

UNIVERSIDADE FEDERAL DE UBERLÂNDIA
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BRUNO DE CARVALHO DORNELAS

PAPEL DOS ACHADOS HISTOPATOLÓGICOS E SOROLÓGICOS NO DIAGNÓSTICO
PRECOCE DE RECIDIVA NA HANSENÍASE E FALÊNCIA TERAPÊUTICA

UBERLÂNDIA

2023

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PRECOCE DE RECIDIVA NA HANSENÍASE E FALÊNCIA TERAPÊUTICA

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Medicina da Universidade Federal de Uberlândia, como requisito parcial para obtenção do título de Doutor em Ciências da Saúde.

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*Aos pacientes com hanseníase, que moldaram
este trabalho, em seu processo de saúde-
adoecimento, com suas vidas, fonte de
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*“Educação não transforma o mundo.
Educação muda as pessoas.
Pessoas transformam o mundo”
Paulo Freire (1979, p. 84).*

RESUMO

Introdução: A hanseníase é uma doença granulomatosa infecciosa milenar que ainda constitui um problema de saúde pública no mundo. Apesar dos avanços, os recursos tecnológicos não são acessíveis universalmente. O tratamento com poliquimioterapia (PQT) reduziu a prevalência da doença, mas sua eliminação não será alcançada facilmente. Ao menos enquanto não se definirem critérios objetivos para a cura real. Outro problema subnotificado são as falências terapêuticas (FT) e recidivas na hanseníase (RH). **Objetivos:** Esta tese foi dividida em dois estudos, cujos objetivos foram detectar fatores laboratoriais histopatológicos, sorológicos e moleculares para a recidiva (Estudo 1) e falência terapêutica (Estudo 2) na hanseníase. **Materiais e métodos:** Tratam-se de estudos caso-controle que recrutaram 756 pacientes pareados e acompanhados em um centro de referência nacional em hanseníase. No Estudo 1, incluíram-se 40 casos de recidiva e 40 casos controle. No Estudo 2, 40 casos de falência terapêutica e 40 casos controle foram incluídos. Variáveis clínicas, epidemiológicas e laboratoriais foram analisadas ao fim do tratamento com PQT, com inferência aos achados histopatológicos de pele e comparadas com os resultados dos testes sorológico (ELISA anti-PGL-I) e molecular. **Resultados:** No estudo 1, nas amostras de pele, infiltrado linfocitário neural/perineural (OR=4,67; $p=0,0076$) e granuloma espumoso (OR=15,55; $p=0,0005$) conferiram chance de 4,7 e 15,6 vezes para RH, respectivamente. Ademais, índice baciloscópico histológico (IBh) $\geq 3+$ acarretou sensibilidade e especificidade de 72% e 65%, respectivamente, para detecção da RH (AUC: 0,72; $p=0,0002$). Demais variáveis histológicas não mostraram associações significativas ($p > 0,05$). O índice ELISA (IE) ≥ 1 acarretou uma chance 4,7 vezes maior (OR=4,67; $p=0,0031$) para RH. Se IE $\geq 3,6$, encontraram-se sensibilidade e especificidade de 71% e 62%, respectivamente, para a detecção de RH (AUC: 0,70; $p=0,0012$). A combinação de infiltrado linfocitário neural/perineural (OR=4,58; $p=0,0350$), granuloma espumoso (OR=7,36; $p=0,0212$), IB ≥ 1 na amostra de pele (OR=1,39; $p=0,0485$) e IE ≥ 1 (OR=1,19; $p=0,0628$) foi mostrou-se capaz de predizer uma probabilidade de até 94,32% para a RH. No estudo 2, granuloma espumoso (OR=7,36; $p=0,0012$) e IBh (OR=1,55; $p=0,0004$) associaram-se à FT conferindo chance de 7,36 e 1,55 vezes, respectivamente. Ademais, IBh $\geq 3+$ acarretou sensibilidade e especificidade de 73% e 78%, respectivamente, para detecção da FT (AUC: 0,75; $p=0,0001$). Demais variáveis histológicas não mostraram associações significativas ($p > 0,05$). O IE ≥ 1 acarretou uma chance 1,4 vezes maior (OR=1,4; $p=0,0019$) para a FT. Se IE $\geq 3,95$, encontraram-se sensibilidade e especificidade de 79% e 59%, respectivamente, para a detecção de FT (AUC: 0,74; $p=0,0001$). A positividade para DNA de *Mycobacterium leprae* por qPCR acarretou uma chance 3,43 (OR=3,43; $p=0,0201$) maior para a FT. A combinação de infiltrado linfocitário neural/perineural (OR=4,06; $p=0,0233$), granuloma espumoso (OR=8,41; $p=0,0002$), IB ≥ 1 na amostra de pele (OR=1,55; $p=0,0030$) e IE ≥ 1 (OR=1,26; IC95% 1,00-1,58; $p=0,0030$) mostrou-se capaz de predizer uma probabilidade de até 95,41% para a FT. **Conclusões:** Os achados histopatológicos, sorológicos e moleculares ao final da PQT são capazes de discriminar aqueles pacientes com maior chance de recaída na hanseníase. Por fim, os resultados reforçam a importância do exame anatomopatológico no monitoramento da resposta terapêutica à PQT. Portanto, propõe-se a adoção de ferramentas laboratoriais como critérios objetivos definidores da real cura na hanseníase.

Palavras-chave: Hanseníase; Recidiva; Falência Terapêutica; Histopatologia.

ABSTRACT

Introduction: Leprosy is an ancient infectious granulomatous disease, and still is a public health problem worldwide. Despite advances, technological resources are not universally accessible. Treatment with multidrug therapy (MDT) has reduced leprosy prevalence, nonetheless elimination will not be achieved easily. At least not until objective criteria for real leprosy healing are defined. Besides, therapy failure (TF) and leprosy relapse (LR) are underreported problem. **Objectives:** We divided this research into two studies. The main objective was to detect histopathological, serological and molecular laboratory factors for relapse (Study 1) and therapy failure (Study 2) in leprosy. **Materials and methods:** These are case-control studies with 756 paired individuals followed up at a national reference center for leprosy. In Study 1, 40 relapse cases and 40 control cases were included. In Study 2, 40 cases of therapy failure and 40 control cases were included. Clinical, epidemiological and laboratory variables were analyzed at the end of MDT, with inference to skin histopathological findings and compared with the results of serological (anti-PGL-I) and molecular tests. **Results:** In study 1, in skin samples, neural/perineural lymphocytic infiltrate (OR=4.67; $p=0.0076$) and foamy granuloma (OR=15.55; $p=0.0005$) resulted in respective increased odds of 4.7 and 15.6 for LR. Furthermore, hBI $\geq 3+$ resulted in sensitivity and specificity of 72% and 65%, respectively, for detecting LR (AUC: 0.72; $p=0.0002$). No associations were found for the other histological surrogates. Concerning serology (anti-PGL-I), the ELISA index (EI) ≥ 1 resulted in 4.7 greater odds (OR=4.67; $p=0.0031$) for LR. Neural/perineural lymphocytic infiltrate (OR=4.58; $p=0.0350$), foamy granuloma (OR=7.36; $p=0.0212$), and histological bacilloscopic index (hBI) ≥ 1 in the skin sample (OR=1.39; $p=0.0485$) and EI ≥ 1 (OR =1.19; $p=0.0628$) were analyzed together and showed significantly higher probability of up to 94.32% for LR. In study 2, foamy granuloma (OR=7.36; $p=0.0012$) hIB (OR=1.55; $p=0.0004$) were associated with TF, increasing odds at 7.36 and 1.55, respectively. Additionally, hIB $\geq 3+$ resulted in a sensitivity and specificity of 73% and 78%, respectively, for detecting TF (AUC: 0.75; $p=0.0001$). No associations were found for the other histological surrogates ($p > 5$). EI ≥ 1 resulted in a increased odds of 1.4 (OR=1.4; $p=0.0019$) for TF. If IE ≥ 3.95 , sensitivity and specificity of 79% and 59%, respectively, were found for TF (AUC: 0.74; $p=0.0001$). *Mycobacterium leprae* DNA positivity by qPCR resulted in an increased 3.43-fold (OR=3.43; $p=0.0201$) for TF. Neural/perineural lymphocytic infiltrate (OR=4.06; $p=0.0233$), foamy granuloma (OR=8.41; $p=0.0002$), hBI ≥ 1 (OR=1.55; $p=0.0030$) and EI ≥ 1 (OR=1.26; 95%CI1.00-1.58; $p=0.0030$) together were capable of predicting a probability of up to 95.41% for the FT. **Conclusions:** Histopathological, serological and molecular findings at the end of MDT are capable of discriminating those patients with a greater chance of leprosy relapse and failure treatment. Finally, the results reinforce the importance of pathological skin examination for monitoring therapeutic response to MDT. Therefore, we propose the use of laboratory tools to objectively defining criteria for the real cure for leprosy.

Keywords: Leprosy; Relapse; Therapy failure; Histopathology.

LISTA DE ILUSTRAÇÕES

Tese

Figura 1 - Fases do caminho para a eliminação da hanseníase	21
Figura 2 - Classificação de Ridley-Jopling.....	26
Figura 3 - Manifestações clínicas decorrentes do padrão de resposta imune ao <i>M. leprae</i>	28
Figura 4 - Interação <i>M. leprae</i> versus hospedeiro	29
Figura 5 - Manifestação cutâneas da hanseníase	30
Figura 6 - Lâmina histológica representativa da pele e da gordura subcutânea	32
Figura 7 - Hanseníase indeterminada	34
Figura 8 - Hanseníase tuberculoide	35
Figura 9 - Hanseníase dimorfo-tuberculoide	36
Figura 10 - Hanseníase dimorfo-dimorfa	37
Figura 11 - Hanseníase dimorfo-vichowiana.....	39
Figura 12 - Hanseníase vichowiana.....	40
Figura 13 - Hanseníase histoide.....	41
Figura 14 - Reação hansênica tipo 1.....	45
Figura 15 - Reação hansênica tipo 2.....	47
Figura 16 - Falência terapêutica na hanseníase	50
Figura 17 - Alterações de persistência de atividade de doença pós-PQT.....	51
Figura 18 - Fenótipos de macrófagos	53
Figura 19 - Índice baciloscópico na biópsia de pele pós-PQT	55

Estudo 1

Figure 1. Selection and pairing of individuals from the treatment failure group, and the control group	67
Figure 2. Values for sensitivity, specificity, and area under the curve referring to the skin histological bacillary index and ELISA index at the end of treatment.	73
Figure 3. Foamy granulomas.	81
Figure 4. Histological bacillary index.	83
Figure 5. Neural/perineural lymphocytic infiltrate.....	84

Estudo 2

.Figure 1. Selection and pairing of individuals from the Relapse Group and Control Group 1.	109
.Figure 2. Histopathological characteristics evaluated in the skin sample at the end of multidrug therapy for leprosy.	111
Figure 3. Sensitivity and specificity values and area under the curve as a function of the skin histological bacillary index and ELISA index at the end of treatment.	114
.Figure 4. Bacilloscopy on the skin sample with Faraco-Fite staining after the end of multidrug therapy.	121
.Figure 5. Lymphocytic infiltration of nerves.....	126
.Figure 6. Involvement of nerves by lymphocytes after leprosy treatment	127
.Figure 7. Skin foamy granuloma after fixed duration treatment	129

LISTA DE TABELAS

Tese

Tabela 1 - Número de casos novos de hanseníase	23
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Estudo 1

Table 1. Histopathological characteristics evaluated in the skin sample at the end of multidrug therapy for leprosy: definitions.	69
Table 2. The protocol adopted for Fite-Faraco staining	70
Table 3. Logarithmic scale of Ridley and Hilson for bacillary index on histological sections	70
Table 4. Histopathological characteristics in skin samples biopsied at the end of multidrug therapy.....	74
Table 5. Statistical significance of the analyzed variables, evaluated by univariate and multivariate logistic regression	75
Table S1. Numerical description of the variables.....	100
Table S2. Laboratory and clinical characteristics.....	101
Table S3. Performance on diagnostic tests.....	102
Table S4. Exposition of multivariate regression variables.....	102

Estudo 2

.Table 1. Histopathological characteristics in skin samples biopsied at the end of multidrug therapy.	115
.Table 2. Statistical significance of the analyzed variables, assessed by univariate and multivariate logistic regression.....	116
.Table 3. Proposal for an objective monitoring therapy response at the beginning, middle, and end of treatment.	130
.Table S1. Numerical description of the variables.....	144
.Table S2. Clinical and laboratory characteristics of the patients.	145
.Table S3. Performance of diagnostic tests.....	146
.Table S4. Exposition of multivariate regression variables.	146

LISTA DE ABREVIATURAS E SIGLAS

C	Grupo Controle
CREDESH	Centro de Referência Nacional em Dermatologia Sanitária e Hanseníase
DD	Dimorfo-Dimorfa
DT	Dimorfo-Tuberculoide
DV	Dimorfo-Virchowiana
EBSERH	Empresa Brasileira de Serviço Hospitalares
ELISA	Ensaio de imunoabsorção enzimática
FF	Faraco-Fite
FT	Grupo Falência Terapêutica
H&E	Hematoxilina e Eosina
HC	Hospital das Clínicas
I	Hanseníase Indeterminada
IB	Índice Baciloscópico
IBh	Índice Baciloscópico histopatológico
IBr	Índice Baciloscópico do raspado intradérmico
IFN- γ	Interferon gama
IgM	Imunoglobulina M
IL	Interleucina
<i>M. leprae</i>	<i>Mycobacterium leprae</i>
MB	Multibacilar
MS	Ministério da Saúde do Brasil
OMS	Organização Mundial de Saúde
PB	Paucibacilar
PCR	Reação em Cadeia da Polimerase
PGL-I	Glicolípido-Fenólico-I
PQT	Poliquimioterapia
qPCR	Reação em Cadeia da Polimerase em Tempo-Real
R	Grupo Recidiva
R&J	Classificação de Ridley-Jopling
RT-PCR	Reação em Cadeia da Polimerase por Transcriptase Reversa
SINAN	Sistema de Informação de Agravos de Notificação

Th1	Célula T auxiliar tipo 1
Th2	Célula T auxiliar tipo 2
TNF- α	Fator de necrose tumoral alfa
TT	Tuberculoide
UAPAT	Unidade de Anatomia Patológica
UFU	Universidade Federal de Uberlândia
VV	Virchowiano

SUMÁRIO

1	INTRODUÇÃO	19
2	FUNDAMENTOS	20
2.1	História.....	20
2.2	<i>Características epidemiológicas.....</i>	22
2.2.1	<i>Casos novos</i>	22
2.2.2	<i>Recidivas na hanseníase</i>	24
2.2.3	<i>Outros reingressos.....</i>	24
2.3	História natural da infecção por hanseníase	25
2.3.1	<i>Hanseníase</i>	25
2.3.2	<i>Classificação.....</i>	25
2.3.2.1	<i>Classificação de Ridley-Jopling.....</i>	26
2.3.2.2	<i>Classificação operacional.....</i>	31
2.4	Diagnóstico da hanseníase	31
2.5	Achados histopatológicos da hanseníase	31
2.5.1	<i>Achados histopatológicos ao início de tratamento.....</i>	32
2.5.1.1	<i>Hanseníase indeterminada.....</i>	33
2.5.1.2	<i>Hanseníase tuberculoide.....</i>	33
2.5.1.3	<i>Hanseníase dimorfo-tuberculoide.....</i>	33
2.5.1.4	<i>Hanseníase dimorfo-dimorfo</i>	33
2.5.1.5	<i>Hanseníase dimorfo-virchowiana</i>	38
2.5.1.6	<i>Hanseníase virchowiana</i>	38
2.5.1.6.1	<i>Hanseníase histoide.....</i>	38
2.5.2	<i>Baciloscopia do raspado intradérmico</i>	38
2.5.3	<i>Sorologia na hanseníase</i>	42
2.5.4	<i>Deteção de DNA do Mycobacterium leprae</i>	43
2.6	Estados reacionais da hanseníase	44
2.6.1	<i>Reação hansênica tipo 1</i>	44
2.6.1.1	<i>Achados histológicos.....</i>	44
2.6.2	<i>Reação hansênica tipo 2</i>	46
2.6.2.1	<i>Achados histológicos.....</i>	46
2.6.3	<i>Reação hansênica tipo 3</i>	46

2.6.3.1	<i>Achados histológicos</i>	46
2.7	Tratamento da hanseníase	48
2.8	Achados histopatológicos ao fim de tratamento	49
2.8.1	<i>Atividade de doença ao fim de tratamento</i>	49
2.8.2	<i>Alterações de regressão de doença ao fim de tratamento</i>	52
2.8.3	<i>Queda do índice bacioscópico ao fim de tratamento</i>	54
2.9	Definições na hanseníase pós-tratamento	54
2.9.1	<i>Alta por cura</i>	54
2.9.2	<i>Insuficiência terapêutica</i>	56
2.9.3	<i>Falência terapêutica</i>	56
2.9.4	<i>Recidiva na hanseníase</i>	57
2.10	Fatores para recorrência na hanseníase	58
2.10.1	<i>Resistência medicamentosa</i>	58
2.10.2	<i>Bacilos persistentes</i>	59
2.10.3	<i>Duração da PQT</i>	59
2.10.4	<i>Tempo de seguimento</i>	60
3	OBJETIVOS	61
3.1	Objetivo geral	61
3.2	Objetivos específicos	61
3.2.1	<i>Estudo 1</i>	61
3.2.2	<i>Estudo 2</i>	61
4	RESULTADOS	62
4.1	Estudo 1 - Role of histopathological, serological and molecular findings for the early diagnosis of treatment failure in leprosy. Part I	62
	Introduction	64
	Materials and methods	66
	<i>Study design and selection of cases</i>	66
	<i>Definition of treatment failure in leprosy</i>	68
	<i>Study variables</i>	68
	<i>Ethical aspects</i>	71
	<i>Statistical analyses</i>	72
	Results	72
	Discussion	77

	<i>Resolution of skin lesions</i>	80
	<i>Decrease in the bacillary index</i>	82
	<i>Decrease in the ELISA index</i>	84
	Conclusion	87
	<i>Limitations of the study</i>	88
	Conflicts of interest	88
	Ethical considerations	88
	Funding / Support	89
	List of abbreviations	89
	Supplemental Supporting Material	100
4.2	Estudo 2 - Role of histopathological, serological and molecular findings for the early diagnosis of leprosy relapse. Part II	103
	Abstract	104
	Introduction	105
	Material and Methods	107
	<i>Study design and selection of cases</i>	107
	<i>Definition of relapse in leprosy</i>	109
	<i>Study variables</i>	110
	<i>Ethical Aspects</i>	112
	<i>Statistical analyses</i>	112
	Results	113
	Discussion	118
	<i>Histological bacillary skin index at treatment discharge due to cure</i>	120
	<i>ELISA index of IgM anti-PGL-I serology</i>	122
	<i>Lymphocytic infiltrate of nerves</i>	126
	Conclusion	131
	Conflicts of interest	132
	Ethical considerations	132
	Financial support	132
	Acknowledgments	133
	List of abbreviations	133
	Supplementary Supporting Material	144
	5 CONSIDERAÇÕES FINAIS	147

REFERÊNCIAS	148
ANEXO A – FICHA DE NOTIFICAÇÃO/INVESTIGAÇÃO DE HANSENÍASE.....	160

1 INTRODUÇÃO

O Centro de Referência Nacional em Dermatologia Sanitária e Hanseníase (CREDESH), do Hospital das Clínicas (HC) da Universidade Federal de Uberlândia (UFU) da Empresa Brasileira de Serviço Hospitalares (EBSERH) é um dos seis centros de referência nacional em hanseníase do Brasil. O CREDESH tem sido cenário de assistência multiprofissional em hanseníase, ensino, pesquisa e extensão há décadas, motivo pelos quais é reconhecido internacionalmente. A Unidade de Anatomia Patológica (UAPAT) do HC/UFU/EBSERH é corresponsável pela assistência diagnóstica em patologia da hanseníase, ensino e pesquisa. Esta Unidade tem experiência na aplicação da classificação de Ridley-Jopling (R&J) e na baciloscopia histopatológica por meio da técnica de Faraco-Fite (FF).

Como não há critérios objetivos para a cura real na hanseníase, isto é, critérios que não se limitem apenas ao cumprimento do tempo de duração da poliquimioterapia (PQT) da Organização Mundial da Saúde (OMS), o CREDESH tem desenvolvido pesquisas para a proposição de protocolos de diagnóstico, monitoramento e cura real dos pacientes com hanseníase através de tecnologias como sorologia, testes moleculares, eletroneuromiografia e ultrassom ao longo da PQT. É nesse sentido, que acreditamos na importância de se regatar o papel da Patologia da Hanseníase na avaliação da resposta terapêutica à PQT.

Refletindo, desenhamos esta pesquisa centrada nos achados histopatológicos da biópsia de pele realizada após o término da PQT e seu valor preditivo para a recorrência da doença. Por tratar-se de uma doença infectocontagiosa e de grande potencial incapacitante, o diagnóstico precoce das recorrências deve ter protagonismo nas medidas de controle da hanseníase.

De fato, a introdução da PQT promoveu alta redução na prevalência mundial da hanseníase. Entretanto, sua eliminação parece ainda utópica. Isso, em parte, deve-se à insuficiência, falência terapêutica (FT) e recidiva de doença que têm como fatores a adesão ao tratamento, a resistência medicamentosa, a persistência de bacilos dentre outros. A ausência de nomenclatura e definições standardizadas na hanseníase certamente dificulta o diagnóstico e encoberta os verdadeiros números na epidemiologia da doença.

Esta tese é o produto desses questionamentos e reflexão e tem por objetivo demonstrar o valor preditivo dos achados histopatológicos, sorológicos e moleculares do momento de alta da PQT no rastreamento precoce das recaídas na hanseníase. Se detectada e tratada precocemente, a doença não causará incapacidades.

2 FUNDAMENTOS

2.1 História

A hanseníase é uma das doenças infecciosas mais antigas da humanidade, um flagelo desde a antiguidade sobre doentes desumanamente tratados durante muitos séculos, obrigados ao isolamento social e a viver em pobreza e solidão (Santacroce *et al.*, 2021). Na Índia, foram encontradas evidências de hanseníase num esqueleto que data de 2.000 a.C. (Robbins *et al.*, 2009).

Sobre a origem da hanseníase, a doença parece ter origem na África Oriental ou no Oriente Próximo e se espalhou com sucessivas migrações humanas sendo introduzida na África Ocidental e nas Américas por europeus ou norte-africanos nos últimos 500 anos (Monot *et al.*, 2005). Na América Latina, sua disseminação está relacionada ao tráfico de escravos africanos, que constituiu a principal via de entrada no continente durante a colonização espanhola e portuguesa (Eidt, 2004).

Somente em 1.873 teve seu agente etiológico, *Mycobacterium leprae* (*M. leprae*), identificado (Vogelsang, 1978). De certa forma, ao excluir a hereditariedade como etiologia, a descoberta do bacilo reduziu o preconceito contra os pacientes com hanseníase, porém mesmo nos dias atuais, esses pacientes ainda são estigmatizados, sobretudo por causa das sequelas neurológicas (Nascimento, 2013). Mais recentemente, em 2008, outra espécie causadora de hanseníase foi descoberta e denominada *Mycobacterium lepromatosis* (Han *et al.*, 2008).

A Declaração de Londres sobre Doenças Tropicais Negligenciadas foi um programa colaborativo de erradicação de doenças lançado em 2012 para erradicar ou prevenir a transmissão de doenças tropicais negligenciadas até o ano 2020 (Kumar; Preston; Phillips, 2022). A Estratégia Global de Hanseníase 2021–2030 “Rumo à zero hanseníase” foi desenvolvida em 2019 e 2020 e visa a eliminação da hanseníase definida como interrupção da transmissão (World Health Organization, 2021d) (Figura 1).

Figura 1 - Fases do caminho para a eliminação da hanseníase

	Phase 1	Phase 2	Phase 3	Phase 4	Remarks
Description	Till 'elimination as public health problem'	Till 'interruption of transmission'	From 'interruption of transmission till elimination of disease'	Post-elimination	To be defined in terms of number or rate of new cases detected in phases 2,3 and 4
Definition	Registered prevalence of <1 per 10,000 population	Zero new autochthonous child cases for five consecutive years	Zero autochthonous cases, to be sustained for at least three years	Sporadic new cases	PHASE 4 Zero case reports to be submitted for ten years Leprosy surveillance part of integrated disease surveillance
Milestones/ cut-offs for moving to the next phase	<1 case on treatment per 10,000 population	Zero new autochthonous child cases for five consecutive years	No new autochthonous cases for at least three years	Sporadic new cases allowed	

Um dos resultados significativos é a distinção entre os vários marcos relacionados com a eliminação. Quatro fases são delineadas para um programa de hanseníase em sua jornada rumo à eliminação e além.

Fonte: World Health Organization (2021c).

2.2 Características epidemiológicas

A hanseníase ainda é um problema de saúde pública especialmente pelo seu potencial incapacitante, efeito de discriminação e estigma sociais que atinge pessoas de ambos os sexos e de todas as faixas etárias (Brasil, 2022). No Brasil, esta doença é de investigação obrigatória que, após a confirmação diagnóstica, precisa ser notificada de forma compulsória através do Sistema de Informação de Agravos de Notificação (SINAN) (Brasil, 2020).

