PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIAS DA SAÚDE

ANGELA MARQUES BARBOSA

INQUÉRITO SOROEPIDEMIOLÓGICO COM O TESTE ML- FLOW NOS CONTATOS DE PACIENTES DE HANSENÍASE EM UM MUNICÍPIO DO ESTADO DE SÃO PAULO

Presidente Prudente - SP
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Dissertação apresentada à Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de mestre. - Área de concentração: Ciências da Saúde

Orientadora: Profa. Dra. Marilda Aparecida Milanez Morgado de Abreu

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Presidente Prudente, 28 de janeiro de 2019

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DEDICATÓRIA

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“O doente de Hansen não precisa de piedade. Não precisa de compaixão. Precisa e precisa muito é de soliedariedade e muita compreensão”. (Malta Tohan-1977)

RESUMO

Inquérito soroepidemiológico com o teste ml- flow nos contatos de pacientes de Hanseníase em um município do Estado de São Paulo

Introdução: A hanseníase é uma doença infecciosa incapacitante, causada pelo *Mycobacterium leprae*. O objetivo foi pesquisar a ocorrência de hanseníase entre contatos domiciliares de pacientes de hanseníase, no município de Presidente Prudente, SP, no período de 2006 a 2016. **Métodos:** Este estudo é um inquérito sorológico de contatos de pacientes tratados ou em tratamento para hanseníase, na cidade de Presidente Prudente, Oeste do Estado de São Paulo, utilizando exame clínico e a pesquisa de anticorpos anti-PGL-I, através da sorologia ML-Flow. **Resultados:** Um total de 263 casos-índices de hanseníase foram localizados no período estudado. Desses, 53 foram abordados e entre os seus contatos domiciliares, 108 foram examinados. O teste ML-Flow foi positivo em 2 (1,85%) indivíduos, mas o exame clínico não revelou sinais ou sintomas de hanseníase, considerando-se, portanto, infecção subclínica. Em nenhum dos outros comunicantes foi confirmado também hanseníase. **Conclusão:** Neste estudo, uma porcentagem baixa dos pacientes apresentaram o teste ML-Flow positivo. O uso do ML-Flow deve ser estimulado para o seguimento de populações de risco e para monitorar os resultados do tratamento.

Palavras-chave: Diagnóstico; Hanseníase; *Mycobacterium leprae*; Testes sorológicos.

ABSTRACT

Soroepidemiologic survey with the ml-flow test in contacts of leprosy patients living in a city in the state of São Paulo

Introduction: Leprosy is a disabling infectious disease caused by *Mycobacterium leprae*. The objective was to investigate the occurrence of leprosy among household contacts of leprosy patients, in the city of Presidente Prudente, SP, from 2006 to 2016. **Methods:** This study is a serological survey of contacts of patients treated or undergoing treatment for leprosy in the city of Presidente Prudente, in the State of São Paulo, using clinical examination and the determination of anti-PGL-I antibodies using ML-Flow test. **Results:** A total of 263 leprosy index-cases were found in the study period. Of these, 53 were investigated and among their household contacts, 108 were examined. The ML-Flow test was positive and 2 (1,85%) individuals, but the clinical examination revealed no signs or symptoms of leprosy, therefore, it was considered a subclinical infection. Leprosy was not confirmed in any of the household contacts. **Conclusion:** A low percentage of patients had the ML-Flow positive test. The use of ML-Flow should be encouraged for the follow-up of at-risk populations and for monitoring treatment outcomes.

Keywords: Diagnosis; Hanseníase; *Mycobacterium leprae*; Serologic tests.

LISTA DE SIGLAS

BCG	- Bacilo de Calmette Guerin
ELISA	- *Enzyme Linked Immunosorbent Assay*
OMS	- Organização Mundial da Saúde
IGM	- Imunoglobulina M
MB	- Multibacilar
M. leprae	- *Mycobacterium leprae*
ML-FLOW	-Teste sorológico de fluxo lateral para *M. leprae*
PB	- Paucibacilar
PGL-I	- glicolipídeo fenólico 1
TCLE	- Termo de Consentimento Livre e Esclarecido

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Periódicos selecionados para provável submissão:

1. Journal of Microbiology, immunology and infection. É uma revista tailândesa de acesso aberto, comprometida com a divulgação de informações sobre as últimas tendências e avanços em microbiologia, imunologia, doenças infecciosas e parasitologia. Artigos sobre investigações clínicas ou laboratoriais de relevância para microbiologia, imunologia, doenças infecciosas, parasitologia e outros campos relacionados que sejam de interesse para a profissão médica são elegíveis para consideração. Os tipos de artigos considerados incluem perspectivas, artigos de revisão, artigos originais, relatórios breves e correspondência. O fator de impacto é 2,094 e Qualis Capes na área de Medicina II é A2. Link de acesso: (https://www.elsevier.com/wps/find/journaldescription.cws_home).
2. Epidemiology and Infection. É uma revista médica, revisada por pares, que contém relatórios e análises originais sobre todos os aspectos da infecção em humanos e animais. Alguns desses aspectos incluem zoonoses, infecções tropicais, higiene alimentar e estudos de vacinas. O fator de impacto é 2,075 e Qualis Capes na área de Medicina II é B1. Link de acesso: (https://www.cambridge.org/core/journals/epidemiology-and-infection)
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RESUMO

Conhecimentos/Objetivos: A hanseníase é uma doença infecciosa incapacitante, causada pelo *Mycobacterium leprae*. O objetivo foi pesquisar a ocorrência de hanseníase entre contatos domiciliares de pacientes de hanseníase, no município de Presidente Prudente, SP, no período de 2006 a 2016.

Métodos: Inquérito sorológico de contatos de pacientes tratados ou em tratamento para hanseníase, na cidade de Presidente Prudente, Oeste do Estado de São Paulo, utilizando exame clínico e a pesquisa de anticorpos anti-PGL-I, através da sorologia ML-Flow.

Resultados: Um total de 263 casos-índices de hanseníase foram localizados no período estudado. Desses, 53 foram abordados e entre os seus contatos domiciliares, 108 foram examinados. O teste ML-Flow foi positivo em 2 (1,85%) indivíduos, mas o exame clínico não revelou sinais ou sintomas de hanseníase, considerando-se, portanto, infecção subclínica. Em nenhum dos outros comunicantes foi confirmado também hanseníase.

Conclusão: Neste estudo, uma porcentagem baixa dos pacientes apresentaram o teste ML-Flow positivo. O uso do ML-Flow deve ser estimulado para o seguimento de populações de risco e para monitorar os resultados do tratamento.

Palavras-chave: Diagnóstico; Hanseníase; *Mycobacterium leprae*; Testes sorológicos; Triagem

INTRODUÇÃO

A hanseníase é uma moléstia infectocontagiosa crônica causada pelo *Mycobacterium leprae* que acomete principalmente a pele e o sistema nervoso periférico[1]. Segundo a classificação operacional da Organização Mundial de Saúde (WHO), multibacilares (MB) são os pacientes com mais de 5 lesões ou índice baciloscópico positivo; paucibacilares (PB) são aqueles com até 5 lesões e índice baciloscópico negativo[2].

Em 2017, 147 países reportaram 210.973 casos novos à WHO, a maioria na Índia[3]. O Brasil ocupa o segundo lugar, com 28.064 casos em registro ativo, sendo 26.875 casos novos[4]. A WHO, desde 1991, busca atingir a meta de eliminação da hanseníase no mundo como problema de saúde pública, definida pela prevalência de menos de um doente para cada 10.000 habitantes, mas isto ainda não ocorreu no Brasil, que apresenta 1,35/10.000 habitantes[5].

A principal via de contágio são as vias aéreas superiores, sendo o contato íntimo e prolongado com doente o principal fator de risco de transmissão da hanseníase. Esse risco é cinco a dez vezes maior se um membro da família já apresentou a doença[6].

Contatos domiciliares compreende um grupo de indivíduos que viveram em intimidade com um paciente de hanseníase antes dele ter sido diagnosticado. Indivíduos com infecção subclínica, especialmente contatos domiciliares, podem participar na disseminação de *M. leprae*. Assim, a monitorização desses contatos promove diagnóstico precoce da doença, auxiliando na interrupção da cadeia de transmissão[6,7].

No Brasil, é recomendado o exame clínico dos contatos domiciliares no momento do diagnóstico do caso índice; se esse exame for normal, eles recebem a vacina Bacillus Calmette-Guérrin (BCG)[8]. Porém, devido ao período de incubação longo (entre 2 a 7 anos), a doença pode se manifestar mais tardiamente, sendo necessários vários anos de seguimento[1].

O glicolipídio fenólico-I (PGL-I) é um trissacarídeo ligado por uma molécula de fenol a lipídios, presente na cápsula do *M. leprae*[1,9,10]. É um antígeno específico do bacilo, que induz à produção de anticorpos da classe imunoglobulina M (IgM), sugerindo infecção pelo *M. leprae*[1,9,10]. Antígenos nativo e sintéticos são empregados em testes sorológicos para o diagnóstico da hanseníase, sendo o mais usado o enzyme-linked immunosorbent assay (ELISA)[9,10]. Um teste imunocromatográfico de leitura rápida, o teste semi-quantitativo de fluxo lateral (ML-Flow), mostrou alta sensibilidade (97,4%) para a hanseníase MB e alta especificidade (90,2%), além de concordância de 91% com o ELISA[11].

