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JONNIA MARIA SHERLOCK ARAÚJO

REAÇÕES HANSÊNICAS: PERFIL CLÍNICO E RESPOSTA IMUNOLÓGICA A ANTÍGENOS RECOMBINANTES DE *MYCOBACTERIUM LEPRAE*

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Dissertação apresentada ao Núcleo de Pós-Graduação em Medicina da Universidade Federal de Sergipe como requisito parcial à obtenção do grau de Mestre em Ciências da Saúde

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Aprovada em: ____/____/____

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RESUMO

Reações hansênicas: perfil clínico e resposta imunológica a antígenos recombinantes de *Mycobacterium leprae*, Jonnia Maria Sherlock Araújo, Aracaju, 2013.

A hanseníase é uma doença infecciosa de grande impacto mundial. A doença se associa a importante morbidade, relacionada tanto à ocorrência das reações hansênicas, quanto ao desenvolvimento de lesões neurológicas periféricas incapacitantes. As reações hansênicas são complicações imunologicamente mediadas, que além de apresentarem quadros clínicos inflamatórios e potencialmente graves, podem se associar também ao surgimento de incapacidades físicas. Ainda existem muitas lacunas quanto aos seus determinantes clínicos e imunológicos, o que dificulta o seu controle. O uso de antígenos recombinantes tem se revelado como importante ferramenta para elucidação de diversos aspectos imunológicos da hanseníase. Esse estudo objetivou avaliar o perfil clínico e a resposta imunológica, a antígenos recombinantes de *Mycobacterium leprae,* associados às reações hansênicas. Os objetivos específicos foram: 1) Avaliar as características clínicas que se associam às reações hansênicas; 2) Avaliar as características clínicas que se relacionam com incapacidades físicas ao final do tratamento; 3) Comparar a resposta imune a antígenos recombinantes de *M. leprae* entre pacientes com e sem reações hansênicas. Para avaliação dos objetivos 1 e 2 foi desenvolvido um estudo retrospectivo, baseado na análise de prontuários de pacientes com hanseníase, atendidos em dois centros de especialidades médicas em Sergipe. Para o objetivo 3, desenvolveu-se um estudo transversal no qual se comparou a resposta imunológica (IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-15, IL-17a, IL-23, IL-27, INF-γ, TNF-α, TGF-β, MCP-1 e MIP-1α) após estimulação de antígenos recombinantes de *M. leprae* (MLCS, ML0276, ML2028, ML2055, ML2258, ML2531, ML2629, ML82F, ML2044, ML2380, ML2331, LID1 e PADL) e de *M. tuberculosis* (ID93, ID83, PPD) entre os pacientes que desenvolveram ou não reações hansênicas. Os resultados do objetivo 1 e 2 revelaram a ocorrência de reações hansênicas em 40% dos pacientes e de incapacidades físicas em 30% dos pacientes. Observou-se que o sexo masculino se associou com formas multibacilares e com reações hansênicas. O uso de subdosagem de corticosteróides para o tratamento das reações se associou de forma independente à presença de incapacidades físicas ao final do tratamento para hanseníase. Os resultados do objetivo 3 demonstraram que quimiocinas inflamatórias como MCP-1, após estímulo dos antígenos recombinantes ID93 e ML2531, foram mais elevadas nos pacientes que desenvolveram reações hansênicas. Assim, ressaltamos a necessidade de maior vigilância do gênero masculino, bem como de tratamento mais adequado aos pacientes que evoluem com episódios reacionais, a fim de prevenir incapacidades físicas. A produção de MCP-1, em resposta aos antígenos ID93 e ML2531, pode ser testada como marcador de reações hansênicas.

Descritores: Hanseníase; Neurite; Imunologia; Antígenos; Corticosteróides.

ABSTRACT

Leprosy reactions: clinical profile and immune response to recombinant antigens from *Mycobacterium leprae*, Sherlock Jonnia Maria Araujo, Aracaju, 2013.

Leprosy is an infectious disease of great global impact. It is associated with significant morbidity, related to both the occurrence of leprosy reactions and the development of peripheral neurological disabling injuries. Leprosy reactions are immunologically mediated complications, which presents inflammatory and potentially serious clinical forms and may also be associated with the onset of physical disabilities. There are still many gaps regarding their clinical and immunological determinants, which hinders its control. The use of recombinant antigens has been shown as an important tool for the elucidation of various immunological aspects of leprosy. This study aimed to evaluate the clinical profile associated with leprosy reactions and the immune response to recombinant antigens from *Mycobacterium leprae* associated with reactions. Specific objectives were: 1) to evaluate the clinical characteristics that are associated with leprosy reactions, 2) to evaluate the clinical characteristics that correlate with physical disabilities at the end of treatment, 3) to compare the immune response to recombinant antigens of *M. leprae* between patients with and without reactions. For evaluation of objectives 1 and 2 was developed a retrospective study based on chart analysis of leprosy patients treated at two centers of medical specialties in Sergipe. For the third objective, we developed a cross sectional study that compared the immune response (IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-15 , IL-17a, IL-23, IL-27, INF-γ, TNF-α, TGF-β, MCP-1 and MIP-1α) after stimulation with recombinant antigens of *M. leprae* (MLCS, ML0276, ML2028, ML2055, ML2258, ML2531, ML2629, ML82F, ML2044, ML2380, ML2331, and LID1 PADL) and M. tuberculosis (ID93, ID83, PPD) among patients who developed or not reactions. The results of objective 1 and 2 revealed the occurrence of leprosy reactions in 40% of patients and physical disabilities in 30% of patients. It was observed that the male sex was associated with multibacillary forms and reactions. The use of low doses of corticosteroid for the treatment of reactions was independently associated with the presence of physical disability at the end of treatment for leprosy. The results of objective 3 showed that inflammatory chemokines such as MCP-1, after stimulation of recombinant antigens like ML2531 and ID93, were higher in patients who developed reactions. Thus, we emphasize the need for greater vigilance of males, as well as most appropriate treatment for patients who develop reactive episodes in order to prevent physical disabilities. The production of MCP-1 in response to antigens ML2531 and ID93 can be assayed as a marker of reactions.

Descriptors: Leprosy; Neuritis; Immunology; Antigens; Corticosteroids.

LISTA DE ABREVIATURAS

M leprae	*Mycobacterium leprae*
OMS	Organização Mundial de Saúde
PGL-1	Glicolipídeo fenólico 1
IL-2	Interleucina 1
IL-4	Interleucina 4
IL-5	Interleucina 5
IL-6	Interleucina 6
IL-10	Interleucina 10
INF-γ	Interferon gama
TNF-α	Fator de necrose tumoral alfa
TGF-β	Fator de transformação do crescimento beta
Treg	Células T reguladoras
PB	Paucibacilar
MB	Multibacilar
TT	Tuberculóide
VV	Virchowiana
DT	Dimorfa tuberculóide
DD	Dimorfa dimorfa
DV	Dimorfa virchowiana
HI	Hanseníase indeterminada
HPN	Hanseníase neural pura
PQT	Poliquimioterapia
ENH	Eritema nodoso hansênico
GIF	Grau de incapacidade física

SUMÁRIO

1 INTROUÇÃO

Doença infectocontagiosa, de ocorrência milenar, a hanseníase ainda se apresenta como um importante desafio para a saúde pública. Sua morbidade relaciona-se principalmente às lesões neurológicas periféricas e às reações hansênicas, que podem resultar em sequelas físicas incapacitantes.

Alguns fatores justificam o difícil controle da doença. Em primeiro lugar, o seu agente etiológico, o *Mycobacterium leprae*, nunca foi cultivado, dificultando a realização de estudos experimentais. Além disso, essa micobactéria apresenta um tropismo peculiar pelo sistema nervoso periférico, o que favorece sua multiplicação em um ambiente pouco permissivo à ação de medicamentos e do sistema imunológico. Soma-se a esses fatores o longo período de incubação da doença, o que dificulta a identificação do paciente transmissor na maior parte dos casos.

Estudos com antígenos recombinantes são fundamentais para suprir a lacuna decorrente da impossibilidade de cultivo do *M. leprae.* O uso desses antígenos pode contribuir para o desenvolvimento de testes diagnósticos, de vacinas, bem como de imunoterapia para a hanseníase.

Dados da Organização Mundial de Saúde (OMS) revelam que 228,474 novos casos de hanseníase foram detectados em todo o mundo em 2011 (WHO, 2011). Neste cenário, o Brasil aponta com uma prevalência de 1,54 casos por 10.000 habitantes (WHO, 2011) e como o segundo país com maior número de casos novos, perdendo apenas para a Índia (WHO, 2011). Em Sergipe, o coeficiente de detecção de casos novos, em 2011, foi de 20,77 casos por 100.000 habitantes (MS, 2011).

As reações hansênicas são complicações agudas, reconhecidas como a principal causa de lesão neurológica periférica na hanseníase, que ocorrem em 30 a 50% dos pacientes (RANQUE, 2007; SCOLLARD, 2006). Alterações imunológicas variadas são fundamentais para o desenvolvimento desses eventos, porém, seus determinantes imunológicos ainda não foram completamente elucidados (GOULART, 2002; SCOLLARD, 2006). A despeito da frequência desses eventos e da significativa morbidade associada a eles, ainda existem muitas lacunas quanto aos seus fatores desencadeantes, o que dificulta a definição de critérios para a operacionalização de seu controle (SCOLLARD, 2006; OLIVEIRA, 2007). Em virtude do exposto, o presente estudo objetivou avaliar o perfil clínico e a resposta imunológica, a antígenos recombinantes de *M. leprae,* associados às reações hansênicas.

2 REVISÃO DA LITERATURA

2.1 Agente etiológico, Transmissão e Imunopatogênese da Hanseníase

O *Micobacterium leprae* (*M. leprae*) é um bacilo gram-positivo, álcool-ácido resistente e não cultivável. Apresenta alta infectividade e baixa patogenicidade e virulência, o que implica que a maioria dos pacientes que entra em contato com o bacilo não manifesta a doença. A transmissão desse micro-organismo ocorre predominantemente pelas vias aéreas superiores e o período de incubação, que compreende o período entre o contágio e a manifestação dos primeiros sinais e sintomas da doença, é longo e variável, em média 2 a 5 anos, o que inviabiliza a identificação do paciente transmissor na maior parte dos casos (ARAUJO, 2003). Pacientes que apresentam grande número de bacilos em seu organismo, ditos multibacilares, são a principal fonte de infecção, sendo os seus contatos domiciliares, independente do gênero, os maiores susceptíveis ao contágio (SALES, 2001). Esses pacientes abrigam grande quantidade de bacilos, tanto nas fossas nasais quanto na pele, e permanecem como fonte de transmissão da doença até que seja instituído o tratamento adequado (ARAUJO, 2003; BAKER, 2006; JOB, 2008; LOCKWOOD, 2005).

Na maioria dos indivíduos, ocorre uma infecção subclínica, limitada por uma resposta imune efetiva, com cura espontânea sem a necessidade de tratamento (MENDONÇA, 2008). Os pacientes que manifestam clinicamente a doença apresentam sinais e sintomas variados que se enquadram em uma das seguintes formas clínicas da hanseníase: hanseníase indeterminada (forma inicial e instável), hanseníase tuberculóide (polo de resistência), hanseníase dimorfa (formas interpolares) e hanseníase virchowiana (polo de susceptibilidade). A manifestação de cada forma clínica é dependente da interação entre o patógeno e o hospedeiro e sofre influência da genética e da resposta imune do hospedeiro. Diversos genes estão relacionados com o controle da resposta imunológica humana contra o *M. leprae,* operando tanto em nível de imunidade inata quanto de imunidade adaptativa, e contribuindo para manifestação dos diferentes fenótipos da doença (PREVEDELO, 2007; SCOLLARD, 2006).

No sítio de inoculação, o bacilo é capturado pelas células apresentadoras de antígenos, representadas na pele principalmente pelas células de Langehans, que o processam expressando antígenos do bacilo em sua superfície. Entre esses antígenos está o glicolipídio

fenólico-1 (PGL-1), específico da parede celular do *M. leprae*. Atribui-se ao PGL-1 a capacidade supressora da atividade macrofágica (BARROS, 2000; FOSS, 1997; SCOLLARD, 2006). Há diferenças quantitativas em relação às células de Langehans entre as formas clínicas da hanseníase, de modo que, nas lesões do polo tuberculóide, há maior número dessas células quando comparadas às do polo virchowiano (MENDONÇA, 2008).

As células de Langehans migram para os linfonodos onde apresentam os antígenos do bacilo às células T CD4+ virgens. Neste momento, ocorre a ativação do linfócito T virgem, o início da produção de IL-2 e a diferenciação deste linfócito em Th0, o qual pode se diferenciar em outros subtipos: Th1, Th2, Th17 ou T regulatório. A diferenciação em Th1 ou Th2 é responsável pela evolução do indivíduo em direção aos polos tuberculóide ou virchowiano, respectivamente (MACHADO, 2004). Artigo recente descreveu também a presença de maior número de células T reguladoras (Treg) na forma virchowiana, indicando a participação destas células na imunossupressão específica e proliferação bacilar observadas nessa forma clínica (PALERMO, 2012). As formas interpolares, ditas dimorfas, são imunologicamente dinâmicas, apresentando oscilações entre as polares.

O padrão de resposta Th1 é caracterizado pela produção de interleucina 2 (IL-2), interferon-γ (IFN-γ) e fator de necrose tumoral-α (TNF-α), entre outras citocinas, responsáveis pela construção de uma imunidade mediada por células (GOULART, 2002). Os indivíduos que desenvolvem uma resposta Th1 são imunologicamente capazes de controlar a multiplicação bacilar e, portanto, apresentam formas clínicas mais brandas, com poucos bacilos nas lesões cutâneas e na linfa, classificadas como paucibacilares (PB) (GOULART, 2002).

O padrão de resposta Th2 caracteriza-se pela produção de IL-4, IL-5, IL-6 e IL-10, responsáveis pela formação de anticorpos, inibição de macrófagos e supressão da resposta imune celular (GOULART, 2002; YEMURI, 2000). Tem-se, desse modo, a construção de uma resposta humoral que é ineficaz contra a replicação do *M. leprae* e favorece disseminação da doença no hospedeiro. As células T reguladoras produzem IL-10 e TGF-β, citocinas que modulam tanto a resposta de células T como a de células fagocíticas e apresentadoras de antígenos. Assim, a predominância de respostas Th2 ou Treg associam-se às manifestações de formas clínicas com múltiplos bacilos, ditas multibacilares (MB) (GOULART, 2002).

2.2 Antígenos recombinantes

A impossibilidade de crescimento do *M. leprae in vitro,* tem motivado a busca constante por diversos antígenos desta micobactéria. Amplificando o DNA de *M. leprae* por PCR, Duthie et al. (2007) construíram uma biblioteca de DNA genômico (Thai-53) desta micobactéria no vetor de expressão pET28a (Novagen, Madison, WI) em *Escherichia coli*. Os clones individuais foram então induzidos a produzir proteínas recombinantes. Essas proteínas recombinantes têm sido utilizadas como antígenos em estudos imunológicos, visando encontrar epitopos do *M. leprae* que estejam relacionados com a imunopatogenia da doença e/ou que possam ser utilizados em testes diagnósticos para identificar a doença nos estágios iniciais tanto de formas clínicas multibacilares quanto de paucibacilares (DUTHIE, 2010; DUTHIE, 2007; REECE, 2006; SAMPAIO, 2011). Contudo, a maioria desses antígenos não foi avaliada em pacientes com hanseníase e muitos deles possivelmente não serão aprovados para utilização em seres humanos devido a grande homologia que apresentam com proteínas humanas (BAUMGART, 1996; GELBER, 1994; MATSUOKA, 1997; NGAMYING, 2001; NOMAGUCHI, 2002).

Duthie, et al. (2007), demonstraram que uma proteína de fusão denominada LID-1, feita a partir de dois antígenos recombinantes de *M. leprae*, ML0405 e ML2331, possibilitou o diagnóstico de hanseníase ainda em fase subclínica. De forma semelhante, a *protein advances diagnostico of leprosy* (PADL), proteína de fusão quimérica complementar de LID-1 (ML0405, ML2331, ML2055, ML0411 e ML0091) apresentou capacidade de diferenciar os pacientes com as formas MB dos controles, podendo ser utilizada em testes diagnósticos para hanseníase (DUTHIE, 2010; DUTHIE, 2011).

Raman, et al. (2009), em estudo experimental, demonstraram que a combinação do antígeno ML0276 com o agonista de TLR-4 (EM005) induz uma resposta Th1, limitando a inflamação local, porém, sem impedir a multiplicação do bacilo em ratos infectados por *M. leprae.* Neste estudo, os autores sugerem que essa combinação pode ser benéfica em pacientes com reações hansênicas pela possibilidade de limitar a resposta inflamatória.

Antígenos recombinantes de *M. tuberculosis,* a exemplo de ID83 (fusão de 3 proteínas de *M. tuberculosis*) e ID93 (fusão de quatro proteínas de *M. tuberculosis*) foram produzidos e utilizados na imunização contra *M. tuberculosis.* Em estudos experimentais ambos demonstraram induzir uma resposta protetora contra o *M. tuberculosis* (BALDWIN, 2010; BERTHOLET, 2011). A homologia existente entre o *M. leprae* e o *M. tuberculosis*

justifica a utilização de antígenos recombinantes de *M. tuberculosis* em estudos com hanseníase.

2.3 Manifestações Clínicas, Diagnóstico e Tratamento

2.3.1 Classificação Clínica e Critérios Diagnósticos

A hanseníase se expressa, clínica e histopatologicamente, por meio de formas polares e espectrais dependentes, sobretudo, da habilidade do hospedeiro de desenvolver uma resposta imune celular contra o *M. leprae*, como descrito anteriormente (FEASEY, 2009; SCOLLARD, 2006). Baseada neste princípio, a classificação de Ridley & Jopling, 1966, que abrange critérios clínicos, histopatológicos e imunológicos, estratifica a hanseníase em cinco subtipos que compreendem as formas tuberculóide (TT), virchowiana (VV), dimorfa tuberculóide (DT), dimorfa dimorfa (DD) e dimorfa virchowiana (DV) (ARAUJO, 2003; LOCKWOOD, 2007). Posteriormente, a forma indeterminada (HI), que corresponde ao estágio inicial da doença, foi adicionada a essa classificação (LOCKWOOD, 2007). A subdivisão da hanseníase em formas clínicas favorece uma melhor vigilância dos pacientes, uma vez que algumas formas estão sob maior risco de desenvolver complicações e eventos adversos (LOCKWOOD, 2007). Muitas vezes é necessário associar os dados clínicos com a histopatologia para melhor classificação da hanseníase (PAVANI, 2008).

A forma indeterminada surge após período de incubação variável e caracteriza-se pela presença de mácula hipocrômica ou eritêmato-hipocrômica associada a distúrbios da sensibilidade, sudorese e vasomotores. A partir desse estágio, a infecção pode ser abortada, por ação da imunidade celular ou de tratamento específico, ou pode evoluir para um dos cinco subtipos da doença (ARAUJO, 2003; SCOLLARD, 2006).

Na forma tuberculóide, as lesões cutâneas são únicas ou em pequeno número e apresentam-se como placas eritêmato-acastanhadas ou róseo-acastanhadas com bordos bem delimitados (ARAUJO, 2003). As lesões neurais são precoces, agressivas e localizadas. Esses pacientes apresentam poucos bacilos no organismo e, portanto, não são fontes de infecção (SCOLLARD, 2006).

A forma virchowiana, descrita como polo anérgico da doença, caracteriza-se clinicamente pelo polimorfismo das lesões cutâneas e pela presença, facultativa, de

manifestações sistêmicas resultantes da infiltração bacilar (rinite, lagoftalmo, conjuntivite, linfonodomegalia, hepatoesplenomegalia, atrofia testicular, osteoporose, etc.) (ARAUJO, 2003; SCOLLARD, 2006). As lesões cutâneas manifestam-se como máculas infiltradas de coloração variável, desde eritêmato-hipocrômicas até mesmo ferruginosas, com ou sem alteração da sensibilidade. Podem ser vistos ainda pápulas, placas ou nódulos. O comprometimento neurológico é menos intenso e mais difuso.

Entre os dois polos, o tuberculóide e o virchowiano, existem as formas intermediárias, "borderline", também denominadas dimorfas, que englobam a maioria dos pacientes (BRITTON, 2004; SCOLLARD, 2006). A variedade dimorfa-tuberculóide (DT) apresenta lesões cutâneas numerosas com aspecto tuberculóide, além de importante comprometimento de troncos nervosos (SCOLLARD, 2006). Já os casos clássicos da forma dimorfa-dimorfa (DD) apresentam lesões anulares com área central hipocrômica e com bordos imprecisos (lesões "em queijo suíço"). O comprometimento neural também é expressivo. Por fim, a forma dimorfa-virchowiana (DV) é caracterizada por numerosas lesões, menos polimorfas que as virchowianas, e por comprometimento neural semelhante ao do polo anérgico.

Aproximadamente 5 a 15% dos casos apresentam uma forma incomum de doença denominada hanseníase primariamente neural (HPN) (GARBINO, 2004; JARDIN, 2007). Caracteriza-se por comprometimento assimétrico de nervos periféricos, com alteração da sensibilidade e da força muscular e sem lesões cutâneas (BRITTON, 2004). Pode levar anos para ser identificada, necessitando, em geral, da avaliação neurológica clínica e de exames complementares como a eletroneuromiografia ou até a biópsia de nervo para ser diagnosticada (GARBINO, 2004; JARDIN, 2007). Com a evolução, os pacientes com hanseníase primariamente neural podem apresentar lesões de pele (GARBINO, 2004).