2.2.1 Casos novos

A hanseníase é uma doença tropical negligenciada e frequente no Brasil que continua a ser uma doença importante mundial, com prevalência de 140.546 casos notificados (taxa de prevalência de 16,9 por milhão de habitantes) em 2021 por 143 países (World Health Organization, 2021a) (Tabela 1). Nos últimos anos, houve redução no diagnóstico de hanseníase na população em geral e em menores de 15 anos que é atribuída à grave pandemia da COVID-19, durante a qual ocorreu a suspensão dos serviços regulares de saúde, dificultando o diagnóstico da doença (Paz *et al.*, 2022).

Globalmente, 8.492 novos casos foram diagnosticados com grau de incapacidade 2 (taxa de detecção de casos grau 2 de incapacidade de 1,1 por milhão de habitantes) (World Health Organization, 2021b). Esses números refletem diagnóstico tardio por deformidades visíveis.

No período de 2001 a 2021, foram notificados no Brasil 763.593 novos casos de hanseníase dos quais 5,13% (39.331/763.593) ocorreram no estado de Minas Gerais e município de Uberlândia apresentou 5,05% (1.980/39.331) das notificações de novos casos nesse período (Brasil, 2020). Nesse contexto, o Brasil ocupa o segundo lugar entre os países com maior número de casos no mundo, atrás apenas da Índia (World Health Organization, 2021a).

Em aspectos gerais, de 2001 a 2021, foram notificados 49.207 casos no país que apresentaram grau de incapacidade 2 no momento do diagnóstico dos quais 8,57% (4.216/49.207) foram identificados em Minas Gerais e Uberlândia, por sua vez, apresentou 14,14% (280/1.980) do total de notificações municipais, 6,64% das estaduais e 0,57% das notificações nacionais com este grau de incapacidade (Brasil, 2020).

Tabela 1 - Número de casos novos de hanseníase

Região	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
África	20.911	18.597	21.219	20.704	20.758	20.590	20.207	16.690	21.201	22.022
Américas*	33.084	33.789	28.806	27.343	29.101	30.956	29.935	19.195	19.826	21.387
Brasil	(31.044)	(31.064)	(26.395)	(25.218)	(26.875)	(28.660)	(27.863)	(17.979)	(18.318)	(19.635)
Europa	0	0	18	41	24	50	42	27	14	53
Oriente Médio	1.680	2.342	2.167	2.865	3.551	4.356	4.211	4.077	3.588	3.770
Pacífico Ocidental	4.570	4.299	3.617	3.879	4.052	4.166	3.984	2.699	2.432	2.450
Sudeste da Ásia	155.385	154.834	156.118	163.095	153.487	148.495	143.787	84.818	93.485	124.377
Total	215.630	213.861	211.945	217.927	210.973	208.613	202.166	127.506	140.546	174.059

* Número de casos novos de hanseníase nas Américas, discriminando entre () aqueles notificados pelo Brasil.

Fonte: World Health Organization (2023c).

A Estratégia Global de Hanseníase 2021–2030 “Rumo à zero hanseníase” foi desenvolvida por meio de um amplo processo de consulta com todas as principais partes interessadas durante 2019 e 2020 e visa a eliminação, entendida como fim da transmissão da hanseníase, até 2030 (World Health Organization, 2021d). Entretanto, novos casos em crianças continuam sendo notificados e indicam transmissão recente. Em 2021, foi observado um aumento de 4,7% (9.052/140.546) no número de novos casos infantis em comparação com 2020 (8.642) (World Health Organization, 2021a). Esses dados mostram que essa Estratégia da OMS ainda está longe de ser alcançada.

2.2.2 Recidivas na hanseníase

Em relação às recidivas, 51 países relataram que 3.201 casos após a conclusão da PQT, sendo o maior no Brasil (1.212), seguido pela Índia (510) em 2012 (World Health Organization, 2021a). Em Uberlândia, observaram-se 691 reingressos, entre os quais 226 eram recidivas, representando 32,71% (226/691) dos casos (Brasil, 2020). As taxas de recidiva na hanseníase são divergentes nas diferentes regiões do globo e variam entre 1% e 68,2% (Nascimento *et al.*, 2022; Rajan *et al.*, 2021; World Health Organization, 2017).

2.2.3 Outros reingressos

A Ficha de Notificação/Investigação utilizada para notificar os casos de hanseníase não permite a notificação dos casos de insuficiência e falência terapêuticas, que acabam sendo englobadas sob o termo “outros reingressos” (Anexo A), motivo pelo qual os dados são escassos na literatura nacional. Entretanto, um trabalho que analisou 50 casos de falência terapêutica diagnosticados num período de seis anos, observou-se taxa de 4,7% (50/1.059) (Nascimento *et al.*, 2022). Urge, portanto, adotar nomenclatura estandardizada pela comunidade científica e políticas públicas dos diversos países a fim de se reconhecer a miríade de situações da hanseníase.

2.3 História natural da infecção por hanseníase

2.3.1 *Hanseníase*

A hanseníase é uma doença infecciosa granulomatosa curável causada pelo *M. leprae* e menos frequentemente pelo *Mycobacterium lepromatosis*, ambos são micobactérias álcool-ácido resistentes (Alrehaili, 2023). Os bacilos demonstram tropismo sobretudo pelas zonas mais frias do organismo, como nervos periféricos, pele, mucosa respiratória e olhos, podendo infectar células epiteliais e mesenquimais (Anderson, 1976; Soares, 2021). Doença que acomete nervos e pele preferencialmente e tem potencial para graves e devastadoras consequências para o paciente (Goulart *et al.*, 2002; Ridley, 1978; Scholtz *et al.*, 2023). As deficiências físicas causadas pela hanseníase e seus efeitos destrutivos nos nervos e na pele geraram preconceito, medo e segregação em todas as sociedades (Santacroce *et al.*, 2021).

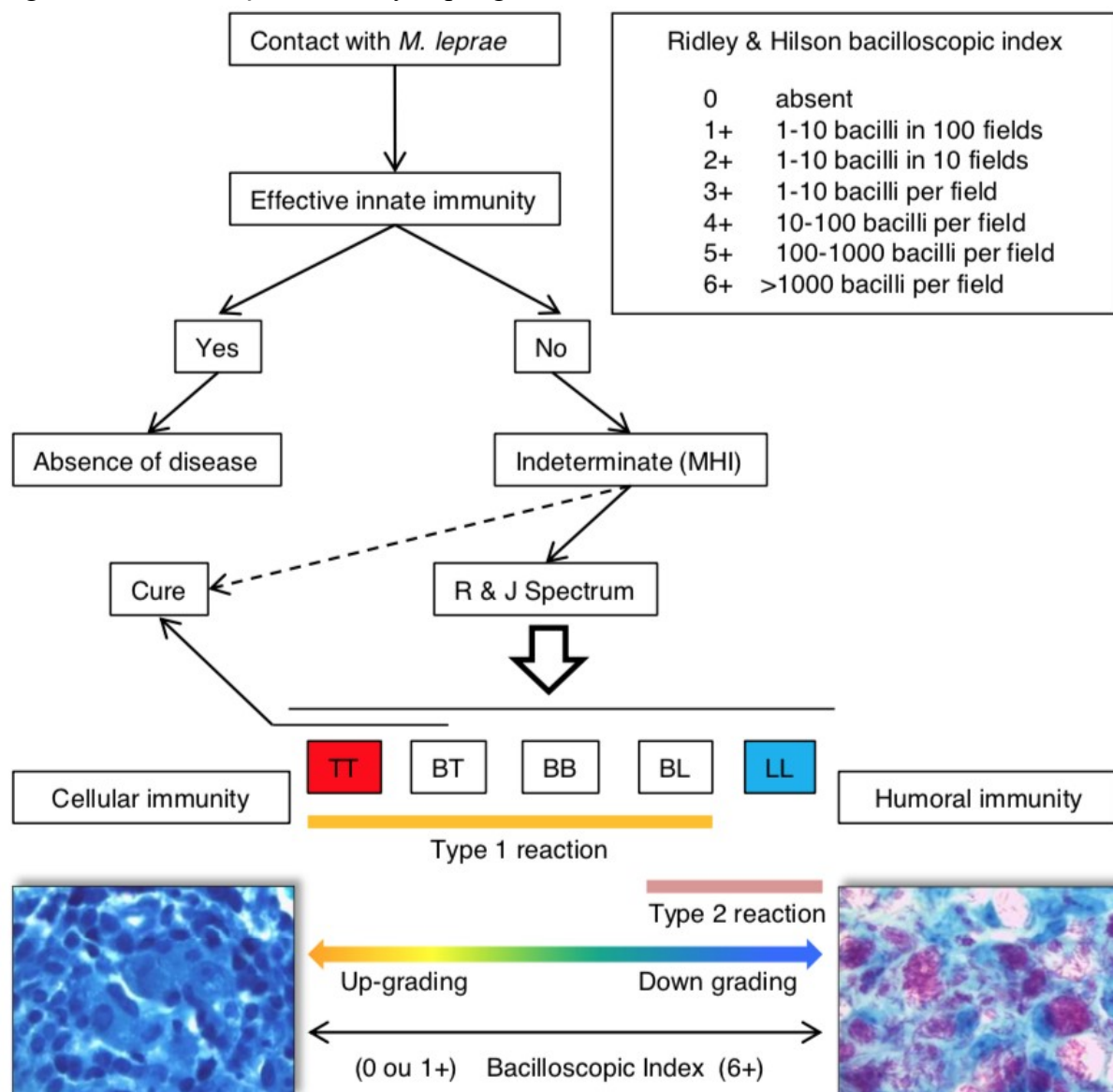
O período de incubação é altamente variável, com as manifestações da doença aparecendo de 6 meses a mais de 10 anos (Taggart *et al.*, 2022; White; Franco-Paredes, 2015). Entretanto, a maioria dos indivíduos não desenvolve a doença após a exposição e quando sintomas aparecem, a hanseníase tende a progredir de forma insidiosa (Frazier, 1943).

2.3.2 *Classificação*

A hanseníase é uma doença espectral suas manifestações clínicas dependem da interação entre o bacilo e o sistema imune do hospedeiro (Goulart *et al.*, 2002; Ridley; Jopling, 1966). Aqueles indivíduos que adocem após a exposição, apresentam inicialmente a forma clínica indeterminada (I), que pode ter remissão espontânea ou evoluir para alguma das formas clínicas descritas por Ridley e Jopling (Burns *et al.*, 2010).

A hanseníase I é caracterizada pela presença de uma ou poucas máculas hipocrômicas e/ou eritematosas, com superfície lisa e margens imprecisas, alopecias, que com o tempo, pode apresentar hipoidrose e perda sensorial geralmente mínima, dissociada, que frequentemente passa despercebida e que a maioria dos casos evolui para cura ou, na minoria dos pacientes evolui para as formas espectrais definitivas da doença (Yawalkar, 2002). Por ser uma forma clínica inicial, a hanseníase I não se inclui no espectro descrito por Ridley e Jopling (Figura 2).

Figura 2 - Classificação de Ridley-Jopling



TT: tuberculoid leprosy; BT: borderline-tuberculoid; BB: borderline-borderline; BL: borderline-lepromatous; LL: lepromatous leprosy

Fonte: Soares (2021).

2.3.2.1 Classificação de Ridley-Jopling

A classificação de Ridley-Jopling (R&J) para a hanseníase foi proposta em 1966 e baseia-se no padrão de resposta imunológica do hospedeiro, achados histopatológicos da lesão cutânea e no IB (Figura 2) (Ridley; Hilson, 1967; Ridley; Jopling, 1966; Soares, 2021). O espectro clínico descrito por Ridley e Jopling inclui duas formas polares e estáveis: tuberculoide (TT) e virchowiana (VV) (Ridley, 1978).

No polo TT, há predomínio da resposta imune celular mediada por linfócitos T, com formação de granulomas epitelioides, cerco à infecção e eliminação do *M. leprae* enquanto no polo VV, há ausência de resposta imune celular, prevalecendo a resposta humoral, permissiva à proliferação e disseminação bacilar (Ridley; Jopling, 1966). Entre os extremos, situam-se as três formas instáveis da hanseníase, dimorfa-tuberculoide (DT), dimorfa-dimorfa (DD) e dimorfa-virchowiana (DV), nas que a resposta é flutuante de acordo com o estado imunológico do doente (Goulart *et al.*, 2002; Ridley; Jopling, 1966) (Figura 3).

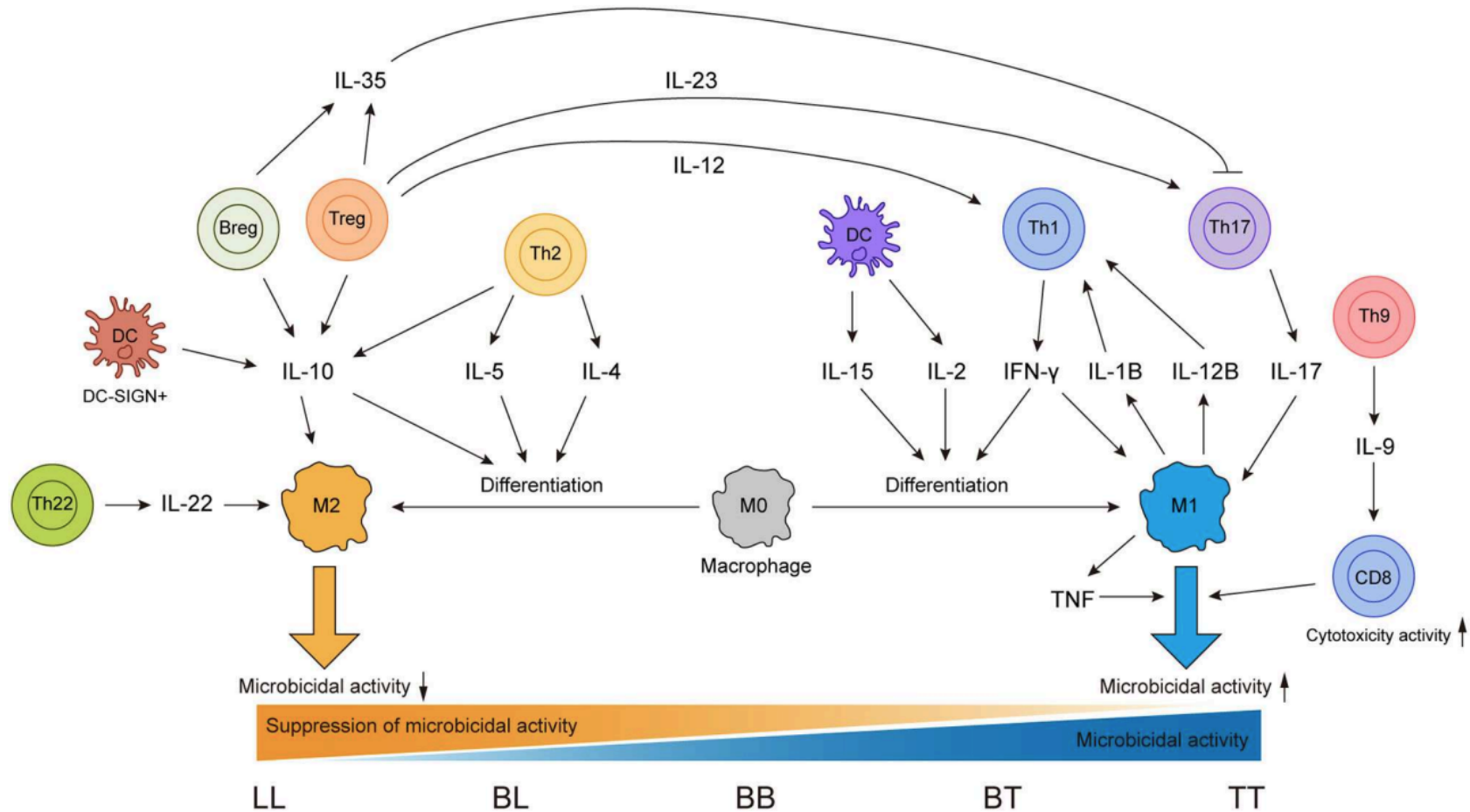
As citocinas são secretadas por várias células do sistema imunológico e desempenham um papel essencial na formação das respostas imunológicas na hanseníase e até mesmo pode conduzir a conversão funcional entre células antagônicas (Mi *et al.*, 2020). A hanseníase TT é o polo de resistência e de acentuada resposta imune celular Th1 com consequente produção de citocinas – interleucina (IL)-2, IL-17 e interferon-gama (IFN- γ) (Alrehaili, 2023; Froes; Sotto; Trindade, 2022; Goulart *et al.*, 2002) (Figura 4).

Figura 3 - Manifestações clínicas decorrentes do padrão de resposta imune ao *M. leprae*



Fonte: Adaptado de Alemu Belachew e Naafs (2019).

Figura 4 - Interação *M. leprae* versus hospedeiro



Possíveis mecanismos para a polarização da resposta imune nas formas clínicas, virchowiana e tuberculoide. IL: interleucina; IFN- γ : interferon-gama; TT: hanseníase tuberculoide; BT: dimorfa-tuberculoide; BB: dimorfa-dimorfa; BL: dimorfa-virchowiana; LL: virchowiana.

Fonte: Mi *et al.* (2020).

O quadro clínico da forma TT apresenta uma ou poucas lesões cutâneas maculares ou infiltradas, anulares, bem delimitadas, hipocrômicas ou eritematosas, de tamanhos variados, anestésicas, com redução de sudorese e pelos (Figura 5A) (Alemu Belachew; Naafs, 2019; Souza, 1997).

Figura 5 - Manifestação cutâneas da hanseníase



A) Hanseníase tuberculoide. B) Fácies leonina da hanseníase virchowiana. C) Hanseníase dimorfo-virchowiana.

Fonte: Material do CREDESH/HC-UFU/EBSERH.

No extremo do espectro, está a hanseníase VV, polo de extenuação e de resposta imune celular Th2 ineficiente e resposta humoral acentuada com produção de citocinas - IL-4, IL-5, IL-6, IL-8 e IL-10 (Alrehaili, 2023; Froes; Sotto; Trindade, 2022; Goulart *et al.*, 2002). As manifestações clínicas são variadas e oriundas da multiplicação e ampla disseminação bacilar. Na pele, manifesta-se como pápulas, tubérculos, nódulos (hansenomas), placas ferruginosas infiltração difusa e rarefação de pelos (Rodrigues; Lockwood, 2011). A típica a fácies leonina se deve a infiltração tegumentar da face e os lóbulos das orelhas (Souza, 1997) (Figura 5B). A ausência de cerco imunológico permite outros achados como perfuração septal nasal, lesões osteoarticulares e periodontais, laringite, comprometimento hepático, esplênico, testicular e dentre outros (Alemu Belachew; Naafs, 2019; Souza, 1997).

As formas dimorfas da hanseníase, DT, DD e DV, situam-se entre os polos estáveis e são as formas de apresentação clínica mais comuns da doença (Alrehaili, 2023). As cutâneas são polimórficas, assimétricas, com bordas eritemato-infiltradas e centro claro (lesões foveolares), com rarefação de pelos e alteração das sensibilidades térmica, tátil e dolorosa, além de comprometimento neural variável (Alemu Belachew; Naafs, 2019; Rodrigues; Lockwood, 2011; Souza, 1997) (Figura 5C).

2.3.2.2 Classificação operacional

Para a OMS, caso de hanseníase é definido como um indivíduo que apresenta uma ou mais das seguintes características e que ainda precisa completar um esquema de tratamento completo: a) lesões cutâneas hipopigmentadas ou avermelhadas com perda de sensibilidade confirmada; b) envolvimento dos nervos periféricos, demonstrado por espessamento com perda de sensibilidade; e, c) esfregaço cutâneo positivo para bacilos álcool-ácido resistentes (World Health Organization, 1988).

Com base no exposto, os casos são classificados em dois tipos para fins de tratamento: caso paucibacilar (PB) e caso multibacilar (MB) (World Health Organization, 2023b):

- a) Caso PB: caso de hanseníase com 1 a 5 lesões cutâneas, sem presença comprovada de bacilos em esfregaço cutâneo.
- b) Caso MB: caso de hanseníase com mais de cinco lesões cutâneas; ou com envolvimento nervoso (neurite pura ou qualquer número de lesões cutâneas e neurite); ou com presença demonstrada de bacilos em esfregaço cutâneo, independentemente do número de lesões cutâneas.

2.4 Diagnóstico da hanseníase

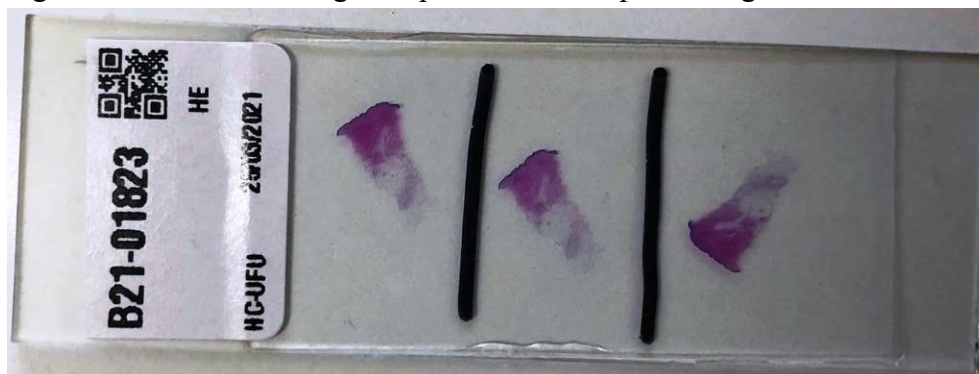
A OMS e o Ministério da Saúde do Brasil (MS) sugerem que o diagnóstico da hanseníase seja feito clinicamente e limita o uso de ferramentas aos casos difíceis de se diagnosticarem (Brasil, 2022; World Health Organization, 2023a). Já, o *Centers for Disease Control and Prevention* reitera que o diagnóstico da hanseníase seja confirmado por histopatologia de pele ou nervo e coloração para bacilo álcool-ácido-resistente (Centers for Disease Control and Prevention, 2017).

2.5 Achados histopatológicos da hanseníase

Para o tratamento apropriado, o diagnóstico da hanseníase deve ser feito com o máximo de precisão com o auxílio das características histopatológicas, IB e correlação clínica (Premalatha *et al.*, 2016). O exame histopatológico das lesões de pele é, ainda, o padrão-ouro para o diagnóstico preciso da hanseníase e permite ir além da confirmação do diagnóstico uma vez que permite classificar a lesão no espectro da hanseníase, quantificar a carga bacilar

tecidual, avaliar a atividade da doença e resposta ao tratamento e diagnosticar reações hansênicas (Massone, 2012; Soares, 2021). Idealmente, a biópsia de pele deve ser realizada por *punch* de 4 a 6 mm, representativa da gordura subcutânea, fixada em solução de formalina tamponada (Figura 6). O Quadro 1 resume os sítios adequados de biópsia.

Figura 6 - Lâmina histológica representativa da pele e da gordura subcutânea



Os cortes histológicos devem ser corados em hematoxilina e eosina (H&E) e FF.

Fonte: Material da UAPAT/HC-UFU/EBSERH.

Quadro 1 - Suspeição clínica da hanseníase e lugar mais adequado para colher a biópsia

Condição clínica	Sítio de biópsia
I	Centro hipoestésico da lesão
TT e DT	Borda infiltrada da lesão
DD e R1	Área mais edematosa e infiltrada da lesão
DV, VV e R2	Centro da mácula ou nódulo

I: hanseníase indeterminada; TT: hanseníase tuberculoide; DT: dimorfa-tuberculoide; DD: dimorfa-dimorfa; DV: dimorfa-virchowiana; VV: virchowiana; R1: reação tipo 1; R2: reação tipo 2.

Fonte: Adaptado de Massone (2012).

2.5.1 Achados histopatológicos ao início de tratamento

Os cortes histológicos devem ser múltiplos, em torno de seis, para ambas as colorações, H&E e FF, e na leitura devem verificar-se presença de infiltrado leucocitário da derme superficial e profunda, especialmente em torno de filetes neurais (perineurite), folículos pilosos, glândulas écrinas e músculos pilo-erectores, na intimidade de nervos (neurite), composição do infiltrado leucocitário (inespecífico ou granulomatoso), presença de elementos característicos como faixa de Unna e, finalmente, determinação de o IBh.

2.5.1.1 Hanseníase indeterminada

O quadro histológico é, geralmente, de uma dermatite crônica inespecífica, com infiltrado predominantemente linfocitário, com poucos macrófagos, que acomete menos de 5 % da derme, perivascular, superficial e profundo, perineural, periécrina e em torno de músculos pilo-erectores. Pode haver linfócitos intraepidérmicos (Figura 7), com IBh = 0 – 1+ (Calonje; Brenn; Lazar, 2011; Massone, 2012; Patterson, 2014; Soares, 2021).

2.5.1.2 Hanseníase tuberculoide

A hanseníase TT se caracteriza pela presença de granulomas epitelioides, bem formados, com espessa orla de linfócitos, com ou sem células gigantes de tipo Langhans, mas sem caseificação. O exsudato leucocitário pode tocar e erodir a epiderme na TT (IBh = 0 – 1+), (Calonje; Brenn; Lazar, 2011; Massone, 2012; Patterson, 2014; Soares, 2021) (Figura 8).

