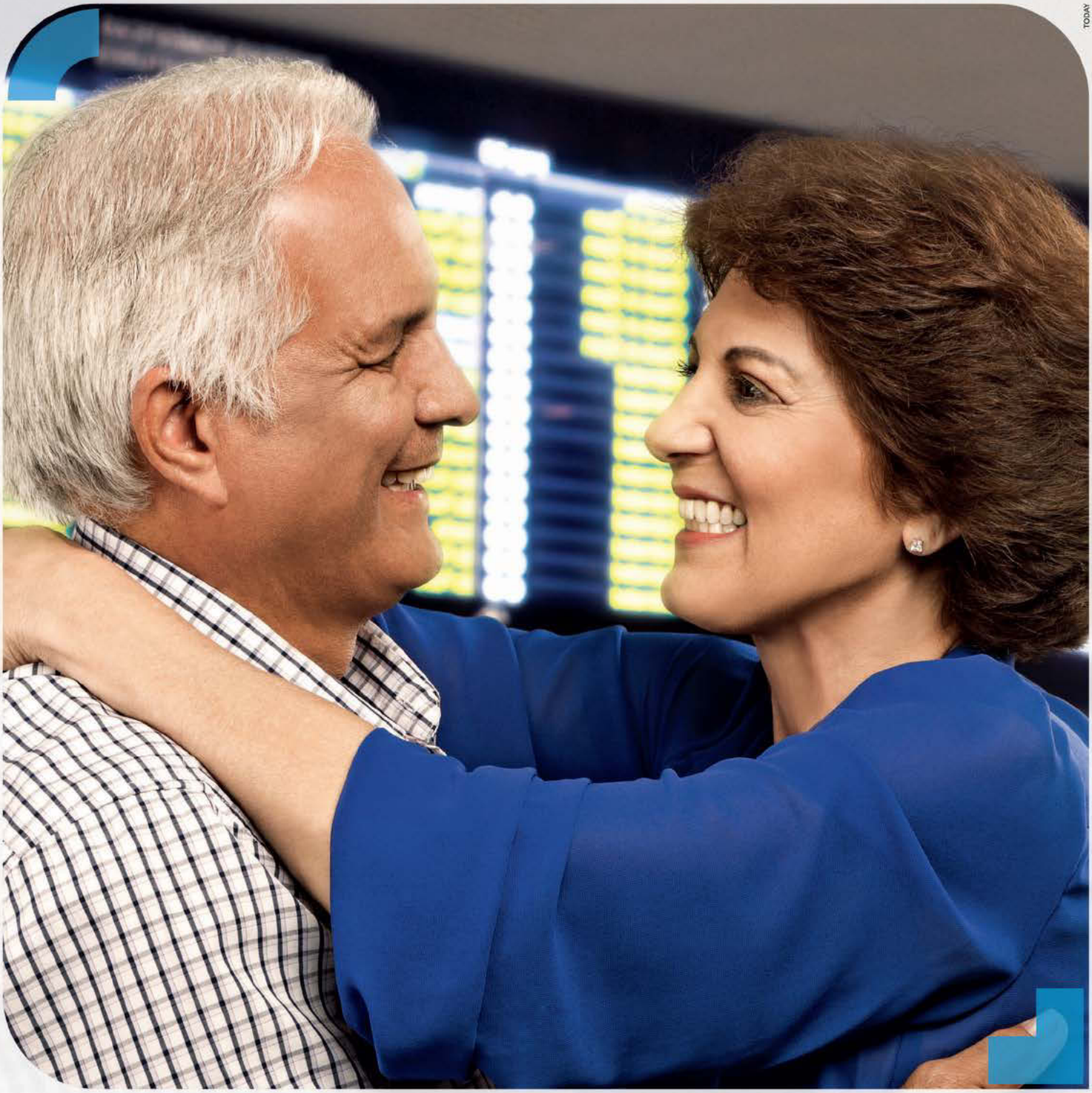

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Metade do preço: em comparação a produtos similares no mercado de planos de saúde individuais (tabela de novembro/2013 – Omint). [1] De acordo com a disponibilidade da rede médica da operadora escolhida e do plano contratado. [2] Conforme condições contratuais. A disponibilidade e as características desse benefício especial podem variar conforme a operadora escolhida e o plano contratado.

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