O Ministério da Saúde define como caso de hanseníase a presença de um ou mais dos seguintes sinais: lesões cutâneas com alteração da sensibilidade, espessamento de nervos periféricos e baciloscopia da linfa positiva (MS, 2002; VILARROEL, 2007). Entre os principais exames complementares para confirmação laboratorial da doença, estão a baciloscopia e a histopatologia (BARROS, 2000). A baciloscopia da linfa é um exame de fácil realização que permite classificar os pacientes em multibacilares ou paucibacilares, de acordo respectivamente com sua positividade ou negatividade. Mostra-se negativa nas formas indeterminada e tuberulóide, fortemente positiva na virchowiana e revela resultado variável nas dimorfas, com tendência a ser negativa na DT e positiva nas DD e DV (ARAUJO, 2003). A histopatologia da pele subdivide a doença em suas principais formas clínicas e é

considerada o padrão ouro para o diagnóstico da doença. A identificação de um infiltrado de células inflamatórias em filetes de nervos cutâneos permite a diferenciação histológica da hanseníase de outras doenças granulomatosas cutâneas (BRITTON, 2004). Já a histopatologia do nervo, só é solicitada em casos especiais quando é necessário o diagnóstico diferencial com outras neuropatias (ARAUJO, 2003). Existem ainda outros métodos laboratoriais, como a dosagem sérica de anticorpos anti-PGL-1 que pode ser usada para a detecção precoce de recidivas e para o acompanhamento terapêutico, como marcador de carga bacteriana (BARROS, 2000; MOURA, 2008; TEIXEIRA, 2008). As formas multibacilares se associam à positividade de anticorpos anti-PGL-1 em 80 a 100% dos casos (BRITTON, 2004; MOURA, 2008).

2.3.2 Tratamento

A instituição da poliquimioterapia (PQT) adequada para o tratamento da hanseníase respeita os critérios determinados pela baciloscopia da linfa ou os preconizados pela classificação operacional estabelecida pela organização mundial de saúde. Segundo essa classificação, é considerado paucibacilar o paciente que apresenta menos de 5 lesões cutâneas e multibacilar o que possui 5 ou mais lesões (MS, 2002). Alguns estudos demonstraram que os critérios operacionais nem sempre estão de acordo com os baciloscópicos, revelando a necessidade de combinar métodos clínicos e laboratoriais para a escolha adequada da PQT (GALLO, 2003; TEIXEIRA, 2008).

A PQT, padronizada pela Organização Mundial de Saúde, tem ação bactericida e bacteriostática e garante a cura quando realizada adequadamente, de modo que, casos de recidiva são raros e ocorrem em torno de 1% dos pacientes (DINIZ, 2009). Em geral as recidivas se associam ao tratamento inadequado ou à resistência bacteriana (DIÓRIO, 2009). Existem dois principais esquemas terapêuticos. O primeiro envolve duas drogas, a dapsona e a rifampicina, e está indicado para o tratamento dos casos paucibacilares por 6 meses. Já o segundo é composto por dapsona, rifampicina e clofazimina que devem ser administradas por 12 meses nos multibacilares. Esses períodos de tratamento podem ser prolongados por mais 6 meses nos paucibacilares ou mais 1 ano nos multibacilares, caso as lesões clínicas ainda permaneçam ativas ao final da PQT. As doses são diferentes para adultos e crianças.

2.4 Complicações

2.4.1 Reações Hansênicas

O curso crônico da hanseníase pode ser afetado, em qualquer momento, seja antes durante ou após o tratamento adequado da doença, por eventos inflamatórios agudos denominados reações hansênicas ou estados reacionais. As reações hansênicas são complicações frequentes, imunomediadas, que ocorrem em 30 a 50% dos pacientes com hanseníase e acarretam grande morbidade (SCOLLARD, 2006). São divididas em reação tipo 1, ou reação reversa, e reação tipo 2, ou eritema nodoso hansênico, cada qual apresentando particularidades inerentes à fisiopatologia, ao quadro clínico e à terapêutica.

A morbidade dos estados reacionais decorre, principalmente, do comprometimento agudo e agressivo dos nervos periféricos. A reação inflamatória neurológica pode desencadear alterações permanentes das funções dos nervos acometidos, denominadas sequelas neurológicas da hanseníase. Clinicamente as sequelas se apresentam de formas variadas, como dor neuropática crônica, paresias ou deformidades físicas incapacitantes. Tanto as reações hansênicas quanto as sequelas neurológicas estigmatizam os pacientes e se associam com significativo prejuízo da qualidade de vida destes (MARTINS, 2008).

Ainda não se conhecem os mecanismos precisos que desencadeiam esses eventos. Contudo, uma série de variáveis clínicas foi associada, em maior ou menor grau, com sua ocorrência, persistindo algumas controvérsias. Fatores como gravidez, infecção, vacinação e estresse psicológico já foram considerados como precipitantes dos estados reacionais, porém, essas associações não se confirmaram em estudos prospectivos (FOSS, 2003; KAHAWITA, 2008).

2.4.1.1 Reação Tipo 1

A reação tipo 1, ou reação reversa, parece estar associada ao aumento súbito da resposta imune celular contra antígenos do *Mycobacterium leprae* (GOULART, 2002). O padrão encontrado de citocinas, tanto séricas quanto nas lesões teciduais, demonstra que nessa reação ocorre uma resposta imunológica do tipo Th1, com aumento da atividade de macrófagos (GOULART, 2002; SCOLLARD, 2006). Conforme demonstrado por estudos de imunohistoquímica, em lesões de pele de reações tipo 1 (KIRKALDY, 2003), há um aumento da expressão da quimiocina MCP-1, que age atraindo macrófagos e linfócitos T para a local da inflamação. O dano neural, presente nessa reação, decorre da invasão de células

mononucleraes e da ação de citocinas Th1 que levam à degeneração axonal (HARBOE, 2005).

Clinicamente se manifesta como inflamação aguda na pele ou em nervos periféricos ou em ambos (KAHAWITA, 2008). Sintomas sistêmicos são incomuns. Na pele, apresenta-se com o surgimento de novas lesões eritematosas e infiltradas em áreas de pele sã ou em locais de lesões antigas da hanseníase. Nos nervos, em função da resposta granulomatosa, ocorre espessamento de um ou mais nervos periféricos, seguido de alteração das funções sensitivas e ou motoras, acompanhadas ou não de dor aguda de intensidade variável. O comprometimento inflamatório dos nervos periféricos, denominado neurite, costuma ser grave e requer intervenção médica imediata, para prevenir o estabelecimento de incapacidades físicas (FOSS, 2003).

É a reação mais frequente e ocorre em qualquer forma clínica da hanseníase, exceto na indeterminada, embora seja mais comum entre os pacientes com as formas dimorfas (BALAGON, 2010; FOSS, 2003; KAHAWITA, 2008; RANQUE, 2007) afetando 15 a 30% destes pacientes (SCOLLARD, 2006) e rara entre os pacientes com a forma virchowiana (FOSS, 2003). Não se conhecem os fatores que precipitam esse evento ou a razão de não afetar todos os pacientes (SCOLLARD, 2006). Raros casos ocorrem após o tratamento para hanseníase e exigem o diagnóstico diferencial com recidiva da doença (KAHAWITA, 2008).

2.4.1.2 Reação Tipo 2

A reação tipo 2, ou eritema nodoso hansênico (ENH), ocorre em pacientes com imunidade celular deficiente contra o *Mycobacterium leprae,* com muitos bacilos e uma forte resposta humoral com produção de grande quantidade de imunoglobulinas circulantes (SCOLLARD, 2006). Envolve a participação de imunocomplexos, de citocinas do padrão Th2 e de TNF-α (GOULART, 2002; KAHAWITA, 2008; SCOLLARD, 2006). O dano neural, nesse evento, é provavelmente induzido pelo depósito local de imunocomplexos e pela ativação do complemento (HARBOE, 2005).

O ENH é quadro um inflamatório sistêmico, com comprometimento potencial de múltiplos órgãos, que tem como critério diagnóstico a presença do eritema nodoso, caracterizado por nódulos subcutâneos eritematosos e espontaneamente dolorosos. Estas lesões têm evolução variável, podendo se apresentar como episódios únicos ou recorrentes com duração de meses (FOSS, 2003; GUERRA, 2002). As manifestações extracutâneas têm intensidade variável e incluem febre, dores no corpo, apatia, irite, hepatoesplenomegalia,

adenomegalia, glomerulonefrite, artrite, neurite e etc. No ENH, a neurite (caracterizada por espessamento dos nervos periféricos acompanhado ou não de dor e de alteração da função neural) tende a ser menos agressiva que a da reação tipo 1 (KAHAWITA, 2008). Contudo, pelo potencial risco de perda da função do nervo acometido, também deve ser identificada e tratada precocemente.

Ocorre com maior frequência durante o tratamento com a PQT, afetando 41% dos pacientes neste período (FEUTH, 2008; GUERRA, 2002). É a primeira manifestação da hanseníase, possibilitando o diagnóstico da doença, em um terço dos pacientes que desenvolvem essa reação (FOSS, 2003; KAHAWITA, 2008).

2.4.1.3 Fatores Associados às Complicações da Hanseníase

Demonstrou-se que algumas formas clínicas da hanseníase são mais susceptíveis aos estados reacionais do que outras (KAHAWITA, 2008; PENNA, 2008; SCOLLARD, 2006). A reação tipo 1 é mais frequente entre os pacientes com formas MB, particularmente os das formas dimorfas (RANQUE, 2007; SCOLARD, 2006), embora alguns autores afirmem que esta seja mais comum entre os pacientes com formas PB (GOULART, 2002; TEIXEIRA, 2010). O ENH também predomina entre os pacientes com formas MB, afetando 15 a 50% dos pacientes com HV (GUERRA, 2002) e em menor frequência os pacientes com HDV (FEUTH, 2008; POCATERRA, 2006).

É também conhecido que, embora esses eventos possam ocorrer em qualquer fase evolutiva da hanseníase, na maioria dos pacientes, eles ocorrem durante o tratamento poliquimioterápico, (PQT) (BRITO, 2008; KAHAWITA, 2008; RICHARDUS, 2004; SCOLLARD, 2006; TEIXEIRA, 2010). De acordo com Shen et al. (2009), em 72,4% dos pacientes que apresentam reações hansênicas, estas ocorrem durante o primeiro ano da PQT. As reações que ocorrem após a alta estão fortemente associadas à positividade de anticorpos anti-PGL-1 (BRITO, 2008).

Mastrangelo et al. (2001), demonstraram uma associação entre estados reacionais em mulheres e o número de contatos domiciliares destas pacientes, sugerindo que uma carga bacilar externa, liberada por membros da casa, possa desencadear as reações. Observou-se ainda, que pacientes com infecções orais e reações hansênicas apresentaram melhora clínica dos episódios reacionais após tratamento do quadro infeccioso (MOTTA, 2010), o que pode significar que há uma relação entre algumas infecções e a manifestação dos estados

reacionais. Alguns autores demonstraram haver associação entre o índice baciloscópico e a ocorrência de reações hansênicas (GUERRA, 2002; GONÇALVES, 2008; PENNA, 2008).

Há controvérsias a respeito da participação da idade e do gênero no desencadeamento das reações hansênicas. Há estudos que relacionam a ocorrência de reações em paciente maiores de 15 anos, mas isso não se sustenta entre os idosos (BRITO, 2008; GUERRA, 2002; RANQUE, 2007). Outros estudos não observaram associação significativa desses eventos com a idade (SOUZA, 2010).

Segundo alguns autores, o sexo masculino parece ser mais susceptível aos estados reacionais (GUERRA, 2002; SIMON, 2012; SHEN, 2009). Contudo, muitos estudos não encontraram associação entre gênero masculino e reações hansênicas (FEUTH, 2008; SMITH, 2009; SOUZA, 2010). Essa associação do gênero também é observada em relação à hanseníase onde verifica-se que homens se apresentam mais com formas multibacilares, como as virchowianas, e em maior frequência com grau 2 de incapacidade ao diagnóstico, o que em parte pode ser justificado pela tendência dos homens de não procurarem os serviços médicos (BRITTON, 2004; GUERRA, 2002; TEIXEIRA, 2010; OLIVEIRA, 2012; PRATA, 2000; VARKEVISSER, 2009).

A susceptibilidade a formas graves, multibacilares, do gênero masculino pode também ser devido a fatores hormonais. De fato, a testosterona exerce alguns efeitos sobre resposta imune, como demonstrado por estudos in vitro e em modelos experimentais (BENTEN, 1999; PINZAN, 2010; ZHANG 2000). Ainda não estão esclarecidos os mecanismos envolvidos na ação da testosterona no sistema imune. De acordo com alguns atores, a modulação de testosterona na infecção por *L. donovani* pode ser causada por efeito direto nos macrófagos, ou indireto sob a resposta de células T (BENTEN, 1999; ZHANG, 2000). Zhang et al. (2000), demonstraram o efeito direto da testosterona nos macrófagos. Pinzan et al. (2010) demonstraram, em estudo desenhado em camundongos, que os hormônios sexuais têm um efeito profundo na biologia do sistema imune, sendo os estrógenos promotores de resposta Th1 e a testosterona de Th2 e da produção de IL-10.

Quanto ao comprometimento neurológico, Leite et al. (2011), demonstraram que a ocorrência de neuropatia silenciosa (perda recente da função motora ou sensitiva do nervo, na ausência de dor, desencadeada por atividade imunológica celular ou por eritema nodoso) é maior entre os pacientes com grau 1 ou 2 de incapacidade ao diagnóstico, do que entre os com grau 0. De modo semelhante, outros autores constataram que pacientes, que ao início do tratamento apresentaram grau de incapacidade maior que zero ou espessamento de nervos, tiveram maior chance de apresentar reações ou progressão no grau de incapacidade

(GONÇALVES, 2008; GONÇALVES, 2009; RICHARDUS, 2004; SMITH, 2009). Schuring et al. (2008), acrescentaram ainda que pacientes que apresentam anticorpos anti-PGL-1 têm maior risco de desenvolver algum dano neural, corroborando com estudo prévio que revelou risco associado entre estes anticorpos e o desenvolvimento de reação tipo 1 (ROCHE, 1991; SCHURING, 2008). A presença de nervos acometidos também já foi associada ao desenvolvimento de reação tipo 1 (KUMAR, 2004).

2.4.1.4 Tratamento das Reações Hansênicas

O tratamento das reações hansênicas, em particular das que se apresentam com neurite, envolve o uso de medicações imunossupressoras e deve ser instituído precocemente para prevenir lesões neurológicas definitivas. Os corticosteróides orais são as drogas de escolha para o controle das reações com neurite, porém nem todos os pacientes respondem bem ao tratamento (VAN VEEN, 2008; LOCKWOOD, 2005). A resposta terapêutica depende da intensidade do dano neural, de modo que sequelas físicas podem persistir em 20 a 40% dos casos tratados com corticoterapia (VAN VEEN, 2008).

A OMS padronizou, para reação tipo 1, o uso de glicocorticóides em doses que variam entre 1 e 2 mg/kg em um período de 12 semanas. Anderson et al. (2005), estudando pacientes com reação tipo 1, demonstraram que o tratamento adequado com prednisona é capaz de reduzir a expressão de citocinas como o TNF-α, IL-1β e IL-10 nas lesões cutâneas, após 1 mês de tratamento, e que, após 6 meses de tratamento, a produção de TNF-α por células mononucleares sanguíneas estimuladas é significativamente menor que a observada com 1 mês de tratamento (ANDERSON, 2005). Esses mesmos autores observaram que apesar da melhora dos sinais cutâneos da reação, só houve melhora da função neurológica em 50% dos pacientes tratados com a prednisona (ANDERSON, 2005).

A despeito da recomendação da OMS, ainda existem controvérsias quanto ao regime ideal de corticoterapia, incluindo a dose ideal e o tempo de tratamento (VAN VEEN, 2008; WALKER, 2011). Walker et al. (2011), observaram que o uso de corticoterapia intravenosa associada à oral, não traz melhores resultados aos sintomas cutâneos e neurais da reação tipo 1 quando comparado com uso isolado de corticoterapia oral. O estudo randomizado de Rao et al. (2006), comparou três regimes diferentes de corticóides em pacientes com reação tipo 1, o primeiro com dose alta e longa duração, o segundo com dose baixa e longa duração e o terceiro com dose alta e curta duração, e concluiu que os tratamentos de longa duração são mais efetivos que os de curta, demonstrando que o tempo de tratamento tem maior influência

na resposta clínica que a dose terapêutica. Neste estudo, os pacientes submetidos aos regimes longos de corticóides apresentaram resposta satisfatória ao tratamento em 69 a 76% dos casos (RAO, 2006).

Um estudo randomizado com 636 pacientes multibacilares avaliou o uso de corticoterapia profilática durante 4 meses (SMITH, 2004). Este estudo demonstrou que o grupo tratado com prednisona teve uma redução do risco de desenvolver reações durante o tratamento de 75%, quando comparado ao grupo placebo. Porém, após 8 meses do término do tratamento profilático o risco passou a ser igual para os dois grupos.

O tratamento da reação tipo 2 deve ser feito com talidomida, na dose de 100 a 400 mg/dia devendo-se manter a dose inicial até a regressão clínica do quadro. A talidomida, por seus efeitos teratogênicos, não deve ser prescrita a mulheres jovens em idade fértil. Os corticosteróides serão indicados para pacientes que apresentem comprometimento neural, irite ou iridociclite, orquiepidimite, nefrite, eritema nodoso necrotizante, fenômeno de Lúcio, mãos e pés reacionais e para mulheres em idade fértil que não podem utilizar a talidomida (MS, 2002).

2.4.2 Incapacidades Físicas

O principal determinante do dano neurológico na hanseníase é a capacidade peculiar do *M. leprae* de se ligar, através de receptores específicos, às células de Schwann do sistema nervoso periférico (HARBOE, 2005). Na reação reversa, observa-se que a hipersensibilidade tardia é direcionada contra determinantes antigênicos da micobactéria, liberados pelas células de Schwann, o que precipita a agressão neural (HARBOE, 2005; SCOLLARD, 2006). Enquanto que no ENH crônico, a lesão neural é devido à deposição local de imunocomplexos, atração de granulócitos e ativação de complemento (HARBOE, 2005; SCOLLARD, 2006).

As incapacidades físicas na hanseníase, ou sequelas neurológicas, decorrem, principalmente, dos estados reacionais com neurites que não são diagnosticados e tratados adequadamente. Essas sequelas se apresentam de variadas formas com paralisia de músculos da face, mãos e pés ou úlceras crônicas, palmar ou plantar, por perda da função sensitiva (RICHARDUS, 2003). Medidas de prevenção das incapacidades neurológicas incluem o seguimento periódico do paciente com exame neurológico e a instituição precoce da corticoterapia logo que o dano neural é identificado.

A realização criteriosa e periódica da avaliação neurológica padronizada pela OMS é fundamental para o diagnóstico da neuropatia silenciosa e de outras alterações neurológicas na haneníase (GONÇALVES, 2009; PIMENTEL, 2004). O exame neurológico, segundo normas da OMS, envolve a palpação de nervos periféricos, os testes de sensibilidade e da força muscular e utiliza escalas como a do grau de incapacidade física (GIF) cuja sensibilidade e o valor preditivo negativo correspondem a 50% e 88% respectivamente (EBENSO, 2007). O grau de incapacidade física é classificado como grau 0 quando não há alteração de sensibilidade em olhos, mãos e pés, grau 1 quando há hipoestesia sem deformidades em olhos, mãos ou pés e grau 2 quando há deformidades em olhos como lagoftalmo, ectrópio, opacidade da córnea e triquíase, e em mãos ou pés como lesões ulcerosas, tróficas, absorções digitais, garras e mãos ou pés caídos (MS, 2002). Existem, contudo, outros métodos diagnósticos, mais específicos, porém mais caros, para identificação precoce da neuropatia hansênica (BRAKEL, 2008).

As incapacidades físicas, identificadas como grau 2 de incapacidade, segundo a OMS, podem estar presentes mesmo ao diagnóstico da hanseníase. Nesses casos, normalmente estão relacionadas com o diagnóstico tardio, com formas virchowianas e com a quantidade de nervos periféricos espessados (MOSCHIONI, 2010; VAN VEEN, 2006).

O tempo de evolução do dano neurológico se relaciona com a resposta terapêutica. Um estudo multicêntrico, realizado no Nepal e em Bangladesh, revelou que pacientes com dano neural crônico, com mais de 6 meses de duração, não apresentam resposta clínica ao tratamento com dose e tempo adequados de corticóides (RICHARDUS, 2003).

Em resumo, a hanseníase ainda constitui um desafio em muitos aspectos, incluindo o diagnóstico precoce da doença e das suas complicações e a imunopatogênese das reações hansênica e das lesões neurológicas. Assim, ressaltamos a importância de se conhecer os fatores clínicos e imunológicos relacionados com o desenvolvimento de reações e de alterações neurológicas, para que se possa estabelecer uma maior vigilância dos pacientes e, desse modo, prevenir as sequelas da doença que se associam a prejuízos físicos e psicossociais para os pacientes.