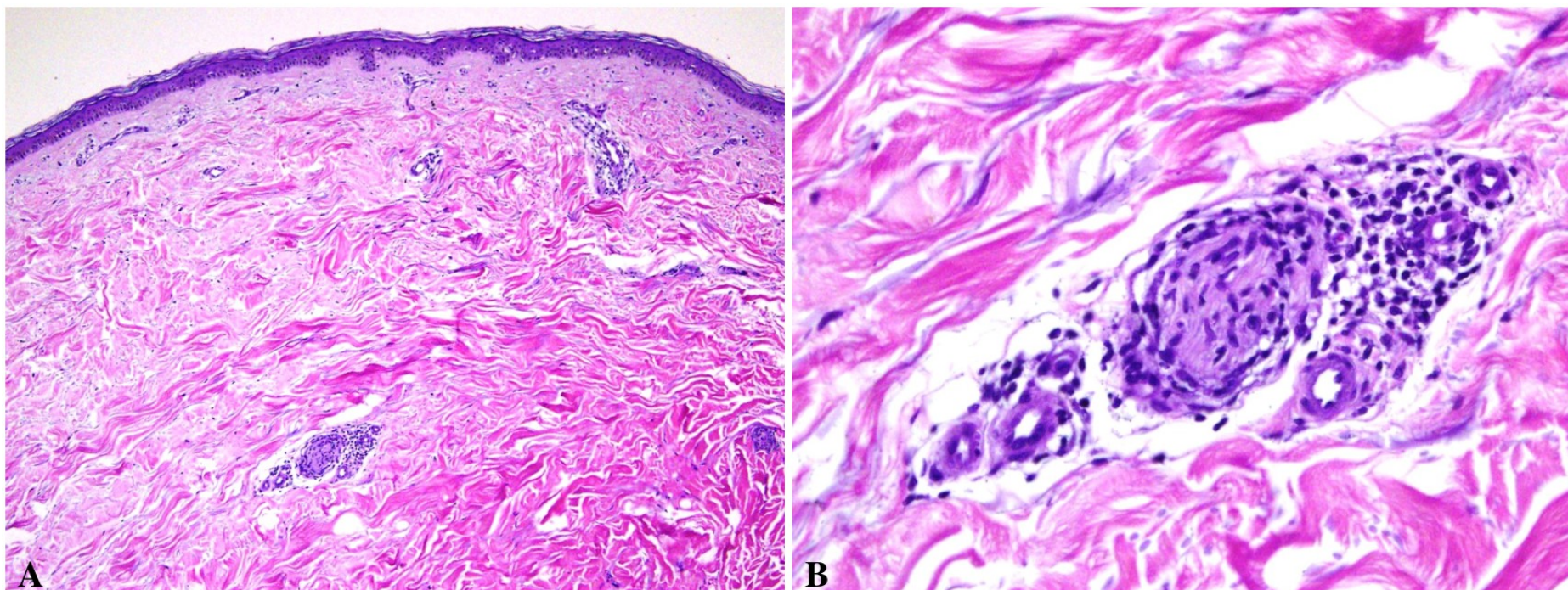
2.5.1.3 Hanseníase dimorfo-tuberculoide

A hanseníase DT possui zona de Grenz, mesmo que fina. Ainda, caracteriza-se pela presença de linfócitos em menor quantidade se comparada à TT, mas em maior quantidade se comparada à DD. Os granulomas são frouxos e as células gigantes de tipo Langhans são poucas (IBh = 0 – 2+) (Calonje; Brenn; Lazar, 2011; Massone, 2012; Patterson, 2014; Soares, 2021) (Figura 9).

2.5.1.4 Hanseníase dimorfo-dimorfo

Na hanseníase DD, há infiltração difusa de células epitelioides, com linfócitos esparsos, granulomas frouxos, mal-formados, com presença de células de Langhans e de Virchow, ademais, há edema dérmico e faixa de Unna (Figura 10), com IBh = 3 – 4+ (Calonje; Brenn; Lazar, 2011; Massone, 2012; Patterson, 2014; Soares, 2021).

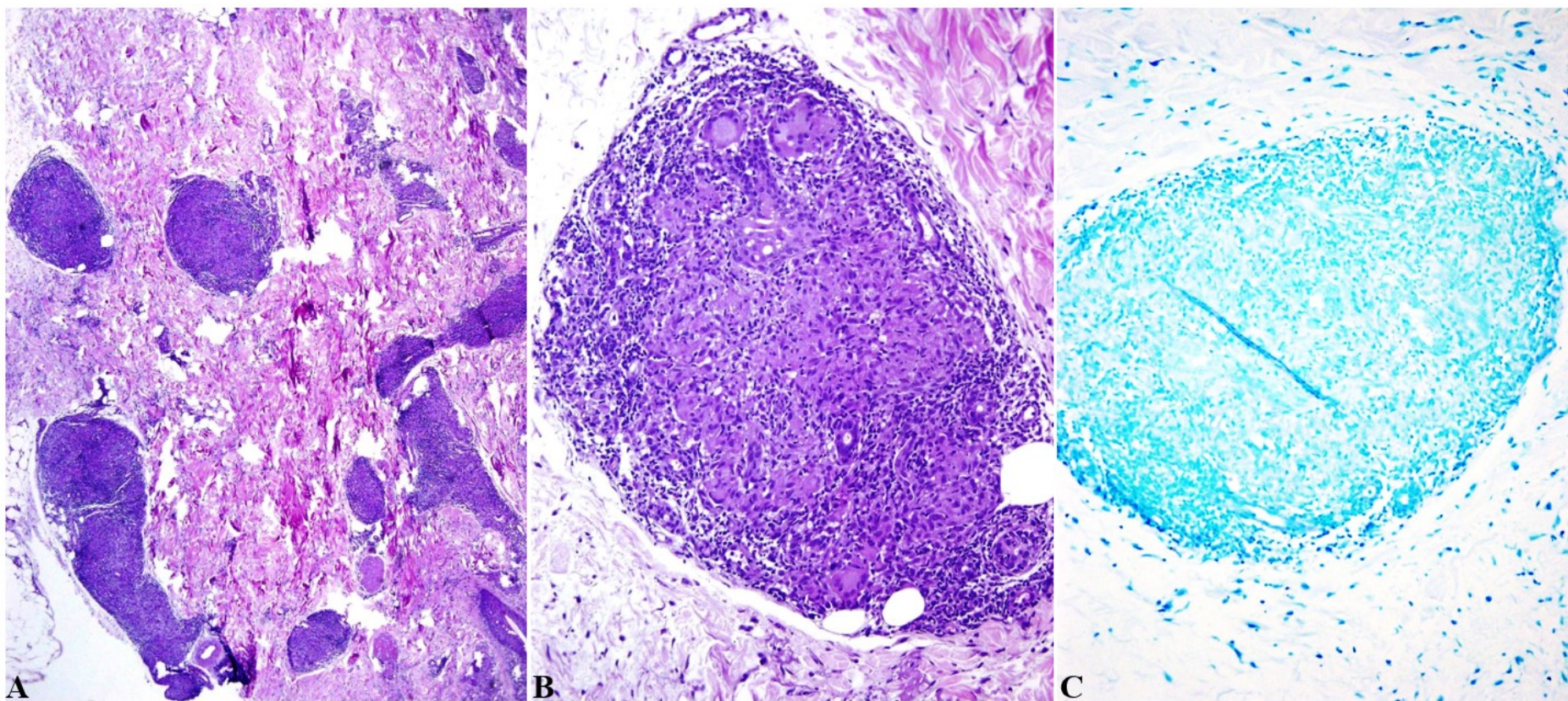
Figura 7 - Hanseníase indeterminada



A) Fragmento de pele com escassas alterações patológicas ao pequeno aumento (H&E, 4 x). C) Neurite e perineurite linfocítica (H&E, 40x).

Fonte: Material da UAPAT/HC-UFU/EBSERH.

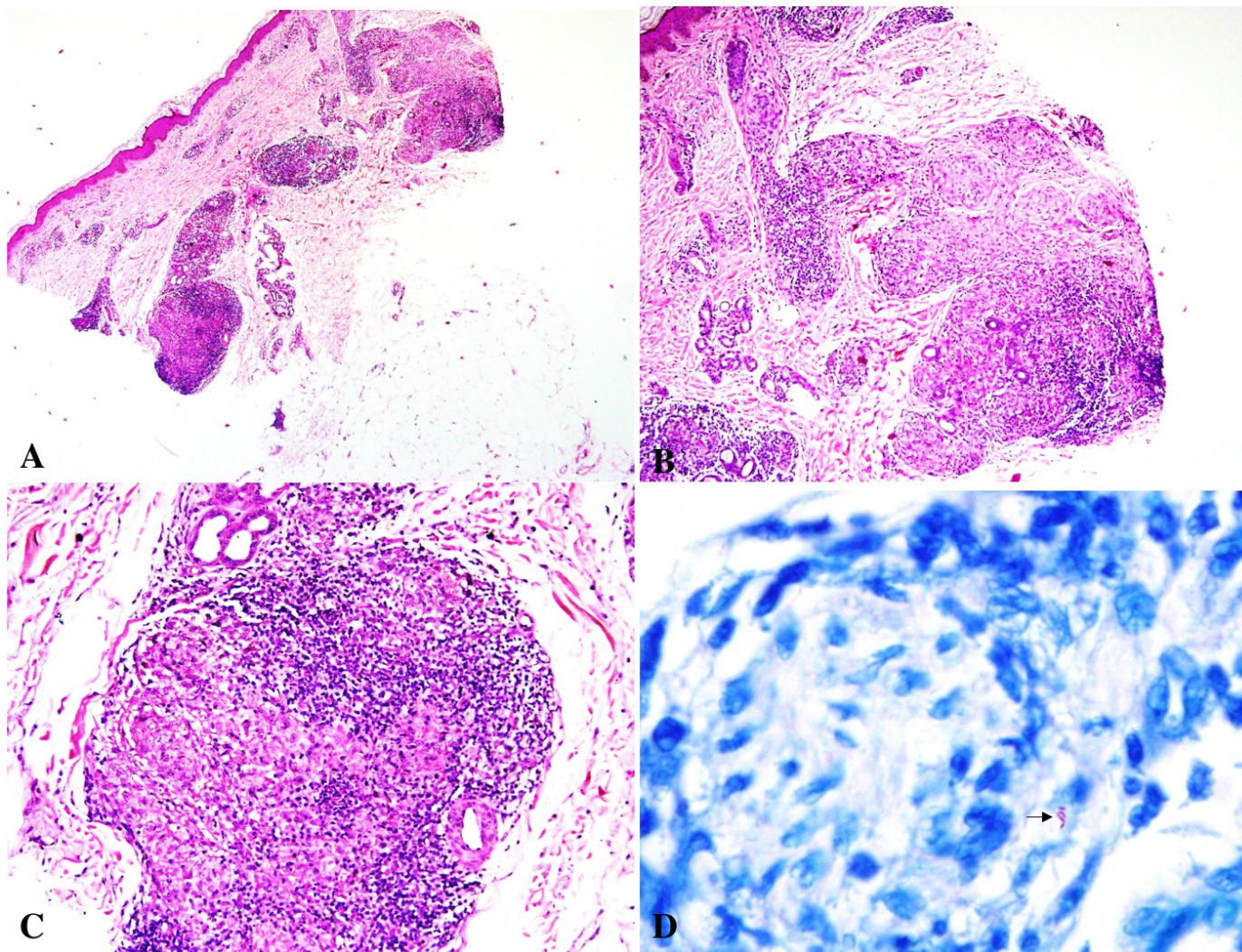
Figura 8 - Hanseníase tuberculoide



A) Dermatite granulomatosa não caseosa com distribuição arboriforme dos granulomas (H&E, 4 x). B) Granuloma epitelioides com espessa orla de linfócitos e frequentes células gigantes multinucleadas de Langhans (H&E, 20x). C) Índice baciloscópico caracteristicamente negativo (FF, 20x).

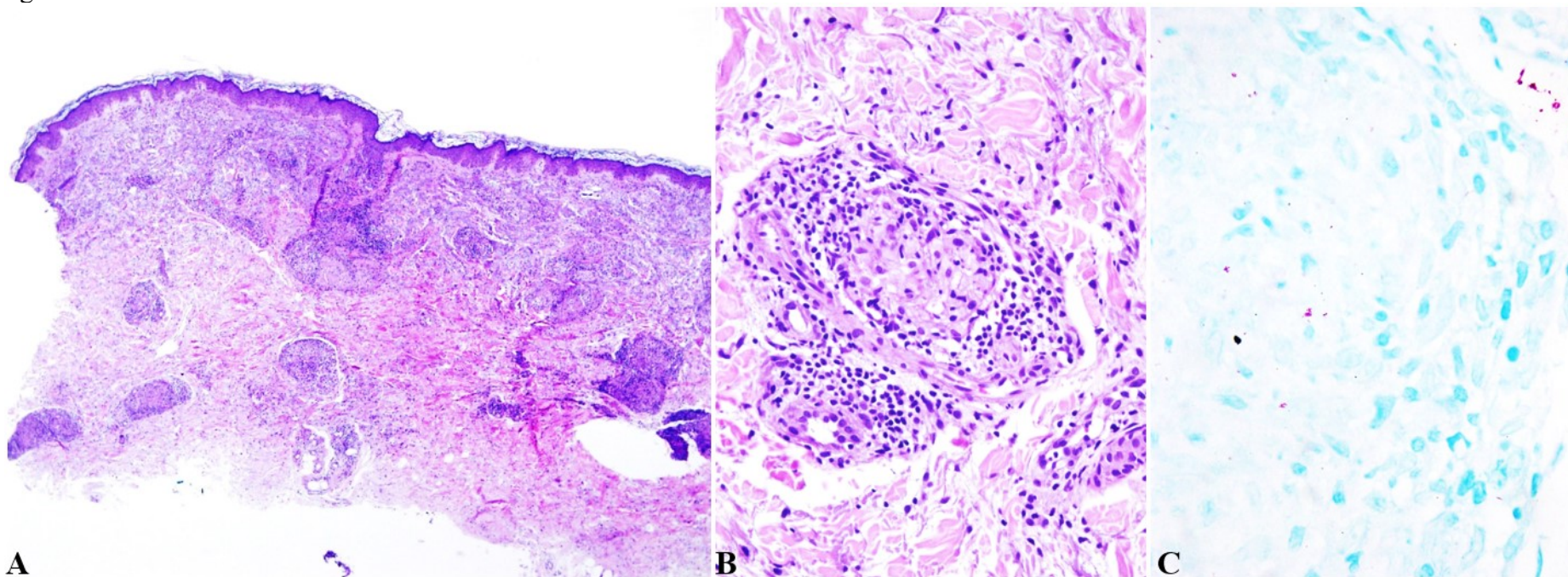
Fonte: Material da UAPAT/HC-UFU/EBSERH.

Figura 9 - Hanseníase dimorfo-tuberculoide



A) Dermatite granulomatosa não caseosa (H&E, 4 x). B) Inflamação granulomatosa com distribuição oblonga e tropismo por anexos cutâneos (H&E, 10x). C) Granuloma epitelioides com espessa orla de linfócitos (H&E, 20x). D) Baixo índice baciloscópico (seta): 2/6+ (FF, original 100x).

Figura 10 - Hanseníase dimorfo-dimorfa



A) A pequeno aumento, observam-se granulomas na derme superficial e profunda (H&E, 4x). B) Pequenos granulomas malformados permeados por poucos linfócitos (H&E, 30x). C) Bacilos íntegros e fragmentados, 4/6+ (FF, 100x).

Fonte: Material da UAPAT/HC-UFU/EBSERH.

2.5.1.5 *Hanseníase dimorfo-virchowiana*

Pequenas coleções de macrófagos com citoplasma granuloso ou finamente vacuolado são vistas na hanseníase DV, na que é variável o número de linfócitos, ademais, faixa de Unna e fibrose perineural “em casca de cebola” são frequentemente encontradas, com IBh = 4 – 5+ (Calonje; Brenn; Lazar, 2011; Massone, 2012; Patterson, 2014; Soares, 2021) (Figura 11).

2.5.1.6 *Hanseníase virchowiana*

Numerosos macrófagos contendo grande quantidade de bacilos são característicos da hanseníase VV, forma na que há poucos linfócitos, mas a faixa de Unna está presente. Existe abundância de bacilos acumulados em globias nos macrófagos, também em células de Schwann, músculos pilo-erectores, células endoteliais e glândulas écrinas. A epiderme está normal ou adelgada. Há completa destruição e perda de anexos cutâneos. Nas lesões mais antigas, observam-se macrófagos vacuolados (células de Virchow), com IBh = 5 – 6+ (Figura 12) (Calonje; Brenn; Lazar, 2011; Massone, 2012; Patterson, 2014; Soares, 2021).

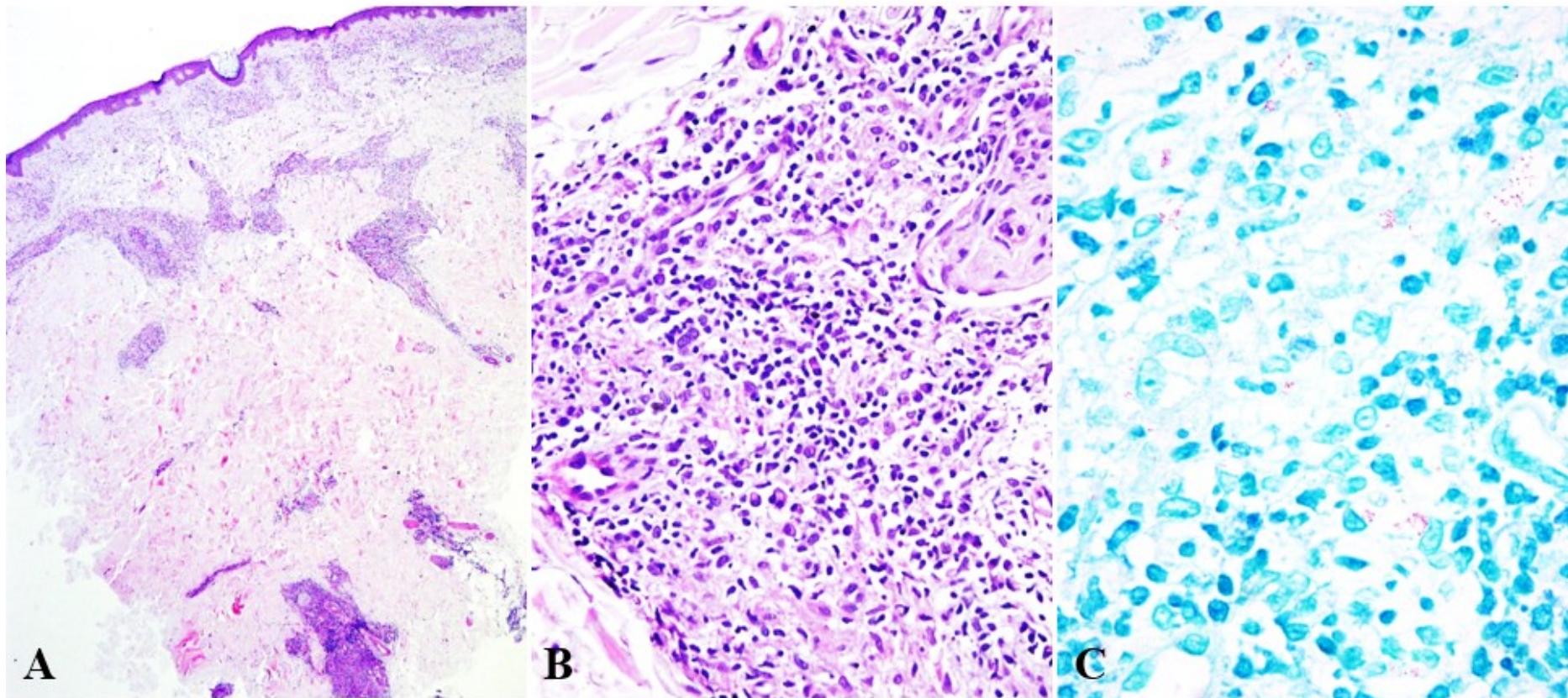
2.5.1.6.1 Hanseníase histoide

A forma histoide é uma variante morfológica da hanseníase VV, na que há infiltração de células predominantemente fusiformes (células dendríticas dérmicas; XIIIa +), dispostas em fascículos, podendo haver pequenas coleções de macrófagos espumosos (Figura 13). Os bacilos são numerosos; IB = 6+ (Calonje; Brenn; Lazar; Massone, 2012; Patterson, 2014; Soares, 2021).

2.5.2 *Baciloscopia do raspado intradérmico*

O diagnóstico clínico pautado nos sinais cardinais da hanseníase e microbiológico arrolado na presença de bacilo em esfregaços dérmicos ou de pele ou nervo têm sensibilidade diagnóstica de 97% e valor preditivo positivo de 98% (Britton; Lockwood, 2004; Ooi; Srinivasan, 2004; World Health Organization, 2018).

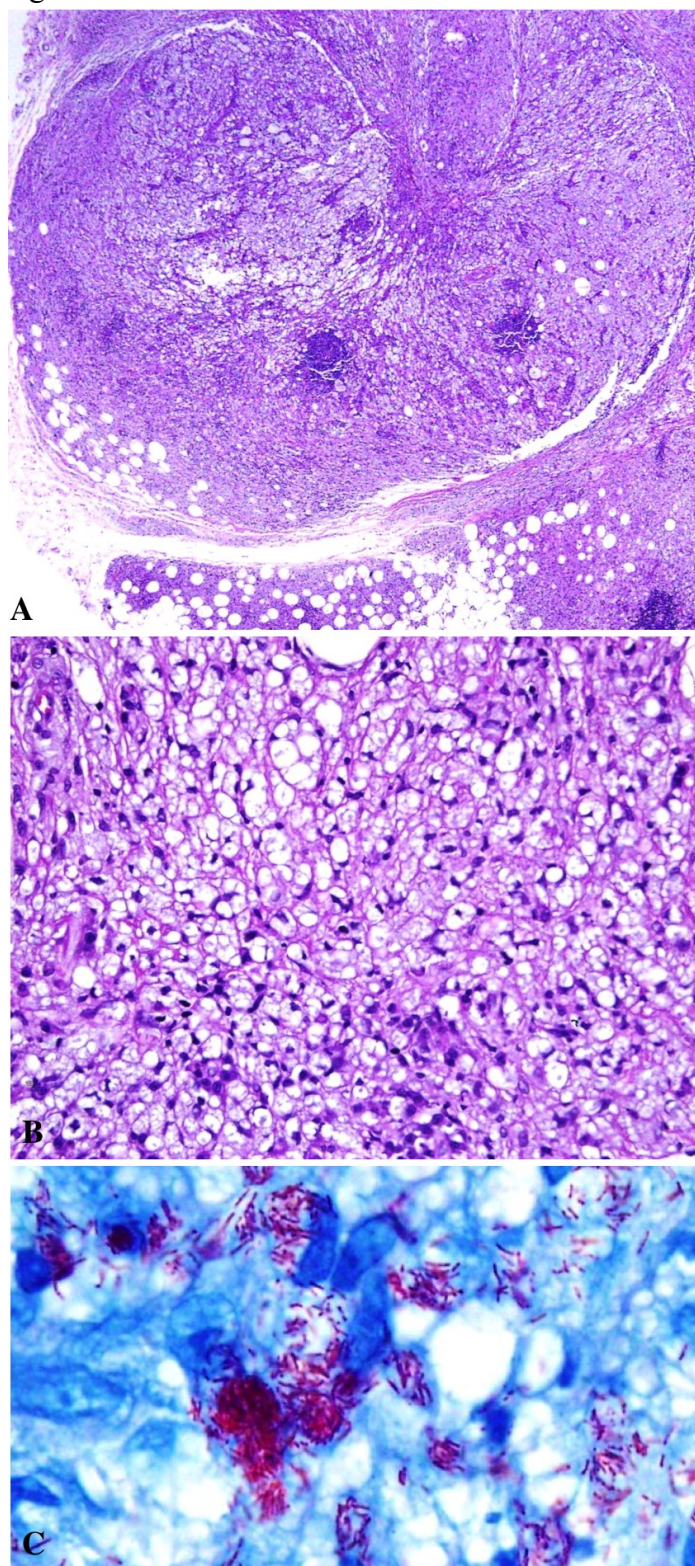
Figura 11 - Hanseníase dimorfo-vichowiana



A) Infiltrado linfocitário moderado a acentuado em torno de vasos superficiais e profundos e de anexos e nervos cutâneo (H&E, 4x). B) Infiltrado linfocitário misto, com linfócitos, plasmócitos e macrófagos (H&E, 40 x). C) Numerosos bacilos, predominantemente, fragmentados dentro de macrófagos (FF, 40x).

Fonte: Material da UAPAT/HC-UFU/EBSERH.