Como o exame clínico é insuficiente para identificar indivíduos infectados no estágio assintomático, mas capazes de disseminar *M. leprae* e com risco de desenvolver a doença, a realização de busca ativa de novos casos entre os contatos de pacientes de hanseníase, utilizando testes sorológicos com PGL-I, pode ser uma estratégia capaz de contribuir para o diagnóstico precoce ao identificar indivíduos com maior risco de desenvolvimento da doença, particularmente de hanseníase MB[7,11-28].

O objetivo deste estudo foi pesquisar a ocorrência de hanseníase entre contatos domiciliares aparentemente saudáveis de pacientes tratados ou em tratamento de hanseníase, no município de Presidente Prudente, SP, no período de 2006 a 2016, através do exame clínico e do teste ML-Flow.

MÉTODOS

Este foi um estudo transversal envolvendo contatos domiciliares de pacientes de hanseníase, que foram tratados ou estavam em tratamento, no período de 2006 a 2016, no município de Presidente Prudente, SP, Brazil. Trata-se de um inquérito soroepidemiológico, onde foi utilizado o exame clínico e o teste ML-Flow.

Um contato domiciliar foi definido como uma pessoa que convive ou conviveu na mesma residência de um paciente de hanseníase nos últimos 5 anos anteriores ao diagnóstico do paciente.

Os pacientes de hanseníase foram identificados a partir do setor de informática e informação em saúde da Secretaria Municipal de Saúde de Presidente Prudente, SP. As informações como telefone, endereço, classificação operacional da doença e vacinação dos contatos com BCG foram extraídas dos prontuários eletrônicos do Palácio da Saúde, local de tratamento e acompanhamento dos pacientes. Foram critérios de inclusão ser residente em Presidente Prudente, SP, e ser contato domiciliar de um caso índice de hanseníase.

Presidente Prudente é um município localizado na região do Oeste Paulista, a 558 km da capital, São Paulo. A população estimada para 2017 foi de 225.271 habitantes e a área territorial é de 560.637 km[29]. Em 2017, 7 casos novos de hanseníase foram detectados, com 11 casos em registro ativo e coeficiente de prevalência de 0,49/10.000 habitantes[30]. De 2013 a 2017, 60 casos novos foram detectados[30].

Teste sorológico ML-Flow

O teste ML-Flow foi empregado em sangue obtido por puntura do dedo indicador dos contatos domiciliares, para detectar anticorpos circulantes da classe IgM contra um

análogo semi-sinético de PGL-I de *M. leprae*, ligado à albumina de soro bovino (NT-P-BSA). O teste apresenta-se como um dispositivo que contém uma fita porosa, marcada em uma de suas extremidades com o anticorpo (representado pelo reagente de detecção, formado de partículas móveis de ouro coloidal). Possui no centro uma linha onde está inserido o antígeno e uma linha de controle marcada com IgM humana. A execução e a interpretação do teste, como positivo ou negativo, foram feitas segundo especificações do fabricante (IPTSP/UFG, GO, Brasil)[11].

Exame clínico

Os contatos domiciliares foram avaliados clinicamente numa visita domiciliar, com aplicação de questionário e exame clínico dermatoneurológico para identificar sinais e sintomas da hanseníase por médico especializado e experiente no diagnóstico de hanseníase. A definição de caso de hanseníase se baseou no encontro de pelo menos um dos seguintes sinais e sintomas: a) lesão(ões) e/ou área(s) da pele com alteração de sensibilidade; b) acometimento de nervo(s) periférico(s), com ou sem espessamento, associado a alterações sensitivas e/ou motoras e/ou autonômicas[8].

Os dados foram analisados usando o teste exato de Fisher para comparar as frequências entre os grupos. O nível de significância estabelecido foi $p < 0.05$.