3 OBJETIVOS

3.1 Geral

Avaliar o perfil clínico e a resposta imunológica, a antígenos recombinantes de *M. leprae,* associados às reações hansênicas.

3.2. Específicos

1- Avaliar as características clínicas que se associam às reações hansênicas.

2- Avaliar as características clínicas que se relacionam com incapacidades físicas ao final do tratamento.

3- Comparar a resposta imunológica a antígenos recombinantes de *M. leprae* entre pacientes com e sem reações hansênicas.

4 MATERIAIS E MÉTODOS

Esse estudo foi aprovado pelo comitê de ética e pesquisa da Universidade Federal de Sergipe (CEP-UFS, 0134.0.107.000-11).

4.1 Materiais e métodos dos objetivos 1 e 2

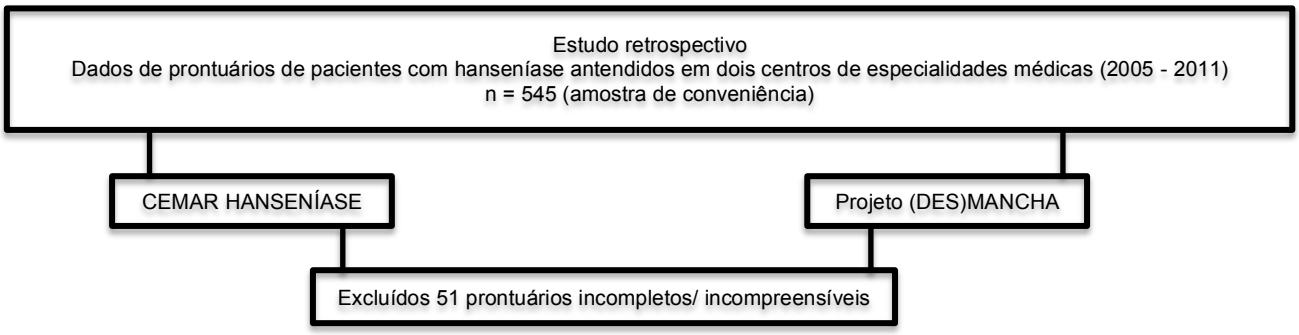

Figura 1: Fluxograma do desenho dos objetivos 1 e 2. n: tamanho da amostra. CEMAR: Centro de especialidades médicas de Aracaju-SE, Projeto (DES)MANCHA: serviço de hanseníase do Hospital Universitário da Universidade Federal de Sergipe.

Coleta dos dados dos prontuários - Foi elaborada uma ficha de avaliação clínica detalhada (APÊNDICE 1), para coletar os dados dos prontuários, que incluiu questões como sexo e idade ao diagnóstico (avaliada de forma categórica: < 15 e > 15 anos), e questões clínicas. Entre as características clínicas destacam-se as formas clínicas da hanseníase, definidas pelo exame histopatológico, a positividade da baciloscopia antes do tratamento, e a presença de reações hansênicas, com determinação do período de ocorrência em relação ao tratamento e o tipo, sendo considerada tipo 1 quando houvesse surgimento agudo de novas lesões cutâneas com ou sem neurite (dor aguda, parestesia súbita, déficit sensorial ou motor), ou neurite isolada, e reação tipo 2 quando houvesse eritema nodoso hansênico. As outras características foram baseadas no exame neurológico, realizado ao diagnóstico e ao final do tratamento, padronizado pelo Ministério da Saúde (ANEXO 1- MINISTÉRIO DA SAÚDE, 2002) com cálculo do grau de incapacidade (grau 0, sem perda de sensibilidade, deformidade ou déficit motor, grau 1, perda de sensibilidade sem deformidade ou déficit motor e grau 2, deformidade ou déficit motor). Foram também levantados dados a respeito do tratamento

instituído para as reações com pesquisa da dose máxima de corticosteróide sistêmico utilizada e do tempo máximo de uso do corticosteróide em cada paciente.

Análise Estatística - Foi criado um banco de dados no programa SPSS na versão 17.0.0. As variáveis categóricas foram descritas como frequências simples e percentagens e as varáveis quantitativas como média e desvio padrão. Para analisar a associação entre as variáveis categóricas utilizou-se o teste do qui-quadrado. Os fatores associados com a gravidade da doença foram avaliados pelo modelo de regressão logística, considerando como variável dependente as "formas multibacilares e/ou episódios reacionais" e como variáveis independentes o gênero e a presença de incapacidade física ao diagnóstico. O modelo de regressão logística também foi usado para avaliar como variável dependente a "presença de grau de incapacidade física > 0 após a cura" e como variáveis independentes o gênero masculino, os episódios reacionais e o uso de subdosagem de corticosteróides para o tratamento de reações (dose menor do que 20 mg por dia por menos de 30 dias). Adotou-se como nível de significância $p \leq 0,05$.

4.2 Material e métodos do objetivo 3

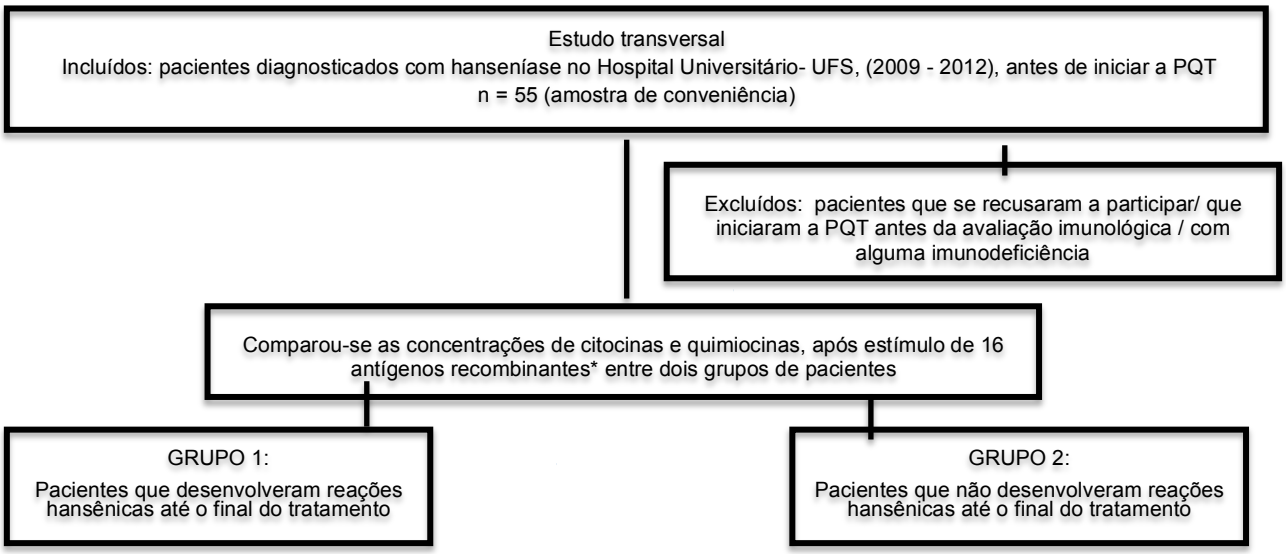

Figura 2: Fluxograma do desenho do objetivo 3. n: tamanho da amostra. * Antígenos recombinantes de *M. leprae* (MLCS, ML0276, ML2028, ML2055, ML2258, ML2531, ML2629, ML82F, ML2044, ML2380, ML2331, LID1 e PADL), antígenos recombinantes de *M. tuberculosis* (ID93, ID83), PPD.

Os antígenos – Os antígenos recombinantes de *M. leprae* e *M. tuberculosis* foram produzidos a partir de uma biblioteca de expressão de DNA genômico descrita por Reece et al. (2006) ou com base na homologia de proteínas segregadas conhecidas do *M. tuberculosis* e foram cedidos por Dr Malcom Duthie e Steven Reed do Infectious Disease Research Institute (IDRI), Seatle, USA (DUTHIE, 2007). Os seguintes antígenos foram utilizados: antígeno sonicado bruto de *M. leprae* (MLCS), antígeno purificado derivado de *M. tuberculosis* (PPD), dez proteínas recombinates individuais de *M. leprae* (ML0276, ML2028, ML2044, ML2055, ML2258, ML2331, ML2380, ML2531, ML2629, ML82F), uma fusão quimérica bivalente, LID1 (ML0405 e ML2331) e outra pentavalente, o PADL (ML0405, ML2331, ML2055, ML0411 e ML0091) e duas proteínas recombinantes de *M. tuberculosis*: ID83 (fusão de dois antígenos) e ID93 (fusão de três antígenos).

Resposta imune aos antígenos recombinantes - Foi coletado sangue heparinizado (15 ml) dos pacientes, antes de iniciar o tratamento para hanseníase (PQT), para estimulação *in vitro* com antígenos de *M. leprae* e *M. tuberculosis.* O sangue total heparinizado foi cultivado em placas de cultura de tecidos com 24 orifícios e, logo após a coleta (até 30 minutos), estimulado com 10 μg/ml de cada antígeno recombinante sendo as placas, em seguida, incubadas em estufa umidificada a 37°C e 5% de CO_2. Após 24 horas, este material foi centrifugado durante 10 minutos e os sobrenadantes das culturas foram coletados e estocados em frezer a -80°C até a posterior dosagem de citocinas (Eutaxinas, IL-2, IL-4, IL-5, IL-6, IL-17, IL-10, IL-12p70, IL-13, IL-15, IL-17, IL-23, IL-27, TNF-α, TGF-β E IFN-γ) e quimiocinas (MCP-1 e MIPI-1α) pela técnica de Luminex. Padronização prévia das concentrações dos antígenos e comparação entre as respostas imunes realizadas com sangue total e com células mononucleares do sangue periférico foi realizada pelos nossos colaboradores do IDRI (DUTHIE, 2007; DUTHIE, 2008; REECE, 2006). Pela dificuldade de obtenção dos antígenos recombinantes, número desigual de antígenos foi utilizado entre os pacientes.

Os grupos – Após o diagnóstico, os pacientes foram seguidos durante o tratamento para hanseníase e avaliados quanto ao desenvolvimento ou não de reações até o final do tratamento. Os episódios reacionais foram caracterizados como reação tipo 1 quando houvesse o aparecimento súbito de novas lesões cutâneas, acompanhado ou não por manifestações de neurite ou pela manifestação de neurite isolada e como reação tipo 2 quando o paciente desenvolvesse nódulos subcutâneos dolorosos (eritema nodoso) acompanhados ou

não de comprometimento sistêmico e de neurite (MS, 2009). Ao final do seguimento os pacientes foram divididos em dois grupos: os que desenvolveram reações hansênicas e os que não apresentaram estes eventos.

Análises estatísticas - Foi criado um banco de dados no programa EXCEL (versão 2011). As concentrações das citocinas produzidas em resposta aos antígenos recombinantes de *M. leprae* e *M. tuberculosis* foram comparadas (PRISMA) entre os grupos de pacientes que tiveram ou não reações hansênicas, pelo teste não paramétrico de Mann-Whitney, uma vez que o teste de normalidade de D'Agostino e Pearson constatou que os valores apresentados não obedeciam à distribuição Gaussiana.

5 RESULTADOS

5.1 Resultados dos objetivos 1 e 2 – Estudo clínico - Artigo submetido à Revista Journal of Tropical Medicine

RESUMO

A hanseníase é uma doença crônica que afeta a pele e os nervos periféricos. Os episódios reacionais e as incapacidades físicas são complicações da doença. O desafio dos programas de hanseníase é a redução do grau 2 de incapacidade. Esse estudo objetiva avaliar os fatores clínicos associados com a ocorrência de incapacidades físicas nos pacientes com hanseníase. Foi realizado um estudo retrospectivo com dados de prontuários de pacientes acompanhados em dois centros de especialidades médicas em Aracaju, Sergipe, de 2005 a 2011. Foi utilizado o teste do qui-quadrado para analisar a associação entre variáveis categóricas: gênero, idade, classificação operacional, formas clínicas, incapacidades físicas ao diagnóstico e após a cura, episódios reacionais e tratamento com corticosteróides. O modelo de regressão logística foi usado para avaliar as variáveis clínicas associadas com "formas multibacilares e/ ou episódios reacionais" além da "presença de grau de incapacidade física > 0 após a cura". Os resultados demonstraram que os homens foram mais afetados por formas multibacilares, episódios reacionais e grau 2 de incapacidade ao diagnóstico. Reações hansênicas foram detectadas em 40% dos pacientes e incapacidades físicas em 30% dos casos. A maioria dos pacientes com reações hansênicas foi tratada com corticosteróides, mas utilizou-se dose e tempo inferiores ao recomendado, perpetuando ou acentuando os danos físicos.

PHYSICAL DISABILITIES ARE FREQUENT AND INADEQUATELY TREATED IN LEPROSY PATIENTS

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ABSTRACT

Leprosy is a chronic disease that affects skin and peripheral nerves. Reactional episodes and physical disability are complications of the disease. The challenge of leprosy programs is to reduce the grade 2 of disability. This study aims to evaluate clinical factors associated with the occurrence of physical disability in leprosy patients. We conducted a retrospective study of data from medical records of patients followed in two important centers for treatment of leprosy in Aracaju, Sergipe, from 2005 to 2011. We used chi-square test to analyze the association between categorical variables: gender, age, operational classification, clinical forms, physical impairment at the diagnosis and after cure, reactional episodes and corticosteroid treatment. Logistic regression model was used to evaluate clinical variables associated with "multibacillary leprosy and/or reactional episodes" and the "presence of any grade of physical impairment after cure". We found that men were more affected by multibacillary forms, reactional episodes and grade 2 of physical impairment at diagnosis. Reactional episodes were detected in 40%, and 30% had physical impairment. The majority of patients with leprosy reactions was treated with corticosteroids, but used a dose and time below the recommended, maintaining or increasing physical impairments.

Key words: Leprosy, leprosy reactions, physical impairment, corticosteroid treament.

INTRODUCTION

Leprosy is a chronic disease caused by infection with *Mycobacterium leprae*. The bacillus affects the skin and Schwann cells of the peripheral nerves leading to cutaneous lesions and neuropathy. Loss of sensory, motor, and autonomic nerve function in the eyes, hands, and feet can result secondary complications as deformity and/or impairment, psychological disturbances and social exclusion [1, 2].

Acute phenomena of immunologic hypersensitivity known as reactional episodes can occur before the diagnosis, during or after treatment and lead to nerve injury if not appropriately treated [3]. There are two primary types: type 1 reaction, also called reversal reaction (exacerbation of cellular immunity) and type 2 reaction, or erythema nodosum leprosum (exacerbation of humoral immunity) [3]. There is a clear recommendation for corticosteroid treatment of the severe reactional episodes by the Brazilian Ministry of Health and International Leprosy Association (ILA): 1 to 2 mg/kg of body weight during at least 90 days.

The assessment of physical impairment in leprosy is employed as an epidemiological indicator to evaluate leprosy programs, determine early/late diagnosis and monitor patient follow-up in the health care over the course of treatment [4]. The global strategy by the World Health Organization (2011 to 2015) aims at reducing the rate of grade 2 of physical impairment [5]. Additionally, early detection of new cases of leprosy and corticosteroid treatment for the severe reactional episodes may prevent future irreversible nerve damage [6].

The state of Sergipe is located in northeastern Brazil and is a priority within the national leprosy control program. In the last seven years 3,039 patients were registered in the Brazilian Information Health System (Sistema de Informação de Agravos de Notificação - SINAN) and the percentage of grade 2 of impairment is increasing (4.8% in 2005 to 8.7% in 2010) [7]. This study aims to evaluate clinical factors associated with the occurrence of

physical impairment in leprosy patients from two centers for treatment of Leprosy patients in the state of Sergipe.

MATERIALS AND METHODS

We conducted a retrospective study of data from medical records of patients followed in two centers for treatment of Leprosy located in the Aracaju city of Sergipe state, from 2005 to 2011. From 545 records of patients diagnosed with leprosy, 51 were excluded because they did not finish specific treatment, or were transferred on the first two months of treatment, and the records did not contain necessary information. The analysis were performed in 494 patients recorded and the variables analyzed were: gender, age (≥ 15 years and < 15 years), the classification of physical impairment at the diagnosis and after treatment (grade 0, 1 and 2), based on the standard exam proposed by the World Health Organization (WHO) and Brazilian Ministry of Health, operational classification (paucibacillary - PB and multibacillary - MB), clinical forms (indeterminate, tuberculoid leprosy, borderline leprosy, lepromatous leprosy and leprosy neural pure), reactional episodes and treatment with corticosteroids. We considered type 1 reaction the episode characterized by acute inflammation in skin lesions or nerves singly or both [8, 9, 10] and type 2 reaction the appearance of inflamed cutaneous nodules with neuritis or not [9].

Nerve involvement in leprosy is considered when at the palpation of the nerves there are signs of pain, or nerve thickening, and when there is loss of sensitivity by the monofilament test, or any motor impairment, but for the analysis we only considered the objective scale of physical impairment by the WHO. These scale ranks physical disability in leprosy as: Grade 0: no anesthesia and no visible impairment to the eyes, hands or feet; Grade 1: anesthesia, but no visible deformities to the eyes, hand or foot; and Grade 2: hands ulcers, absorption of the digits or contractures and/ or feet plantar ulcers, callosities, foot drop and claw. This assessment should be conducted at the beginning and at the end of treatment or at any time during treatment if the patient has a complaint.

The severity of the disease is associated with clinical forms with high bacilli loads (multibacillary forms – MB) [11] and/ or occurrence of reactional episodes [12]. We then investigated the demographic and clinical data associated to these two variables and also to physical impairment.

Statistical analysis - We created a database in SPSS statistical program version 17.0.0. Categorical variables were described as simple or frequency counts and percentages and quantitative variables as means and standard deviations. To analyze the associations between categorical variables we used the chi-square test. To assess the factors associated with MB leprosy and/or reactional episode we used logistic regression model considering as dependent variable "MB leprosy and/or reactional episode" and as independent variables, gender and physical impairment at diagnosis. Logistic regression model was also used to evaluate as dependent variable the "presence of any degree of physical impairment after treatment", and as independent variables, male gender, reactional episodes and underdosing of corticosteroids (dose lower than 20 mg for a period less than 30 days). We adopted as the level of significance $p \leq 0.05$ and two-tailed tests.

RESULTS

We analyzed the medical records of 494 patients 268 (54%) were men and 226 (46%) were women of the two major centers for treatment of leprosy patients in the state of Sergipe from 2005 to 2011. We found similar percentages of PB forms 50.8% (251/494, 95% CI [46.8 to 55.1]) and MB forms 49.2% (243/494, 95% CI [44.9 to 53.2]), but men presented higher frequency of MB forms. From the 268 men, 162 were affected by MB forms (60,4%) as compared to 81 from the 226 women (35,8%), $p < 0.001$. Physical impairment was evaluated and recorded in 396 from the 494 (80,2%) at diagnosis (200 men and 196 women). Men presented twice as much grade 2 of physical impairment at diagnosis [12.0% (24/200)] than women [6.1% (12/196); $p = 0.04$] and reactional episodes were more frequently observed in MB forms [57.2% (139/243); $p < 0.0001$] and among men [45.1% (121/268)] than in women [33.6% (76/226); $p = 0.009$] (Table 1).

Ages were registered in 488 patients and ranged from 3 to 85 years (mean ± SD, 41 ± 18.2). Eight percent of the patients (39/488) were children aged less than 15 years. Clinical forms were registered in 424 patients. The predominant clinical form of leprosy was tuberculoid (TT) [40.1% (170/424)], following by borderline (BB) [20.1% (85/424)], indeterminate (IL) [18.4% (78/424)], lepromatous (LL) [17.2% (73/424)], pure neural (PN) [4.2% (18/424)].

Reactional episodes were detected in 40.0% (197/494; 95% CI [35.6 to 44.1]) of the patients and were more frequently observed in MB forms [57.2% (139/243); $p < 0.0001$]. The reversal reaction (RR) or type 1 reaction was the most common [75.1% (148/197)] and more frequent during the treatment for leprosy.

Table 1. Clinical characteristics according to gender of leprosy patients attended from 2005 to 2011 in Sergipe, Brazil.

Variable	Male % (n/total)	Female % (n/total)	p[3]
Operational Classification			
PB[1]	39.6 (106/268)	64.2 (145/226)	
MB[2]	60.4 (162/268)	35.8 (81/226)	< 0.001
Reactional Episode	45.1 (121/268)	33.6 (76/226)	0.009
Neurological impairment at Diagnosis			
Grade 0	61.0 (122/200)	66.8 (131/196)	
Grade 1	27.0 (54/200)	27.0 (53/196)	
Grade 2	12.0 (24/200)	6.0 (12/196)	0.040

1: Paucibacillary form; 2: Multibacillary form; 3: Chi-square test.

Figure 1A highlight the frequency of recorded physical impairment exams before and after treatment and the frequency of any degree of physical impairment found in examined patients. From the 396 patients evaluated for physical impairment at diagnosis, most patients had grade 0 [63.9% (253/396)] followed by grade 1 [27.0% (107/396)] and grade 2 [9.0% (36/396)]. After treatment, physical impairment was recorded in only 37.4% (185/494) of patients. Grade 0 physical impairment was detected in 70.3% (130/185) patients, grade 1 in 24.9% (46/185), and grade 2 [4.9% (9/185)] (Figure 1B).