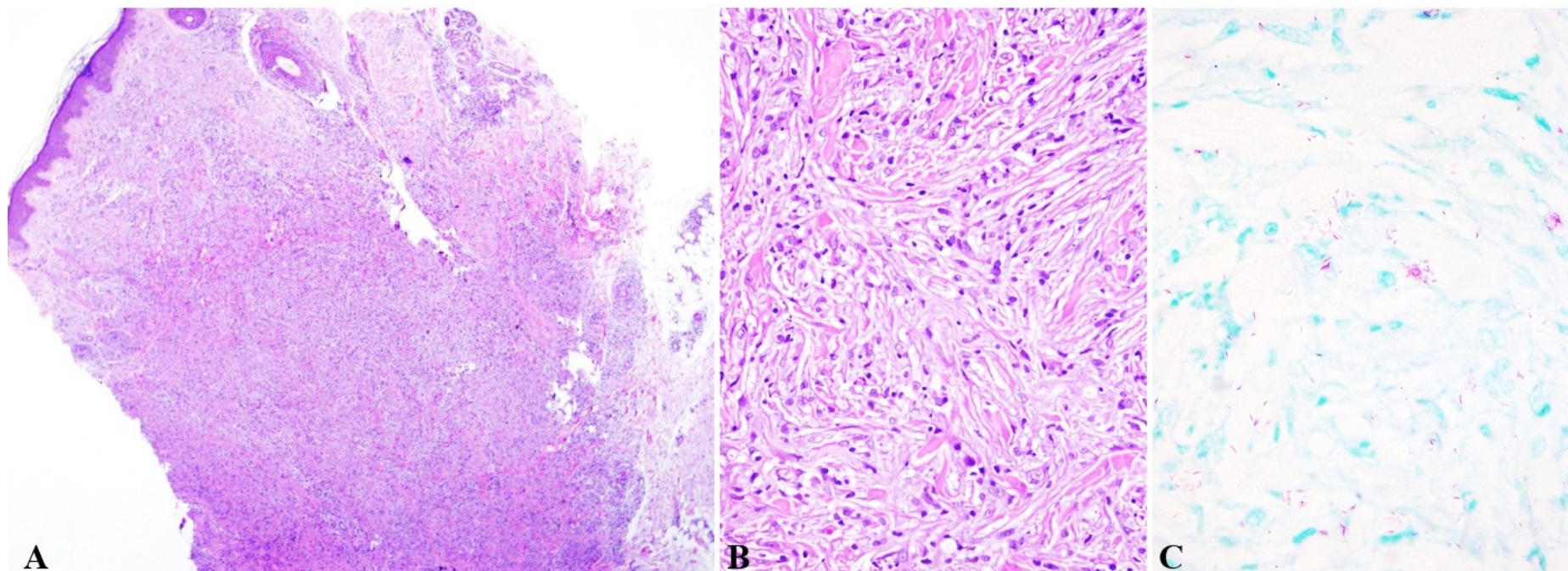
Figura 12 - Hanseníase vichowiana



A) Infiltrado linfocitário acentuado, com formação de granulomas espumosos na derme e no tecido gorduroso subcutâneo (H&E, 4x). B) Macrófagos vacuolados (H&E, 20 x). C) Numerosos bacilos, predominantemente, fragmentados dentro de macrófagos (FF, original 100x).

Fonte: Material da UAPAT/HC-UFU/EBSERH.

Figura 13 - Hanseníase hísticoide



A) Infiltração difusa da derme por macrófagos (H&E, 4 x). B) Macrófagos fusocelulares organizados em feixes (H&E, 10x). C) Alta carga bacilar, IBh = 6/6+ (FF, 100 x).

Fonte: Material da UAPAT/HC-UFU/EBSERH.

O raspado intradérmico tem papel central na Classificação Operacional da OMS segregando pacientes PB através da baciloscopia negativa com no máximo cinco lesões de pele e/ou apenas um tronco nervoso acometido e os MB com baciloscopia positiva, mais de cinco lesões de pele e/ou dois ou mais troncos nervosos acometidos (World Health Organization, 1988, 2021a). A positividade do IB requer de ao mínimo 10.000 bacilos/grama de tecido para detecção confiável, mas a sensibilidade é baixa, principalmente em pacientes com características TT da doença, onde os bacilos são raros ou ausentes (Shepard; McRae, 1968).

O exame baciloscópico deve ser feito idealmente através da coleta de material nos lóbulos auriculares, cotovelos e joelhos e do raspado de lesões cutâneas (Brasil, 2010). A baciloscopia do esfregaço dérmico é um dos critérios definidores de caso conforme a OMS (World Health Organization, 2018). Entretanto, o MS é incisivo em indicar o tratamento da PQT mesmo quando a baciloscopia estiver indisponível (Brasil, 2022).

2.5.3 Sorologia na hanseníase

Em 1981, foi publicado o trabalho que descreveu o glicolípido fenólico-I (PGL-I), uma molécula constituinte da parede celular do *M. leprae* com alta capacidade antigênica na produção de imunoglobulina do tipo M (IgM) (Hunter; Brennan, 1981). Desde então, o antígeno mais amplamente utilizado no diagnóstico sorológico da hanseníase, classificação de pacientes, monitoramento de tratamento, risco de recidiva e na seleção dos contatos com maior risco de adoecer é o PGL-I (Hunter; Brennan, 1981; Moura *et al.*, 2008; Spencer; Brennan, 2011).

Sabe-se que o PGL-I também está envolvido em mecanismos de invasão das células de Schwann e na regulação negativa da resposta imunológica permitindo a persistência de *M. leprae* no organismo (Ng *et al.*, 2000; Spencer; Brennan, 2011).

A detecção do anticorpo IgM anti-PGL-I pelo ensaio de imunoabsorção enzimática (ELISA) é ainda mais útil em casos de hanseníase MB, que estão associados à presença de maior quantidade de bacilos na pele (Cho *et al.*, 1991; Eichelmann *et al.*, 2013; Nascimento *et al.*, 2022; Premalatha *et al.*, 2016; Spencer; Brennan, 2011). Entretanto, a sorologia IgM anti-PGL-I é capaz de detectar anticorpos não somente entre os pacientes MB, mas também no rastreamento ou detecção de contatos (Araujo *et al.*, 2016; Douglas *et al.*, 2004; Goulart; Goulart, 2008). A soropositividade dos contatos indica exposição ao bacilo, infecção de uma célula fagocítica e estimulação humoral traduzindo uma infecção subclínica (Lobato *et al.*, 2011). A detecção anticorpos anti-PGL-I apresenta positividade de 13% a 25% no grupo dos

contatos o que se associa a um risco maior de adoecer, reforçando a importância da utilização da sorologia ELISA anti-PGL-I como uma estratégia de monitoramento da doença (Goulart; Goulart, 2008; Moura *et al.*, 2008; Penna, 2014).

2.5.4 Detecção de DNA do *Mycobacterium leprae*

Uma das grandes limitações no estudo da hanseníase sempre foi a impossibilidade da cultura de *M. leprae*, o que motivou novas pesquisas, sobretudo no final da década de 80, quando se demonstrou a possibilidade do uso da reação em cadeia da polimerase (PCR) para detecção específica do DNA de *M. leprae* (Hartskeerl; Wit; Klatser, 1989; Woods, 1989). A PCR permite, de forma rápida, específica e sensível, a identificação do DNA de *M. leprae* em amostras contendo quantidades mínimas de bacilo podendo auxiliar no diagnóstico de hanseníase, na classificação dos pacientes e no monitoramento do tratamento com PQT (Goulart *et al.*, 2007). Para o diagnóstico da hanseníase, nas biópsias de pele, a qPCR apresenta uma sensibilidade de 50-90% e uma especificidade de 94-100% (Araujo *et al.*, 2016; Matsuoka, 2010).

Além disso, estudos relatam o uso da PCR para determinar a presença do DNA de *M. leprae* em *swabs* nasais de pacientes e contatos (Jamil *et al.*, 1993; Patrocínio *et al.*, 2005), podendo indicar dispersão do bacilo em regiões endêmicas. Outros estudos inferem a existência de portadores sadios ou infecção subclínica com presença transitória de bacilos na secreção nasal (Araujo *et al.*, 2016), indicando que esses indivíduos podem exercer um papel ativo na transmissão para pessoas susceptíveis.

Um estudo que descreveu a técnica de PCR em tempo-real quantitativa (qPCR) para detecção do gene repetitivo RLEP do *M. leprae*, indicou o potencial de detecção de apenas 10 bacilos em uma amostra e permitiu quantificar a carga bacteriana pela interpolação com o número de moléculas de DNA de um controle padrão conhecido (Truman *et al.*, 2008).

Tanto na definição diagnóstica, principalmente nos casos PB, quanto na avaliação da eficácia da PQT, a detecção de DNA do *M. leprae* por PCR tem papel primordial ao lado da determinação da taxa de recidiva (Hatta, 1997; Jamil *et al.*, 1993; Kaimal; Thappa, 2009; Manta *et al.*, 2019; Martinez *et al.*, 2014). Ensaios de polimerase por transcriptase reversa (RT-PCR) permitem avaliar a viabilidade dos bacilos após o fim do tratamento (Devita *et al.*, 2019).

2.6 Estados reacionais da hanseníase

As formas reacionais ou estados reacionais são surtos inflamatórios agudos ou subagudos, com agravamento das lesões cutâneas e neurais, e manifestações gerais como febre, adinamia, artralgia *etc.*, que podem ocorrer durante a história natural da doença, durante ou após o fim da PQT e são classificados em reações tipo 1 (reação reversa) e tipo 2 (eritema nodoso hansênico) (Brasil, 2022). As reações podem estar presentes em todas as formas clínicas da doença, com exceção da hanseníase I, e geralmente são decorrentes dos efeitos da PQT e redução da carga bacilar (Foss *et al.*, 2003; Naafs; van Hees, 2016). As reações hansênicas podem ser entendidas como exacerbações agudas resultantes de alterações no balanço imunológico entre o hospedeiro e o *M. leprae* (Pinheiro *et al.*, 2022; Sampaio; Sarno, 1998), e afetam principalmente a pele e nervos, constituindo a principal causa de morbidade e incapacidades relacionadas à doença (Brasil, 2022; Foss *et al.*, 2003).

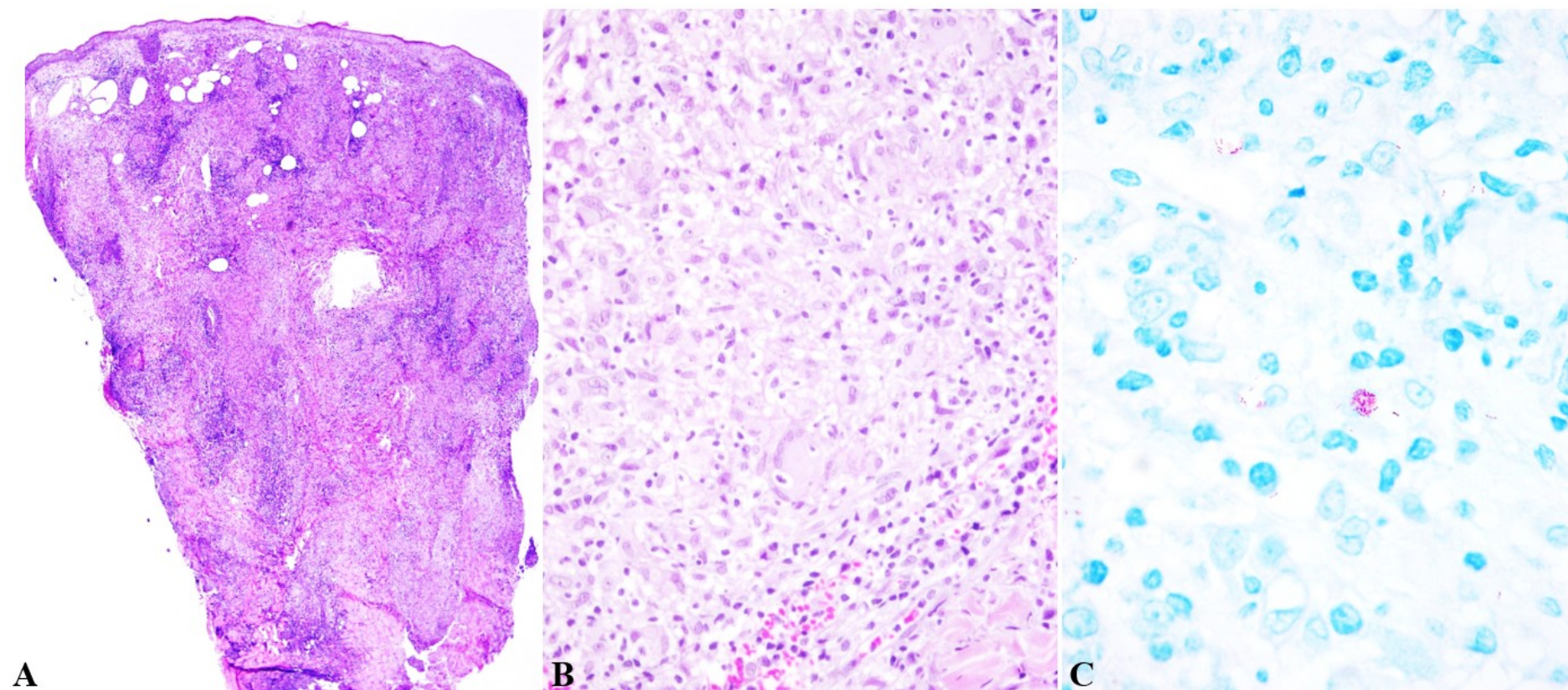
2.6.1 Reação hansênica tipo 1

Reação Tipo 1 ou reação reversa ocorre principalmente nas formas dimorfas instáveis da hanseníase dimorfa (DT, DD e DV) e, geralmente, com desvio para o polo tuberculoide (reação reversa ou ascendente; *upgrading reaction*), por aumento da hipersensibilidade retardada. Incomumente, há desvio para o polo virchowiano (reação descendente; *downgrading reaction*) (Foss *et al.*, 2003; Naafs; van Hees, 2016). Notam-se edema e eritema cutâneos, às vezes, desenvolvem-se úlceras e novas lesões e pode haver neurite e manifestações sistêmicas (Antunes *et al.*, 2016; Naafs; van Hees, 2016).

2.6.1.1 Achados histológicos

A reação do tipo *upgrading* mostra edema da derme, com maior número de linfócitos e, por vezes, células gigantes de Langhans, grupos de células epitelioides e necrose fibroide ou caseosa em nervos periféricos, quadro histológico semelhante ao da hanseníase TT ou DT; já na reação tipo *downgrading*, predominam os acúmulos de macrófagos espumosos com maior carga bacilar (Figura 14) (Massone, 2012; Soares, 2021).

Figura 14 - Reação hansênica tipo 1



A) Edema da derme papilar e acentuado infiltrado leucocitário difuso, que se estende à gordura subcutânea (H&E, 4 x). B) Granulomas epitelioides com células gigantes tipo Langhans (H&E, 20x). C) Bacilos fragmentados, IBh = 5-6+ (FF, 100 x).

Fonte: Material da UAPAT/HC-UFU/EBSERH.

2.6.2 Reação hansênica tipo 2

A reação tipo 2 ocorre usualmente durante o tratamento, mas pode ser precipitada por gravidez, *stress*, infecções intercorrentes, vacinas *etc.*, na hanseníase VV (25 a 70% dos pacientes com a forma) e, ocasionalmente, na DV. As manifestações clínicas englobam placas e nódulos eritematosos e/ou violáceos, dolorosos, especialmente nos membros. Ocasionalmente, ocorrem púrpura, vesículas e úlceras. Pode haver manifestações sistêmicas intensas (Foss *et al.*, 2003; Naafs; van Hees, 2016).

Nestas reações, existe uma associação com a resposta imune do tipo Th2, envolvendo a produção de fator de necrose tumoral alfa (TNF- α), IL-6, IL-8 e IL-10 (Antunes *et al.*, 2016; Foss *et al.*, 2003; Naafs; van Hees, 2016) que resulta na vasculite por imunocomplexos e, menos, da ação de citocinas (imunidade celular). Pode ocorrer ainda a deposição de imunocomplexos em diversos órgãos, levando a quadros de artrite, glomerulonefrite, orquiepididimite, miosite, iridociclite (Foss *et al.*, 2003).

2.6.2.1 Achados histológicos

A histopatologia da pele, na R2, caracteriza-se por edema, acúmulos de macrófagos associados a exsudatos neutrofílico e linfocitário, vasculites necrosante ou leucocitoclástica, paniculite lobular e septal e bacilos fragmentados em macrófagos (Figura 15). Necrose, abscessos e úlceras são vistos em formas graves. A imunofluorescência mostra positividade para C3 e IgG nas paredes vasculares (Fava *et al.*, 2012).

2.6.3 Reação hansênica tipo 3

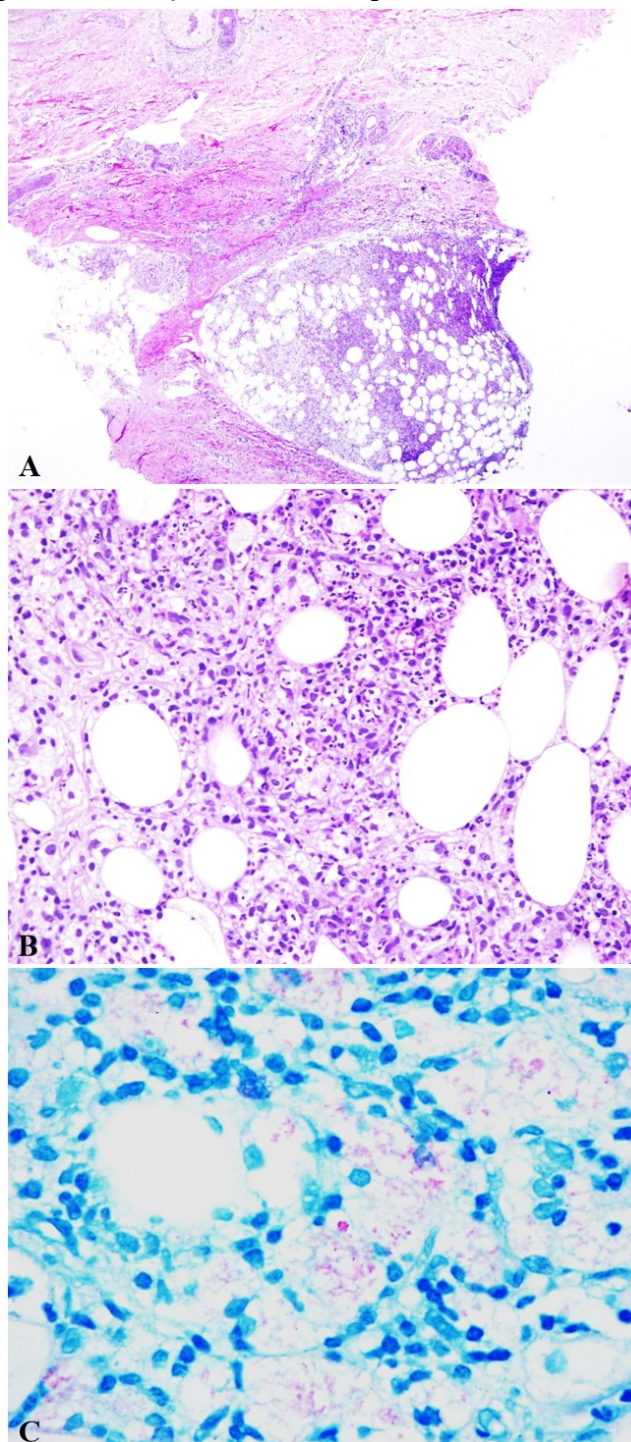
A reação hansênica tipo 3, fenômeno de Lúcio, ocorre na hanseníase virchowiana difusa (não-nodular), que se observa no México e na América Central. Clinicamente, há placas e úlceras hemorrágicas na ausência de febre ou leucocitose (Walker, 2020).

2.6.3.1 Achados histológicos

A reação tipo 3 se caracteriza por proliferação exacerbada dos bacilos de Hansen, que invadem a parede dos vasos sanguíneos e agridem as células endoteliais, causando proliferação endotelial e diminuição do lúmen vascular, trombose, infarto epidérmico e úlceras (Monteiro *et*

al., 2012). A inflamação é usualmente mínima, com menos neutrófilos que na reação tipo 2 (Soares, 2021).

Figura 15 - Reação hansênica tipo 2



A) Panniculite lobular (H&E, 4x). B) Infiltrado leucocitário misto acentuado, rico em histiócitos e neutrófilos (H&E, 40x). C) Numerosos bacilos fragmentados e granulados, IBh = 6/6+ (FF, 100 x).

Fonte: Material da UAPAT/HC-UFU/EBSERH.

2.7 Tratamento da hanseníase

Medidas de prevenção para a hanseníase estão contidas no Código de Manu de 1.500 a.C. (Dharmendra, 1947). O isolamento compulsório foi a única forma de controle da transmissão da hanseníase e perdurou muito depois de ter sido provado que era cientificamente desnecessário, causando privação das liberdades civis, promoção de estigmas sociais e uso ineficiente dos recursos de saúde (Sato; Frantz, 2005). O óleo de chaulmoogra foi primeiro medicamento descrito para a doença em 600 a.C. e permaneceu um pilar do tratamento até a introdução das sulfonas (Jacob; Franco-Paredes, 2008).

Na década de 1940, as sulfonamidas revolucionaram o tratamento da doença, oferecendo esperança de que a hanseníase desapareceria rapidamente, contudo resistência bacteriana à dapsona e número crescente de casos ameaçavam pôr em risco todo o esforço de controle por volta de 1960 (Aubry *et al.*, 2022). Em 1982, a OMS passou a recomendar o tratamento com PQT a todos os pacientes e a prevalência mundial da hanseníase caiu acentuadamente (Lazo-Porras *et al.*, 2020; World Health Organization Study Group, 1982). Apesar da redução na prevalência, a notificação de casos novos ainda permanece alta, persistindo como problema de saúde pública.

O MS, em cumprimento às recomendações da OMS, regulamenta o tratamento ambulatorial da hanseníase através de esquemas terapêuticos padronizados de acordo com a Classificação Operacional. Atualmente, a associação dos fármacos rifampicina, dapsona e clofazimina, na apresentação de blisters, é denominada poliquimioterapia única (PQT-U).

Os casos PB devem ser tratados com regime de 6 doses e os casos MB com regime de 12 doses: rifampicina (600 mg), dapsona (100 mg) e clofazimina (30 mg) com administração supervisionada mensal. A dose diária autoadministrada é de 100 mg para a dapsona e 50 mg para a clofazimina. Na última dose, os pacientes PB e MB devem ser submetidos ao exame dermatológico, avaliação neurológica e do grau de incapacidade física e receber alta por cura. Em crianças ou adultos com peso inferior a 30 Kg, deve-se ajustar a dose de acordo com o peso (Brasil, 2022).

O tratamento dessa doença espectral é realizado de acordo com essa classificação operacional simplista adotada pelos programas dos mais diversos países que divide os pacientes em apenas dois grupos, hanseníase PB e MB (Gelber; Grosset, 2012; World Health Organization, 1994; World Health Organization Study Group, 1982). A escolha do esquema terapêutico por meio da contagem de lesões cutâneas indicada pela classificação operacional da OMS pode resultar num diagnóstico pouco sensível e na atribuição de regimes de tratamento PB a uma elevada quantidade de doentes MB (Kumar; Dogra, 2009; Pardillo *et al.*, 2007).

Pacientes MB que não apresentarem melhora clínica ao final do tratamento devem ser encaminhados para avaliação nas unidades de maior complexidade para verificar a necessidade de um segundo ciclo de tratamento com 12 doses. Em casos de intolerância ou contraindicação, esquemas terapêuticos substitutivos com a combinação de ofloxacina e/ou minociclina são recomendados (Brasil, 2022).

2.8 Achados histopatológicos ao fim de tratamento

De acordo com as recomendações vigentes, a PQT da hanseníase segue um esquema de duração fixa, independentemente do desaparecimento de lesões cutâneas ou do IB na pele (Brasil, 2022; World Health Organization, 2018). Nesse contexto, muitos pacientes são submetidos à biópsia de pele para avaliar a persistência de atividade de doença após o tratamento (Joshi, 2011) (Figura 16).

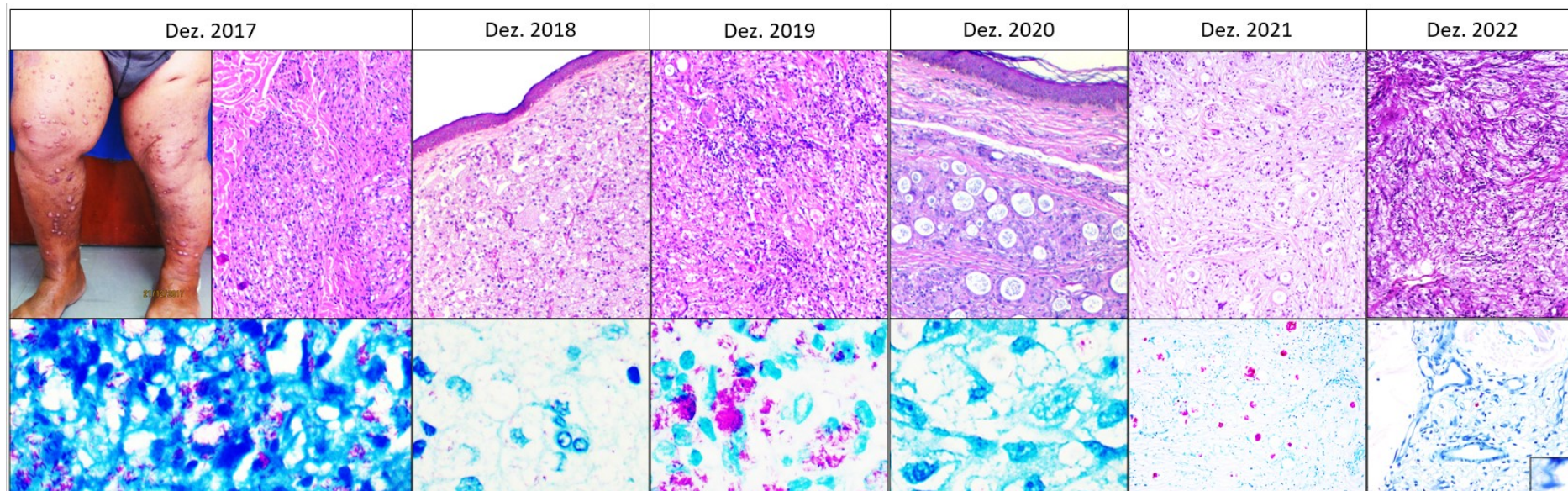
Conhecer as alterações histopatológicas que ocorrem nas lesões cutâneas da hanseníase após a conclusão da PQT pode ajudar o patologista a determinar se as lesões continuam ativas ou se tornaram inativas histologicamente (Joshi, 2011; Massone, 2012; Soares, 2021).