RESULTADOS

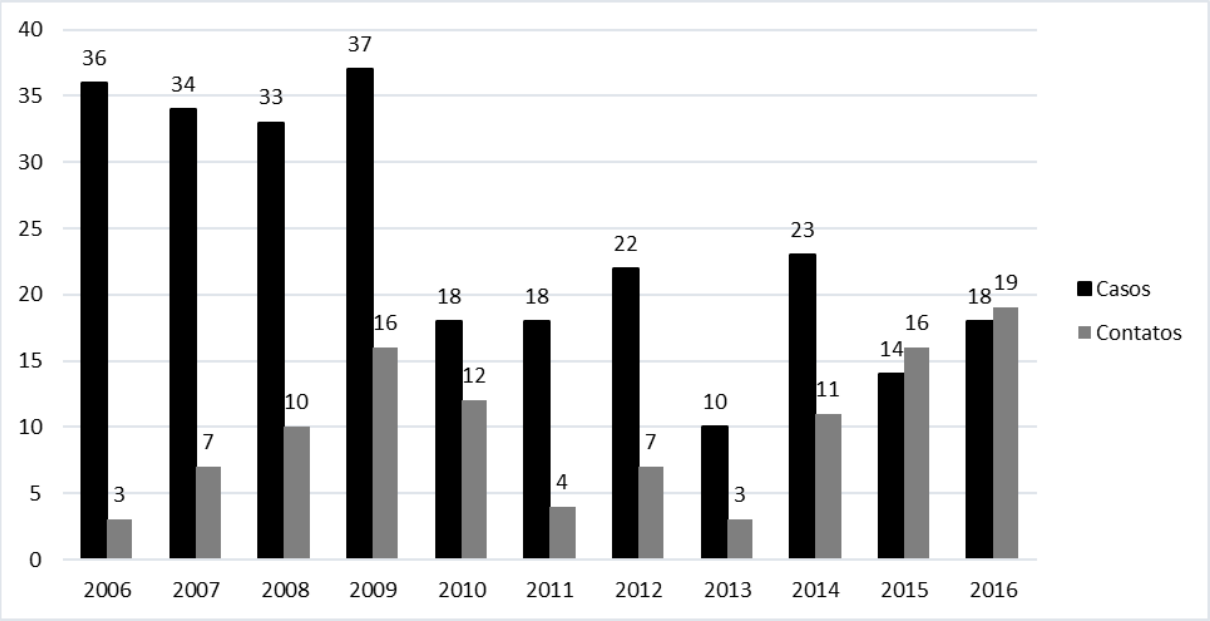
Um total de 263 pacientes de hanseníase foram localizados no período estudado. Desses casos-índices, 210 por vários motivos não participaram (Tabela 1). Dos 53 participantes, entre os seus contatos domiciliares, 108 foram localizados.

Tabela 1 – Justificativa da não participação dos casos índice no estudo

Motivo	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**TOTAL**
Não atendeu fone	9	6	7	4	2	6	5	3	5	3	4	54
Fone inexistente	17	21	14	12	6	6	5	4	8	-	2	95
Não quis participar	4	2	5	5	2	3	5	1	3	2	2	34
Não deu certo a visita	3	1	1	2	-	-	1	1	1	1	1	12
Não havia contato	1	1	1	5	1	-	3	-	-	1	2	15
Total	34	31	28	28	11	15	19	9	17	7	11	210

A distribuição dos contatos examinados e os casos-índices relacionados nos anos do período em estudo é ilustrada na figura 1.

Figura 1 – Distribuição das quantidades de casos e contatos examinados nos anos do período em estudo, de 2006 a 2016.



Na análise da figura 1 pode-se verificar que o número de contatos examinados nos últimos anos foi proporcionalmente maior em relação aos anos anteriores. Por outro lado, é nítida a maior quantidade de casos de hanseníase nos anos do início do período estudado.

Conforme os dados resumidos na Tabela 2, a maioria dos contatos eram do gênero feminino, da cor branca, vacinados pelo BCG na época do diagnóstico do caso-índice e apresentaram resultado do teste ML-Flow negativo. Para dois contatos (1,85%), o ML-flow foi positivo. Um deles era uma mulher, de 100 anos de idade e da cor parda. Ela havia tomado a vacina BCG e o seu caso-índice relacionado foi um homem com hanseníase MB, do ano de 2015. O segundo contato positivo foi outra mulher, de 27 anos de idade, da cor parda, que havia tomado BCG e cujo caso-índice relacionado foi um homem com hanseníase PB, do ano de 2009.

Tabela 2 – Quantidades e porcentagens de contatos examinados e casos-índices segundo as variáveis estudadas.

	Dados dos contatos examinados N=108	
Variável	**Opções**	**N (%)**
Gênero	Feminino	67 (62,04%)
	Masculino	41 (37,96%)
Idade (anos)	Média ± desvio padrão	44,6 (21,2)
Cor	Branca	65 (60,19%)
	Preta	6 (5,55%)

	Parda	37 (34,26%)
Vacina BCG	Não	10 (9,26%)
	Sim	98 (90,74%)
ML-Flow	Positivo	2 (1,85%)
	Negativo	106 (98,15%)
	Dados dos casos-índices N=53	
Variável	**Opções**	**N (%)**
Gênero	Feminino	35 (66,04%)
	Masculino	18 (33,96%)
Idade (anos)	Média ± desvio padrão	53,8 ± 19
Cor	Branca	34 (64,15%)
	Preta	2 (3,77%)
	Parda	17 (32,08%)
Classificação operacional	MB	27 (50,94%)
	PB	26 (49,01%)

Segundo a classificação operacional, os contatos eram quase que igualmente relacionados a caso-índices MB e a caso-índices PB. Os dois contatos que tiveram o teste ML-Flow positivo, 1 (50%) era contato de caso-índice MB e 1 (50%) de PB. Dentre os 106 contatos que tiveram o teste ML-Flow negativo, 54 (50,94%) era contato de caso-índice MB e 52 (49,06%) de PB (p=1).