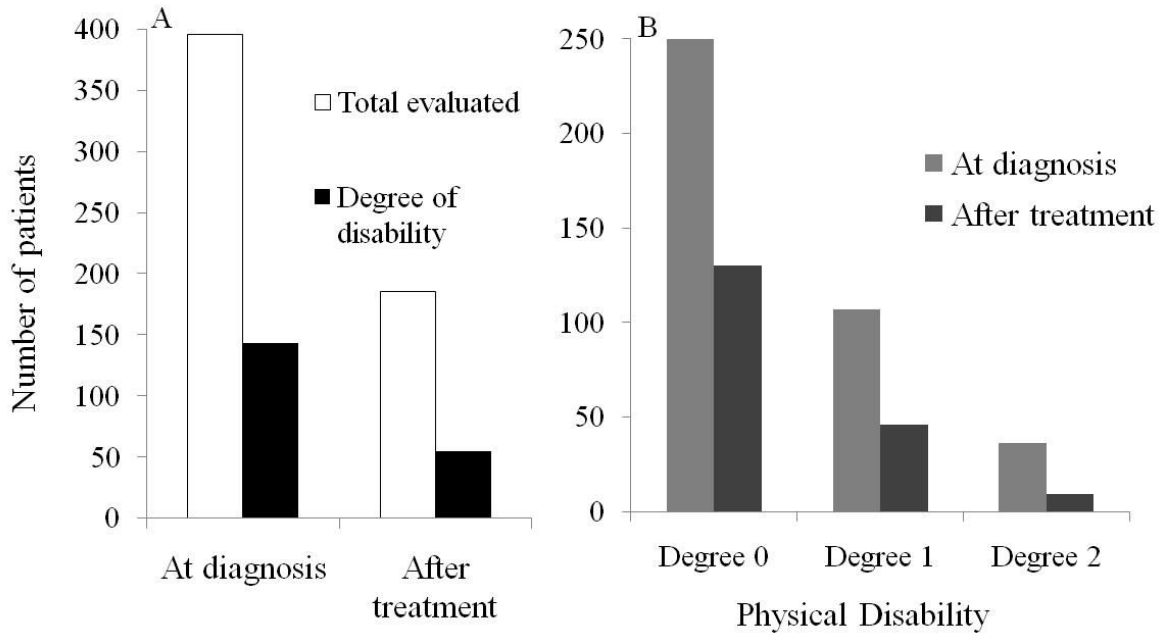

Figure 1. Number of leprosy patients with recorded neurological exams and with any degree of physical disability detected at diagnosis and at the end of treatment (A) and degrees of physical impairment at diagnosis and after treatment (B) attended in the two major leprosy centers in Sergipe, Brazil from 2005 to 2011.

Since a majority of patients, 309/494, (62.5%) did not have their evaluation of grade of physical impairment exam recorded after treatment, considering the frequency of grades 1 and 2 of physical impairment found after treatment of 30%, we could have detected physical impairment in more 93 patients if they had been examined.

Of the 494 patients included in this study, 40.0% (197/494, 95% CI [35.6 to 44.1]) had reactional episodes, and all received corticosteroid treatment. Additionally, 162/197 (82.2%) patients received a dose and time below the dose recommended by the Brazilian Ministry of Health and International Leprosy Association (ILA) (1 to 2 mg/kg of body weight for at least 90 days). Of these 162 patients that were underdosed (<20mg/daily for < 30 days of treatment), only 54 underwent neurological assessment at the beginning and at the end of the treatment, and 50% (27/54) of them remained or increased their grade of physical impairment. For instance, from these 54 patients, 5 had grade 2 of physical impairment and after

corticosteroid treatment 4 remained with grade 2, while 1 improved to grade 1. Of the 23 patients presenting with grade 1 of physical impairment, 14 remained degree 1, one progressed to grade 2 and only 8 improved to grade 0. From the 26 patients who had grade 0 of physical impairment 18 had no signs of nerve injury, 7 progressed to grade 1 and one to grade 2 of physical impairment.

To assess the factors associated with "MB leprosy and/or reactional episode" we used logistic regression model considering as dependent variable "MB leprosy and/or reactional episode" and as independent variables, gender and grades 1 and 2 of physical impairment at diagnosis. Table 2 shows an independent association between "MB leprosy and/or reactional episode" and factors such as male gender (OR 2.42, 95% CI [1.49 to 3.95]; p<0.001) and grade 2 of physical impairment (OR 6.32, 95% CI [1.98 to 20.15]; p= 0.002) or 1 (OR 2.72, 95% CI [1.50 to 4.92]; p= 0.001) at diagnosis. When evaluated as dependent variable "presence of any degree of physical disability after cure" and as independent variables, male gender, age, reactional episodes and underdosing of corticosteroids we observed an independent association of "presence of any degree of physical disability after cure" with treatment with underdosing of corticosteroids (OR 4.94, 95% CI [2.49 to 9.82]; p<0.001). No associations were detected with male gender, age and leprosy reactions.

Table 2. Analysis of factors associated with "disease severity" and "bad prognosis" in leprosy patients attended from 2005 to 2011 in Sergipe, Brazil.

Dependent Variables	Independent Variables	OR[1]	CI (95%)[2]	p[3]
Multibacillary/ leprosy reaction)	Gender			
	Male	2,42	1.49-3.45	<0.0001
	Female	1		
	Physical impairment at diagnosis			
	Grade 2	6,32	1.98-2.15	<0.002
	Grade 1	2,72	1.50-4.92	<0.001
	Grade 0	1		
Physical impairment after treatment	Underdosing of corticosteroids			
	Yes	4.94	2.49-9.82	<0.001
	No	1		

Model: Logistic regression after adjustment of the model (Forward Stepwise). Variables that were not associated independently with physical impairment after treatment: gender (p = 0.44), age (p = 0.17) and reactional episode (p = 0.24).

DISCUSSION

The morbidity associated with leprosy is related to multiple skin lesions observed in MB forms [11], leprosy reactions [12] and or physical impairment [2], the main complications of the disease. It is important to identify the clinical characteristics that can predict these deleterious outcomes. In this study we show independent associations of male gender with MB forms, reactional episodes and physical impairment. A higher prevalence of leprosy in men is reported by others [13, 2, 14], as well as associations between male gender with leprosy reactions [15] and physical impairment [16]. This distribution might be related to socio-cultural issues, such as lower pursuit of health care by men, the fear of losing their jobs because of the stigma of leprosy, or higher exposure due to more intense social or work activities [2].

Interestingly, in leishmaniasis, a disease also caused by an intracellular organism, epidemiological data and experimental animals studies also suggest a higher susceptibility of males [17]. A study in experimental model of infection by *Paracoccidioides braziliensis* showed the influence of sex hormones on the immune response, demonstrating that testosterone promotes an increased production of IL-10 and less protection against this infection [18]. There is no study in leprosy investigating this issue.

The global strategy for control of leprosy from 2011 to 2015 aims at reducing the rate of new cases with grade 2 of physical impairment worldwide in more than 35% by the end of 2015 compared with the baseline at the end of 2010 [6]. The proportion of patients evaluated by the neurological exam and diagnosed with physical impairment allows an indirect assessment of the effectiveness of programs for prevention of impairment [19]. The number of thickened nerves is significantly associated with increased risk for physical disabilities [2, 20]. Furthermore, physical impairment at diagnosis has been associated with a worst prognosis for deformities [20, 21]. The Brazilian Ministry of Health recommends that any

member of the multidisciplinary team can perform the neurological assessment. In the present study, we show that leprosy reaction and physical impairment are frequent in this population. However, there are still patients in these medical centers who are not tested for impairment at diagnosis and an even larger number that are not assessed at the end of treatment. These results may reflect the existence of problems related to professional training for prevention of physical impairment of leprosy. It is known that some patients develop subclinical nerve damage that is not detected with the standard clinical tests (monofilaments and voluntary muscle testing). In this situation, sensory nerve conduction test is more sensitive to detect earliest affected nerves [22]. Nevertheless, it is essential to develop strategies that emphasize the importance of the neurological exam to prevent physical impairment.

Early identification coupled with the proper treatment of leprosy reactions can be an effective strategy for prevention of physical impairment in leprosy [13, 23]. Prednisone is the drug of choice not only for the treatment of leprosy reactions and silent neuritis events [21] but also to reduce swelling and prevent further physical impairment [24]. The Ministry of Health guidelines suggest administration of 1 to 2 mg/kg of prednisone per day for at least 90 days to treat reactions [21], however, in this study, patients received smaller doses of steroids over a shorter period of time. This may be insufficient to reverse the nerve damaged, resulting in a high frequency of physical impairment after treatment. In fact, in the present study we demonstrated an independent association between persistence of physical impairment after treatment and had been treated with underdosing of corticosteroids. A multicenter study on Bangladesh and Nepal investigated whether the risk of leprosy related reaction and associated impairment of nerve function could be reduced by prophylactic oral corticosteroids. Prophylaxis with oral prednisolone (20mg) was given for MB patients for 4 months and reduction in primary outcome events was observed. However this significant effect was not

sustained at one year [24]. A clinical trial using oral corticosteroids for neuropathy observed, during the first month, that higher doses of steroids produced better results but earlier treatment with lower dose was also effective [25]. The administration of the correct dose of prednisone improved the clinical and electrophysiological condition of the pure neural patients, contributing to the prevention of further neurological damage [26].

In conclusion, there is an association of male gender with MB forms and reactional episodes and those patients present twice as much grade 2 of physical impairment at diagnosis. The majority of patients treated with corticosteroids were treated with a dose and duration below that recommended by the leprosy control program and 50% of the examined patients remained with physical impairment. The data described here suggest that a more careful control of neurological injury plus the use of adequate regimes of corticosteroid to treat leprosy reactions should be clearly stated in order to achieve the WHO recommendation of reducing physical impairment.

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5.2 Resultados do objetivo 3 - Estudo imunológico

Avaliação imunológica inicial foi realizada em 55 pacientes. A ocorrência de reações hansênicas foi avaliada apenas nos 41 pacientes que finalizaram o tratamento durante o período do estudo, considerando que a maioria dos casos de reação ocorre durante a poliquimioterapia. Destes, 39% (16/41) eram homens e 61% (25/41) mulheres. Quanto à baciloscopia, 66% (27/41) dos pacientes eram PB e 34% (14/41) MB. Reações hansênicas ocorreram em 61% (25/41) dos pacientes avaliados.

A comparação da resposta imunológica, antes do tratamento, entre pacientes que desenvolveram reações hansênicas com pacientes que não apresentaram esses eventos, revelou alterações significativas nas concentrações da quimiocina MCP-1. As concentrações de MCP-1, tanto em resposta ao ML2531 (14 pacientes avaliados) quanto ao ID93 (12 pacientes avaliados), foram maiores nos pacientes que apresentaram reações hansênicas (Figuras 1 e 2). Entre os 8 pacientes com reações hansênicas, que produziram maiores concentrações de MCP-1 em resposta ao antígeno ML2531, 7 eram PB e dos 8 com reações, que responderam ao ID93 metade era PB. Não houve diferenças na produção de MCP-1 em resposta a estes respectivos antígenos em pacientes com diferentes formas cínicas da doença. Não houve alteração significativa de outras quimiocinas ou citocinas em resposta a outros antígenos recombinantes.

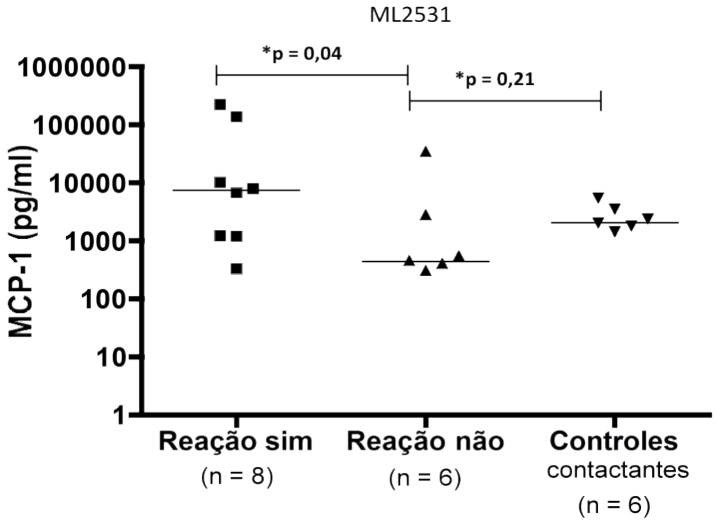

Figura 2. Comparação entre as concentrações de MCP-1 em sobrenadantes de sangue total, estimulados com o antígeno recombinante ML2531 de pacientes que desenvolveram reações hansênicas e que não desenvolveram esses eventos e controles contactantes dos doentes. * Mann-Whitney.

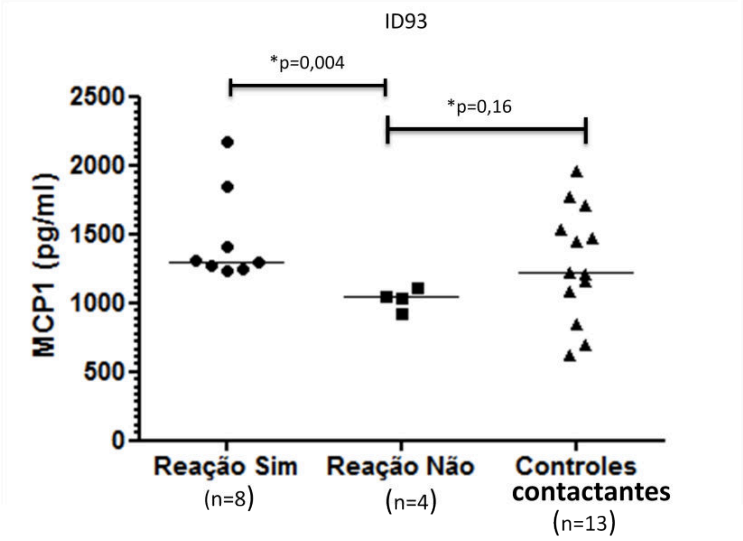

Figura 3. Comparação entre as concentrações de MCP-1 em sobrenadantes de sangue total, estimulados com o antígeno recombinante ID93 de pacientes que desenvolveram reações hansênicas e que não desenvolveram esses eventos e controles contactantes dos doentes *Mann-Whitney.

6 DISCUSSÃO

Os resultados dos objetivos 1 e 2 mostram que o sexo masculino apresentou, em maior frequência, formas multibacilares, reações hansênicas e grau de incapacidade inicial alterado. Esses dados sugerem que os homens se apresentam com formas clínicas mais graves ao diagnóstico, estando assim mais sujeitos a manifestarem complicações da doença. Alguns autores descreveram a associação entre sexo masculino e susceptibilidade a formas mais graves da hanseníase, incluindo as reações hansênicas (GUERRA, 2002; SHEN, 2009; SIMON, 2012), as formas multibacilares, bem como o grau 2 de incapacidade ao diagnóstico (BRITTON, 2004; GUERRA, 2002; TEIXEIRA, 2010; OLIVEIRA, 2012; VARKEVISSER, 2009). Isso pode ser, em parte, justificado pela tendência dos homens a não procurarem os serviços médicos (PRATA, 2000), pela maior exposição a uma carga bacilar maior, devido a atividade social ou de trabalho, ou ainda por maior susceptibilidade ligada a fatores hormonais. De qualquer forma, estes dados reforçam a importância de se estabelecer ações direcionadas ao diagnóstico precoce e ao seguimento clínico mais rigoroso desse grupo populacional.

Enquanto a OMS preconiza como meta a redução do grau de incapacidade física na hanseníase, nossos resultados revelam uma inadequada frequência de avaliação do grau de incapacidade, no início do tratamento e, principalmente, no final deste. Em relação ao tratamento das reações hansênicas, complicação inflamatória que pode levar ao dano neural, há uma associação independente entre subdosagem de corticosteróides para tratamento dos estados reacionais e presença de alterações no grau de incapacidade física ao final do tratamento. Este estudo demonstra que o tratamento utilizado, dessa forma, não é suficiente para reverter e tratar o dano neurológico nos pacientes com reações hansênicas, pois a maioria dos pacientes mantém os mesmos graus de incapacidade inicias ou progridem para graus mais elevados.

A OMS e o MS determinam o uso de corticosteróides orais, em doses entre 1 e 2 mg/kg, por um período de 12 semanas, para tratamento de reações com neurite (MS, 2002). Contudo, sabe-se que, embora os corticosteróides orais sejam as drogas de escolha para o controle das reações com neurite, nem todos os pacientes respondem bem ao tratamento (LOCKWOOD, 2005; VAN VEEN, 2008). Alguns autores demonstraram que o tratamento de reações tipo 1 com doses adequadas de prednisona reduziu consideravelmente a expressão de citocinas inflamatórias nas lesões cutâneas, porém a melhora da função neurológica só ocorreu em 50%

dos pacientes tratados (ANDERSON, 2005). A resposta terapêutica mostra-se depende do tempo de evolução do dano neurológico (RICHARDUS, 2003) e da intensidade deste dano, de modo que sequelas físicas podem persistir em 20 a 40% dos casos tratados com corticoterapia (VAN VEEN, 2008). No estudo atual há manutenção de percentuais semelhantes de incapacidade física 1 e 2 ao final do tratamento, nos pacientes que foram examinados.

Mesmo com limitações na resposta clínica da reversão do dano neural, regimes adequados de corticosteróides são fundamentais na tentativa de controle do dano neurológico e de prevenção de incapacidades físicas na hanseníase. Um estudo multicêntrico concluiu que os tratamentos de longa duração com corticosteróides são mais efetivos que os de curta, demonstrando que o tempo de tratamento tem influência na resposta clínica (RAO, 2006). Neste estudo, os pacientes submetidos a regimes longos de corticosteróides apresentaram resposta satisfatória ao tratamento em 69 a 76% dos casos (RAO, 2006). Demonstrou-se também que além do benefício potencial nos nervos afetados, regimes de corticosteróides podem proteger outros nervos por prevenir novos episódios de neurite (JARDIM, 2007; SMITH, 2004). Desse modo, os corticosteróides revelam-se benéficos tanto para tratamento como para profilaxia da lesão neurológica da hanseníase.

Durante a infecção pelo *M. leprae*, antígenos específicos deste bacilo são reconhecidos pelas células apresentadoras de antígenos e podem, assim, influenciar na ativação da resposta adaptativa para os padrões Th 1, Th2, Th17 ou T reguladora. É o que ocorre com o PGL-1 (glicolipídeo fenólico específico do *M. lepare*) que induz IL-10, suprime a atividade macrofágica e pode favorecer a diferenciação da resposta T reguladora (BARROS, 2000; SCOLLARD, 2006). De modo semelhante ao PGL-1, outros antígenos de *M. leprae* podem ser capazes de induzir uma resposta que seja protetora ou não contra o desenvolvimento da hanseníase (REECE, 2006).

Nossos resultados revelaram a produção de maiores concentrações de MCP-1 em resposta aos antígenos ML2531 e ID93 entre os pacientes que desenvolveram reações. Essa é uma quimiocina que atrai macrófagos e linfócitos T para os tecidos (FLORES-VILLANUEVA, 2005). Conforme demonstrado por estudos de imunohistoquímica, a expressão de MCP-1 está elevada em lesões de pele de reações tipo 1 (KIRKALDY, 2003). Essa alteração é compatível com a fisiopatologia destas reações, uma vez que a atividade macrofágica é fundamental para formação do granuloma (KIRKALDY, 2003), alteração histológica encontrada nas lesões de reação tipo 1 (SCOLLARD, 2006). A maioria dos pacientes que produziram MCP-1 em resposta aos antígeno ML2531 e ID93 eram PB. É

possível que o aumento de MCP-1 esteja relacionado ao desenvolvimento da reação hansênica tipo 1 nos pacientes avaliados, já que esta é a mais comum entre os PB.

Além disso, sabe-se que alguns polimorfismos de MCP-1 estão relacionados com a progressão da infecção em tuberculose bem como com uma maior susceptibilidade ao desenvolvimento de leishmaniose mucosa após infecção por *L. braziliensis,* possivelmente pela produção aumentada de TNF-α (FLORES-VILLANUEVA, 2005; RAMASAWMY, 2010). As reações hansênicas são manifestações de hipersensibilidade onde se encontram concentrações elevadas de TNF-α. O aumento de MCP-1 observado entre os pacientes com reações reforça o estado inflamatório que se desenvolve nos pacientes durante esses eventos.

7 CONCLUSÕES

1. O sexo masculino apresentou maior frequência de formas clínicas mais graves e de reações hansênicas, sugerindo que este grupo é mais susceptível às complicações da hanseníase.

2. O baixo percentual de avaliação neurológica dos pacientes com hanseníase e a inadequada condução do tratamento das reações hansênicas com corticosteróides podem favorecer o desenvolvimento de incapacidades físicas.

3. As maiores concentrações de MCP-1 em resposta aos antígenos ML2531 e ID93 entre os pacientes que desenvolveram reações, sugerem que esses antígenos recombinantes possam estar favorecendo o desencadeamento das reações hansênicas.

8 PERSPECTIVAS

1. Estudos futuros envolvendo a avaliação imunológica antes e durante os episódios reacionais e o acompanhamento de pacientes responsivos aos antígenos ID93 e ML2531 com produção de MCP-1 podem ser realizados para avaliar se estes antígenos são marcadores de reações hansênicas.