2.8.1 Atividade de doença ao fim de tratamento

A literatura mostra que alterações histomorfológicas ao final do tratamento da hanseníase que traduzem atividade de doença incluem fundamentalmente persistência de granulomas bem-formados com células epitelioides, infiltrado linfocitário neural/perineural, presença de bacilos dentro do endotélio e infiltrado linfo-histiocitário perianexial (Jain *et al.*, 2016; Joshi, 2011; Nair; Pariath, 2018; Soares, 2021) (Figura 17).

Acredita-se que, na doença ativa, na qual há uma rápida renovação celular, os macrófagos tenham citoplasma abundante e eosinofílico (macrófagos epitelioides), que são frequentemente vistos na hanseníase recidivada (Ridley, 1980). Considerando ainda a presença de granulomas epitelioides como marcador de atividade de doença, esse achado esteve pouco presente, na minoria dos participantes de um estudo já citado que analisou a resposta histopatológica à PQT na hanseníase PB (Nair; Pariath, 2018).

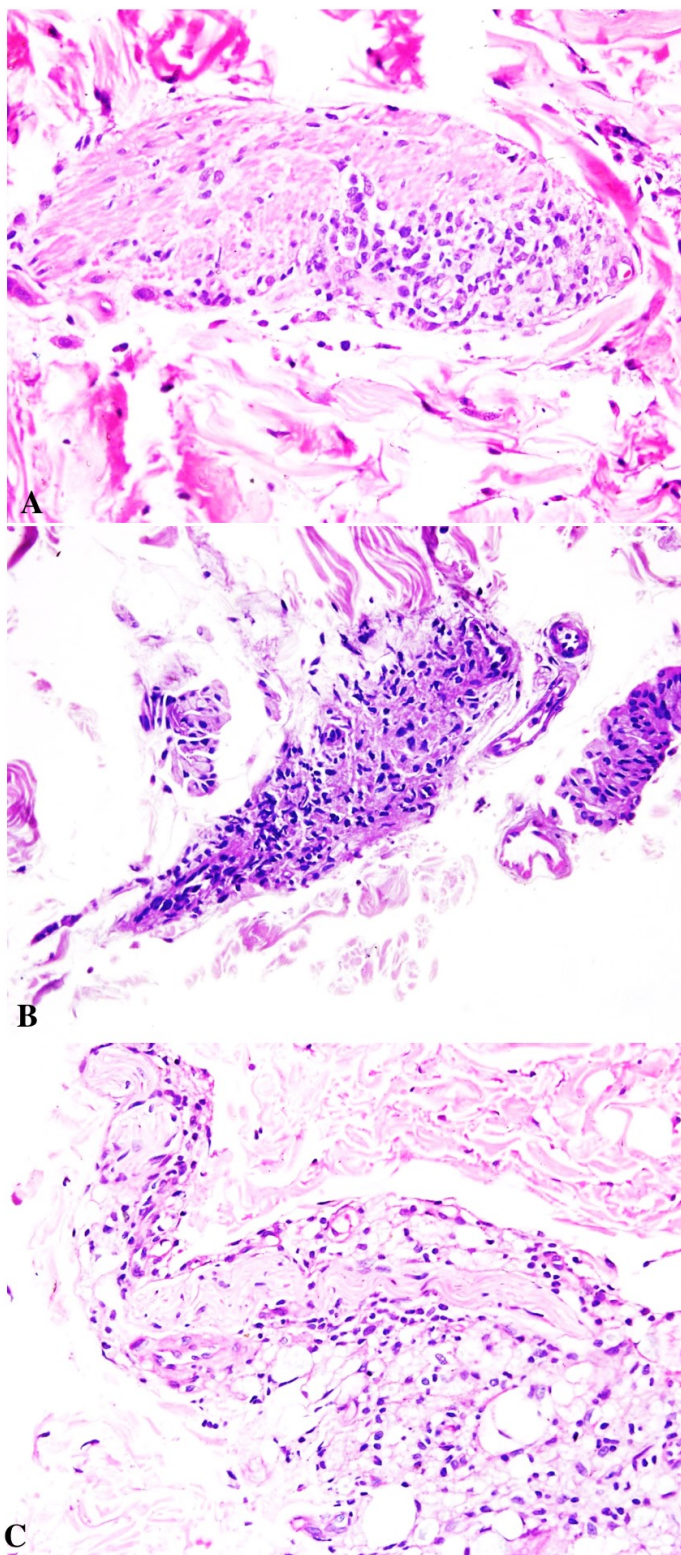
Figura 16 - Falência terapêutica na hanseníase



Evolução histopatológica e de carga bacilar ao longo dos anos. O início de tratamento com PQT deu-se em 2017, desde então o paciente se encontra medicado. As lesões de pele têm se mantido ricas em macrófagos espumosos. O IB tem oscilado e permanece com bacilos sólidos, persistentes, até o último controle em 2022.

Fonte: Material do CREDESH e da UAPAT/HC-UFU/EBSERH.

Figura 17 - Alterações de persistência de atividade de doença pós-PQT



A) Acometimento linfocitário de músculo pilo-erector (H&E, 40x). B) Pequena coleção de macrófagos epitelioides com destruição de ramúsculo neural (H&E, 40x). C) Neurite/perineurite linfocitária (H&E, 40x).

Fonte: Material da UAPAT/HC-UFU/EBSERH.

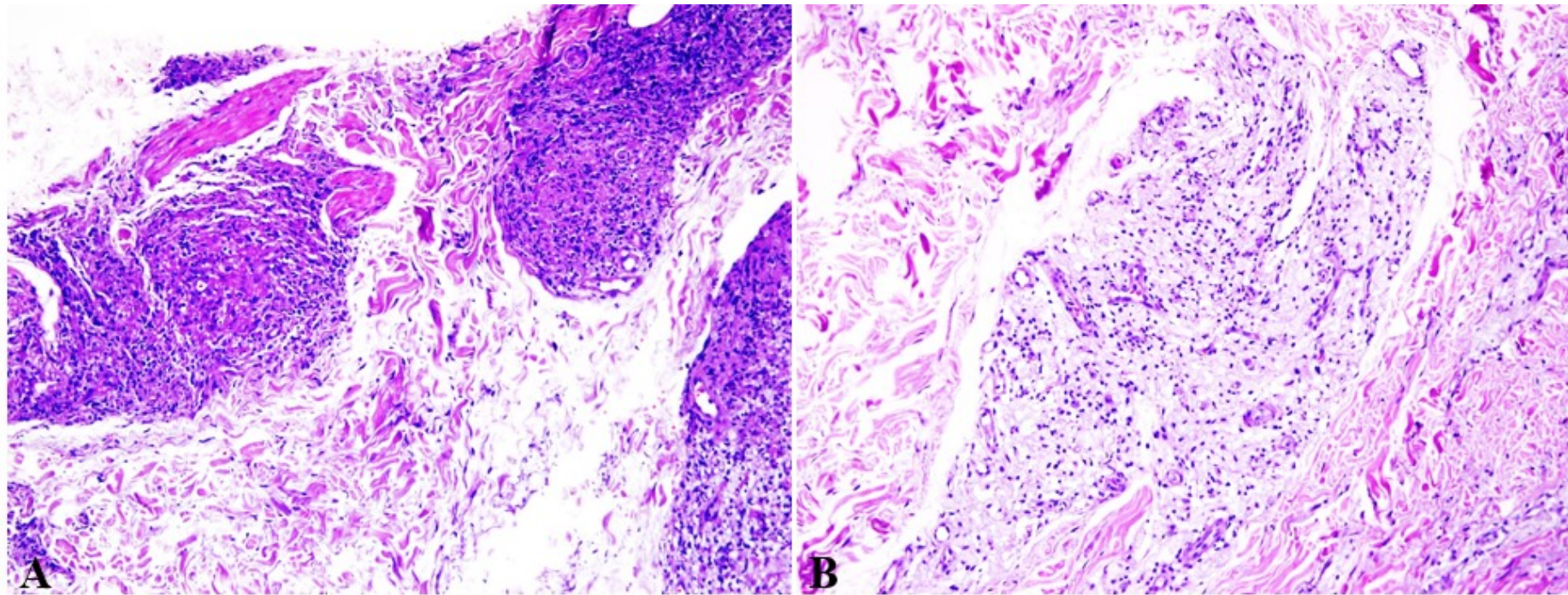
Lesões cutâneas ativas e persistentes são observadas apesar da conclusão da PQT em poucos pacientes do espectro TT e DT e devem ser causadas por bacilos resistentes às drogas ou a um fenômeno autoimune desencadeado e mantido por antígenos bacilares persistentes (Narang *et al.*, 2020). Estudos sugerem que os pacientes PB devem estar sob vigilância por pelo menos 3 anos e os pacientes MB por 9 anos, para que a maioria das recorrências na hanseníase possa ser detectada (Kaimal; Thappa, 2009).

2.8.2 Alterações de regressão de doença ao fim de tratamento

Nas formas TT e DT, os granulomas epitelioides desaparecerem progressivamente, com perda gradual de células epitelioides, sem fibrose, e assumem progressivamente o quadro de um infiltrado linfocitário perivascular superficial ao longo de 1 a 3 anos após a PQT (Massone, 2012) (Figura 18).

Nas formas DV e VV, a degeneração gordurosa dos macrófagos (Figura 18b), com aparecimento de vacúolos citoplasmáticos, caracteriza as lesões regressivas, que desaparecem com lentidão, ao longo de 2–5 anos ou mais (Massone, 2012; Sarno *et al.*, 1984; Soares, 2021). Entretanto, sabe-se que em alguns pacientes, algumas células espumosas podem perdurar por décadas (Lockwood *et al.*, 2008; Soares, 2021). Enquanto alguns autores defendem que granulomas constituídos por macrófagos uniformemente espumosos e contendo debris de bacilos fragmentados e granulares são alterações morfológicas observadas na hanseníase tratada e denotam regressão (Joshi, 2011), outros estudos têm se focado na homeostase lipídica e seu papel na fisiopatologia da infecção; o desequilíbrio homeostático pode levar à formação de células espumosas (Reiss; Glass, 2006).

Figura 18 - Fenótipos de macrófagos



A) Macrófagos epitelioides ativados (H&E, 20x). B) Macrófagos espumosos, xantomizados (H&E, 20x).

Fonte: Material da UAPAT/HC-UFU/EBSERH.

2.8.3 Queda do índice bacioscópico ao fim de tratamento

O IB retrata a concentração de bacilos em esfregaços ou tecidos e inclui bacilos vivos e mortos (Naik *et al.*, 2022). Na maioria dos casos PB, o IB médio é maior na biópsia de pele do que nos esfregaços intradérmicos (Premalatha *et al.*, 2016; Soares, 2021). A razão sugerida é que as bactérias na PB são mais comuns nos tecidos mais profundos (particularmente nos nervos) do que nos estratos dérmicos superficiais e, portanto, provavelmente não são detectadas no índice baciloscópico do raspado intradérmico (IBr) (Kaur *et al.*, 2003). Por outro lado, a correlação entre IBh e IBr é excelente nos casos MB (Naik *et al.*, 2022; Premalatha *et al.*, 2016).

Após o início do tratamento, o IB reduz cerca de 1 log ao ano (Levy, 1976) (Figura 19). A taxa de declínio do IBr por ano é um método mais apropriado para avaliar a remoção de bacilos do que a negatividade do esfregaço, o que é em grande parte um reflexo da classificação e do IBr inicial (Saunderson, 2016). Isto é, aqueles pacientes que inicialmente apresentam um alto IB correm maior risco de recidiva após a PQT de duração fixa em comparação com os pacientes com IB negativo ou baixo no momento do diagnóstico de hanseníase (Kaimal; Thappa, 2009).

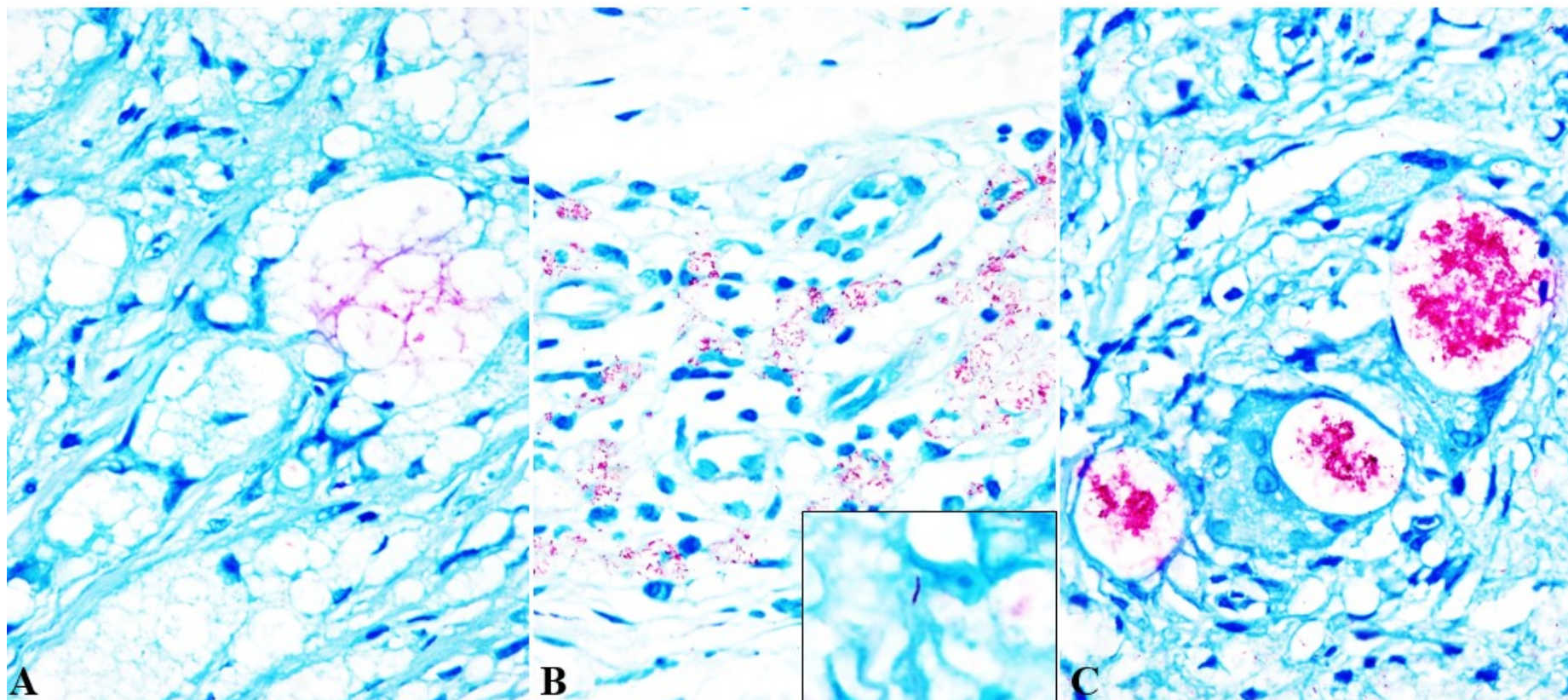
2.9 Definições na hanseníase pós-tratamento

2.9.1 Alta por cura

Um estudo teórico norteado no referencial metodológico da análise de conceitos concluiu que “alta do paciente em hanseníase” inscreve-se como uma condição clínica na qual o paciente inicialmente infectado pelo *M. leprae* e diagnosticado como caso de hanseníase iniciou e completou o tratamento, seja para um caso PB, seja para um caso MB dentro do período estimado pela OMS (Pinheiro *et al.*, 2018). Do mesmo modo, a OMS define alta por cura como:

The currently recommended treatment regimen consists of three drugs: dapsone, rifampicin and clofazimine. The combination is referred to as multi-drug therapy (MDT). The duration of treatment is six months for PB and 12 months for MB cases. MDT kills the pathogen and cures the patient (World Health Organization, 2023b).

Figura 19 - Índice baciloscópico na biópsia de pele pós-PQT



A) Bacilos fragmentados e granulados, com perda da afinidade tintorial, dentro de macrófagos (FF, 40x). B) Alto índice bacilar residual ao fim do tratamento (FF, 40x) No detalhe, bacilo sólido, persistente (FF, 100x). C) Alta carga bacilar e numerosos bacilos intensamente corados depois do fim do tratamento (H&E, 40x).

Fonte: Material da UAPAT/HC-UFU/EBSERH.

Ressalta-se que o esquema preconizado com PQT é administrado por um tempo fixo, independentemente do desaparecimento das lesões de pele ou do estado bacilífero do indivíduo (Nair; Pariath, 2018). Urge, portanto, estabelecer critérios objetivos que definam a real cura na hanseníase.

2.9.2 Insuficiência terapêutica

O MS define insuficiência terapêutica como caso especial por problemas de biodisponibilidade, interações medicamentosas e/ou falhas de absorção que ocorre em:

Situação especial em que o paciente, por diferentes motivos, não recebeu tratamento adequado e suficiente, tais como: casos com irregularidade ao tratamento padrão PQT; casos que foram erroneamente classificados como PB quando deveriam ser classificados como MB; casos MB tratados com esquemas nos quais os pacientes receberam apenas clofazimina diária e rifampicina mensal, por inviabilidade de utilizar a dapsona do esquema padrão; casos geralmente muito avançados e anérgicos, com muitos hansenomas e infiltrações, com índice baciloscópico maior que 3+ e/ou ELISA anti-PGL-I elevados que receberam doze doses PQT/MB e após avaliação clínica e/ou baciloscópica na referência terão necessidade de doze doses adicionais (Brasil, 2015) .

Assim, a insuficiência terapêutica contradiz os atributos essenciais para se definir o conceito de alta do paciente em hanseníase. Embora iniciado o tratamento com apresentação de melhora clínica, o paciente não completa o curso completo da PQT, não alcançando, portanto, a cura da hanseníase (Pinheiro *et al.*, 2018).

2.9.3 Falência terapêutica

Falência terapêutica pode ser compreendida como persistência de atividade de doença ou piora das lesões de pele e falha na queda do IB para a hanseníase MB (Lazo-Porras *et al.*, 2020; Levy, 1976). São aqueles casos que não respondem como esperado, sem arrefecimento de lesões de pele e clareamento de bacilos ao fim da PQT (Kaimal; Thappa, 2009). Já o MS entende como falência terapêutica como situação rara que deve ser suspeitada quando:

O paciente diagnosticado com hanseníase não apresenta sinais de melhora clínica durante o tratamento regular e adequadamente indicado de seis ou doze doses PQT. Situação em que o paciente MB recebeu até vinte e quatro doses

de PQT/MB, portanto já ultrapassou o critério de insuficiência terapêutica, e que, na alta por cura, apresenta sinais de atividade clínica e/ou presença de bacilos íntegros bem definidos no raspado dérmico e/ou exame histopatológico de pele e, quando disponível, manutenção de altos níveis de ELISA anti-PGL1 (IgM) (Brasil, 2015).

Outros autores estabelecem diferentes critérios para falência terapêutica como ou lesões ativas persistentes ou surgimento de novas após completar 12 meses de PQT, positividade ou valor crescente do índice morfológico e aumento de 2 log no IB depois de 12 meses de PQT (Lobo, 1992; Narang *et al.*, 2020). Um aspecto clínico de utilidade diagnóstica é o aparecimento de novas lesões e *downgrading* do paciente na falência terapêutica, enquanto na recidiva há reaparecimento das lesões antigas, mas o paciente raramente apresenta *downgrading* (Singh *et al.*, 2011).

A recorrência de doença em até 9 meses após a alta por cura é provavelmente um sinal de falência terapêutica, em vez de recidiva, que ocorre mais tardiamente após a PQT (Nery *et al.*, 2021). Assim, a falência terapêutica deve ser entendida como um evento precoce, próximo à alta já que a maioria acontece nos primeiros 3 anos após o fim do tratamento (Kaimal; Thappa, 2009). Entretanto, não há critério claro ou universalmente aceito para falência terapêutica na hanseníase, considerada como resultado de baixa adesão ao tratamento ou de resistência medicamentosa (Penna, 2014).

2.9.4 Recidiva na hanseníase

Na literatura, existem variadas definições propostas para recidiva na hanseníase (Becx-Bleumink, 1992; Nair; Pariath, 2018; Rajan *et al.*, 2021; World Health Organization, 1995). A OMS define recidiva na hanseníase como a situação na que o paciente que finalizou o esquema fixo da PQT e volta a manifestar novos sinais e sintomas da doença durante a vigilância de dois anos para a hanseníase PB e de cinco anos para a hanseníase MB (World Health Organization, 1995). Outros critérios para recidiva da hanseníase citados na literatura englobam reativação ou aparecimento de novas lesões de pele, IBr $\geq 2+$, nova atividade neural periférica, evidência histopatológica de recidiva na pele ou nervo e atividade hansênica ocular (Becx-Bleumink, 1992; Rajan *et al.*, 2021). Enquanto o MS define como recidiva como:

Todos os casos de hanseníase, tratados regularmente com esquemas oficiais padronizados e corretamente indicados, que receberam alta por cura, isto é, saíram do registro ativo da doença no SINAN, e que voltam a apresentar novos

sinais e sintomas clínicos de doença infecciosa ativa. Os casos de recidiva em hanseníase geralmente ocorrem em período superior a cinco anos após a cura (Brasil, 2015).

O diagnóstico clínico de recidiva pode ser desafiador (Opromolla, 1994). O Quadro 2 resume as características para se estabelecer o diagnóstico diferencial entre reação hansênica e recidiva na hanseníase.

Quadro 2 - Comparação entre as características clínicas de reação e recidiva na hanseníase

CARACTERÍSTICAS	REAÇÃO	RECIDIVA
Período de ocorrência	Frequente durante a PQT e/ou menos frequente no período de dois a três anos após término do tratamento	Em geral, período superior a cinco anos após término da PQT
Surgimento	Súbito e inesperado	Lento e insidioso
Lesões antigas	Algumas ou todas podem se tornar eritematosas, brilhantes, intumescidas e infiltradas	Geralmente imperceptíveis
Lesões recentes	Em geral, múltiplas	Poucas
Ulceração	Pode ocorrer	Raramente ocorre
Regressão	Presença de descamação	Ausência de descamação
Comprometimento neural	Muitos nervos podem ser rapidamente envolvidos ocorrendo dor e alterações sensitivo-motoras	Poucos nervos podem ser envolvidos com alterações sensitivo-motoras de evolução mais lenta.
Resposta a medicamentos antirreacionais	Excelente	Não pronunciada

Fonte: Brasil (2016).

2.10 Fatores para recorrência na hanseníase

2.10.1 Resistência medicamentosa

A falência terapêutica deve alertar para a investigação molecular de resistência medicamentosa aos fármacos da PQT (Rosa, 2011; World Health Organization, 2017). Em 2021, oito países comunicaram dados sobre resistência antimicrobiana. Dos 3.452 pacientes

testados, 51 foram diagnosticados com cepas de *M. leprae* resistentes à rifampicina, 49 à dapsona e 3 à ofloxacina; 4 tinham cepas resistentes a mais de um antimicrobiano (World Health Organization, 2021b).

As taxas de resistência medicamentosa variam entre 8,0% e 43,24% cepas de *M. leprae* mutadas conferindo resistência às drogas da PQT (Cambau *et al.*, 2018; Diório *et al.*, 2009; Rosa *et al.*, 2020). As taxas atuais de resistência aos medicamentos podem estar subestimadas pela nossa falta de conhecimento dos mecanismos moleculares subjacentes (Aubry *et al.*, 2022). A instituição imediata do tratamento, a detecção precoce de casos e a monitorização eficiente da resistência à PQT são pilares da estratégia global de controlo da hanseníase (World Health Organization, 2021a). Contudo, é necessário desenvolver estratégias para avaliar melhor as causas da falência e identificar prontamente casos de resistência (Rosa, 2011).

2.10.2 Bacilos persistentes

Os bacilos persistentes são organismos permanente ou parcialmente dormentes capazes de sobreviver apesar da PQT adequada e estão presentes em cerca de 10% dos pacientes MB e esse número pode ser ainda maior nos casos com alto IB (Levy, 1976; Saunderson, 2016; Kaimal; Thappa, 2009). Isso porque os bacilos adormecidos estão em áreas imunologicamente favoráveis, como nervos dérmicos, músculo liso, linfonodos, íris, medula óssea e fígado, de modo que não podem ser alcançados pelos medicamentos durante o tratamento (Maghanoy *et al.*, 2011). Um centro de referência terciário para a hanseníase conduziu uma pesquisa com pacientes MB, de duração média de 9,41 anos entre a conclusão da PQT e a recidiva e detectou 68,2% (15/22) de casos de recidiva após o intervalo maior ou igual a 6 anos, sendo a causa mais provável a presença de *M. leprae* persistentes (Rajan *et al.*, 2021).