O exame clínico dermatoneurologico não evidenciou sintomas ou lesões suspeitas de hanseníase nos 108 contatos examinados. Assim, os 2 (1,85%) contatos que

apresentaram o teste ML-Flow positivo foram considerados "portador de infecção subclínica" e serão acompanhados anualmente, por um período de cinco anos, a fim de verificar o possível desenvolvimento da doença. Os outros 106 (98,15%) contatos, que apresentaram teste ML-Flow negativo e exame clínico normal, foram considerados como "contato normal". Assim, não foi confirmado nenhum “caso novo de hanseníase".

DISCUSSÃO

A detecção precoce de pacientes com hanseníase é uma prioridade para se conseguir o controle e a eliminação da doença. Para isso, a estratégia mais eficaz é o monitoramento dos contatos domiciliares dos pacientes. No entanto, é preconizado apenas o exame clínico dos contatos no momento em que o caso índice é diagnosticado, o que nem sempre é suficiente para a detecção da hanseníase no estágio inicial da infecção, pois o diagnóstico é feito somente quando há lesões na pele e/ou danos nos nervos, ocasião em que já pode ter ocorrido transmissão e sequelas incapacitantes[12,13,16,18,21,24,25].

O uso de outras ferramentas pode contribuir para a identificação de indivíduos com hanseníase subclínica. No presente estudo, na tentativa de melhorar a detecção precoce, foi realizada a pesquisa de anticorpos anti-PGL-1, pelo teste ML-Flow, em contatos domiciliares de pacientes de hanseníase, encontrando índice de 1,85% de positividade, resultado semelhante ao relatado por Soares et al. (1994), que obtiveram 1% na sua casuística. Porém, o índice foi baixo em comparação com a maioria dos estudos semelhantes publicados na literatura, que relata soropositividade para o PGL-I desde 4,1% até 39%[7,11-28].

Contatos infectados que apresentam boa imunidade contra *M. leprae*, e que desenvolverão hanseníase PB no futuro, podem não apresentar níveis detectáveis de anticorpos anti-PGL-I[11,19], o que pode ser o caso deste estudo. Também, contatos de casos índices MB, que apresentam uma alta carga bacilar, têm maior chance de se infectarem que contatos de casos índices PB[7,11,15,22], o que poderia ser outra justificativa para a baixa positividade aqui encontrada, pois metade dos casos índices era PB.

Outro aspecto a ser ressaltado é que a grande maioria dos contatos domiciliares havia recebido profilaticamente uma dose da vacina BCG na época do diagnóstico do caso-índice. Sabe-se que a vacina BCG induz à ativação de clones de células T que reconhecem epítopos específicos do *M. leprae,* conferindo efeito protetor contra a progressão da doença, inclusive levando à negativação de testes sorológicos com PGL-I antes positivos[23,31].

Um maior número de contatos precisaria ser examinado, porém a dificuldade para abordagem foi grande, devido à recusa na participação no estudo e dificuldades na localização dos casos-índices.

É possível concluir que para os dados deste estudo não houve relação significativa entre as variáveis. No entanto, como foram observados apenas dois contatos com teste ML-Flow positivo, tal resultado indica apenas que não houve evidência suficiente para identificar uma relação, caso ela exista. Assim, estudos futuros, com um tamanho amostral maior, devem retificar ou ratificar tais resultados.

Como o Ml-Flow é um teste rápido, de fácil execução, baixo custo e não requer estrutura laboratorial, pode ser empregado por trabalhadores da saúde em condições de campo ou em diferentes níveis da atenção. Assim, o seu uso deve ser

estimulado para o seguimento de populações de risco, como é o caso dos contatos domiciliares, constituindo uma ferramenta auxiliar para identificar uma possível prevalência oculta, com o intuito de sustentar a eliminação da hanseníase em regiões onde foi alcançada a meta, como é a situação da região aqui estudada.