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APÊNDICE 1

Ficha de avaliação clínica

FICHA DE ACOMPANHAMENTO Aplicar mensalmente a cada retorno						
1. Nº. DO ESTUDO: DATA DA ADMISSÃO:				2. NOME DO PACIENTE:		
3. IDADE (ANOS) _____ 4. SEXO ☐ M (1) ☐ F (2)						
5. COR	☐ Preto (1)	☐ Pardo(2)	☐ Branco (3) ☐ NA (4)	6. MORADIA	☐ Rural (1) ☐ NA (99)	☐Urbana(2)
7. ESCOLARIDADE ☐ Analfab (1)	☐ 1-4 incomp EF (2) ☐ E Méd incomp (6)	☐ 4 completa EF (3) ☐ E Méd comp (7)		☐ 5-8 incomp EF (4) ☐ E Sup incomp (8) ☐ E Sup comp (9)	☐ EF comp (5) ☐ NA (99)	
8. FORMA CLÍNICA	☐ HV (1)	☐ HT(2)	☐HD (3)	☐HI (4) NA(99)	☐ H neural(5)	☐
9. BACILOSCOPIA ADMISSIONAL	☐POSITIVA (1)	☐NEGATIVA(2)	☐NA (99)	10. ÍNDICE BACILOSCÓPICO	____	☐ NA (99)
11. BACILOSCOPIA FINAL	☐ POSITIVA (1)	☐ NEGATIVA (2)	☐NA (99)	12. TEMPO TOTAL DE TRATAMENTO		_____
13. TEMPO ABANDONO TTO MESES	_____	14. PQT		☐ MB (1)		☐ PB (2)
15. SURTO REACIONAL ☐TIPO 1(1) ☐ TIPO 2 (2) ☐ Neurite Pura (3) ☐ TIPO I + NeurR(4) ☐ TIPO 2 + Neu(5) ☐ NÃO(6)						
16. PERÍODO DO SURTO ☐ANTES DO TTO (1) ☐DURANTE TTO (2) ☐APÓS TTO (3) ☐NA(99)						
17. CASO 16 SEJA 2, PERÍODO DE OCORRÊNCIA DO SURTO EM MESES, EM RELAÇÃO AO TTO _____						

FATORES DE RISCO (EM QUALQUER MOMENTO)			**LEGENDA: (1) SIM | (2) NÃO | (99) NÃO DETERMINADO**	
18. ( ) STRESS	19.( ) VACINAÇÃO	20. ( ) GESTAÇÃO	21. ( ) INFECÇÕES	22. ( ) CIRURGIAS RECENTES

QUEIXAS GERAIS (EM QUALQUER MOMENTO) *			**LEGENDA: (1) SIM | (2) NÃO | (99) NÃO DETERMINADO**
23. ( ) ASTENIA	24. ( ) DOR NOS OLHOS	25. ( ) ARTRALGIA	26. ( ) FEBRE

QUEIXAS ESPECÍFICAS (EM QUALQUER MOMENTO) *					
	MÃOS (1)	PÉS (2)	MÃOS E PÉS (3)	OUTROS (4)	NÃO (5)
27. FRAQUEZA MUSCULAR	☐	▱	☐	☐	☐
28. PARESTESIAS	☐	▱	☐	☐	☐

EXAME FÍSICO GERAL (EM QUALQUER MOMENTO) *		**LEGENDA: (1) SIM | (2) NÃO | (99) NÃO DETERMINADO**	
( ) 29. EDEMA MÃOS	( ) 30. LINFONODOMEGALIA	( ) 31. LESÕES NOVAS NA PELE	( ) 32. ARTRITE
( ) 33. EDEMA FACIAL	( ) 34. HEPATOESPLENOMEGALIA	( ) 35. NÓDULOS NA PELE	( ) 36. LAGOFTALMO
( ) 37. EDEMA PÉS	( ) 38. PÉ CAÍDO	( ) 39. PERDA DE PESO	

EXAME FÍSICO (PELE E GÂNGLIOS) **LEGENDA: (1) SIM | (2) NÃO**

40. LESÕES ANTIGAS (ao final do tratamento ou antes do surto)

( ) SIM (1)
( ) NÃO (2)
( ) NÃO SE APLICA (99)

Se a resposta for SIM, qual o(s) tipo(s) de lesão(ões)? Se NÂO ou NÃO SE APLICA, ir para Item 47.

( ) Clareou (41)	( ) Infiltradas (42)	( ) Ulceradas (43)	( ) Menos Elevadas (44)	( ) Inalteradas (45)
( ) Escureceu (46)	( ) Descamativas (47)	( ) Eritemato-sas/Edematosas (48)	( ) Mais Elevadas (49)	
50. N° DE LESÕES INICIAIS	☐ ___ ____ (até 4)	☐ > 5 lesões ( )	☐ Ausentes 6)	

51. LESÕES NOVAS (no surto)

( ) SIM (1)
( ) NÃO (2)
( ) NÃO SE APLICA (99)

Se a resposta for SIM, qual o(s) tipo(s) de lesão(ões)? Se NÂO ou NÃO SE APLICA, ir para Item 63.

( ) Eritematosas (52)	( ) Hemorrágicas (53)	( ) Placas Delimitadas (54)	( ) Nódulos Indolores (55)	( ) Descamativas (56)
( ) Ulceradas (57)	( ) Planas Indolores (58)	( ) Nódulos Dolorosos (59)	( ) Nódulos Eritematosos (60)	( ) Pustulosas (61)
( ) Vesículo-Bolhosas (62)	( ) Planas doloridas (63)	( ) Nódulos Ulceradas (64)		
65. N° LESÕES NOVAS	☐ _____ (até 4)	☐ > 5 lesões (5)	☐ Ausentes (6)	

EXAME FÍSICO (PALPAÇÃO DOS NERVOS)
PREENCHER APENAS NO INíCIO DO TRATAMENTO
LEGENDA: (1) SIM | (2) NÃO

66. **ESPESSAMENTO** NEURAL (**Lado D**)

( ) SIM (1)

( ) NÃO (2)

( ) NÃO SE APLICA (99) ou NÃO AVALIADO?

Se a resposta for SIM, em qual (s) nervo (s)? Se a resposta for NÃO ou NÃO SE APLICA ir para Item 67.

☐ Ulnar (67)	☐ Mediano (68)	☐ Radial (69)	☐ Fibular (70)	☐ Tibial Posterior (71)

72. **DOR** NEURAL (**Lado D**)

( ) SIM (1)

( ) NÃO (2)

( ) NÃO SE APLICA (99)

Se a resposta for SIM, em qual (s) nervo (s)? Se a resposta for NÃO ou NÃO SE APLICA ir para Item 75.

☐ Ulnar (73)	☐ Mediano (74)	☐ Radial (75)	☐ Fibular (76)	☐ Tibial Posterior (77)

78. **ESPESSAMENTO** NEURAL (**Lado E**)

( ) SIM (1)

( ) NÃO (2)

( ) NÃO SE APLICA (99)

Se a resposta for SIM, em qual (s) nervo (s)? Se a resposta for NÃO ou NÃO SE APLICA ir para Item 74.

☐ Ulnar (79)	☐ Mediano (80)	☐ Radial (81)	☐ Fibular (82)	☐ Tibial Posterior (83)

84. **DOR** NEURAL (**Lado E**)

( ) SIM (1)

( ) NÃO (2)

( ) NÃO SE APLICA (99)

Se a resposta for SIM, em qual (s) nervo (s)? Se a resposta for NÃO ou NÃO SE APLICA ir para Item 87.

☐ Ulnar (85)	☐ Mediano (86)	☐ Radial (87)	☐ Fibular (88)	☐ Tibial Posterior (89)

EXAME FÍSICO (SENSIBILIDADE)				
90. **SENSIBILIDADE (Lado D)**	☐ Diminuída na mão (1)	☐ Diminuída no pé (2)	☐ Diminuída na mão e pé (3)	☐ Sem alterações (4)
91. **SENSIBILIDADE (Lado E)**	☐ Diminuída na mão (1)	☐ Diminuída no pé (2)	☐ Diminuída na mão e pé (3)	☐ Sem alterações (4)

EXAME FÍSICO- FORÇA MUSCULAR				
MÃO D	92. Abdução do 5º dedo	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	93. Abdução do polegar	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	94. Extensão do punho	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
MÃO E	95. Abdução do 5º dedo	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	96. Abdução do polegar	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	97. Extensão do punho	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
PÉ D	98. Extensão do Hálux	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	99. Dorsiflexão do pé	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)

PÉ E	99. Extensão do Hálux	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	100. Dorsiflexão do pé	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)

101. **GRAU DE INCAPACIDADE**	☐ Grau 0 (zero) (1)	☐ Grau 1 (um) (2)	☐ Grau II (dois) (3)	☐ Não Avaliada (99)

EXAME COMPLEMENTAR E REABILITAÇÃO (SE LESÃO NEUROLÓGICA AO EXAME FÍSICO)			
	Sim (1)	**Não (2)**	**Sem lesão neurológica (3)**
102. Realizou eletroneuromiografia	☐	☐	☐
103. Realiza fisioterapia	☐	☐	☐
104. Caso resposta anterior **Sim** observou melhora com a reabilitação?	☐	☐	

EXAME FÍSICO (PALPAÇÃO DOS NERVOS)
PREENCHER APENAS NO FINAL DO TRATAMENTO

LEGENDA: (1) SIM | (2) NÃO

105. **ESPESSAMENTO** NEURAL (**Lado D**)
( ) SIM (1)
( ) NÃO (2)
( ) NÃO SE APLICA (99) ou NÃO AVALIADO?
Se a resposta for SIM, em qual (s) nervo (s)? Se a resposta for NÃO ou NÃO SE APLICA ir para Item 109.

☐ Ulnar (106)	☐ Mediano (107)	☐ Radial (108)	☐ Fibular (109)	☐ Tibial Posterior (110)

111. **DOR** NEURAL (**Lado D**)
( ) SIM (1)
( ) NÃO (2)
( ) NÃO SE APLICA (99)
Se a resposta for SIM, em qual (s) nervo (s)? Se a resposta for NÃO ou NÃO SE APLICA ir para Item173.

☐ Ulnar (112)	☐ Mediano (113)	☐ Radial (114)	☐ Fibular (115)	☐ Tibial Posterior (116)

117. **ESPESSAMENTO** NEURAL (**Lado E**)
( ) SIM (1)
( ) NÃO (2)
( ) NÃO SE APLICA (99)
Se a resposta for SIM, em qual (s) nervo (s)? Se a resposta for NÃO ou NÃO SE APLICA ir para Item 121.

☐ Ulnar (118)	☐ Mediano (119)	☐ Radial (120)	☐ Fibular (121)	☐ Tibial Posterior (122)

123. **DOR** NEURAL (**Lado E**)
( ) SIM (1)
( ) NÃO (2)
( ) NÃO SE APLICA (99)
Se a resposta for SIM, em qual (s) nervo (s)? Se a resposta for NÃO ou NÃO SE APLICA ir para Item 80.

☐ Ulnar (124)	☐ Mediano (125)	☐ Radial (126)	☐ Fibular (127)	☐ Tibial Posterior (128)

EXAME FÍSICO (SENSIBILIDADE)				
129. **SENSIBILIDADE (Lado D)**	☐ Diminuída na mão (1)	☐ Diminuída no pé (2)	☐ Diminuída na mão e pé (3)	☐ Sem alterações (4)

130. **SENSIBILIDADE (Lado E)**	☐ Diminuída na mão (1)	☐ Diminuída no pé (2)	☐ Diminuída na mão e pé (3)	☐ Sem alterações (4)

EXAME FÍSICO- FORÇA MUSCULAR				
MÃO D	131. Abdução do 5º dedo	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	132. Abdução do polegar	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	133. Extensão do punho	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
MÃO E	134. Abdução do 5º dedo	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	135. Abdução do polegar	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	136. Extensão do punho	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
PÉ D	137. Extensão do Hálux	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	138. Dorsiflexão do pé	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
PÉ E	139. Extensão do Hálux	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)
	140. Dorsiflexão do pé	☐ Sem alterações (1)	☐ Diminuída (2)	☐ Paralisado (3)

141. **GRAU DE INCAPACIDADE**	☐ Grau 0 (zero) (1)	☐ Grau 1 (um) (2)	☐ Grau II (dois) (3)	☐ Não Avaliada (99)

EXAME COMPLEMENTAR E REABILITAÇÃO (SE LESÃO NEUROLÓGICA AO EXAME FÍSICO)			
	Sim (1)	**Não (2)**	**Sem lesão neurológica (3)**
142. Realizou eletroneuromiografia	☐	☐	☐
143. Realiza fisioterapia	☐	☐	☐
144. Caso resposta anterior **Sim** observou melhora com a reabilitação?	☐	☐	

PROCEDÊNCIA ☐ Transf em TTO (1) ☐ Transf em TTO + surto(2) ☐ Transf após TTO + surto(3) ☐ Caso novo (4) recidiva(5)

APÊNDICE 2

Consentimento informado para o estudo imunológico

NOME DO PACIENTE: _____

Registro.HU:_____ Nº: __ __ __ - __ __

Investigadores: Jonnia M Sherlock Araujo, Tel: (79)8855 0098 e Amélia R de Jesus, Tel: (79) 8823 7245. Hospital Universitário Rua Cláudio Batista S.N, Bairro Sanatório.

Convite e Objetivo:

Você é convidado(a) a participar de um estudo que tem como objetivo entender porque as pessoas têm Hanseníase. Este estudo incluirá 90 pessoas com esta doença que apresentam formas diferentes de feridas na pele. Além das informações deste documento você pode perguntar tudo sobre o estudo ao seu médico. Caso decida participar do estudo você será solicitado(a) assinar este formulário de consentimento.

Participação voluntária: A sua participação é voluntária. Você pode decidir não participar do estudo em qualquer momento, sem perder os benefícios dos cuidados médicos prestados e de seu tratamento. Caso, após aceite participar, resolva descontinuar sua participação, isto será feito sem qualquer prejuízo para você. Participando ou não do estudo você receberá o medicamento utilizado para o tratamento da Hanseníase.

Finalidade do estudo: Este estudo vai estudar como o seu corpo se defende quando atacado pela bactéria que causa esta doença. Para isto estudaremos o seu sangue e uma parte do exame de biópsia de sua ferida na pele.

Procedimentos: Caso você concorde em participar do estudo, além de ser examinado por um médico clínico, realizar biópsia da lesão, teste intradérmico e exame de secreção de sua orelha, métodos que são necessários para o diagnóstico da doença, você doará 40ml de sangue (mais ou menos 3 colheres de sopa) para a pesquisa dos mecanismos de defesa do organismo. A retirada do pedaço da pele ou da ferida para diagnóstico da sua doença será feita com anestesia para você não sentir dor e parte deste material poderá ser utilizado para os estudos da defesa do seu corpo contra a bactéria que causa a doença. Caso o diagnóstico de Hanseníase não seja confirmado, todo o material obtido para pesquisa será destruído.

Duração do estudo: Após a assinatura do termo de consentimento sua participação no estudo é de 5 anos, a contar do primeiro dia de tratamento, caso você tenha Hanseníase. Periodicamente, você será examinado para determinar a cura da doença ou necessidade de utilização de novo tratamento, que também lhe será fornecido gratuitamente.

Confidencialidade: Qualquer informação obtida durante este estudo só será do conhecimento da equipe médica e do órgão que protege o indivíduo em pesquisas (Comitê de ética do Hospital Universitário) Você e qualquer participante desse estudo não será identificado por nome nas publicações dos resultados do estudo. Apenas os representantes do Comitê de Ética em Pesquisa poderão ver sua ficha clínica.

Análises de riscos e benefícios: A retirada de seu sangue e de um pedaço da ferida são feitos se você tiver ferida, ainda antes do tratamento, para confirmar o diagnóstico da doença. Dor leve na retirada de sangue devido à punção com agulha pode ocorrer. Em casos raros a retirada de sangue provoca sangramento ou mancha roxa na pele. Como anestesia local é utilizada, a retirada de um pedaço da ferida não é acompanhada de dor. O tratamento que você receberá é igual ao que todos os pacientes receberão participando ou não do estudo. A participação lhe trará como benefício um acompanhamento clínico mais freqüente. Um médico lhe visitará em sua casa para examinar também sua família. Você deve retornar às

consultas médicas regularmente de acordo com marcação de seu cartão do Ambulatório do HU

Retorno de benefícios para o sujeito e para a sociedade: A Hanseníase é relacionada a reação do seu organismo contra a bactéria que causa a doença e o conhecimento destas reações do seu corpo pode contribuir não só para o entendimento da doença como para o aparecimento de novas formas de tratamento ou controle os sintomas e também formas de previnir a doença.

Custos: Você não terá custos com o tratamento. Você não receberá pagamento por sua participação neste estudo.

Esclarecimentos: Caso você precise de atendimento médico durante o estudo, você pode contactar um dos seguintes Médicos pelo telefone (79)3237-7353: Dra. Amélia Ribeiro de Jesus Dr. Emerson Ferreira da Costa ou Dr. Roque Almeida. Caso você queira saber alguma coisa sobre seus direitos e de seu filho, como paciente, você pode procurar o Comitê de Ética do Hospital Universitário, cujo endereço encontra-se no inicio deste consentimento ou pelo telefone (79) 3218-1805.

Consentimento: Se você leu o consentimento informado ou este lhe foi explicado e você concorda em participar do estudo, favor assinar o nome abaixo. A você será entregue uma cópia deste formulário para guardar.

_____	_____
Assinatura do participante	Data
_____	_____
Assinatura do pesquisador	Data
_____	_____
Assinatura da testemunha (apenas analfabetos)	Data

APÊNDICE 3

Consentimento informado para menores de 18 anos

NOME DO PACIENTE: _____

Registro.HU:_____ Nº: __ __ __ - __ __

Investigadores: Jonnia M Sherlock Araujo, Tel: (79)8855 0098 e Amélia R de Jesus, Tel: (79) 8823 7245. Hospital Universitário Rua Cláudio Batista S.N, Bairro Sanatório.

Convite e objetivo: Você está sendo convidado a participar de um estudo científico para determinar as razões porque pessoas desenvolvem Hanseníase. Nós perguntaremos a você sobre a sua saúde. Um médico fará exame físico em você, incluindo boca e nariz. Isto não causará dor em você. Então, nós tiraremos um pouco de sangue (cerca de duas colheres de sopa) de seu braço usando uma seringa e agulha descartáveis para realizar alguns exames que ajudarão a explicar a doença. Nós também iremos fazer um teste na pele, onde nós injetaremos uma pequena quantidade de líquido (duas gotas) no seu braço usando uma agulha fina. Nós também vamos precisar remover um pequeno pedaço da pele ou do nariz para confirmar se você tem a doença. Isso será feito por um médico no hospital, com anestesia local para evitar dor. Nós esperamos através deste estudo esclarecer mais sobre a doença, entendê-la e assim poderemos preveni-la no futuro.

Você pode não participar deste estudo. Se você quer nos ajudar, por favor, assine ou coloque sua impressão digital abaixo.

_____	_____
Assinatura ou impressão do paciente	Data
_____	_____
Assinatura ou impressão do responsável	Data
_____	_____
Testemunha	Data
_____	_____
Pesquisador	Data

APÊNDICE 4

Artigo de Revisão Publicado

Simon M, Sherlock J, Duthie MS, Jesus AR. Clinical, Immunological, and Genetic Aspects in Leprosy. Drug Development Research 72: 509–527 (2011).

DRUG DEVELOPMENT RESEARCH 72:509–527 (2011)

DDR

Disease Overview

Clinical, Immunological, and Genetic Aspects in Leprosy

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Leprosy is a chronic infection caused by *Mycobacterium leprae*. It affects the skin and peripheral nerves and can cause irreversible chronic disabilities. The worldwide registered number of cases in 2009 was 213,036. This review discusses clinical aspects of the disease, including leprosy reactions and neuronal damage, as well as immunological and immunogenetic aspects influencing disease susceptibility and outcome. The cardinal signs of leprosy are skin lesions with altered sensation, thickened peripheral nerves, and presence of alcohol acid-resistant bacilli in skin biopsy or lymph. Confirmatory examinations include (1) bacteriological examination, which allows patients' classification into two operational groups, multibacillary (MB) and paucibacillary (PB); and (2) histopathological examination, which permits stratification in different clinical forms. These clinical forms differ not only by histopathology but also according to the host's immune response to *M. leprae*. These forms comprise the extremes of (1) tuberculoid leprosy (TT), with a specific Th1 response and control of *M. leprae* multiplication; (2) lepromatous leprosy (LL) without Th1 response and preserved Th2 response; and (3) the interpolar clinical forms, borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL), and indeterminate form (IL). Appropriate treatment is based on smear examination or the number of lesions at diagnosis. In the evolution of leprosy, acute inflammation, known as reactions, may occur during or after treatment. These reactions are classified into two main types: the type I reaction or reversal reaction (RR), and the type II reaction or erythema nodosum leprosum (ENL). The role of innate immune response to control the infection is supported by immunological and genetic studies. Drug Dev Res 72:509–527, 2011.