2.10.3 Duração da PQT

A recidiva na hanseníase é influenciada por outros fatores além do tempo de acompanhamento, como classificação de hanseníase, duração da PQT, episódios reacionais e IB (Shetty *et al.*, 2005). Em relação à duração da PQT, um estudo que acompanhou por um período maior ou igual a 12 anos pacientes tratados com 24 doses de PQT, reportou uma taxa de recidiva de 9% (13/142) (Gelber; Balagon; Cellona, 2004). Outro estudo acompanhou 300 pacientes com hanseníase MB que completaram um ano de PQT, nele não foram encontradas

recaídas em menos de 5 anos após o final do tratamento, mas 3% (8/300) de recidivas foram detectadas entre 6 e 9 anos depois, enquanto 5% (15/300) de casos de recidiva só foram identificados 10 anos ou mais após a conclusão da PQT (Maghanoy *et al.*, 2011).

Pacientes MB (162) foram tratados com 12 doses de PQT e seguidos por 4,08 anos, desses indivíduos 8% (13/162) apresentaram recaída da doença em um período de até dois anos, o que foi atribuído a insuficiência ou falência terapêutica (Kumar *et al.*, 2013). A OMS afirma que um regime mais curto (6 doses) de PQT implica em um aumento potencial no risco de falência terapêutica (World Health Organization, 2018). Quando a duração do tratamento não é longa o suficiente para que o organismo elimine os bacilos, a resposta inflamatória contínua resulta em atividade clínica persistente por 12 a 18 meses, que às vezes pode resultar em falência terapêutica (Malathi; Thappa, 2013).

Ao mesmo tempo em que existem trabalhos que afirmem que as recorrências na hanseníase sejam pouco frequentes, outros as relatam como comuns. Essa discussão tem fundamental pertinência à atualidade, já que as variações nas estimativas das taxas de recidiva são grandes entre os diferentes países (Kaimal; Thappa, 2009), mas, certamente, nos indivíduos tratados com PQT não são baixas (Gelber; Balagon; Cellona, 2004). Ademais, a taxa de recidiva na hanseníase é a medida mais importante de eficácia da PQT (World Health Organization, 1995).

2.10.4 Tempo de seguimento

Um tempo médio de 10 anos de seguimento atribui uma maior e melhor vigilância pós-alta (Maghanoy *et al.*, 2011; Nascimento *et al.*, 2022). Assim, a taxa de recidiva na hanseníase tende a aumentar consideravelmente quanto maior for o tempo de seguimento dos pacientes, porém torna-se mais difícil de se acompanhar após o sexto ano (Pattyn; Grillone, 2002; Waters, 1995). Um trabalho mostrou uma taxa de recidiva de 4% (6/136) aos seis anos da alta por cura, que se elevou a 13% (17/136) após 8 anos de acompanhamento (Pattyn; Grillone, 2002). Isso prova que os números variam em dependência do tempo em que os pacientes são seguidos.

3 OBJETIVOS

3.1 Objetivo geral

O objetivo geral deste trabalho foi detectar fatores laboratoriais histopatológicos, sorológicos e moleculares para a recidiva e falência terapêutica na hanseníase.

3.2 Objetivos específicos

3.2.1 Estudo 1

- Estabelecer o perfil clínico, histopatológico, sorológico e molecular de casos recidiva na hanseníase;
- Conhecer a taxa de recidiva na hanseníase no período estudado;
- Correlacionar os achados laboratoriais com dados epidemiológicos, clínicos, terapêuticos neste grupo de doentes;
- Sugerir critérios de alta por cura do esquema terapêutico padrão de poliquimioterapia;
- Definir valor preditivo dos achados histopatológicos na biópsia de pele pós-alta;
- Definir valor preditivo dos achados sorológicos pós-alta;
- Definir valor preditivo dos achados moleculares pós-alta;
- Correlacionar o valor preditivo dos achados laboratoriais.

3.2.2 Estudo 2

- Estabelecer o perfil clínico, histopatológico, sorológico e molecular de casos falência terapêutica na hanseníase;
- Conhecer a taxa de falência terapêutica no período estudado;
- Correlacionar os achados laboratoriais com dados epidemiológicos, clínicos, terapêuticos neste grupo de doentes;
- Definir valor preditivo dos achados histopatológicos na biópsia de pele pós-alta;
- Definir valor preditivo dos achados sorológicos pós-alta;
- Definir valor preditivo dos achados moleculares pós-alta;
- Correlacionar o valor preditivo dos achados laboratoriais.

4 RESULTADOS

4.1 Estudo 1 - Role of histopathological, serological and molecular findings for the early diagnosis of treatment failure in leprosy. Part I

Role of histopathological, serological and molecular findings for the early diagnosis of treatment failure in leprosy. Part I

Running title: Early Detection of Leprosy Treatment Failure

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Summary

Objectives: This case-control study aimed to identify predictive laboratory factors for treatment failure (TF) in leprosy patients completing multidrug therapy (MDT/WHO).

Methods: We analyzed 80 individuals from a national leprosy reference center, comprising 40 TF cases (mean relapse at 13.0 months) and 40 controls (mean 113.1 months without disease signs). Epidemiological and clinical-laboratory parameters were assessed post-MDT. Results Foamy granuloma (OR=7.36; $p=0.0012$) and histological bacillary index (hBI) (OR=1.55; $p=0.0004$) in skin samples correlated with TF, with hBI $\geq 3+$ showing 73% sensitivity and 78% specificity (AUC: 0.75; $p=0.0001$). Anti-phenolic glycolipid I (anti-PGL-I) serology ($p=0.0024$) indicated a 1.4-fold increase in TF odds for ELISA index (EI) ≥ 1 , achieving 79% sensitivity and 59% specificity with EI ≥ 3.95 (AUC: 0.74; $p=0.0001$). Real-time quantitative PCR positivity for *Mycobacterium leprae* DNA resulted in 3.43-fold greater TF odds ($p=0.0201$). Multivariate analysis revealed a joint occurrence of neural/perineural lymphocytic infiltrate, foamy granuloma, BI $\geq 1+$, and EI ≥ 1 significantly associated with TF (predictive probability up to 95.41%).

Conclusion: Nerve-selective lymphocytic infiltrate persistence, foamy granulomas, and bacilli in skin samples, along with elevated EI post-MDT, may discriminate individuals at higher risk of TF.

Keywords: glycolipid-phenolic-1, leprosy, pathology, real-time polymerase chain reaction, treatment failure

Introduction

150 years after the discovery of *M. leprae*, leprosy continues to be a concern for the medical community and public health in developing countries (1–3). And, almost two decades ago, a new species was described as causing this granulomatous disease, with a predilection for peripheral nerves and skin, namely *Mycobacterium lepromatosis* (4). These alcohol-acid-resistant bacilli go far beyond Schwann cells and macrophages, parasitizing diverse other cell types throughout the body tissues and organs (5).

According to the Ridley and Joplin (R&J) classification and resulting from the interaction between the bacillus and the host's immune system, leprosy is a spectral disease that has two polar forms, tuberculoid (TT) and lepromatous leprosy (LL), and three unstable and changing forms, namely borderline-tuberculoid (DT), borderline-borderline (DD) and borderline-lepromatous (BL) (6). The R&J classification reflects the complexity of the disease, correlating its clinical presentations with distinct immunopathological patterns, from a vigorous cellular immune response, with a Th1 pattern in the tuberculoid pole, to an absence of a specific cellular response to bacillary antigens in the lepromatous pole, with a predominance of the Th2 response and exacerbation of the humoral mechanism (3). On the contrary, the treatment of this spectral disease, since 1981, has been carried out under the simplistic WHO operational classification adopted by programs in most countries, which divides patients into just two groups, paucibacillary (PB) and multibacillary (MB) leprosy (7–9). Thus, patients are treated with the same three-drug MDT, comprised of rifampicin, dapsone, and clofazimine, with a fixed-duration treatment of six months for PB and 12 months for MB leprosy (10).

The scientific community has discussed and expressed concerns about the choice of treatment using the WHO operational classification, given its understanding that the

indication of MDT may inappropriately assign PB treatment regimens to MB patients (11–13). Thus, the R&J spectral classification would support, at least potentially, the choice of the more indicated treatment, resulting in a minimum relapse rate (14–16).

Inadequate treatment of leprosy can result in treatment insufficiency for different reasons, such as irregularity in taking MDT, erroneous operational classification of MB patients as PB, unfeasibility of using dapsone, and bioavailability issues or drug interactions (17). However, TF is a rare situation in which the patient does not show signs of clinical improvement during adequate and well-indicated MDT and presents solid bacilli or maintenance of high EI levels after the end of therapy and for less than five years (17–19). The numbers referencing insufficiency and TF are little known due to the lack of standardization of the defining criteria for these cases. In the literature, the TF rate varies from 4.72% to 31.68% during the first five years after treatment with MDT (20–22).

TF must alert the clinician to perform a molecular investigation of drug resistance to the MDT drugs (23,24). Drug resistance rates concerning mutated *M. leprae* strains conferring resistance to MDT drugs vary between 8.0% and 43.24% (25–27). Even though, the current rate of drug resistance may be underestimated (28). Immediate institution of treatment, early detection of cases, and efficient monitoring of resistance to MDT are pillars of the global leprosy control strategy (29).

Clinically, the resolution of skin lesions and, objectively, the decrease or negative results of BI, EI, and qPCR are of great use in evaluating the effectiveness of MDT and in distinguishing between TF and leprosy reaction throughout and at the end of treatment (30–32). It is in these situations when combined clinical and laboratory evaluation becomes essential to the early diagnosis of TF cases.

Little has been dedicated to the identification of predictive clinical and laboratory factors and almost nothing to the value of post-MDT cutaneous histopathological findings associated with TF. The main objective of this work was to identify histopathological, serological, and molecular predictive factors at discharge from the first treatment of patients who were subsequently diagnosed with TF, within a period of less than five years from treatment discharge.

Materials and methods

Study design and selection of cases

We carried out a case-control study of leprosy cases diagnosed at CRESDEH/HC-UFU/EBSERH, Minas Gerais, Brazil, between 2008 and 2020. Among the 1,869 cases reported by CREDESH during this period, the initial selection was 567 cases based on analysis of secondary data, obtained from medical records, specific software, and the National Reference Center and the Notifiable Diseases Information System (SINAN) of the Brazilian Ministry of Health (MS).

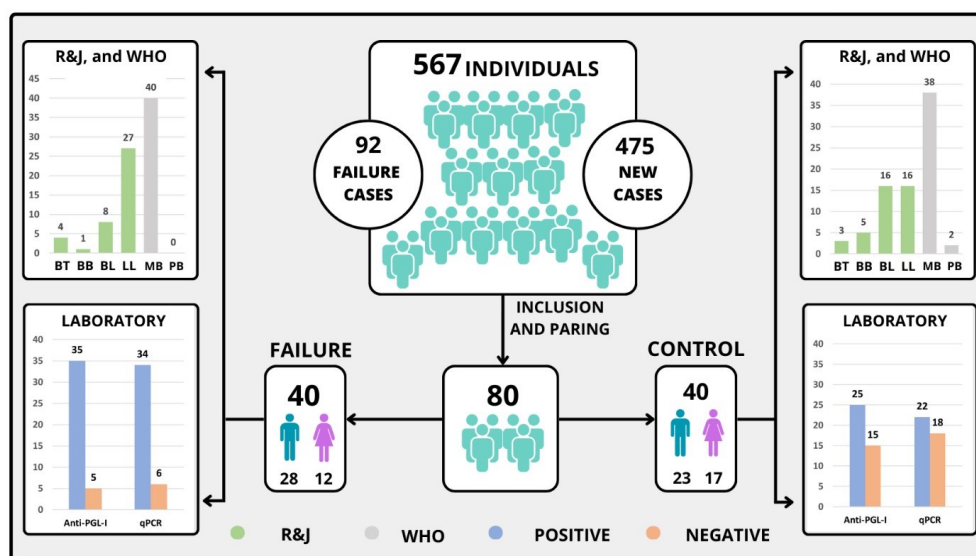
The patients included in this study were adults (age ≥ 18 years) regularly treated with a fixed MDT regimen of six, 12, or 24 doses. All patients were diagnosed, classified, and treated by experienced leprologists at CREDESH. According to clinical, histopathological, bacillary, serological, and molecular characterization, patients were classified following the R&J spectrum (6) into borderline-tuberculoid (BT), borderline-borderline (BB), borderline-lepromatous (BL) or lepromatous leprosy (LL) and, for treatment purposes, into PB or MB according to the WHO operational classification (8).

We divided the sample into two comparative groups: a) Treatment Failure Group: we selected all 92 cases reported as TF cases in our specific software and SINAN in the

period between 2013 and 2020; and, b) Control Group: we selected 475 new cases of leprosy between 2008 and 2013, and included those cases identified as having no notifications of other reentries in our specific software and SINAN after treatment discharge in a period ≥ 60 months since the end of the first treatment.

We included patients who completed the treatment regimen and had results from bacilloscopic exams, IgM anti-PGL-I serology, and qPCR for *M. leprae* DNA at the end of treatment. We excluded patients with other infectious comorbidities such as tuberculosis, HIV/AIDS, and viral hepatitis. Furthermore, we excluded those cases in which formalin-fixed paraffin-embedded blocks were insufficient or unavailable in the Pathology Unit files of HC/UFU/EBSERH for new histological sections. Finally, the cases selected from Group TF and Group C were paired, resulting in 40 patients in both groups (Figure 1).

Figure 1. Selection and pairing of individuals from the treatment failure group, and the control group



Epidemiological and clinical data and laboratory results at the end of the first multidrug therapy for leprosy. Ridley and Jopling (R&J) classification. BT: borderline-tuberculoid

leprosy; BB: borderline-borderline leprosy; BL: borderline-lepromatous leprosy; LL: lepromatous leprosy. World Health Organization (WHO) standard operational classification. PB: paucibacillary leprosy; MB: multibacillary leprosy. Anti-PGL-I serology (IgM), positive if ELISA index (EI) ≥ 1 . qPCR: real-time quantitative polymerase chain reaction.

Definition of treatment failure in leprosy

TF is defined as all cases of leprosy that do not show signs of clinical improvement during regular and appropriately indicated treatment of six or 12 MDT doses or a situation in which the MB patient has received up to 24 MDT doses, therefore having already exceeded the criteria for treatment insufficiency, and which, upon treatment discharge, presents: signs of clinical activity and/or the presence of well-defined solid bacilli in the dermal smear, and/or histopathological examination of the skin and maintenance of high levels of anti-PGL-I (IgM) (17).

In this study, the diagnosis of TF was established by experienced leprologists from CREDESH based on clinical and laboratory findings, ruling out reactionary states and complying with the TF criteria from the MS.

Study variables

Epidemiological and clinical variables. The hospital records of each patient were consulted to obtain the following data points: sex, age, R&J clinical form, WHO operational classification, number of doses, time (in months) between treatment discharge and TF for Group TF and time (in months) elapsed since treatment discharge for Group C.

Laboratory variables. Intradermal smear bacilloscopy. The smear bacillary index (sBI) was determined by collecting samples from at least six skin sites (earlobes, elbows, and knees). The smears were stained using the cold Ziehl-Neelsen technique according to the MS protocol (33).

Histopathological analysis. The skin lesion sample was fixed in 10% buffered formalin, embedded in paraffin and the 3 μ m histological sections were stained with hematoxylin-eosin (H&E) and Fite-Faraco (FF) staining, which were utilized to evaluate the histopathological variables (Table 1).

Table 1. Histopathological characteristics evaluated in the skin sample at the end of multidrug therapy for leprosy: definitions.

Characteristic	Criteria
Epithelioid granuloma	Aggregates of macrophages with abundant eosinophilic cytoplasm, with occasional multinucleated giant cells, without central caseation/necrosis
Neural/perineural lymphocytic infiltrate	Aggregates of polymorphic lymphocytes, with a predominance of small forms, in variable quantities, around and/or delaminating the perineurium and/or within the nerve
Lymphocytes in the epidermis and/or eccrine ductal epithelium	Aggregates of polymorphic lymphocytes, with a predominance of small forms, in variable quantities, above the basement membrane of the epidermis and/or eccrine duct
Atrophy of the epidermis	Epidermal thickness diminished due to the reduced number of keratinocytes and flat at its base with loss of the papillary pattern
Perivascular and/or perianexial edema	Extracellular accumulation of fluids around vessels and/or skin appendages
Fibrosis of the dermis	Elevated amount of fibroblasts and collagen causing thickening of the dermis in a morphea pattern
Non-cohesive lymphocytic infiltrate	Disperse lymphocytes along the dermis, forming no aggregates or follicles
Foamy granulomas	Prominent aggregates of macrophages with cytoplasmic lipid vacuoles of variable size

To quantify the bacillary load, we performed staining by the modified FF technique (Table 2) (34) and applied the logarithmic histological bacillary index (hBI) (Table 3) (35).

Table 2. The protocol adopted for Fite-Faraco staining

Modified Fite-Faraco technique	
	1. Remove the paraffin in mineral oil, at 60° to 80°, for 1 hour*
	2. Clean the excess oil from the backside of the slide
	3. Dry the section very gently with fine filter paper, three times
	4. Wash in running tap water for 5 minutes
	5. Rinse in distilled water
	6. Perform carbol fuchsin staining for 30 minutes
	7. Wash in running tap water for 2 minutes
	8. Differentiate to purplish-pink color⁺
	9. Immerse for five to six dips in 0.15% methylene blue
	10. Wash under running tap water for about 3 minutes to light blue
	11. Quickly dehydrate in absolute alcohol, for three changes
	12. Immerse in xylene for two changes and assemble

*Slides should only be deparaffinized in oil and never in xylene.

⁺ Differentiate with 1% acid alcohol and intersperse with distilled water at least 3 times.

Adapted from C. Massone (1).

Table 3. Logarithmic scale of Ridley and Hilson for bacillary index on histological sections

0	Absence of bacilli in 100 fields examined*
1+	1–10 bacilli/100 fields examined
2+	1–10 bacilli/10 fields examined
3+	1–10 bacilli/ field examined
4+	10–100 bacilli/field examined
5+	100–1.000 bacilli/field examined
6+	>1,000 bacilli/field examined (globy)

* The slides must be observed at 100x objective in immersion oil. Adapted from Ridley

(1–3)

The analysis of slides from cases in both groups, FT and C, was carried out blindly by four observers simultaneously using a multi-head optical microscope (Nikon Eclipse 80i, Nikon Corporation, Japan). Three of the four observers were pathologists with experience in R&J classification.

IgM anti-PGL-I serology. IgM anti-PGL-I serum antibodies were detected by immunoassay (ELISA) performed against native PGL-I purified from the *M. leprae* cell wall. The following reagent was obtained by donation from BEI Resources, NIAID, NIH: *Mycobacterium leprae* Phenolic Glycolipid-I (PGL-I), NR-19342 and Monoclonal Anti-*Mycobacterium leprae* PGL-I, Clone CS-48 (produced *in vitro*), NR-19370. The titer of anti-PGL-I antibodies was expressed as an ELISA index. EI values ≥ 1.0 were considered positive (36).

DNA extraction and quantitative polymerase chain reaction. DNA was extracted from skin fragments and frozen in liquid nitrogen to amplify the specific genomic region of the bacillus (RLEP3) in a qPCR system (ABI 7300, Applied Biosystems, Foster City, CA, United States) (37, 38).

Ethical aspects

The research followed the principles of the Declaration of Helsinki. Informed consent was waived and the research was approved by the Research Ethics Committee of the Federal University of Uberlândia (CAAE: 42878620.0.0000.5152) on 16/04/2021. The data were obtained from medical records, specific software, and SINAN, and were blinded for analysis. The informed consent waiver was also requested due to the difficulty of obtaining written consent from patients in a long-term, retrospective study.

Statistical analyses

Quantitative variables were described as the mean, median, maximum, and minimum standard deviation. Qualitative variables were described (frequency and percentage) using double-entry tables. The associations of qualitative variables were evaluated using the likelihood ratio test (39).

The variables that differentiate Groups C and R were evaluated by univariate logistic regression, as well as multiple logistic regression followed by variable selection using the stepwise method (40). The ROC (Receiver Operating Characteristic) curves for the hBI variables in skin biopsy and anti-PGL-I serology were constructed to evaluate the performance of these tests in detecting Group R. All tests were applied using a significance level of 5% ($p < 0.05$). The procedures were performed using the Statistical Package for Social Sciences (SPSS) version 22.0 (IBM, Armonk, NY, U.S.A.).

Results

The mean time elapsed between treatment discharge and TF was 13.0 months (Min. 1, Max. 42 months) in the Group TF. For Group C, the mean disease-free period was 113.1 months (Min. 83, Max. 158 months) (Table S1). The TF rate was 6.7% (92/1,385) between 2013 and 2020.

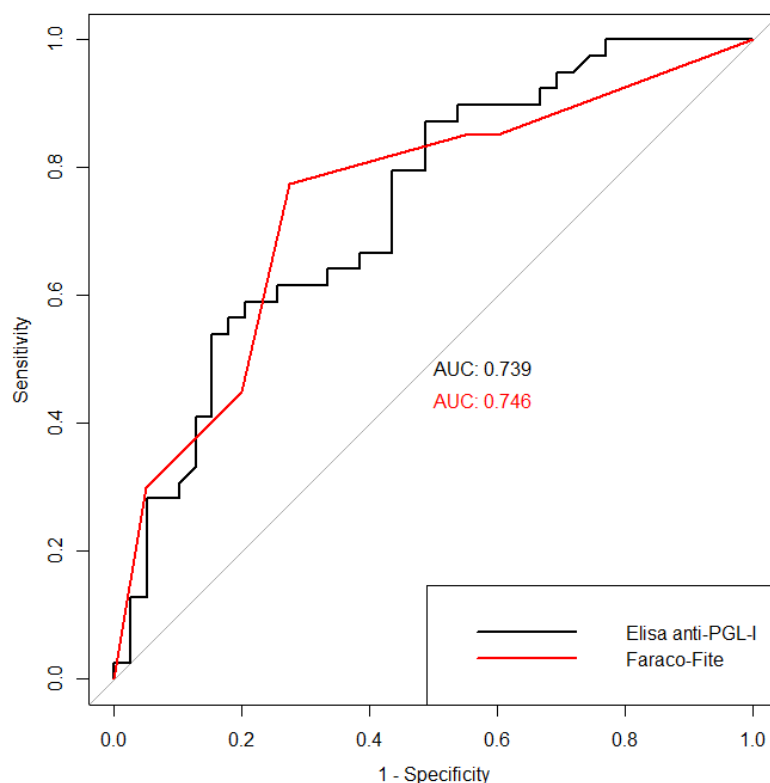
To evaluate the associations between categorical surrogates and groups, we employed the G Test (likelihood ratio), which demonstrated no statistical difference for the epidemiological and clinical variables ($p > 0.05$) (Table S2).

Regarding the number of doses of MDT administered to Group TF, six patients (15%) received 12 doses and 34 patients (85%) received 24 doses. The numbers of MDT

doses administered to Group C were: two patients (5%) received 6 doses, 20 patients (50%) received 12 doses and 18 patients (45%) received 24 doses.

The mean hBI in the skin sample of patients included in Group TF was 3.02+ (SD $\pm$ 2.02) and the median was 2+ (Min. 0 Max. 6), while the mean hBI in Group C was 1.8+ (SD $\pm$ 1.88) and the median 2+ (Min. 0 Max. 6) ($p=0.004$). The ROC curve was utilized to detect cutoff point values and optimize sensitivity as a function of specificity. When a cutoff value for hBI $\geq$ 3+ in skin biopsy was used, a sensitivity of 73% and a specificity of 78% were found to discriminate TF (AUC: 0.75; cutoff of $\geq$ 3+; $p = 0.0001$) (Figure 2) (Table S3).

Figure 2. Values for sensitivity, specificity, and area under the curve referring to the skin histological bacillary index and ELISA index at the end of treatment.



In Group TF, the mean EI for anti-PGL-I was 4.48 (SD $\pm$ 2.80) and the median 4.41 (Min. 0.43 Max. 14.6), while in Group C, the mean was 2.44 (SD $\pm$ 2.30) and the

median 1.43 (Min. 0.02 Max. 9.94) ($p = 0.0024$). The EI showed a sensitivity of 79%, and a specificity of 59% for discriminating TF (AUC: 0.74; cutoff of ≥ 3.95 ; $p = 0.0001$).

Regarding the histopathological characteristics of the skin fragments biopsied at the end of MDT (Table 4), there was an association between Group TF and the surrogates neural/perineural lymphocytic ($p = 0.0402$), and infiltrate foamy granuloma ($p = 0.0003$), and skin hBI ($p = 0.0111$). For the other characteristics analyzed, there was no difference among the proportions of these categories in the two groups ($p > 0.05$).

Table 4. Histopathological characteristics in skin samples biopsied at the end of multidrug therapy.