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ANEXOS

ANEXO A

Parecer do Comitê de Ética em Pesquisa

UNOESTE - UNIVERSIDADE DO OESTE PAULISTA

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: INQUÉRITO SOROEPEPIDEMIOLÓGICO COM O TESTE ML- FLOW NOS CONTATOS DE PACIENTES DE HANSENIASE NO PERÍODO DE 2006 A 2016 EM MUNICÍPIOS DO OESTE PAULISTA, ESTADO DE SÃO PAULO.

Pesquisador: Marilda Aparecida Milanez Morgado de Abreu

Área Temática:

Versão: 2

CAAE: 69516017.2.0000.5515

Instituição Proponente: UNOESTE - Universidade do Oeste Paulista

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.310.423

Apresentação do Projeto:

Segundo Parecer. A Hanseníase é um problema se saúde pública no Brasil. As ações de controle estão baseadas no diagnóstico e tratamento dos doentes e na vigilância de seus contatos. O teste ML Flow é um teste de fluxo lateral, para a detecção de IgM contra o PGL-1, cujos resultados são obtidos entre 5 a 10 minutos usando sangue total ou soro. Está relacionado à presença do Mycobacterium leprae no hospedeiro sem
necessariamente o mesmo apresentar a doença. No presente trabalho, será realizado um inquérito soroepidemiológico nos contatos de pacientes de hanseníase, nos municípios do Oeste Paulista, nos anos de 2006 a 2016.

Objetivo da Pesquisa:

Analisar a ocorrência de hanseníase entre contatos aparentemente saudáveis de indivíduos tratados ou em tratamento de hanseníase, no município de Presidente Prudente, SP, no período de 2006 A 2016, através do exame clínico e emprego do teste ML FLOW, como método auxiliar e diagnóstico.

Avaliação dos Riscos e Benefícios:

Não há riscos previstos pelos pesquisadores e foi comentado no TCLE, após primeiro parecer,

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Continuação do Parecer: 2.310.423

sobre o desconforto mínimo decorrente de leve picada para coleta de sangue realizada por profissional capacitado. No entanto, mesmo que mínimos, precisam ser descritos na coleta de uma gota de sangue no dedo do paciente, conforme previsto na Norma 466/12. Os pesquisadores estabeleceram os benefícios o encinhamento e cuidados necessários dos casos diagnosticados., bem como orientações, atendendo à sugestão dos pareceristas.

Comentários e Considerações sobre a Pesquisa:

Adequadamente proposta em termos científicos e relevante para a área e para a sociedade.

Considerações sobre os Termos de apresentação obrigatória:

Presentes e corretamente assinados .

Recomendações:

Verificar a linguagem e vocabulário dos TCLEs e Assentimento.

Conclusões ou Pendências e Lista de Inadequações:

NÃO HÁ PENDÊNCIAS.

Considerações Finais a critério do CEP:

Em reunião realizada no dia 02/10/2017, o Comitê de Ética em Pesquisa da Universidade do Oeste Paulista (CEP-UNOESTE), concordância com o parecerista, considerou o projeto APROVADO.

Solicitamos que sejam encaminhados ao CEP:

1. Relatórios anuais, sendo o primeiro previsto para 30/12/2018.
2. Comunicar toda e qualquer alteração do Projeto e Termo de Consentimento Livre e Esclarecido. Nestas circunstâncias a inclusão de participantes deve ser temporariamente interrompida até a aprovação do Comitê de Ética em Pesquisa.
3. Comunicar imediatamente ao Comitê qualquer Evento Adverso Grave ocorrido durante o desenvolvimento do estudo.
4. Os dados individuais de todas as etapas da pesquisa devem ser mantidos em local seguro por 5 (cinco) anos, após conclusão da pesquisa, para possível auditoria dos órgãos competentes.
5. Este projeto está cadastrado na CPDI-UNOESTE sob o número 3933.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação

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Continuação do Parecer: 2.310.423

Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_939114.pdf	06/09/2017 14:24:46		Aceito
Outros	Sujeito_da_pesquisa_ok.pdf	06/09/2017 14:24:25	Marilda Aparecida Milanez Morgado de Abreu	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_menores.doc	06/09/2017 14:22:36	Marilda Aparecida Milanez Morgado de Abreu	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_contatos.docx	06/09/2017 14:22:29	Marilda Aparecida Milanez Morgado de Abreu	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_assentimento.docx	06/09/2017 14:22:13	Marilda Aparecida Milanez Morgado de Abreu	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.docx	06/09/2017 14:22:01	Marilda Aparecida Milanez Morgado de Abreu	Aceito
Projeto Detalhado / Brochura Investigador	Projeto.docx	06/09/2017 14:21:50	Marilda Aparecida Milanez Morgado de Abreu	Aceito
Declaração de Instituição e Infraestrutura	Infraestrutura.pdf	06/09/2017 14:21:39	Marilda Aparecida Milanez Morgado de Abreu	Aceito
Outros	Uso_de_prontuario.pdf	07/06/2017 15:30:24	Marilda Aparecida Milanez Morgado de Abreu	Aceito
Outros	Termo_de_responsabilidade.pdf	07/06/2017 15:30:03	Marilda Aparecida Milanez Morgado de Abreu	Aceito
Declaração de Pesquisadores	Termo_de_compromisso.pdf	07/06/2017 15:28:04	Marilda Aparecida Milanez Morgado de Abreu	Aceito
Folha de Rosto	Folha_de_rosto.pdf	07/06/2017 15:26:43	Marilda Aparecida Milanez Morgado de Abreu	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