Key words: leprosy; clinical; immunopathogenesis; immunogenetics

INTRODUCTION

Leprosy is a human chronic infectious disease caused by *Mycobacterium leprae*. It affects the skin and peripheral nerves and can cause irreversible impairment of nerve function and consequent chronic disabilities [Lockwood, 2007; Scollard et al., 2006a]. Despite widespread control efforts, leprosy still poses a significant health and economic burden on the developing countries. In 2009, a total of 121 countries or territories reported leprosy to the World Health

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Organization (WHO): 31 from Africa, 25 from the Americas, 10 from the Southeast Asia, 22 from the Eastern Mediterranean, and 33 from the Western Pacific. The number of new cases reported worldwide during 2008 was 249,007; at the beginning of 2009, the number of leprosy cases globally was 213,036. Although multidrug therapy is succeeding in reducing the number of active cases, the number of new cases reported each year is no longer significantly declining. Furthermore, patients "cured" of the infection usually require further care, as they can present with tissue-damaging leprosy reactions, may have permanent neurological deficits, or can relapse [Goulart and Goulart, 2008; Lockwood, 2004; Meima et al., 2008; Scollard 2008b,c, 2009].

Leprosy presents as a spectrum of diseases, making it an attractive model with which to investigate the regulation of immune responses to infection. Only a small percentage ($<1\%$) of the population that comes into contact with *M. leprae* will develop the disease, providing an extraordinary opportunity to study resistance versus susceptibility to a microbial pathogen. Additionally, the large spectrum of disease is highly dependent on the type of immune response developed by the host to the infection, providing a good model to understand pathogenesis mechanisms [Gulia et al., 2010; Massone et al., 2010].

This review will outline the current epidemiological and clinical situation regarding leprosy, with particular emphasis on a leprosy referral center in Sergipe, Brazil. We will also assess the current understanding of the general immune and pathogenic responses that present during various forms or phases of leprosy. Finally, we will summarize some of the published reports linking particular genetic factors with leprosy and will speculate on the potential candidate genes yet to be examined during leprosy, considering data from other infections.

DIAGNOSTICS AND CLINICAL FORMS OF LEPROSY

The cardinal signs of leprosy are skin lesions with altered sensation, thickened peripheral nerves, and the presence of alcohol acid-resistant bacilli revealed during skin biopsy [Villarroel et al., 2007b]. The conventional criteria for laboratory confirmation of disease include (1) bacteriological examination, which allows the classification of patients into two operational groups, multibacillary (MB) and paucibacillary (PB), and (2) histopathological examination that further divides the disease into five to six clinical forms. The appropriate treatment for each case is determined by smear examination or by the number of lesions at diagnosis, based on the operational classification established by the WHO. Additional laboratory methods, such as the ML-Flow test or serum anti-phenolic glycolipid (PGL)-I enzyme-linked immunosorbent assay (ELISA), can be used as indicators of bacterial load and for therapeutic monitoring [Moura et al., 2008; Teixeira et al., 2008].

Leprosy is expressed clinically and pathologically through polar and spectral clinical forms depending mainly on the host's immune response to the bacillus. This capacity can be evaluated with the Mitsuda test. A positive or negative test will be observed in patients whose cellular immune response against *M. leprae* is either preserved or absent, respectively [Abulafia and Vignale, 1999; Scollard et al., 2006a]. According to the Ridley and Jopling classification, covering clinical, histopathological, immunological, and bacteriological criteria, leprosy is stratified into five subtypes: the extremes of true tuberculoid leprosy (TT) and lepromatous leprosy (LL), and the interpolar clinical forms, which include borderline tuberculoid (BT), borderline boderline (BB), and borderline lepromatous (BL) [Ridley and Jopling, 1966]. The indeterminate form, indeterminate leprosy (IL), may also be added to this classification [Lockwood, 2007].

The IL form, considered an initial stage of the disease, is characterized by the presence of macula, hypochromic or erythematous, associated with disturbances of sensation, as well as sweating and vasomotor symptoms. Histologically, there is a nonspecific perineural infiltrate, in which bacilli are rarely found. From this stage, the infection can either become controlled and resolved by cellular immunity or specific treatment, or it may evolve into one of five overt subtypes of the disease [Lockwood et al., 2007].

In the TT form, skin lesions consist of erythematous brownish or pinkish brown plaques, with well-defined edges, single or in small numbers, and with various shapes. The neural lesions resulting from the granulomatous response appear early and are very aggressive. Histological analysis shows well-defined granulomas invading nerve fibers with rare bacilli. These patients are smear-negative and therefore are not considered significant reservoirs of *M. leprae* [Lockwood et al., 2007].

The LL form, often described as an anergic pole of the disease, is clinically characterized by multiple and polymorphous cutaneous lesions and by the presence of some systemic manifestations resulting from bacterial infiltration (e.g., rhinitis, lagophthalmos, conjunctivitis, lymphadenopathy, hepatosplenomegaly, testicular atrophy, anemia, osteoporosis, and osteitis). The lesions display imprecise limits and variable color (from hypochromic to reddish ferruginous), and may or not be accompanied by hyposensitivity or anesthesia. Papules, plaques, or nodules can be seen in LL

patients. As a result of intense infiltration, progressive alopecia occurs in different regions and may result in madarosis. The appearance of "lion" face is not uncommon, because of the presence of multiple infiltrated lesions on the face accompanied by an enhancement of the natural folds. Histopathology shows macrophages with large numbers of bacilli and microcolonies of bacilli. Smear examination shows a large number of bacteria, which are also responsible for the strong positivity of serum antibodies against PGL-I (80–100%). LL patients possess a large number of bacilli in the nasal passages and can represent a source of transmission and perpetuation of the disease until adequate and full treatment is provided [Bakker et al., 2006; Britton and Lockwood, 2004; Lockwood et al., 2007; Moura et al., 2008; Penna et al., 2008].

Between the two polar forms described above (TT and LL), the intermediate forms or borderline forms (BT, BB and BL) are immunologically dynamic. A progressive reduction of the cell-mediated response from the BT to the BL forms is accompanied by an increased number of neurocutaneous lesions, increased bacterial load, and increased antibody titers to PGL-1. Patients with the BT form have numerous skin lesions with a tuberculoid appearance, and a major commitment of the nerve trunks. The smear is often positive, and the histopathological picture shows tuberculous granulomas. Classical BB leprosy is found in the middle of the spectrum of annular lesions, with a well-defined central area that is hypochromic or apparently normal, and with imprecise edges (lesions "bumpy," "comb honey," or "Swiss cheese"). The neural involvement is important. The smear is positive for bacilli, and the histopathology shows granuloma and infiltration of nerve fibers by macrophages with many bacilli. Finally, numerous lesions characterize the BL form, although with fewer polymorphonuclear cells than are found in the lepromatous form, and neural involvement similar to the anergic pole. The smear is always positive and the histopathology reveals infiltration around the skin appendages, as well as nerve damage and granuloma with lymphocytes and macrophages filled with bacilli [Lockwood, 2007; Scollard, 2004; Scollard et al., 2006a].

Primary neural leprosy is an unusually difficult to diagnose form of the disease; it can occur in 5–15% of patients. It is characterized by the presence of asymmetric involvement of peripheral nerves but an absence of cutaneous manifestations. Dysesthesia or change in muscle strength is commonly associated with this form and can be evaluated by neurological examination, electromyography (EMG), or nerve biopsy. These patients may have a positive or negative Mitsuda reaction (which measures the immune response to intradermal administration of lepromin and has a high prognostic value for susceptibility or resistance to the lepromatous form of leprosy) and can later develop cutaneous manifestations [Jardim et al., 2007].

TREATMENT

Over the past 15 years, leprosy treatment has been provided gratis through a drug fund provided by the Nippon Foundation, and now through the multidrug therapy (MDT) donation provided by Novartis and the Novartis Foundation for Sustainable Development. It is a cocktail of antibiotics (rifampicin, dapsone, and clofazimine) in the form of MDT, in a regimen standardized by the WHO [2009]. MDT has bactericidal and bacteriostatic activity, preventing the development of disease and interrupting the transmission cycle. The duration of treatment and the combination of medications are set according to the operational classifications of MB (>5 skin lesions, or positive smear) or PB (<5 skin lesions or negative smear) [WHO, 2009]. The use of MDT is intended to prevent drug resistance, which can emerge more readily when only a single drug is used [WHO, 2006a,b].

For PB patients, the MDT regimen consists of rifampicin in a monthly dose of 600 mg with supervised administration, combined with dapsone in a supervised monthly dose of 100 mg and self-administered daily doses. The duration of treatment is 6 months [WHO, 2009]. For MB patients, the MDT regimen consists of rifampicin in a supervised monthly dose of 600 mg, clofazimine in a supervised monthly dose of 300 mg and a 50-mg self-administered daily dose, and dapsone in a supervised monthly dose of 100 mg and self-administered daily doses. For MB patients, treatment is recommended for 12 months. However, in the case of MB patients with numerous lesions or large areas of skin infiltration that may have a slow regression of the lesions, treatment is often extended for another 12 months. For children, the doses are adjusted according to weight. Cure is arbitrarily determined upon completion of the number of doses recommended for the operational classification. PB patients can receive 6 supervised doses in up to 9 months; MB patients can receive 12 supervised doses in ≤ 18 months [WHO, 2006a,b].

COMPLICATIONS OF LEPROSY

Leprosy Reactions

In the complex evolution of leprosy, acute inflammation phenomena known as reactions may occur during or after treatment. An estimated 30–50% patients present with reactional episodes at some time during the course of the disease [Motta

et al., 2010; Scollard et al., 2006a]. Reactions are classified into two main types: type I reaction, or reversal reaction (RR), and type II reaction, or erythema nodosum leprosum (ENL). Despite their high frequency, there are no laboratory tests or clinical parameters with adequate accuracy to predict which patients will develop these episodes or when they might occur. However, studies suggest that the presence of high bacterial load after discharge by cure is a risk factor for the development of post-treatment reactions [Brito Mde et al., 2008]. Other risk factors attributed to the development of reactional states are occurrence of borderline subtypes [Lockwood, 2007; Penna et al., 2008; Ranque et al., 2007]; extensive disease, estimated by the number of body areas involved; number of lesions; neurological involvement; and positive smear [Motta et al., 2010; Roche et al., 1991; Van Brakel et al., 1994]. Although these risk factors are known, the mechanisms responsible for the reactions are not yet fully understood.

Reactions may occur spontaneously or following some triggering factors such as concomitant infections, pregnancy or postpartum, immunizations, medications, and either physical or emotional stress. Patients with reactions can present with intense neural inflammation, painful or not, resulting in sudden and even permanent loss of sensory, autonomic, and motor functions. In the absence of early intervention, consequent sequelae responsible for strong social stigma and significant impairment of quality of life may occur. Disabling injuries, such as paralysis, facial deformity, loss of extremities due to progression of joint contracture and bone resorption, as well as chronic ulcers resulting from trauma palmoplantar not identified by the patient due to the loss of sensitivity, can occur secondary to irreversible nerve damage [Harboe et al., 2005; Moschioni et al., 2010; Motta et al., 2010; Pimentel et al., 2004; Richardus et al., 2003; Roche et al., 1997].

Reversal reaction, or type 1 reaction

Reversal reaction (RR) is the most frequent of leprosy reactions, occurring in 30% of susceptible patients and representing the main cause of nerve damage in leprosy. Although it may appear at any evolutionary stage of the disease, a higher risk of RR occurs after the initiation of chemotherapy. Some investigators have identified the first 2 months of treatment as the most common period for the development of RR [Britton, 1998; Britton and Lockwood, 2004; Ranque et al., 2007; Shen et al., 2009; Walker and Lockwood, 2008].

Swelling of the hands and feet, and neural involvement characterized by thickening of one or more peripheral nerves presenting mainly as an extremely painful neuritis, are frequent if preventative measures are not established early. Some investigators also consider that these reactions can appear only with neurological impairment, with no alterations in skin lesions [Kahawita and Lockwood, 2008; Ranque et al., 2007]. In rare cases, type I reactional lesions, regardless of the clinical type of leprosy, can ulcerate. Clinical conditions, such as claw hand, foot drop, and facial palsy, can accompany the neural thickening and loss of sensitivity of the affected areas as the sequelae of acute reaction; these manifestations urgently require appropriate treatment. RR must be differentiated from relapses, especially in the BT and TT forms of leprosy. It is usually considered that relapse cases occur within a year of MDT completion. Unlike RR, relapse is insidious and may be accompanied by mild erythema of some old lesions, usually with mild neurological impairment and no significant changes in patient's general condition [Kar et al., 2009; Lienhardt and Fine, 1994; Richardus et al., 2004; Siddaraju et al., 2009; Siddiqui et al., 2002; van Veen et al., 2009].

Treatment of the type I reaction aims at controlling the pain and inflammation of the skin lesions and preventing further neurological damage and sequelae. Patients with signs of neuritis or skin inflammation should be treated with oral corticosteroids such as prednisone at doses of 1–2 mg/kg/day. A randomized controlled trial comparing the ability of different corticosteroids regimens with control RR suggested that the duration of prednisone therapy is more important than the dose [Rao et al., 2006]. MDT should also be initiated in patients with RR who are not already in treatment and should be continued as if they are beginning treatment afresh.

Erythema nodosum leprosum, or type 2 reaction

Erythema nodosum leprosum (ENL) affects patients with poor cellular immune responses, but with preserved humoral responses against *M. leprae* and thus presents with high levels of circulating immunoglobulins. Therefore, there is a predominance of ENL in patients classified as having the lepromatous pole (LL, BL), and it is more common in patients with a high bacillary index. Although ENL tends to occur during treatment, commonly later during MDT (after the sixth month of chemotherapy), there are reports of ENL either preceding diagnosis or emerging only after the completion of MDT [Feuth et al., 2008; Kahawita and Lockwood, 2008; Sales et al., 2007; Saunderson et al., 2000; Shen et al., 2009; Teixeira et al., 2010; Walker et al., 2007]. Penna and colleagues [2008], in a study that included 1,124 leprosy patients from a state in Brazil, documented 328 (29.3%) leprosy reactions, and ENL was the most commonly observed (192 from 328 cases, 58.5%). ENL were more

prevalent in LL and BL forms (176 from 192 cases, 91%). For reasons still unknown, there is an association between ENL and the male gender, although it may simply be linked to the higher incidence of lepromatous disease among men or to other risk factors more frequently linked to male gender (e.g., alcohol use) [Kahawita and Lockwood, 2008].

Although pre-existing lesions tend to remain unchanged, patients present an abrupt onset of erythematous nodules, which are typically but not necessarily painful. These nodules may develop into pustules and blisters, with subsequent ulceration and necrosis, classified as severe ENL (also called necrotizing erythema nodosum). Patients with ENL often present with swollen hands, feet, and face. Overall health is typically poor, with common features being fever and toxemia, with or without lymph node infarction, generalized muscle and bone pain (usually tibial pain), arthritis, iritis, iridocyclitis, orchitis, epididymitis, neuritis, glomerulonephritis, and hepatosplenomegaly. Neuritis is less aggressive than that observed in RR [Kahawita and Lockwood, 2008], but deterioration of nerve function also occurs. Lucio's phenomenon and erythema multiforme are reported variants of severe ENL [Feuth et al., 2008; Scollard et al., 1992, 2006a; Walker et al., 2007].

ENL can persist for years as chronic and recurrent forms in most patients [Kahawita and Lockwood, 2008]. Intermittent reactions with a duration of 1–2 weeks occur, followed by periods of 1–2 months without reaction. Very rarely, ENL reactions may be confused with the papular-nodular rashes, which are relapse manifestations of MB. ENL leads to worsening of pre-existing lesions, appearance of new smear-positive lesions, and slow progression. The differential diagnosis of ENL should take other causes under consideration. Infections (bacterial, viral, and fungal), drug reactions, sarcoidosis, and lymphoma are examples of possible confounding factors.

Consistent with the immunopathogenesis of ENL (reviewed below), immunomodulators that regulate tumor necrosis factor-α (TNF-α) gene expression can be used for therapy [Kaplan et al., 1989; Penna et al., 2008; Scollard et al., 2006a]. Thalidomide, by acting on TNF, is effective in controlling cutaneous and systemic symptoms of ENL [Kahawita and Lockwood, 2008]. However, because of its well-documented teratogenic effects, thalidomide should be avoided in young women of childbearing age. Other therapeutic options for these individuals include pentoxifylline, clofazimine, azathioprine, methotrexate, corticosteroids, and anti-TNF monoclonal antibodies [Hagge et al., 2009; Kahawita and Lockwood, 2008; Tadesse et al., 2006]. Corticosteroids are recommended for patients with impaired neural iritis or iridocyclitis, orquiepidimite, nephritis, necrotizing erythema nodosum, Lucio's phenomenon, and hand and foot reaction.

Neuropathy

Nerve damage is present in all forms of leprosy. The major determinant of neurological damage in leprosy is the peculiar ability of *M. leprae* to bind to and infect Schwann cells of the peripheral nervous system, through specific receptors. In RR, a delayed-type (type IV) hypersensitivity directed against *M. leprae* antigens released by Schwann cells triggers neural aggression and damage. The neuronal injury observed in ENL is caused by local deposition of immune complexes and consequent tissue damage. The main nerves involved in leprosy are ear nerves, radial, ulnar, median, common peroneal, and posterior tibial. These nerves are frequently affected in an area near the body surface, rendering it possible to examine them by palpation [Kahawita and Lockwood, 2008; Schuring et al., 2008; Scollard, 2008a].

Patients with the tuberculoid forms of leprosy present with sudden onset and asymmetrical involvement of nerve trunks, while patients with lepromatous forms present an usually insidious and symmetrical neuropathy. The neural damage is clinically identified through the pain in response to palpation of the affected nerves, paresthesia or numbness, and reduced muscle strength and/or inaccuracy of movements. As mentioned earlier, some patients may present with silent neuritis, in which there are no neurological symptoms. Risk factors for nerve damage include smear positivity, positive anti-PGL-I responses, leprosy reactions, and previous neurological involvement [Harboe et al., 2005; Pimentel et al., 2004; Schuring et al., 2008; Smith et al., 2004].

Physical Disabilities

Neurological functional disability is defined as any reduction in sensory or motor functions. Because neurological involvement is inherent in all forms of leprosy, disability is sadly a frequent complication resulting either from the natural course of leprosy or from reactions [Kahawita and Lockwood, 2008].

Epidemiological studies indicated that MB patients and those with neuritis have a higher risk of developing disabilities. Pimentel et al. [2004] found that 11.7% of patients had neurological deterioration as measured by the degree of disability worsening at the end of treatment, and 91.7% of these showed leprosy reactions. Neurological involvement, even if subclinical, is a predictor of permanent neural injury in MB patients [Smith et al., 2004]. In addition to smear positivity, the presence of anti-PGL-I antibodies can

also predict the risk of neural injury in both MB and PB patients [Schuring et al., 2008]. Delayed diagnosis, neuritis, or nerve damage at diagnosis and the occurrence of RR are all considered important risk factors for the development of neurological disabilities. Silent or symptomatic neuritis are responsible for rapid functional deterioration. Affected patients can develop claw hand, drop foot, facial palsy, lagophthalmos, and even blindness, as well as sensory loss and focal or segmental neural thickening. The identification of risk factors and signs of neural damage are key to avoid irreversible nerve damage [Saunderson et al., 2000; Van Veen et al., 2006, 2008].

Neurological examinations of patients can be performed using the Maximum Degree scale of disability established by the WHO, whose sensitivity and negative predictive values were 50% and 88%, respectively [Koelewijn et al., 2003; Scollard et al., 1996; Villarroel et al., 2007a,b]. This examination is performed at the beginning, middle, and the end of MDT treatment, or any time, if the patient shows neurological symptoms. Prolonged corticosteroid therapy is the treatment of choice for reactions that are accompanied by neurological damage. Reversal or regression of the neural damage can occur, and is most frequently observed in early lesions (<6 months) [Rao et al., 2006; Richardus et al., 2003; van Veen et al., 2008].

ANALYSIS OF CLINICAL DATA FROM LEPROSY PATIENTS IN A REFERENCE CENTER IN SERGIPE, BRAZIL

To assess the local leprosy situation in Sergipe, Brazil, we conducted a retrospective study based on data collected from medical records of 165 patients treated in a referral hospital for leprosy between 2003 and 2009. Demographic data indicated that patients during this period were mostly women, in contrast with previous reports in the literature, which generally shows a predominance of leprosy in men [Feuth et al., 2008; Penna et al., 2008; Ranque et al., 2007]. However, another study did not find differences in the prevalence of the disease in relation to gender [Penna et al., 2008]. The average age was 41 ± 19.6 years for women and 37 ± 19.3 years for men, consistent with existing data in the literature [Feuth et al., 2008]. Regarding the clinical forms of leprosy, large numbers of patients with indeterminate leprosy (20.6%), and a higher frequency of PB forms (68.4%) were reported, in contrast to a previous study conducted in another Brazilian referral center (University Hospital of Brasilia, Brazil), which found a predominance of MB and lepromatous patients [Penna et al., 2008]. The high prevalence of early stages of disease suggests that the leprosy control program developed at the University Hospital of Sergipe, Brazil, (DES)MANCHA-SE, has been effective. This program actively searches for contacts of leprosy patients, and provides early diagnosis for many patients. These differences may also be explained by genetic differences within the studied populations, particularly given the large admixture of the Brazilian population. Consistent with the reviewed literature, in which the occurrence of leprosy reactions is estimated in 30–50% of patients (with the exception of the indeterminate clinical form [IL]), data from Sergipe revealed that 35.6% of patients developed leprosy reactions. Both reaction types (RR and ENL) were more common among MB than PB patients and occurred predominantly during MDT and as type 1 (RR) reactions [Foss et al., 1993; Scollard et al., 1994].