Variables		Therapeutic failure	Control	<i>p-value</i>
Epithelioid granuloma	Negative	34 (85)	38 (95)	0.1281
	Positive	6 (15)	2 (5)	
Neural/perineural lymphocytic infiltrate	Negative	30 (75)	35 (87.5)	0.0402
	Positive	10 (25)	5 (12.5)	
Intraepithelial lymphocytes	Negative	36 (90)	37 (92.5)	0.6919
	Positive	4 (10)	3 (7.5)	
Epidermal atrophy	Negative	10 (25)	15 (37.5)	0.2266
	Positive	30 (75)	25 (62.5)	
Perivascular and perianexial edema	Negative	27 (67,5)	24 (60)	0.4851
	Positive	13 (32,5)	16 (40)	
Fibrosis of the dermis	Negative	31 (77,5)	37 (92.5)	0.0554
	Positive	9 (22,5)	3 (7.5)	
Non-cohesive lymphocytic infiltrate	Negative	21 (52,5)	22 (55)	0.8226
	Positive	19 (47,5)	18 (45)	
Foamy granulomas	Negative	4 (10)	18 (45)	0.0003
	Positive	36 (90)	22 (55)	
Histological bacillary index	Negative	6 (15)	16 (40)	0.0111
	Positive	34 (85)	24 (60)	

The data were submitted to logistic regression analysis, the results of which are displayed in Table 5.

Table 5. Statistical significance of the analyzed variables, evaluated by univariate and multivariate logistic regression

		Univariate Analysis				Multivariate Analysis			
		P value	Odds Ratio (OR)	(CI 95% para OR)		P value	Odds Ratio (OR)	(CI 95% for OR)	
Sex		0.2467	0.58	0.23	1.46	-	-	-	-
Age		0.1072	0.98	0.95	1.00	-	-	-	-
Initial Operational Classification		0.9922	1.00	-	-	-	-	-	-
Operational Classification of Failure		0.9996	1.00	-	-	-	-	-	-
Epithelioid granuloma		0.1546	3.35	0.63	17.74	-	-	-	-
Neural/perineural lymphocytic infiltrate		0.0624	3.00	0.94	9.53	0.0153	5.24	1.37	19.98
Lymphocytes in the epidermis and/or eccrine ductal epithelium		0.6933	1.37	0.29	6.56	-	-	-	-
Epidermal atrophy		0.2302	1.80	0.69	4.70	-	-	-	-
Perivascular and/or perianexial edema		0.4859	0.72	0.29	1.80	-	-	-	-
Non-cohesive lymphocyte aggregates		0.9999	1.00	0.41	2.41	-	-	-	-
Dermal fibrosis		0.0723	3.58	0.89	14.39	-	-	-	-
Foamy granulomas		0.0012	7.36	2.20	24.60	-	-	-	-
Histological bacilloscopic index		0.0004	1.55	1.22	1.99	0.0030	1.55	1.16	2.07
Anti-PGL-I serology		0.0019	1.40	1.13	1.74	0.0467	1.26	1.00	1.58
qPCR of skin biopsy		0.0201	3.43	1.21	9.69	-	-	-	-
qPCR of dermal smear		0.0534	2.95	0.98	8.82	-	-	-	-
Initial clinical form	BT	0.6933	1.37	0.29	6.56	-	-	-	-
	BB	0.1251	0.18	0.02	1.61	-	-	-	-
	BL	0.0546	0.38	0.14	1.02	-	-	-	-
	LL	0.0150	3.12	1.25	7.78	-	-	-	-
Clinical form of failure	BT	0.9999	1.00	-	-	-	-	-	-
	BB	0.9999	1.00	-	-	-	-	-	-
	BL	0.9999	1.00	-	-	-	-	-	-
	LL	0.9999	1.00	-	-	-	-	-	-

§ World Health Organization classification; PB: paucibacillary leprosy; MB: multibacillary leprosy.

* Classification of Ridley and Jopling; BT: borderline-tuberculoid leprosy; BB: borderline-borderline leprosy; BL: borderline-lepromatous leprosy; LL: lepromatous leprosy.

In the univariate logistic regression analysis, the associations were significant with the LL initial clinical form (OR=3.12; 95%CI 1.25-7.78; $p=0.0150$), giving 3.12-fold greater odds for TF.

Regarding histopathological variables, the associations were significant ($p<0.05$) for the presence of foamy granuloma, and hBI. At the end of treatment, when the presence of foamy granuloma was verified, the odds were 7.4-fold greater (OR=7.36; 95% CI 2.20-24.60; $p=0.0012$) for Group TF, compared to Group C. When $\text{hBI} \geq 1+$, the odds were 1.6-fold greater (OR=1.55; 95%CI 1.22-1.99; $p=0.0004$) for the occurrence of TF. These odds increase 55% per log, reaching up to 330%, if $\text{hBI} = 6+$. For the other histopathological characteristics, there were no associations between the two groups, which do not appear to be associated with risk or protective factors.

Among the laboratory findings, the associations were significant for the IgM anti-PGL-I serology surrogate. According to the analysis, when at the end of treatment, $\text{EI} \geq 1$, the odds of TF occurrence were 1.4-fold greater (OR=1.40; 95%CI 1.13-1.74; $p=0.0019$). It is important to highlight that these odds increase by 40% per EI unit. Concerning qPCR of skin biopsy, when positive for the presence of *M. leprae* DNA, the TF odds were 3.4-fold greater (OR=3.43; 95% CI 1.21-9.69; $p=0.0201$).

Multivariate regression analysis was employed to test whether clinical, histopathological, and laboratory characteristics could predict TF. The data showed that the joint presence of the variables neural/perineural lymphocytic infiltrate (OR=5.24;

95%CI 1.37-19.98; $p=0.0153$), hBI (OR=1.55; 95%CI 1.16-2.07; $p=0.0033$) and EI (OR=1.26; 95%CI 1.00-1.58; $p=0.0467$) significantly predicted TF. Furthermore, when combining the histopathological variables in a statistical model, the presence of lymphocytic neural/perineural infiltrate, $\text{hBI} \geq 3+$, and $\text{EI} \geq 3.95$ indicated a high probability (up to 95.41%) for TF (Table S3).

Discussion

In the present case-control study, after the initial selection of 567 individuals, 80 participants were distributed into two paired groups, with 40 participants in Group TF and 40 individuals in Group C. This final sample was due to the application of strict research inclusion criteria and caution in pairing between groups, which allowed the exclusion of statistical differences between the samples ($p > 0.05$). All patients were analyzed for their therapeutic response to the fixed-duration MDT regimen with 6, 12, or 24 doses of rifampicin, dapsone, and clofazimine, at the time of treatment discharge. Given the scarcity of literature on the clinicopathological and bacteriological evaluation of patients with persistent signs of disease activity in skin lesions after the end of MDT (41), this study sought to understand the predictive value of cutaneous histopathological and serological findings for TF in leprosy.

TF can be understood as the persistence of activity or worsening of skin lesions and failure to reduce the BI in MB leprosy (18,19). These patients do not respond as expected in terms of the disappearance of skin lesions and elimination of bacilli at the end of therapy or may show disease progression throughout MDT (22). For this research, TF is understood as a) any cases of leprosy that do not show signs of clinical improvement during regular and appropriately indicated treatment of six or 12 doses of MDT (17,42); b) every MB patient who received up to 24 doses of MDT, and who, upon treatment discharge, presents signs of clinical activity and/or the presence of well-defined solid

bacilli in the dermal smear and/or histopathological examination of the skin, and maintenance of high anti-PGL-I (IgM) levels (17). Other studies establish their criteria for TF, such as persistent active lesions or the appearance of new ones after completing 12 months of MDT, positivity or increasing value of the morphological index, and a 2-log increase in BI after 12 months of MDT (43, 44). Another clinical aspect of diagnostic utility is the appearance of new lesions and downgrading of the patient in TF, while in leprosy relapse there is a reappearance of old lesions, but the patient rarely presents downgrading (45). However, there is no clear or universally accepted criterion for TF in leprosy, considered as a result of low adherence to treatment or drug resistance (46). Thus, it is necessary to develop strategies to better evaluate the causes of TF and promptly identify cases of drug resistance (23).

In this research, over a 12-year follow-up period, we found a mean TF time of 13.0 months and a TF rate of 6.7% (92/1,385). In a previous study, we analyzed 50 cases of TF diagnosed within a period of six years, and a similar TF rate was observed, namely 4.7% (50/1,059) (21). Disease recurrence within 9 months after treatment discharge is probably a sign of TF, rather than leprosy relapse, which occurs later after MDT (47). Therefore, TF should be understood as an early event, near the treatment discharge, as the majority occurs in the first 3 years after the end of MDT (22).

In Group TF, 100% (40/40) of cases were classified as MB before starting treatment with MDT. For treatment, the WHO classifies leprosy into PB and MB based on the number of skin lesions, neural involvement, and BI results (10). There is a small but significant risk of undertreatment of so-called PB cases that have a high bacillary load (48). Among those who were classified as PB in a study that included 264 untreated leprosy patients, 38% to 51% (100-134/264) of patients had MB leprosy according to classical criteria and were therefore at risk of undertreatment by WHO recommendations

(11). In another series of PB patients with up to 5 skin lesions and with bacterio-histopathological confirmation, it was concluded that 24.2% (15/62) had the MB form of leprosy (49).

Due to the underlying symptoms of leprosy, there is a difference between clinical and environmental forms and perceptions. Thus, the correlation of clinical, histopathological, and bacteriological findings could be more useful in diagnosing leprosy than considering just a single parameter in isolation (50).

The majority of patients (34/40) included in Group TF received 24 doses of MDT, that is, patients who did not improve during multidrug therapy or who exceeded the criteria for treatment insufficiency. The WHO states that a shorter regimen (six doses) of MDT implies a potential increase in the risk of TF (10). When the duration of treatment is not long enough to eliminate the bacilli, the continuous inflammatory response results in persistent clinical activity for 12 to 18 months, which can sometimes result in TF (51). A study that evaluated 25 patients with MB leprosy treated with MDT, of which 11 patients were BL and 14 LL, concluded that *M. leprae* persists in tissues after 2 years of treatment (52). The group of MB patients presents a high risk of recurrence after 2 years of MDT (53).

In the present study, those diagnosed at the beginning of treatment as LL had 3.12-fold greater odds of developing TF. Relapse and treatment failure are largely confined to BL or LL patients with high initial BI (9). In this sense, a study with 189 MB patients treated with four different drug regimens and monitored for up to 12 years after the start of treatment found relapse rates of up to 3% with MDT in BL and LL patients with a high bacterial load (54).

There is a lack of a precise definition of a true leprosy cure, beyond the simple completion of the fixed-duration therapy (46). The therapeutic response of patients treated

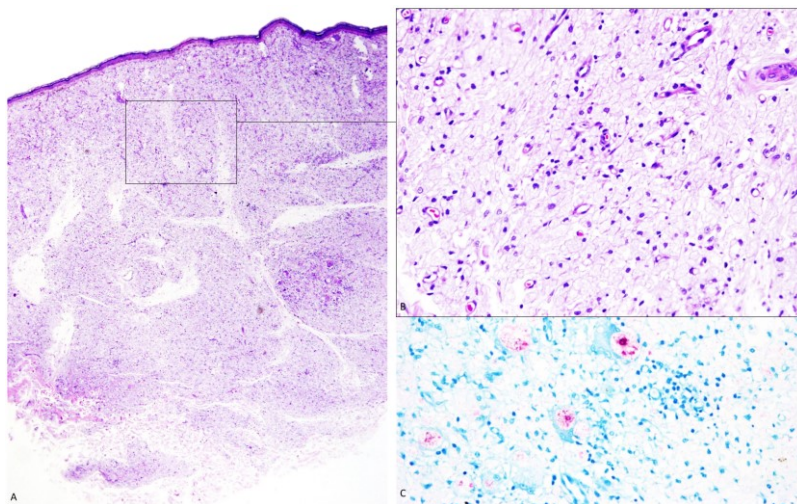
with MDT can be measured by three parameters: 1) resolution of skin lesions; 2) decrease in BI and 3) recurrence rate (30).

Resolution of skin lesions

For appropriate treatment, the diagnosis of leprosy must be made with maximum precision with the help of histopathological characteristics, BI, and clinical correlations (55). The histopathological examination of skin lesions remains the gold standard for the accurate diagnosis of leprosy, which allows: for classifying leprosy and its reactions, estimating the BI, monitoring the response to treatment and disease activity, and differentiating TF from leprosy reaction (45,50,56). Active and persistent skin lesions are observed despite the completion of MDT in a few patients in the TT and BT spectrum and must be caused by drug-resistant bacilli or an autoimmune phenomenon triggered and maintained by persistent bacillary antigens (41). Studies suggest that PB patients should be under surveillance for at least 3 years and MB patients for 9 years so that the majority of leprosy recurrences can be detected (22). In our national reference center, patients are followed up annually for 10 or 20 years depending on clinical and laboratory conditions at the end of MDT (21).

The data from this work showed that the presence of foamy granuloma (Figure 3) gives 7.36-fold greater odds for TF. We believe that this is due to multiple factors in the agent-versus-host interaction, such as high bacillary load, persistent bacilli, clinical form, and duration of MDT.

Figure 3. Foamy granulomas.



A 62-year-old woman with BT leprosy was treated with 12 doses of MDT. She presented treatment failure after 3 months of follow-up. A) Foamy granuloma throughout the dermis and subcutaneous fat (H&E, 4x). B) Collection of foamy macrophages (H&E, 40x). C) Numerous granular and fragmented bacilli (Fite-Faraco, 40x).

Whether or not foamy macrophages can recognize antigens from dead *M. leprae* is not the key question to elucidate the role of foamy granulomas in TF, but rather it is the answer as to whether foamy macrophages can hide and protect live bacilli. *M. leprae* promotes changes in lipid metabolism that modulate inflammatory and immunological responses to support the survival and dissemination of the bacillus and host tolerance (57). Several studies have postulated that differentiation to foamy macrophages creates an anti-inflammatory environment, restrictive of the bactericidal response, in the skin lesions of LL patients, ideal conditions for the evasion of *M. leprae* and an increase in the number of skin lesions in this pole of the disease (58–62). Therefore, the idea of the foamy appearance being produced by the degradation of dead *M. leprae* (63) becomes fanciful. Therefore, the presence of foamy granulomas on skin biopsy at the end of treatment should be seen as a warning sign for TF.

Decrease in the bacillary index

The BI depicts the concentration of bacilli in smears or tissues and includes live and dead bacilli (50). In most PB cases, the average BI is higher in skin biopsy than in intradermal smears (5,55). The suggested reason was that bacteria in PB are more common in deeper tissues (particularly nerves) than in superficial dermal strata and are therefore unlikely to be detected in sBI (64). On the other hand, the correlation between hBI and sBI is excellent in MB cases (50,55).

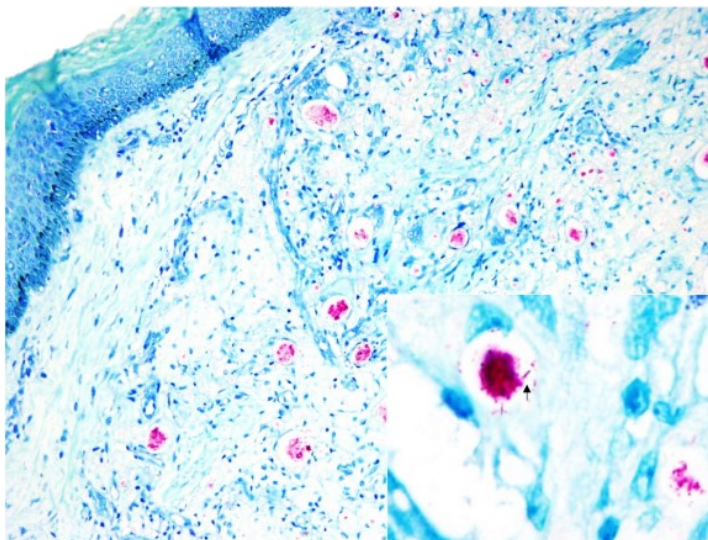
The data from this research showed that $\text{hBI} \geq 1+$ in the skin sample at the end of MDT increased the odds of TF by 1.55-fold per log, that is, up to 9.3-fold, if $\text{hBI} = 6+$. A study that included a total of 46 cases of TF and relapse found that 20.8% of cases had $\text{BI} \geq 4+$ (65). However, it is important to correctly classify the patient through bacilloscopy and biopsy since the BI allows adequate monitoring and is particularly useful in suspected cases of TF or leprosy reactions (66). Nevertheless, there are still authors who affirm that a low positive sBI at the time of discharge in a patient whose initial smear was negative at the diagnosis, but who improved clinically, may not indicate TF (67).

After starting treatment, BI reduces by approximately 1 log a year (19). The rate of declining sBI is more appropriate for assessing bacillary clearance than smear negativity itself, which is largely a reflection of initial classification and sBI (68). That is, those patients who initially have a high BI are at greater risk of recurrence after fixed-duration MDT compared to patients with negative or low BI at the time of leprosy diagnosis (22). Contrary to those who say that not even the rate of decline or negative BI can predict the success or failure of a therapeutic regimen (9), our work supports the idea that the downward trend or negativity in hBI should be used as an objective criterion for leprosy cure.

Furthermore, there may be confusion between TF and reactions, especially in the first 3 to 5 years after treatment, when hBI is a useful way to define the diagnosis in those patients with granulomatous reactivation at the end of MDT (Figure 4) (5,69). In a disease recurrence, the type 1 reaction can be seen as a partially effective immune reconstitution (upgrading reaction) or as a hypersensitivity reaction (downgrading) (70).

The data from this study detected moderate sensitivity (73%) and specificity (78%) to discriminate TF when using an hBI cutoff $\geq 3+$ in skin biopsy at the end of MDT.

Figure 4. Histological bacillary index.



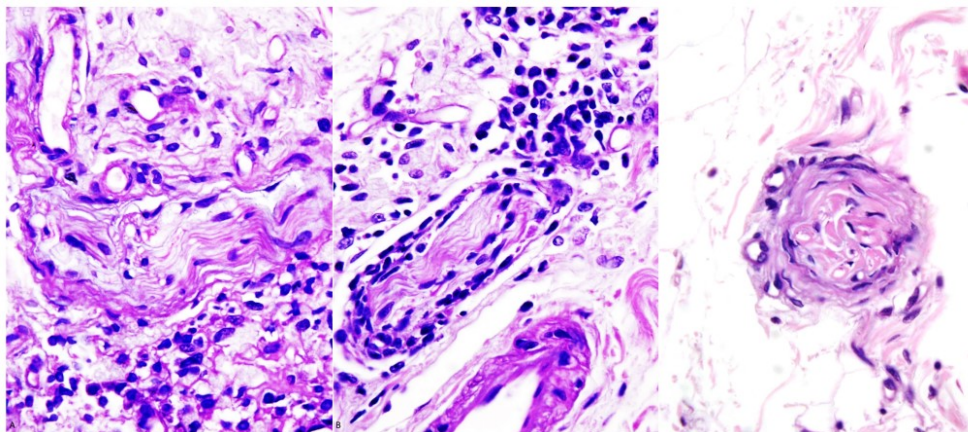
A 41-year-old man with LL was treated with 24 doses of MDT. He presented treatment failure after 2 months of discharge follow-up. At the treatment discharge, there were numerous granular and fragmented bacilli (Fite-Faraco, 40x). In detail, solid bacillus (arrow) (Fite-Faraco, original 100x).

Persistent bacilli are permanently or partially dormant organisms capable of surviving despite adequate chemotherapy and are present in approximately 10% of MB patients and this number may be even higher in cases with high BI (19,22,69). This is

because the dormant bacilli are in immunologically favorable areas, such as dermal nerves, smooth muscle cells, lymph nodes, iris, bone marrow, and liver, so they cannot be reached properly by medications during treatment (71).

The post-inflammation productive/reparative process generates perineural and endoneurial fibrosis of the skin nerves (Figure 5). It is in these places that *M. leprae* is buried alive and where it can revert to a normal division rate after MDT (46,56). Therefore, some authors advocate regular skin biopsies and dermal smears, at least once every 6 months, during the treatment period and in the following 5 years after achieving BI negativity (22).

Figure 5. Neural/perineural lymphocytic infiltrate.



A woman aged 56, with BL leprosy was treated with 24 doses of MDT. She presented treatment failure after 14 months of follow-up since treatment discharge. A) Mild perineural lymphocytic infiltrate, B) Lymphocytic delamination of the perineurium, and C) hyaline fibrosis of the nerve (H&E, 100x).

Decrease in the ELISA index

After MDT, the main differential diagnoses for leprosy activity are leprosy reactions, treatment insufficiency, TF, relapse, and reinfection. Depending on the

scenario, in addition to histopathological findings, serological findings are useful (22). During the treatment of MB patients, repeated ELISA tests can be used as an additional tool to measure the effectiveness of therapy while increased antibody levels can indicate a recurrence (31,72,73). In a study involving LL patients, anti-PGL-I was detected in 94.1% (64/68) of new cases ($BI = 3.2-5.8+$) and 78.8% (26/33) of patients with relapsed leprosy ($rBI = 3.0-5.3+$) (74). Likewise, other authors detected anti-PGL-I in 93.1% (27/29) of patients with $hBI \geq 4.0+$ and 68.2% (15/22) of those patients with $hBI = 3.0 - 3.9+$, but in none of the 37 patients with $hBI < 1.9+$ (75).

A study that evaluated 115 cases of leprosy found a high agreement of 82% (94/115) between serology and sBI (31). Other authors demonstrated higher levels of IgM anti-PGL-I in the serum of MB patients than in those of PB, which once again strongly correlates with bacillary load (76,77). Similarly, another study showed that patients with higher BI were 3.4 times more likely to produce antibodies compared to healthy controls (78).

In this study, at the end of MDT treatment, serological results with EI values ≥ 1 pointed to 1.40-fold greater odds per each unit increase favoring TF. When using an EI cutoff value ≥ 3.9 for the anti-PGL-I serology result, moderate sensitivity (79%) and specificity (59%) were found to discriminate Group TF. It is worth noting that the positivity or elevation of EI levels after MDT tends to occur early in the recurrence of the disease, even before the bacilli can be detected in the skin (31,79).

Considering the rapid disappearance of PGL-I antigen and the gradual decrease in IgM anti-PGL-I antibodies resulting from treatment, EI can be useful in monitoring the effectiveness of treatment both in the early stages and subsequently (74). For this reason, seronegative conversion of anti-PGL-I is considered useful for monitoring the elimination of bacterial load, thus measuring the effect of MDT (80). Our study advocates the use of

EI to define a cure for leprosy through the downward trend or negativity in serological levels of anti-PGL-I.

Real-time polymerase chain reaction (qPCR) of skin biopsy

Clinically, leprosy is called “the great imitator” because it has a clinical and histopathological profile similar to other skin diseases, such as pityriasis alba, pityriasis versicolor, vitiligo, tinea circinata, psoriasis, and others (5,32,34,81). Both in the diagnostic definition, especially in PB cases, and in the evaluation of the effectiveness of MDT, the detection of *M. leprae* DNA by PCR plays a primary role alongside determining the recurrence rate (22,82–85).

In our research, when the qPCR of the skin biopsy at the end of MDT was positive, the odds of TF were 3.43-fold greater. One study evaluated samples from 38 patients with undetermined leprosy after 4 to 6 years of treatment with MDT and without signs of active infectious disease and detected positivity for *M. leprae* DNA in 54.5% (18/38) suggesting the presence and circulation of live bacilli much longer than expected, although reinfection of individuals cannot be excluded (86). It is possible to infer that individuals with suspected leprosy, with positive qPCR, may be carriers of subclinical infection and potential transmitters of *M. leprae* to more susceptible individuals (87).

An RT-PCR assay evaluated different samples from 24 PB patients, all with sBI = 0, and showed positive results in 21% (5/24), 25% (6/24), and 96% (23/24) for intradermal smear examinations of the earlobe and cutaneous lesions and skin biopsy, respectively, increasing the diagnostic capacity of the histopathological examination (79%) by up to 17% (32). For the diagnosis of leprosy, in skin biopsies, qPCR presents a sensitivity of 50% and a specificity of 94% (88).

No data could be found that assessed the odds of TF in leprosy based on histopathological findings in skin samples at the end of MDT. The data we present show, for the first time, that the joint presence of neural/perineural lymphocytic infiltrate in skin biopsy and $\text{hBI} \geq 3+$ and $\text{EI} \geq 3.95$ at the end of MDT results in a 95.41% probability of TF.

Conclusion

In an unprecedented way, this research allowed us to elucidate the predictive value of histopathological findings in post-MDT skin biopsy in TF, which will be useful in monitoring leprosy cases treated with WHO fixed-duration regimens. Unlike relapse, TF should be understood as an early event that is closer to the end of MDT, with the LL patient population facing the greatest odds of TF.

At the time of discharge upon completion of the MDT regimen, foamy granulomas, and $\text{hBI} \geq 1+$ increase the TF odds in patients regularly treated with MDT. Likewise, predictive factors for TF include seropositivity for IgM anti-PGL-I ($\text{IE} \geq 1$) and qPCR detection of *M. leprae* DNA, at the end of treatment. When associated with the parameters, neural/perineural lymphocytic infiltrate, foamy granulomas, $\text{hBI} \geq 3+$, and $\text{EI} \geq 9.5$, the probability of TF at the end of MDT is significantly increased. This, in our opinion, is a fertile ground for new research studies assessing a greater number of individuals.