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ANEXO B

Normas de publicação da revista científica a qual o artigo será submetido

Guide for Authors

Author information pack

Introduction

Aims and Scope

Journal of Microbiology, Immunology and Infection, launched in 1968, is the official bi-monthly publication of the Taiwan Society of Microbiology, the Chinese Society of Immunology, the Infectious Diseases Society of Taiwan and the Taiwan Society of Parasitology.

The journal is an open access journal, committed to disseminating information on the latest trends and advances in microbiology, immunology, infectious diseases and parasitology. Articles on clinical or laboratory investigations of relevance to microbiology, immunology, infectious diseases, parasitology and other related fields that are of interest to the medical profession are eligible for consideration. Article types considered include perspectives, review articles, original articles, short communication and correspondence.

The Editorial Board of the Journal comprises a dedicated team of local and international experts in the field of microbiology, immunology, infectious diseases and parasitology. All members of the Editorial Board actively guide and set the direction of the journal. With the aim of promoting effective and accurate scientific information, an expert panel of referees constitutes the backbone of the peer-review process in evaluating the quality and content of manuscripts submitted for publication.

JMII is open access and indexed in SCIE, PubMed, MEDLINE, EMBASE, Scopus, AIDS & Cancer Reseach, CABI, BIOSIS Previews, Biological Abstracts, EBSCOhost, CancerLit, Reactions Weekly (online), Chemical Abstracts, HealthSTAR, Global Health, ProQuest.

The Editorial Board requires authors to be in compliance with the *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (URMs)*; current URMs are available at http://www.icmje.org.

Types of article

1. PerspectivesThese are comments on recent news or groundbreaking work and should provide a short review of the current state of research and explain the importance of the new findings. Perspectives on papers previously published in the JMII should add a different viewpoint to the research and should not merely be a repetitive summary of the original paper. Although many of the Perspectives published in the Journal are normally invited, unsolicited Perspectives are welcome and will be given due consideration. As these are meant to express a personal commentary, with rare exceptions, Perspectives should have no more than 3 authors.

Format guide:

• Word limit: 1000 words (excluding the abstract and references). • References: 10 or less.
• Tables/Figures: 1 table or figure.

2. Review ArticlesThese should aim to provide the reader with a balanced overview of an important and topical subject in the field, and should be systematic and critical assessments of literature and data sources. They should cover aspects of a topic in which scientific consensus exists as well as aspects that remain controversial and are the subject of ongoing scientific research. All articles and data sources reviewed should include information about the specific type of study or analysis, population, intervention, exposure, and tests or outcomes. All articles or data sources should be selected systematically for inclusion in the review and critically evaluated. We welcome viewpoints that present the opinions of the authors rather than new experimental data or literature reviews.

Format guide:

• Word limit: 3500 words (excluding the abstract and references).• References: 50 or less.
• Abstract: Up to 250 words, unstructured.• Tables/Figures: Data in the text should not be repeated extensively in tables or figures.

4. Original ArticlesThese articles typically include randomized trials, intervention studies, studies of screening and diagnostic tests, laboratory and animal studies, cohort studies, cost-effectiveness analyses, case-control studies, and surveys with high response rates, which represent new and significant contributions to the field.Section headings should be: Abstract, Introduction,

Methods, Results, Discussion, Conflicts of Interest Statement (if any), Acknowledgments (if any) and References.

5. The Introduction should provide a brief background to the subject of the paper, explain the importance of the study, and state a precise study question or purpose.
6. The Methods section should describe the study design and methods (including the study setting and dates, patients/participants with inclusion and exclusion criteria, or data sources and how these were selected for the study, patient samples or animal specimens used, explain the laboratory methods followed), and state the statistical procedures employed in the research.
The Results section should comprise the study results presented in a logical sequence, supplemented by tables and/or figures. Take care that the text does not repeatdata that are presented in tables and/or figures. Only emphasize and summarize the essential features of any interventions, the main outcome measures, and the main results.
The Discussion section should be used to emphasize the new and important aspects of the study, placing the results in context with published literature, the implicationsof the findings, and the conclusions that follow from the study results.