These data indicated an association between males and smear positivity, odds ratio (OR) 3.2 (95% confidence interval [CI] 1.43–7.25), which was previously shown in a large epidemiological study [Varkevisser et al., 2009]. A large epidemiological study conducted in China examining the occurrence of reaction showed a higher frequency of leprosy reactions among men [Shen et al., 2009]. Our data also show a predominance of leprosy reactions among men (52.2%), as compared with women (47.8%; OR 2.1 [95% CI 1.03–4.97]). This relationship may be explained in part by the fact that most lepromatous and borderline patients from Sergipe were men.

IMMUNE RESPONSE TO *M. leprae* INFECTION

Leprosy presents as a spectrum of diseases and immunological profiles. Patients with tuberculoid leprosy (TL) display a resistant response that restricts the growth of the pathogen. Few lesions and bacilli are observed in TL patients. The development of an effective innate immune response, coupled with the low virulence of *M. leprae*, can mediate the destruction of *M. leprae* and activate the adaptive cellular immune response, inducing resistance to disease. Although efficient in bacilli control, this strong response accounts for the destruction of the peripheral nerves. Conversely, lepromatous leprosy (LL) patients exhibit specific cellular unresponsiveness to *M. leprae* antigens associated with a T-helper 2 (Th2) immune response and high mycobacterial loads in the skin and nerves. However, most leprosy patients display an intermediate response and are classified as either borderline tuberculoid, borderline borderline, or borderline lepromatous. How the immune response positions itself within this wide spectrum of disease is an important point that remains unclear [Britton, 1993; Cooper et al., 1989; Lockwood et al., 2007; Scollard, 2008a; Scollard et al., 2006a].

Infectious agents invade, proliferate, and produce tissue harmful substances during the promotion of disease. During the process of invasion, microorganisms bypass several natural barriers that are important for preventing pathogen colonization and penetration. After overcoming these barriers, pathogens can trigger an inflammatory reaction in tissues, activating the immune system. Innate immunity is the initial response to infection, which involve phagocytic cells, natural killer (NK) cells, complement, acute-phase proteins, and cytokines. Phagocytes recognize specific pathogen-associated molecular patterns (PAMP), by binding to lower diversity receptors (rPAMP), such as Toll-like receptors, manose binding lectin (MBP), and NOD2, secreted, or located at the cell surface or in intracellular compartments. The binding of pathogens to these receptors induces phagocyte activation, which increases their microbicidal capacity. As a result, the activation of Toll-like receptors (TLRs) releases chemical mediators of immunity (chemokines and cytokines), and induces the expression of phagocyte activation markers (B7.1/B7.2), as well as MHC class I/class II molecules. The signals induced by TLR activation play an important role on the initial events of specific immune responses, because the expression of the co-stimulatory signals (B7.1/B7.2) needed for T-cell activation and cytokine production, is important for T-cell differentiation. Innate immune response can eliminate the infectious agent and some cells can also function as antigen-presenting cells, which can process the pathogen and carry the antigens to lymphoid organs, where they can activate T cells, providing the link between the innate and adaptive immune responses [Beutler, 2003; Hottat et al., 1988].

TLRs are crucial for the recognition of pathogens by macrophages and dendritic cells. Dendritic cells (DCs) can take up *M. leprae*; subsequent local production of cytokines and chemokines regulates inflammation and influences the course of the adaptive cell-mediated immunity into a Th1, Th2, or T-regulatory response, and also possibly a Th17 response, which has not yet been reported during leprosy. Although DCs are effective presenters of *M. leprae* antigens, major histocompatibility complex (MHC) class I and II expression is downregulated in monocyte-derived DCs infected with *M. leprae* bacilli. In contrast, DCs stimulated with *M. leprae* membrane antigens upregulate both MHC class II and CD40 ligand-associated interleukin-12 (IL-12) production, suggesting that whole live bacilli may suppress the interaction of DCs and T cells [Goulart and Goulart, 2009; Gulia et al., 2010].

The interaction between the pathogen and host acquired immune response is a determinant of clinical outcome of infection with any disease. Regulating the function of other immune cells, by either increasing or reducing the inflammatory response, is critically important in controlling infection and limiting pathology. The adaptive immune response is highly specific and is capable of protecting against reinfection by inducing and expanding memory cells. Several effector cells are involved in the adaptive immune response, with the principal cells being $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T cells that not only exert direct effector activities but also modulate innate immune response. Helper $CD4^+$ T cells are subdivided into four distinct types: Th1, Th2, Th17, and regulatory T (Tregs) cells. Each subtype is involved in protection against specific types of microorganisms. Th1 cells are essential in the response against intracellular microbes such as mycobacteria (by activating inflammatory responses in macrophages); Th2 cells are involved in the immune response against extracellular and multicellular agents such as helminths (by supporting humoral responses and antibody-mediated cytotoxicity by eosinophils); Th17 cells are effective for rapid control of bacterial and fungal pathogens (by inducing epithelial cell secretion of granulopoietic factors such as granulocyte colony-stimulating factor (G-CSF) and CCL20, which recruit large numbers of neutrophils, and also synergizes with other cytokines, such as IL-1, IL-6, and TNF, that promote activation of tissue infiltrating neutrophils to eliminate extracellular pathogens); and regulatory T cells downregulate immune response to self-antigens and persistent pathogens, preventing chronic immune damage to the host [Cua and Tato, 2010; Pace et al., 2010; Tato and O'Shea, 2006].

Pathogens have, in turn, evolved mechanisms to counter these immune defenses, including methods to circumvent the natural barriers, production of suppressive substances to alter innate responses, interruption of antigen presentation, and induction of suppressive cytokines, e.g., IL-10 and TGF-β, to promote their survival. The imbalance between the adaptive hosts' mechanisms and the pathogen can lead to both excessive proliferation of the infectious agents or to an excessive host immune response, causing an inflammatory response and promoting disease [McNicholl et al., 2000].

M. leprae has been interacting with humans for a long time and has developed several adaptive mechanisms that enables its persistence in the human body without, in most cases, causing significant tissue damage. Infected individuals may be asymptomatic or have mildly symptomatic disease presenting with different degrees of clinical severity. In leprosy, the pattern of the individual cytokines secreted in response to infection with *M. leprae* influences the clinical form.

The cytokine profile present in the lesion appears to be correlated with TLR function: Th1-type cytokines are associated with TLR1 and TLR2 activation, and Th2-type cytokines are associated with inhibition of activation. The expression of TLR1 and TLR2 is more pronounced in monocytes and DCs in TT lesions than in the LL counterparts. In addition, in vitro studies indicate that the *M. leprae* 19-kDa and 33-kDa lipoproteins activate monocytes and monocyte-derived DCs through TLR2 [Krutzik et al., 2003; Sieling et al., 2008]. In *Mycobacterium tuberculosis* infection, although TLR signaling enhances both the innate and adaptive immune responses, it can also downregulate some immune functions. TLR2, in particular, has been implicated in the downregulation or deviation of the immune response through the induction of interleukin-10 (IL-10) and T-helper 2 cell or T-regulatory cell responses. Prolonged TLR signaling might provide homeostatic feedback regulation that limits the extent of the induced responses. Although TLR signaling in APCs induces microbicidal and inflammatory effectors, de novo MHC class II antigen processing and presentation, these functions are inhibited by prolonged signaling with agonists of TLR2, TLR9, and TLR4 [Ferwerda et al., 2005; Pathak et al., 2005; Stenger and Modlin, 2002; Yoshida et al., 2009]. Downregulation of antigen presentation is not specific to *M. tuberculosis*, as it can be induced by components from many microorganisms; however, it could be especially pronounced during persistent infection with intravacuolar pathogens that survive microbicidal mechanisms and that can persistently co-localize with TLRs in phagosomes for prolonged TLR signaling, such as *M. leprae* (TLR2 can be recruited to phagosomes as well as reside on the plasma membrane) [Harding and Boom, 2010; Lancioni et al., 2010; Simmons et al., 2010].

Considering that *M. leprae* is a low-virulence pathogen, the reduction of antigen presentation might reflect a general mechanism of negative feedback that prevents excessive T-cell-mediated inflammation, which has been used by the bacteria to create a niche for survival within infected macrophages to permit evasion of $CD4^+$ T cells [Harding and Boom, 2010]. Montoya et al. [2009] have investigated the regulation of macrophage functions of phagocytosis and microbicidal activities in leprosy by studying cells from leprosy patients and lesion biopsies. To compare the ability of two key innate immune cytokines, IL-10 and IL-15, to trigger macrophage functional programs, they cultured human peripheral blood monocytes with IL-10 or IL-15 for 2 days. Both cytokines induced expression of CD209, a C-type lectin receptor previously present in tissue macrophages. IL-10 induced the phagocytic pathway, increasing phagocytic capability by 10-fold, resulting in phagocytosis of mycobacteria and oxidization of low-density lipoprotein while IL-15 induced the vitamin D-dependent antimicrobial pathway, but IL-15-activated cells were less phagocytic. The differential regulation of functional programs within macrophages was confirmed by the analysis of leprosy lesions: the phagocytosis pathway was prominent in the clinically progressive form of the disease (MB), whereas the vitamin D-dependent antimicrobial pathway was more frequent in the self-limited PB form. These data indicate that programs for phagocytosis and antimicrobial responses within macrophage are distinct and differentially regulated [Montoya et al., 2009].

Th1 cells are essential for the protective response against intracellular microbes, such as mycobacteria, through production of IFN-γ and TNF-α. These cytokines induce macrophage activation and microbicidal activities that help eradicate intracellular microorganisms. They also promote the development of cytotoxic T cells that kills nonphagocytic infected cells [Goulart and Goulart, 2009].

Individuals who develop a Th1 response have milder leprosy forms, with few bacilli in the presence of skin lesions and lymph. Therefore, the clinical forms originating if a Th1 response is induced are usually classified as PB. Histopathologically, these clinical forms are characterized by the formation of granulomas. The granulomas are commonly found in infections with intracellular microbes; they are formed when the cellular immune response cannot quickly eliminate the infecting organism, leading to an accumulation of macrophages and T cells surrounding the infected macrophages. Thus, Th1 cells are pro-inflammatory and may be also involved in the pathogenesis and tissue damage in some clinical forms of infectious disease, as demonstrated in leishmaniasis [Carvalho et al., 2007]. In leprosy, this response is involved in neurological damage, an important complication of this disease.

If the immune response to *M. leprae* infection induces a Th2 response, the patient does not mount an effective cell-mediated immune response, leading to multiplication of the mycobacteria and the development of lepromatous disease. The differentiation of Th2 cells is influenced by IL-4, which is secreted by T lymphocytes, mast cells, eosinophils, and natural killer (NK) cells [Fallon et al., 2002; Flesch et al., 1997; Mochizuki et al., 1998]. Th2 cells, via secretion of IL-4, IL-5, and IL-13, promote antibody production. The Th2 response leads to macrophage inhibition and suppression of cellular immune response [Huber et al., 2010; Khan et al., 2008]. IL-10 is an immunosuppressive cytokine, which plays a major role in the inefficient *M. leprae*-specific immune response.

Both IL-10 and IL-5 stimulate B cells and inhibit activation of macrophages, resulting in progressive infection [Goulart and Goulart, 2009]. Thus, the clinical forms with predominance of Th2 response are classified as MB, because this response is not effective in killing *M. leprae*, Th2 responses are strongly promoted by some parasitic infections with helminth infection downregulating the Th1 immune response to vaccination [Sabin et al., 1996], and increasing the severity of leishmaniasis [O'Neal et al., 2007].

An association between intestinal helminth infections and lepromatous leprosy has been demonstrated with the frequency of intestinal helminths correlating strongly with the mycobacterial index [Diniz et al., 2010]. Evidence that Th1 downmodulation occurs during intestinal helminth infection was provided by the fact that intracellular IFN-γ levels in both tuberculoid and lepromatous, helminth free leprosy patients, were approximately 2-fold higher than in helminth infected leprosy patients. Conversely, lepromatous patients harboring intestinal helminths produced close to 2-fold more IL-4 and IL-10 than helminth-free leprosy patients. These results suggest that a pre-existing infection by intestinal helminths may facilitate initial *M. leprae* infection or its progression to a more severe clinical form [Diniz et al., 2010].

Tregs have been described in many diseases, either participating in the pathogenesis or controlling inflammation and tissue damage. Among the different types of Treg cells, some are formed in the thymus ($CD4^+$ $CD25^+$ $Foxp3^+$) and others differentiate in the periphery, where two subtypes have been described, Tr1 and Th3 [Banham et al., 2006; Powrie, 2004; Thompson and Powrie, 2004; Uhlig et al., 2006]. Although this process remains to be clarified, the presence of IL-10 appears to be important in inducing the differentiation of peripheral Tregs. Tregs participate in immunological tolerance by regulating other T cells through the production of regulatory cytokines (IL-10 and TGF-β) and signaling via cell–cell contact. To examine the role of Tregs during leprosy, Attia et al. [2010] evaluated the frequency and FoxP3 expression of circulating Tregs in 38 patients and 38 healthy controls using flow cytometry. Mean frequency of Treg and the percentage of FoxP3 expression were significantly elevated in patients (particularly TT) compared with controls ($3.8 \pm 2.5\%$ vs. $2.5 \pm 0.8\%$ and $78.8 \pm 56.2\%$ vs. $55.8 \pm 15.7\%$, respectively) ($P < 0.05$). Comparing the four disease groups, the percentage of Treg cells was significantly different (median 5.3% in TT patients, 3.4% in BT patients, 2.8% in BB and BL patients, and 1.2% in LL patients; $P = 0.005$). Notably FoxP3 expression was higher in ENL than in controls. In contrast with expected results, these data suggest that circulating Tregs are elevated in TT patients. Patients with LL and ENL display significantly lower frequencies of Tregs, consistent with disease progression and immune hyperactivation in these disease categories. Thus, rather than being detrimental to immunity, intact Treg activity may be beneficial to leprosy patients [Attia et al., 2010]. One could argue that the regulatory cells could be localized at the site of skin lesions and therefore removed from the circulation. However, in determining the frequency and distribution of natural Treg, $CD4^+$ $CD25^+$ $FoxP3^+$ in 20 retrospective leprosy biopsies using immunohistochemistry, Massone et al. [2010] found that $FoxP3^+$ cells were present in 95% of biopsies, comprising approximately 2.9% of the infiltrate. Their distribution was not related to granulomatous structures or special locations. There was no difference of FoxP3 expression between the studied clinical forms (TL, BTL, BLL, and LL). A significantly higher frequency of T-regs in patients affected by reversal leprosy reactions (BT-RR and BB-RR) compared with patients affected by ENL and patients with nonreactional disease forms (BL, LL, BT, TL) was observed [Massone et al., 2010].

The immunopathogenesis of nerve damage during leprosy varies with the type of immune response. In TT patients, the activated Th1 immune response leads to granuloma formation and necrosis, which may culminate with complete destruction of the nerves [Scollard, 2008a; Scollard et al., 2006a] There is an association between IFN-γ production and neural damage, and the level of this cytokine is directly proportional to the number of damaged nerves. Thus, the presence of the Th1 cytokine at high levels would explain the presence of neural lesions in patients with tuberculoid clinical forms [Belgaumkar et al., 2007]. In turn, in MB patients (lepromatous clinical forms), with a predominance of the Th2 response, the neuropathy is related to the infection of peripheral nerves by the bacillus. The mycobacterium has a predilection for Schwann cells, surrounding myelinated fibers of these nerves. Infected cells in the peripheral nerves are able to process and present antigens to T cells, which may be targets of the immune response. Therefore, an immune-mediated inflammatory response is probably also responsible for the appearance of neural lesions, since involvement in neural function occurs more rapidly and more severely in patients with a strong cellular immune response, as occurs in patients of the tuberculoid clinical forms [Scollard, 2008a; Scollard et al., 2006a].

Leprosy reactions are typical examples of immune-mediated disorders. RR can be interpreted as a type IV delayed hypersensitivity reaction [Coombs, 1971; Gell, 1967]. Th1 responses are activated, leading to systemic

and local production of IL-2, IL-12, INF-γ, and tumor necrosis factor-α (TNF-α), pro-inflammatory cytokines that are important mediators of nerve damage. Increased numbers of $CD4^+$ T cells are also observed in reactional cutaneous lesions. RR is clinically characterized by erythema and infiltration of pre-existing lesions, although new lesions may also develop [Andersson et al., 2005; Belgaumkar et al., 2007; Mendonca et al., 2008; Scollard et al., 2006a,b; Walker and Lockwood, 2008].

ENL is a type III hypersensitivity reaction [Coombs, 1971; Gell, 1967], mediated by immune complexes. The deposition of immune complexes in capillaries leads to the activation of the complement system, as well as chemotaxis and activation of immune cells, triggering an acute inflammatory process. ENL is, thus, associated with high IL-12 concentrations, neutrophil infiltration, and complement activation [Brito Mde et al., 2008; Modlin et al., 1986; Scollard et al., 1992, 2006a; Silva et al., 2007]. Increased expression of IL-6, IL-8, and IL-10 mRNA can be observed in lesions of ENL [Belgaumkar et al., 2007; Scollard et al., 2006a]. The most severe reactions are associated with increased production of TNF-α and INF-γ that could possibly be explained by the temporary recovery of the microbicidal capacity of macrophages [Kaplan et al., 1991; Motta et al., 2010; Partida-Sanchez et al., 1998]. In agreement with the immunopathogenesis of ENL, INF-γ injections can trigger ENL lesions and immunomodulators that downregulate TNF-α gene expression can provide therapy [Kaplan et al., 1989; Penna et al., 2008; Scollard et al., 2006a].

Despite current knowledge of leprosy, there are still some gaps in understanding of the determinants involved in the establishment of immunological disease phenotypes, including the role of Th17 cells, and cytokines described more recently to be important in other infectious diseases, including in intracellular pathogens (IL-22, IL-23, IL-27) [Fedele et al., 2008; Gafa et al., 2006; Kastelein et al., 2007; Pitta et al., 2009].

Th17 cells secrete IL-17A and IL-17F, which induce production of proinflammatory cytokines and chemokines and contribute to the recruitment of neutrophils. Therefore, they appear to be involved in the rapid response to many extracellular pathogens, including bacteria and fungi. *Leishmania* induces IL-17 production, which stimulates the production of CSF, increasing the production of neutrophils, monocytes, and chemokines that have potent chemotactic action for Th1 cells [Pitta et al., 2009]. During infection with *M. tuberculosis*, it is believed that IL-17 could control the inflammatory process by modulating the accumulation of mononuclear and polymorphonuclear cells. The absence of memory Th17 cells results in loss of the protective Th1 response [Khader and Cooper, 2008; Scriba et al., 2008; Torrado and Cooper, 2010]. However, these cells may also participate in the immunopathogenesis of leprosy, as they do in some autoimmune diseases (e.g., arthritis, systemic lupus erythematosus, psoriasis, and uveitis), with their potentially highly inflammatory response being the main mechanism determining tissue injury [Cua and Tato, 2010].

GENETIC INFLUENCE OF DISEASE OUTCOME IN LEPROSY

Analysis of the genetic basis of susceptibility to infectious diseases is potentially the most complex area of the multifactorial genetic diseases. The differences in disease prevalence and disease expression found in epidemiological studies among populations exposed to infectious agents reinforce the relevance of the genetic component. Several studies have identified genetic susceptibility to infectious disease and polymorphic genes that influence susceptibility to immune and inflammatory responses [Sachidanandam et al., 2001; Clementi and Di Gianantonio, 2006].

Genetic polymorphisms, small variations in gene structure, occur in more than 1% of the population, giving individuals different allelic forms for the same gene and providing the population with a large degree of genetic diversity. Polymorphisms may consist of variations in either a single nucleotide (SNPs) or larger nucleotide sequences, for example, repetitive DNA, represented by variable numbers of consecutive repetitions [Sha et al., 2010]. SNPs are the most common genetic variation and occur at a frequency of ~1 in 1,000 base pairs (bp) throughout the human genome [Aguillon et al., 2006]. These polymorphisms may be located in coding regions of genes or intergenic regions that can affect, or not affect, the protein sequence or their levels of expression. The observation of the existence of genetic polymorphisms in regulatory regions of immune response genes suggests that individual differences in the phenotypic in vitro expression of chemokines, cytokines, and other products of the immune response to pathogens could be attributable, at least in part, to these functional polymorphisms.

Many studies have described genes related to control of the innate and adaptive immune response against *M. leprae* [Prevedello, 2007; Scollard, 2006]. Various data sets from epidemiological studies suggest that genetic factors influence susceptibility and clinical response to both *M. tuberculosis* and *M. leprae* [Alcais et al., 2005, 2007; Alter et al., 2008, 2010; Berrington and Hawn, 2007; Blackwell, 2001a,b]. In the case of *M. leprae*, susceptibility appears to be associated with complex inheritance patterns determined by the

combined effect of the variation among many genes, with a modest contribution of each individual polymorphism. Many of these genes are associated with the immune response and operate on two levels: either in the innate immune response, influencing the resistance mediated by monocyte lineage cells, or in the acquired immune response, influencing the quantity and quality of specific cellular immunity generated by the infected individuals [Blackwell et al., 1994; Casanova and Abel, 2002; Plancoulaine et al., 2002; Scollard et al., 2006a; Zhang et al., 2009b].

A gene controlling susceptibility or resistance to intracellular pathogens has been identified [Skamene, 1994; Vidal et al., 1993]. Originally called natural resistance associated macrophage protein 1 (NRAMP 1), it is currently known as SLC11A1 and is located at a single locus on chromosome 1 in mice. The murine protein influence pathogen viability and/or replication in macrophages by transporting iron and other divalent cations across phagosomal membranes. In humans, a gene highly homologous to SLC11A1 is located on chromosome 2q35. SLC11A1 has many pleiotropic effects on macrophage activation, including regulation of the CXC chemokine KC, interleukin-1β (IL-1β), inducible nitric oxide synthase (iNOS), major histocompatibility complex (MHC) class II molecules, TNF-α, NO) release, L-arginine flux, oxidative burst, and tumoricidal as well as antimicrobial activity [Blackwell, 2001b; Blackwell et al., 1994]. Polymorphisms in this gene is associated with susceptibility to various infectious diseases. In Malawi, Blackwell and Searle [1999] described significant SLC11A1 haplotype associations with IFN-γ responses to *M. tuberculosis* antigen. Polymorphism at SLC11A1 also influences immune response to "priming/vaccinating" exposures to mycobacteria. The effectiveness of BCG in protecting against leprosy is associated with polymorphism at SLC11A1 in the Malawian population. Polymorphism at SLC11A1 is also associated with a Mitsuda-type skin test reactivity to leprosy antigens [Alcais et al., 2000, 2005; Blackwell and Searle, 1999].

Variants in the shared PARK2 and PACRG regulatory region are common risk factors for *M. leprae* infection [Mira et al., 2004]. A strong association (i.e., OR 3–5), was demonstrated between the PARK2_e01(2599) polymorphism and leprosy. This locus is a specific promoter region of PARK2 and the co-regulator gene, PACRG, located on chromosome 6q25–q27, which encodes a specific ligase that acts on the ubiquitin-proteasome degradation of intracellular proteins. It should be noted, however, that a recent genome-wide scan study in a large sample of Chinese population did not observe an association of the disease "risk" with the loci: PARK2–PACRG [Zhang et al., 2009b]. Although discrepant results have arisen, it remains appealing to speculate a role for PARK2–PACRG in leprosy, given that this pathway regulates the processing of antigenic proteins within macrophages, thus affecting the antigen presentation to lymphocytes and induction of acquired immune response. Needless to say, the precise mechanism by which the gene influences susceptibility to leprosy, however, remains to be determined [Scollard et al., 2006a].

TLRs play an important role in the recognition of pathogens and subsequent cytokine production and T-cell activation [Heine and Lien, 2003]. Studies of leprosy patient cells indicate that TLR2 controls the production of cytokines that act in cell signaling and other aspects of resistance to *M. leprae* [Bochud et al., 2003]. A TLR2 mutation, Arg677Trp, has been associated with signicantly lower IL-12 and TNF-α production, but higher IL-10, in comparison with PBMC from those with the TLR2 wild-type gene, suggesting that this TLR2 gene mutation can provide a molecular mechanism for the poor cellular immune response associated with lepromatous leprosy [Kang et al., 2004].

Similar to TLRs, NOD2 is also a pathogen-associated molecular pattern that binds to molecules of intracellular pathogens. An association between the SNPs of genes NOD2, RIPK2, and MB leprosy has been found suggesting that variants of genes in the NOD2 signaling pathway are associated with susceptibility to *M. leprae* infection [Zhang et al., 2009b]. The MHC is the most polymorphic genetic system in mammals and has been studied in relation to a wide variety of diseases of different etiologies, including infectious diseases. Located on chromosome 6p21, the main role of different molecules within the MHC (or HLA) is the antigen presentation to the T-cell receptor (TCR), which is crucial for the induction of cell-mediated immune response [Germain and Margulies, 1993]. Susceptibility to an infectious disease may be attributable to a particular combination of HLA alleles that are not linked to the peptide in an appropriate manner, or for which the HLA-binding peptide does not develop an adequate response of the T cells [Klein and Sato, 2000; Germain and Margulies, 1993]. Previous studies have documented that HLA-related genes are involved in controlling the clinical outcome of infectious diseases and leprosy at both class I and class II regions [Alcais et al., 2005; Geluk and Ottenhoff, 2006; Klein and Sato, 2000a,b; Ottenhoff et al., 1986, 2005; Vanderborght et al., 2007]. Variants of HLA genes, in particular HLA-DRB1, are associated with leprosy [Ottenhoff et al., 1986], and both protective and risk alleles have been described [Geluk and Ottenhoff, 2006; Ottenhoff et al., 1986, 2005]. A strong association within the HLA (chromosome

6p21) and chromosome 16q12 and additional associations of 13q14 with leprosy within the HLA region. One was within the HLA-B–HLA-C locus (encoding classes I; B and C) and the other was within the HLA-DR–DQ locus, allele A/G (encoding class II; DR and DQ) [Zhang et al., 2009a]. These associations of leprosy with the HLA-DR–DQ locus and the previously described association with HLA-DR-B1 are likely the result of an extensive linkage disequilibrium within the HLA region.

More recently, Sunny and colleagues [2010] conducted an association analysis of more than 1,500 individuals from different case-control and family studies. These investigators observed consistent associations between genetic variants in both TLR1 and the HLA-DRB1/DQA1 regions with susceptibility to leprosy (TLR1 I602S, case-control $P = 5.761028$, OR = 0.31, 95% CI = 0.20–0.48, and HLA-DQA1 rs1071630, case-control $P = 4.9610214$, OR = 0.43, 95% CI = 0.35–0.54). These associations suggest that TLR1 and HLA-DRB1/DQA1 are major susceptibility genes in susceptibility to leprosy. Further population differentiation analysis shows that the *TLR1* locus is extremely differentiated and that the protective dysfunctional 602S allele is rare in Africa but expands to become the dominant allele among individuals of European descent. This supports the hypothesis that this locus may be under selection by Mycobacteria or other pathogens recognized by TLR1 and its co-receptors [Wong et al., 2010]. These observations provide insight into the long-standing host–pathogen relationship between human and Mycobacteria and highlight the key role of the TLR pathway in infectious diseases.

The VDR gene, located on chromosome 12q12, encodes an intracellular receptor protein that binds to the active metabolite of vitamin D, 1α, $25(OH)_2D_3$. The active form of vitamin D not only regulates calcium and bone metabolism but also has an immunoregulatory role mediated through binding to the vitamin D receptor (VDR) in monocytes, macrophages, and activated lymphocytes [Rigby, 1988; Rigby et al., 1990; Rigby and Waugh, 1992]. VDR ligation leads to activation of these cells and subsequently influences the function of T $CD4^+$ and $CD8^+$ lymphocytes [Hayes et al., 2003]. The association between vitamin D physiology and infectious disease is supported by genetic studies implicating polymorphisms in the gene encoding the VDR in disease susceptibility [Uitterlinden et al., 2004]. A *Taq*I restriction fragment length polymorphism defines a single base change, C to T, in codon 352 at the 3′ end of the VDR gene. This site is in very strong linkage disequilibrium with a neighboring *Bsm*I site polymorphism, and in less strong linkage disequilibrium with a cluster of other sequence changes at the 3′ end of this gene. The less common allele of the *Taq*I site, designated "t," has been associated with higher levels of mRNA expression in transient transfection assays. The tt genotype (or tightly linked BB *Bsm*I genotype) has been associated with lower bone mineral density.

In a case-control study of 2,015 African subjects, homozygotes for the Taq1 polymorphism (genotype tt) were signicantly underrepresented in TB patients [Bellamy et al., 1999]. In a study performed with 231 leprosy patients (107 tuberculoid and 124 lepromatous) from Calcutta, India, the tt genotype was found at significantly higher frequency in tuberculoid leprosy (odds ratio [OR] = 3.22 [95% CI 1.47–7.13]) than in the controls. In contrast, the TT genotype was found at increased frequency in the lepromatous leprosy group compared with the controls (OR 1.67 [95% CI 1.02–2.75]). Heterozygotes of genotype Tt were found less frequently in both leprosy types than in controls (for leprosy vs. controls: OR 0.58 [95% CI 0.38–0.89]) [Goulart et al., 2006; Hill, 1998; Pereira et al., 2009; Roy et al., 1999; Velarde Felix et al., 2009].

Migration of monocytes from the bloodstream across the vascular endothelium is required for routine immunological surveillance of tissues, as well as in response to inflammation, and monocyte chemoattractant protein-1 (MCP-1/CCL2) is a key chemokine that regulates this process. Flores-Villanueva et al. [2005] found a strong association of pulmonary tuberculosis with a common promoter polymorphism of MCP1/CCL2 -2518G/A in two geographically and ethnically distinct populations (Mexico and Korea). The odds of developing tuberculosis were 2.3- and 5.4-fold higher in carriers of MCP-1 genotypes AG and GG than in homozygous AA. Cases of homozygous GG had the highest plasma levels of MCP-1 and the lowest plasma levels of IL-12p40, and these values were negatively correlated. The data of Flores-Villanueva and colleagues [2005] also demonstrated that persons bearing the MCP-1 genotype GG produce high concentrations of MCP-1, which inhibits production of IL-12p40 in response to *M. tuberculosis*. These data suggest that high MCP-1 increases the likelihood that *M. tuberculosis* infection progresses to active pulmonary tuberculosis [Flores-Villanueva et al., 2005]. The same polymorphism has also been associated with Chagas cardiomyopathy and mucosal leishmaniasis in Brazil [Ramasawmy et al., 2006, 2010]. Although this polymorphism has not been studied in leprosy patients, based on these data in other diseases caused by intracellular agents, we speculate that associations might be found.

A conserved region on mouse chromosome 11/human 17q11–q21 carries a susceptibility gene(s) for intramacrophage pathogens. The region is rich in candidate genes, including NOS2A, CCL2/MCP-1,

CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCR7, STAT3, and STAT5A/5B. Jamieson and colleagues [2004] studied the equivalent region in man in 92 multicase tuberculosis families (627 individuals) and 72 multicase leprosy families (372 individuals) from Brazil. Multipoint nonparametric analysis (ALLEGRO) using 16 microsatellites showed two peaks of linkage for leprosy at D17S250 (Z(lr), score 2.34; $P = 0.01$) and D17S1795 (Z(lr) 2.67; $P = 0.004$), and a single peak for tuberculosis at D17S250 (Z(lr) 2.04; $P = 0.02$). To determine whether one or multiple genes contributed to these effects, 49 informative SNPs were typed in candidate genes. Family-based allelic association testing (FBAT) demonstrated significant associations with tuberculosis susceptibility at four loci (NOS2A, CCL18, CCL4, Mb-STAT5B). Stepwise conditional logistic regression analysis showed that the four genes contributed to the main effects, consistent with a cluster of susceptibility genes across 17q11.2 [Jamieson et al., 2004].

In patients with resistant forms of the disease (tuberculoid) and type 1 reaction, the serum levels of TNF-α are elevated; expression of this cytokine is also increased in skin lesions. The TNF-α gene is located in the MHC class III on chromosome 6p21. Several polymorphisms in this gene have been identified, especially in the promoter region. Because of the wide range of influence of TNF-α on cellular immunity, these promoter region polymorphisms are of great interest as possible modulators of the degree of host response and therefore of clinical forms of leprosy. Several studies have described genetic associations of TNF-α alleles with different types of leprosy [Franceschi et al., 2009; Goulart and Goulart, 2009; Roy et al., 1997; Sapkota et al., 2010; Sarno et al., 2000; Vejbaesya et al., 2007]. An association of TNF-308 G/A polymorphism with lepromatous leprosy was observed in an Indian population [Roy et al., 1997]. In a sample of the Brazilian population, a higher frequency of GG genotype (85.5% vs. 74.1% in healthy controls, $P = 0.009$) of the TNF-308G/A, along with a decreased frequency of GA/AA genotypes was observed among leprosy patients as compared with the control group (14.5% vs. 25.9%, $P = 0.009$). The GG genotype was particularly higher in patients with tuberculoid (TT) and borderline (BB) leprosy (90.5% and 89.8%, respectively) [Franceschi et al., 2009], suggesting that the TNF-α gene is actively involved in resistance to *M. leprae*.

Given the critical role of IFN-γ in limiting mycobacterial infections, SNPs in the *IFNG* gene have been evaluated in several genetic epidemiological studies. The SNP +874T/A, more specifically the +874T allele, has been associated with protection against infectious diseases, including tuberculosis and leprosy [Amim et al., 2008; Cardoso et al., 2010; Pacheco et al., 2008; Rossouw et al., 2003]. In leprosy, the association of the *IFNG* locus was evaluated in case-control studies examining 2,125 Brazilian subjects from the state of São Paulo, and in 1,370 individuals from Rio de Janeiro using the +874 T/A (rs2430561), +2109 A>G (rs1861494), and rs2069727 SNPs. Results of the case-control studies indicate a protective effect for +874T carriers (OR adjusted = 0.75; $P = 0.005$ for both studies combined). The polymorphisms affected spontaneous IFN-γ release by PBMC, being higher among +874T carriers. Not surprisingly, combining data from tuberculosis and leprosy, the results indicate that the SNP +874T/A plays a role in resistance to mycobacterial diseases [Cardoso et al., 2010].

Malhotra and colleagues [2005] evaluated the association of six SNPs in the IL-10 promoter in 282 Indian leprosy patients and 266 healthy controls by direct polymerase chain reaction (PCR) sequencing and showed that the extended haplotype: -3575T/-2849G/-2763C/-1082A/-819C/-592C was associated with resistance to leprosy, using either a binomial (controls vs cases, $P = 0.01$, OR 0.58 [CI 0.37–0.89]) or ordinal (controls vs PB vs MB, $P = 0.004$) model, whereas the IL-10 haplotype -3575T/-2849G/-2763C/-1082A/-819T/-592A was associated with the risk of development of severe form of leprosy ($P = 0.0002$) [Malhotra et al., 2005]. In a case-control study that included 374 patients and 380 controls, and with meta-analysis (5 studies; 2702 individuals), Pereira and colleagues [2009] also found an association between -819T allele with leprosy susceptibility. Haplotypes combining promoter SNPs also implicated a haplotype carrying the -819T allele in leprosy susceptibility (OR 1.40; $P = 0.01$). Functional analyses revealed that -819T carriers produced lower levels of IL-10 when compared with noncarriers in PBMC stimulated with *M. leprae* antigens [Pereira et al., 2009]. These data strongly suggest the involvement of the IL-10 locus in the outcome of leprosy, at least in the Brazilian and Indian populations.

Interleukin-12 (IL-12) is secreted by macrophages and dendritic cells and is a potent inducer of IFN-γ production by T-helper cells 1 (Th1), which is partly dependent on the degree of expression of IL-12 receptor (IL-12R) on the cell surface [Trinchieri, 1994]. IL-12R is composed of two protein subunits, designated β1 and β2; β2 chain expression is a crucial determinant of Th1/Th2 balance, as STAT4 is activated by interaction with a tyrosine residue in the cytoplasmic domain of IL-12Rβ2 [Carr et al., 1997; Naeger et al., 1999]. Expression of IL-12Rβ2 is greater in tuberculoid lesions than in lepromatous lesions, whereas the expression of IL-12Rβ1 is similar in both [Kim et al., 2001]. It is believed that susceptibility to

various diseases related to mycobacterial pathogens could be determined by the degree of expression of IL-12Rβ2, which can be regulated by genetic factors, including polymorphism IL12Rβ2 [Ohyama et al., 2005, 2008]. The reported lack of expression of IL-12Rβ1 caused by mutations in the human gene, resulting in immunodeficiency with infections by *Mycobacteria* species as predominant disease, demonstrates the essential role of IL-12 in resistance to infections caused by intracellular parasite [Altare et al., 2001; de Jong et al., 1998]. Polymorphisms in the 5′ flanked region of the gene for the IL12Rβ2 receptor can affect the expression of the receptor β2 chain of the IL-12, resulting in individual differences in the intensity of cell-mediated immune response to mycobacteria, leading to lepromatous and tuberculoid types of disease [Ohyama et al., 2005].

SUMMARY

Leprosy remains an elusive, yet highly instructive, disease in which to examine a multitude of clinically and scientifically relevant questions. From the immunological point of view, leprosy is a rare model of human disease, as its wide spectral presentation allows for direct and controlled comparison of innate and adaptive responses and their development. Genetically, the spectral outcome allows for powerful analyses of the impact of, or association with, particular SNPs or allelic variants and disease development. Although considerable progress has been achieved in understanding leprosy and the factors involved with its clinical outcomes, much still needs to be learned.

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ANEXO 1
Ficha de avaliação neurológica

FORMULÁRIO PARA AVALIAÇÃO NEUROLÓGICA SIMPLIFICADA

Nome _____ DataNasc. ____/____/____
Ocupação: _____ Sexo: M ☐ F ☐
Município: _____ Unidade Federada: _____
Classificação Operacional: PB ☐ MB ☐ Data Inicio PQT: ___/____/___ Data Alta PQT: ___/____/____

FACE	1ª / /		2ª / /		3ª / /	
Nariz	**D**	**E**	**D**	**E**	**D**	**E**
Queixa principal						
Ressecamento (S/N)						
Ferida (S/N)						
Perfuração de septo (S/N)						
Olhos	**D**	**E**	**D**	**E**	**D**	**E**
Queixa principal						
Fecha olhos s/ força (mm)						
Fecha olhos c/ força (mm)						
Triquíase(S/N) / Ectrópio(S/N)						
Dimin. sensib. córnea (S/N)						
Opacidade córnea (S/N)						
Catarata (S/N)						
Acuidade Visual						

Legenda: N = não S = Sim

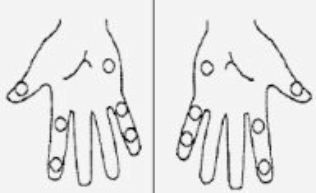
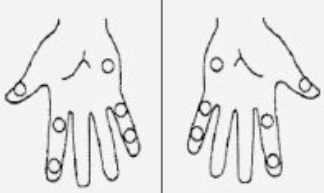
Membros Superiores	1ª / /		2ª / /		3ª / /	
Queixa principal						
Palpação de nervos	**D**	**E**	**D**	**E**	**D**	**E**
Ulnar						
Mediano						
Radial						

Legenda: N = normal E = espessado D = dor

Avaliação da Força	1ª / /		2ª / /		3ª / /	
	D	**E**	**D**	**E**	**D**	**E**
Abrir dedo mínimo Abdução do 5º dedo (nervo ulnar) 						
Elevar o polegar Abdução do polegar (nervo mediano) 						
Elevar o punho Extensão de punho (nervo radial) 						

Legenda: **F**=Forte **D**=Diminuída **P**=Paralisado **ou** **5**=Forte, **4**=Resistência Parcial, **3**=Movimento completo, **2**=Movimento Parcial, **1**=Contração, **0**=Paralisado

Inspeção e Avaliação Sensitiva

1ª / /		2ª / /		3ª / /	
D	**E**	**D**	**E**	**D**	**E**
					

Legenda: Caneta/filamento lilás(2g): Sente ✓ Não sente **X** **ou** Monofilamentos: seguir cores
Garra móvel: M Garra rígida: R Reabsorção: ▨ Ferida: ⬠

MEMBROS INFERIORES	1ª / /		2ª / /		3ª / /	
Queixa principal						
Palpação de nervos	D	E	D	E	D	E
Fibular						
Tibial posterior						

Legenda: N = normal E = espessado D = dor C = choque

Avaliação da força	1ª / /		2ª / /		3ª / /	
	D	E	D	E	D	E
Elevar o hálux Extensão de hálux (nervo fibular)						
Elevar o pé Dorsiflexão de pé (nervo fibular)						

Legenda: F = Forte D=Diminuída P=Paralisado ou 5=Forte, 4=Resistência Parcial, 3=Movimento completo, 2=Movimento parcial, 1=Contração, 0=Paralisado

Inspeção e Avaliação Sensitiva

1ª / /		2ª / /		3ª / /	
D	E	D	E	D	E
					

Legenda: Caneta filamento lilás (2g) Sente✓ Não sente X ou Monofilamentos: seguir cores

Garra móvel: M Garra rígida: R Reabsorção: //// Ferida: ⬭

CLASSIFICAÇÃO DO GRAU DE INCAPACIDADE (OMS)

DATA DA AVALIAÇÃO	OLHOS		MÃOS		PÉS		MAIOR GRAU	ASSINATURA
	D	E	D	E	D	E		
Aval. diagnóstico / /								
Aval. de alta / /								

LEGENDA PARA PREENCHIMENTO DO GRAU DE INCAPACIDADES

GRAU	CARACTERÍSTICAS
0	Nenhum problema com os olhos, mãos e pés devido à hanseníase.
I	perda da sensibilidade nos olhos. perda da sensibilidade nas mãos e/ou pés. (não sente 2g ou toque de caneta)
II	Olhos: lagoftalmo e/ou ectrópio; triquíase; opacidade corneana central; acuidade visual menor que 0,1 ou não conta dedos a fim. Mãos: lesões tróficas e/ou lesões traumáticas; garras; reabsorção; mão caída. Pés: lesões tróficas e/ou traumáticas; garras; reabsorção; pé caído; contratura do tornozelo

MONOFILAMENTOS

COR	Gramas
Verde	0,05
Azul	0,2
Lilás	2,0
Verm. Fechado	4,0
Verm. Cruzado	10,0
Verm. Aberto	300,0
Preto	s/resposta