Format guide:

• Word limit: 3000 words (excluding the abstract and references).
• References: 40 or less.
• Abstract: Up to 250 words, structured.
• Tables/Figures: Data in the text should not be repeated extensively in tables or figures.

4. Short Communications

Short Communications should present unusual aspects of common problems or novel perspectives upon, or solutions to, clinically relevant issues.

Format guide:

• Word limit: 1500 words (excluding the abstract and references).
• References: 10 or less.
• Abstract: Up to 50 words, unstructured format
• Tables/Figures: 1 table and figure.

5. Correspondence

Correspondences include letter to Editor, and comments that respond to a recently published article in JMII or address an issue of interest to JMII readers. Replies will be published in the same issue as the letter, and are invited at the discretion of the Editor.

Format guide:

• Word limit: 500 words.
• Tables/Figures: 1 figure or table.
• References: 5 or less.
• No subheadings.
• Begin with 'Dear Editor'.

Editorial Office

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Before You Begin

Basic Criteria

Articles should be written in English (using American English spelling) and meet the following basic criteria: the material is original, the information is important, the writing is clear and concise, the study methods are appropriate, the data are valid, and the conclusions are reasonable and supported by the data.

Manuscript Submission Online submission

Authors may submit manuscripts to EES at http://ees.elsevier.com/jmii/. If assistance is needed, the Editorial Office can be contacted for any help necessary.

Important Information

- The corresponding author will be notified by the editorial office when the manuscript is accepted and sent to the Publisher. The corresponding author will receive a PDF proof by e-mail from the Publisher within the next 2 months. JMII reserves the right to rescind our provisional decision of acceptance if no response is received from the author by the dategiven by the Publisher.
- Articles submitted should be in Microsoft Word document format and prepared in the simplest form possible. We will add in the correct font, font size, margins and so on according to the journal's style.
- You may use automatic page numbering, but do NOT use other kinds of automatic formatting such as footnotes, endnotes, headers and footers.
- Put text, references, and table/figure legends in one file.
- Figures must be submitted as separate picture files, at the correct resolution of a minimum of 600 dpi. The files should be named according to the figure number and format, e.g. "Fig1.tif", "Fig2.jpg".

Please ensure that the following documents are included (refer also to the checklist that follows these author instructions):

(1) A cover letter. It must include your name, address, telephone and fax numbers, and e-mail address (both of the first author and corresponding author), and state that the manuscript has never been submitted, in whole or in part, to other journals. Your signature and those of ALL your coauthors must be included.

(2) (2) An authorship and conflicts of interest statement. Each author's contribution to the manuscript should be listed. Any and all potential and actual conflicts of interest should also be listed (see relevant section below for more information). Please use the JMII Authorship and Conflicts of Interest Statement form that follows these author instructions. The corresponding author must sign on behalf of all the listed authors in the manuscript.

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(4) (4) An ethics statement. Articles covering the use of human or animal samples in research, or human or animal experiments must be accompanied by a letter of approval from the relevant review committee or authorities (see relevant section below).

(5) (5) Articles where human subjects can be identified in descriptions, photographs or pedigrees must be accompanied by a signed statement of informed consent to publish (in print and online) the descriptions, photographs and pedigrees from each subject who can be identified (see relevant section below).

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Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Studies in humans and animals

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The manuscript should be in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals and aim for the inclusion of representative human populations (sex, age and

ethnicity) as per those recommendations. The terms sex and gender should be used correctly.

Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

All animal experiments should comply with the ARRIVE guidelines and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed. The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study.

Disclosure of Conflicts of Interest A conflict of interest occurs when an individual's objectivity is potentially compromised by a desire for financial gain, prominence, professional advancement or a successful outcome. *JMII* Editors strive to ensure that what is published in the Journal is as balanced, objective and evidence-based as possible. Since it can be difficult to distinguish between an actual conflict of interest and a perceived conflict of interest, the Journal requires authors to disclose all and any potential conflicts of interest.

Conflicts of interest may be financial or non-financial. Financial conflicts include financial relationships such as honoraria; educational grants; participation in speakers bureaus; membership, employment, consultancies, stock ownership, or other equity interest; expert testimony or patent-licensing arrangements. Non-financial conflicts include personal or professional relationships, affiliations, academic competition, intellectual passion, knowledge or beliefs that might affect objectivity.

Please ensure that any conflicts of interest and sources of funding are fully declared on page 2 of the *JMII Authorship and Conflicts of Interest Statement form*.

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