Currently, there is still a need to standardize universal criteria for leprosy cure, treatment insufficiency, and TF and leprosy relapse that are feasible in different regions of the world, objective criteria based on the combination of clinical and laboratory findings. To this end, the R&J classification and skin biopsy are essential in our opinion. Active surveillance must be maintained and reinforced in patients with high odds of TF after MDT.

Limitations of the study

A possible limitation of this research is its relatively small sample size. However, this sample reflects the occurrence of HR in seven years in a national reference center for leprosy. New studies may corroborate the conclusions stated herein.

Concerning qPCR, associations could only be made quantitatively, given that, over the 12 years studied, the values of the results were expressed in different units.

Contributions by the authors

BCD, DFS, and IMBG developed the conceptualization. BCD, WVTC, and IMBG collected the data. FAR and DROC performed the histological techniques. BCD, WVTC, JPFA, and JSD analyzed the histological sections. BCD, LBA, DEA, and IMBG evaluated the data. BCD, DEA, DFS, IMBG assisted with study design and methodology. BCD and IMBG wrote the manuscript. IMBG and CTS reviewed the manuscript. IMBG obtained resources and funds. BCD, WVTC, FAR, JPFA, JSD, DFS, and IMBG carried out the investigation. IMBG administered and supervised the project. BCD, WVTC, FAR, DROC, JPFA, JSD, LBA, DEA, and IMBG validated the work.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical considerations

Ethical questions were completely addressed by the authors.

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List of abbreviations

Anti-PGL-I: phenolic glycolipid I antibody

BB: borderline-borderline leprosy

BI: bacillary index

BL: borderline-lepromatous leprosy

C: control

DT: borderline-tuberculoid leprosy

EI: ELISA index

hBI: histological bacillary index

LL: lepromatous leprosy

MB: multibacillary

MDT: multidrug therapy

PB: paucibacillary

qPCR: real time quantitative polymerase chain reaction

sBI: smear bacillary index

TF: treatment failure

TT: tuberculoid leprosy

WHO: World Health Organization

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Supplemental Supporting Material

Table S1. Numerical description of the variables

	Treatment Failure Group								Control Group							
	n	Mean	Standard deviation	Median	Q1	Q3	Minimum	Maximum	n	Mean	Standard deviation	Median	Q1	Q3	Minimum	Maximum
Age	40	48.25	16.64	46	34.75	60.75	17	77	40	54.45	17.23	58	45.75	68	13	85
Number of doses*	40	22.2	4.34	24	24	24	12	24	40	17.1	6.46	12	12	24	6	24
Time^{&}	40	13.08	11.56	12	3	17.25	1	42	40	113.1	14.14	113.5	103	125	83	158
Bacilloscopic Index in skin biopsy¹	40	3.03	2.02	2.00	2.00	5.00	0.00	6.00	40	1.88	1.96	2	0	3	0	6
Anti-PGL-I²	40	4.48	2.81	4.41	2.46	5.94	0.43	14.60	40	2.44	2.29	1.43	0.51	3.77	0.02	9.94
Bacilloscopic Index of intradermal scraping¹	40	2.55	4.53	2.00	0.57	3.33	0.00	28.00	40	1.70	1.44	1.85	0	2.78	0	4.42

*Number of doses of multidrug therapy administered throughout the treatment.

[&] Time. For the Failure Group, it signifies the number of months between discharge from treatment and treatment failure. For the control group, the follow-up period in months from discharge from treatment until this work was carried out.

¹Bacilloscopic Index for the logarithmic scale of Ridley & Jopling.

²Anti-PGL-I serology (IgM) by the ELISA method, considered positive if ≥ 1 .

Table S 2. Laboratory and clinical characteristics.

Clinical characteristics		Failure	Control	<i>p-value</i>
Sex	Males	28 (70)	23 (57.5)	0.2440
	Females	12 (30)	17 (42.5)	
Initial clinical form	DD	1 (2.5)	5 (12.5)	0.0348
	DT	4 (10)	3 (7.5)	
	BL	8 (20)	16 (40)	
	LL	27 (67.5)	16 (40)	
Initial operational classification	PB	0 (0)	2 (5)	0.0929
	MB	40 (100)	38 (95)	
Clinical form of failure	DD	1 (2.5)	-	0.0004
	DT	3 (7.5)	-	
	BL	7 (17.5)	-	
	LL	29 (72.5)	-	
Operational classification of failure	MB	40 (100)	-	0.9999
Laboratory characteristics				
qPCR of skin biopsy	Negative	7 (17.5)	16 (42.1)	0.0162
	Positive	33 (82.5)	22 (57.9)	
Qualitative anti-PGL-I	Negative	4 (10.3)	14 (35.9)	0.0060
	Positive	35 (89.7)	25 (64.1)	

Table S3. Performance on diagnostic tests

	Skin biopsy IB¹	Anti-PGL-I serology
<i>Cutoff</i>	3	3.95
AUC	0.75	0.74
Sensitivity	0.77	0.87
Specificity	0.72	0.51
Accuracy	0.75	0.69
Positive predictive value	0.74	0.64
Negative predictive value	0.76	0.80

¹Bacillary index using the logarithmic scale of Ridley & Hilson.

Table S4. Exposition of multivariate regression variables.

Neural/perineural lymphocytic infiltrate	IB¹ skin biopsy	Anti-PGL-I	Failure Probability (%)
+	≥3	≥3.95	95.41
+	<3	<3.95	87.45
-	≥3	≥3.95	79.51
+	≥3	≥3.95	69.53
-	<3	<3.95	56.52
+	<3	<3.95	43.32
-	≥3	≥3.95	29.86
-	<3	<3.95	12.48

¹Bacillary index using the logarithmic scale of Ridley & Hilson.

4.2 Estudo 2 - Role of histopathological, serological and molecular findings for the early diagnosis of leprosy relapse. Part II

Role of histopathological, serological and molecular findings for the early diagnosis of leprosy relapse. Part II

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Abstract

Objectives: To identify predictive laboratory factors for leprosy relapse (LR) post-multidrug therapy (MDT). **Methods:** In a 12-year case-control study at a national leprosy reference center, 80 individuals received World Health Organization (WHO) MDT. The Relapse Group (R, n=40) experienced LR after a mean of 89.2 months, while the Control Group (C, n=40) remained asymptomatic for a mean of 113.1 months. Parameters were assessed at MDT completion. **Results:** In skin samples, neural/perineural lymphocytic infiltrate (OR=4.67; p=0.0076) and foamy granuloma (OR=15.55; p=0.0005) correlated with LR. Mean histological bacillary index (hBI) was higher in the R group (3.23 vs. 1.8 in C). hBI $\geq 3+$ exhibited 72% sensitivity and 65% specificity for LR (AUC: 0.72; p=0.0002). Epithelioid granulomas, intraepithelial lymphocytes, epidermal atrophy, perivascular/periadnexal edema, dermal fibrosis, and lymphocytic infiltrate showed no significant associations. Anti-phenolic glycolipid I (anti-PGL-I) serology (p=0.0031) indicated 4.7-fold greater LR odds for ELISA index (EI) ≥ 1 . EI ≥ 3.6 yielded 71% sensitivity and 62% specificity (AUC: 0.70; p=0.0012). Multivariate analysis revealed joint occurrences of neural/perineural infiltrate, foamy granuloma, hBI $\geq 1+$, and EI ≥ 1 significantly associated with LR, providing a predictive probability of up to 94.32%. **Conclusion:** Persistent neural infiltrate, foamy granulomas, high hBI, and elevated EI post-MDT may identify individuals at increased risk of LR.

Keywords: glycolipid-phenolic-1, leprosy, pathology, real-time polymerase chain reaction, relapse

Introduction

Leprosy is a chronic infectious disease of dermato-neurological involvement caused by *Mycobacterium leprae*, an alcohol-acid-resistant bacillus that primarily infects Schwann cells in the peripheral nervous system (1,2). However, the bacillus is capable of parasitizing both mesenchymal cells and epithelial cells of different organs and tissues (3). For treatment purposes, the WHO recommends the same three-drug regimen: rifampicin, dapsone, and clofazimine, for all leprosy patients, with a fixed duration of six months for paucibacillary leprosy (PB) and 12 months for multibacillary leprosy (MB); but this is a conditional recommendation, with very low-quality supporting evidence (4).

Choosing the therapeutic regimen by counting skin lesions indicated by the WHO operational classification, which has been applied in several national programs, may result in an insensitive diagnosis and the attribution of PB treatment course for a large number of MB patients (5,6). Thus, the WHO classification may mask the true relationship between the immunological response and genetic factors of the disease, limiting the information necessary for epidemiological data, treatment, and leprosy control strategies (7,8).

The Ridley and Jopling (R&J) spectral classification is important, not only for a better understanding of the disease but also for selecting the most appropriate therapy (9). Inadequate treatment of leprosy can result in insufficiency, failure, and LR that hinder epidemiological control (1). It should be emphasized that the excessive simplification of leprosy feeds the illusion of a simpler disease that is easier to understand and eliminate (10). It is expected that the best classification for leprosy would be the one that provides the lowest LR rate (11,12).

Various patients develop new skin lesions after the end of MDT, which should be considered as LR or late reactions (13). The Brazilian Ministry of Health (MS) defines

LR as those cases that received regular and correctly indicated treatment, but that, return to manifesting active disease after five years or longer after being discharged due to cure (14).

Even with 51 countries reporting treatment completion rates above 89% (15,16), variations in estimates of relapse rates are large (17). The WHO reported 3,201 LR cases in 2021, of which 38% (1,212/3,201) occurred in Brazil and 16% (510/3,201) in India, and considered the global LR rate to be low, around 1% (15,16,18). Official data from the MS show that 5.1% of all cases reported in Brazil were LR cases between 2016 and 2020 (19). In a previous work, we demonstrated an LR rate of 11.89% (126/1,059) which was recognized by broader and better surveillance and notification (20).

This difficulty in recognizing LR is attributed to the pathophysiology of the disease, the slow growth characteristics of the mycobacteria, and the host response. In this context, clinical and laboratory evaluations become essential for detecting LR cases. Therefore, the spectral classification of leprosy should be standardized in health services (21) while completion of the fixed-duration MDT should not be the only criterion for real cure adopted by the WHO and the MS. Thus, it is necessary to establish objective criteria on account of leprosy cure, such as, for example, negative smear BI (sBI), at least in those with elevated sBI, also aiming to prevent or reduce early LR (22).

The identification of predictive factors associated with LR and its prognosis can support the adoption of actions for prevention, control, and early diagnosis, to minimize the morbidity of those with LR. If LR is detected only late, the risk of systemic and neurological morbidity and developing deformity increases significantly (23). Few published research studies have focused on the description of cutaneous histopathological findings after leprosy treatment, without allusion to judging their predictive values. Acquiring a better understanding of the histopathological and laboratory findings of

patients who have completed MDT, but who are at greater odds of LR, is essential for public health.

In this context, according to the R&J histopathological classification (24), our study intends to contribute to the evaluation of skin histopathology as a necessary tool not only for diagnosis but also for monitoring and discharge from leprosy treatment. Likewise, the U.S. Centers for Disease Control and Prevention reiterated that the diagnosis of leprosy is confirmed by skin or nerve histopathology and staining for alcohol-acid-resistant bacillus (25). The main objective of this work was to identify histopathological, serological, and molecular predictive factors for LR within at least five years after the discharge from MDT.

Material and Methods

Study design and selection of cases

We carried out a case-control study with leprosy cases diagnosed at CRESDEH/HC-UFU/EBSERH, Brazil, between 2008 and 2020. During this period, 1,869 leprosy cases were reported by CREDESH. Among these, the initial selection was 673 cases based on the analysis of secondary data, obtained from medical records, specific software from the National Reference Center, and the Disease Notification Information System (SINAN) of the MS.

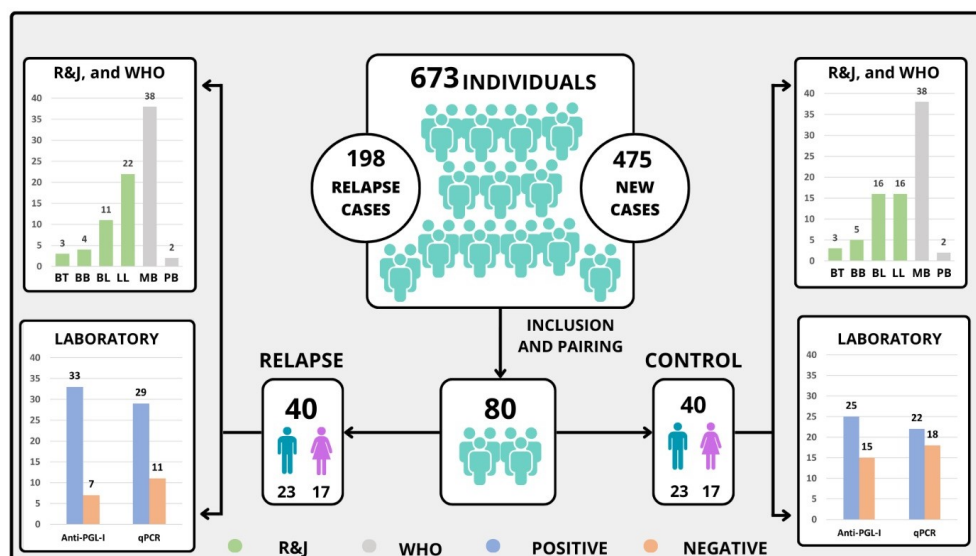
The patients included in this study were adults (age ≥ 18 years) regularly treated with a fixed MDT course, with six, 12, or 24 doses. All patients were diagnosed, classified, and treated by experienced leprologists. According to clinical, histopathological, bacillary, serological, and molecular characterization, patients were classified according to the R&J spectrum (26) into borderline-tuberculoid leprosy (BT),

borderline-borderline (BB), borderline-lepromatous (BL) or lepromatous (LL) and, for treatment purposes, into PB or MB according to the WHO operational classification (27).

We divided patients into two comparative groups: a) Group R: we selected all 198 cases reported as LR cases in the specific software and SINAN between 2013 and 2020 were; and, b) Group C: we selected 475 new leprosy cases between 2008 and 2013, among which we identified those cases that had no notifications of recurrence in the specific software and SINAN after discharge due to cure in a period ≥ 60 months since the end of MDT.

Patients who completed the treatment regimen and who had results from bacillary exams (sIB and hIB), IgM anti-PGL-I serology, and qPCR for *M. leprae* DNA at the end of MDT were included. Patients with other infectious comorbidities were excluded, such as tuberculosis, HIV/AIDS, and viral hepatitis. We also excluded those cases in which the skin fragment fixed in formalin and embedded in paraffin was insufficient or unavailable in the files of the Pathology Unit of HC/UFU/EBSERH. Finally, the cases selected from Group R and Group C were paired, resulting in 40 patients in both groups (Figure 1).

.Figure 1. Selection and pairing of individuals from the Relapse Group and Control Group 1.



Epidemiological and clinical data and laboratory results at the end of MDT for leprosy. LR: relapse of leprosy. Ridley and Jopling (R&J) classification. BT: borderline-tuberculoid leprosy; DD: borderline-borderline leprosy; BL: borderline-lepromatous leprosy; LL: lepromatous leprosy. World Health Organization (WHO) standard operational classification. PB: paucibacillary leprosy; MB: multibacillary leprosy. Anti-PGL-I serology (IgM), positive if ELISA index (EI) ≥ 1 . qPCR: quantitative polymerase chain reaction.

Definition of relapse in leprosy

Relapse in leprosy is defined as all cases of leprosy, regularly treated with standardized and correctly indicated official regimens, which were discharged from treatment due to cure and which returned to presenting new clinical signs and symptoms of an active infectious disease in a period ≥ 60 months later (14).

In this study, the LR diagnosis was established by experienced leprologists at CREDESH based on clinical and laboratory findings, ruling out reactions and complying with the LR criteria of the MS.

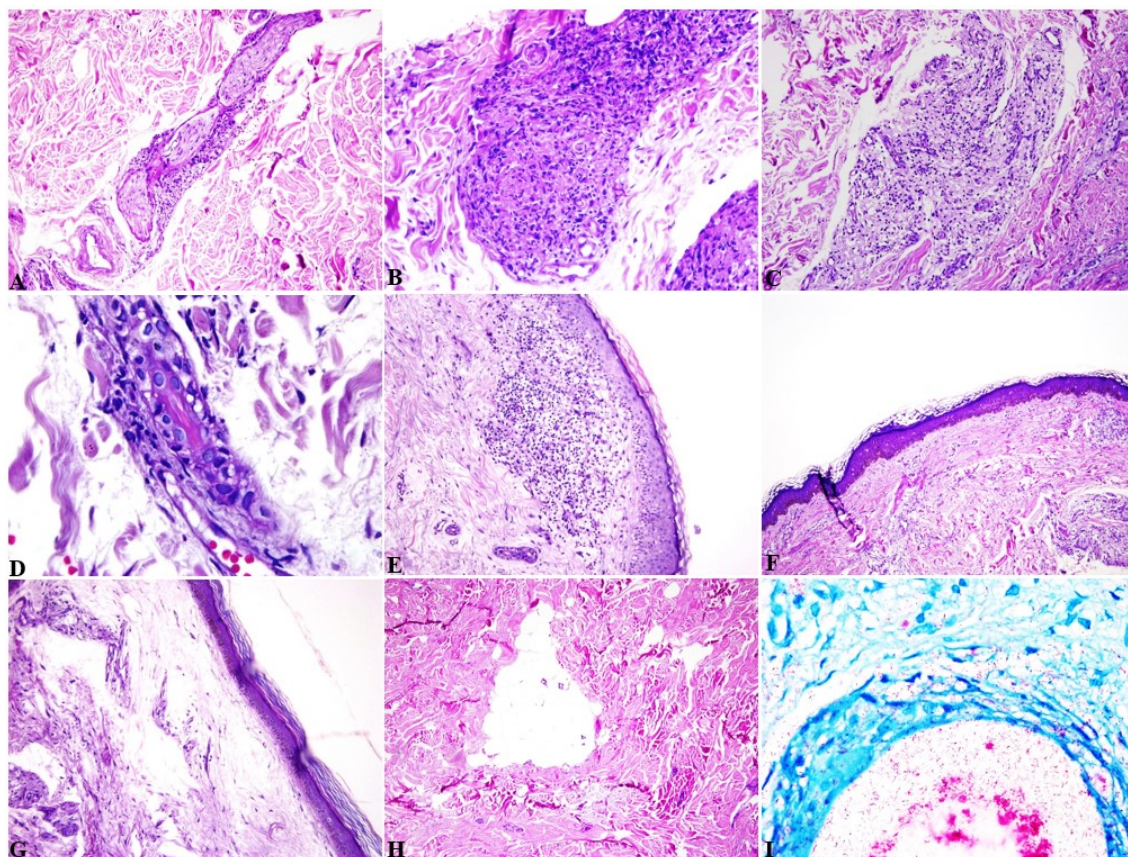
Study variables

Epidemiological and clinical variables. We consulted the records of each patient to obtain the following data points: sex, age, clinical form of R&J, WHO operational classification, number of MDT doses, time (in months) between treatment discharge on account of cure and disease relapse for Group R and time (in months) elapsed since treatment discharge for Group C.

Laboratory variables. Intradermal smear bascilloscopy. The sBI was determined by collecting samples from at least six skin sites (earlobes, elbows, and knees). The smears were stained using the cold Ziehl-Neelsen technique according to the MS protocol (28).

Histopathological analysis. The skin lesion sample was fixed in 10% buffered formalin, embedded in paraffin and the 3 μ m histological sections were stained with hematoxylin-eosin (H&E) and Fite-Faraco (FF) staining, which were utilized to evaluate the histopathological variables (Figure 2). To quantify the bacillary load, we performed staining by the modified FF technique (29), and applied the logarithmic hBI (30). The analysis of slides from cases in both groups, R and C, was carried out blindly by four observers simultaneously using a multi-head optical microscope (Nikon Eclipse 80i, Nikon Corporation, Japan). Three of the four observers were pathologists with experience in R&J classification.

.Figure 2. Histopathological characteristics evaluated in the skin sample at the end of multidrug therapy for leprosy.



Histopathological variables. A, selective lymphocytic infiltrate of neural dermal branches (H&E, 10x). The presence of lymphocytes in mild, moderate, and pronounced quantities around nerves and/or within nerves was considered. B, epithelioid granuloma comprised of macrophage aggregates with abundant and acidophilic cytoplasm (H&E, 10x); C, foamy granuloma consisting of large collections of xanthomized macrophages (H&E, 10x); D, presence of intraepithelial lymphocytes in the eccrine ducts and/or epidermis (H&E, 40x); E, presence of non-cohesive lymphocytic infiltrate (H&E, 10x); F, the atrophic epidermis (H&E, 4x); G, perivascular and/or periadnexal edema (H&E, 4x); H, sclerodermiform/morphea pattern fibrosis (H&E, 10x); I, logarithmic bacillary index (FF, 40x).

IgM anti-PGL-I serology. We detected IgM anti-PGL-I serum antibodies by immunoassay (ELISA) performed against native PGL-I purified from the *M. leprae* cell wall. The following reagent was obtained by donation from BEI Resources, NIAID, NIH: *Mycobacterium leprae* Phenolic Glycolipid-I (PGL-I), NR-19342 and Monoclonal Anti-*Mycobacterium leprae* PGL-I, Clone CS-48 (produced *in vitro*), NR-19370. The titer of anti-PGL-I antibodies was expressed as an ELISA index. EI values ≥ 1.0 were considered positive (31).

DNA extraction and quantitative polymerase chain reaction. DNA was extracted from skin fragments and frozen in liquid nitrogen to amplify the specific genomic region of the bacillus (RLEP3) in a qPCR system (ABI 7300, Applied Biosystems, Foster City, CA, United States) (32, 33).

Ethical Aspects

The research followed the principles of the Declaration of Helsinki. Informed consent was waived and the research was approved by the Research Ethics Committee of the Federal University of Uberlândia (CAAE: 42878620.0.0000.5152) on 16/04/2021. The data were obtained from medical records, specific software, and SINAN, and were blinded for analysis. The informed consent waiver was also requested due to the difficulty of obtaining written consent from patients in a long-term, retrospective study.

Statistical analyses

Quantitative variables were described as the mean, median, maximum, and minimum standard deviation. Qualitative variables were described (frequency and percentage) using double-entry tables. The associations of qualitative variables were

evaluated using the likelihood ratio test (34).

The variables that differentiate Groups C and R were evaluated by univariate logistic regression, as well as multiple logistic regression followed by variable selection using the stepwise method (35). The ROC (Receiver Operating Characteristic) curves for the hBI variables in skin biopsy and anti-PGL-I serology were constructed to evaluate the performance of these tests in detecting Group R. All tests were applied using a significance level of 5% ($p < 0.05$). The procedures were performed using the Statistical Package for Social Sciences (SPSS) version 22.0 (IBM, Armonk, NY, U.S.A.).

Results

The mean return time, the time elapsed between treatment discharge due to cure and LR, was 89.2 months (Min. 60, Max. 152 months) in Group R. For Group C, the average time of being free from illness was 113.1 months (Min. 83, Max. 158 months) (Table S1). The relapse rate was 10.7% (198/1,385) for the period between 2013 and 2020.

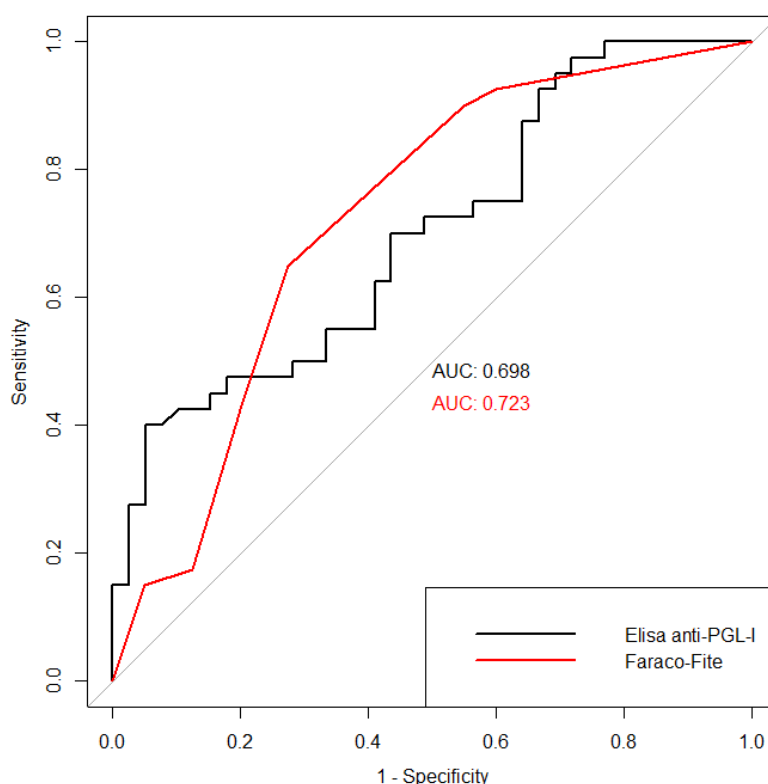
To evaluate the association between categorical variables and Groups R and C, the G Test (likelihood ratio) was used, which demonstrated no statistical difference for epidemiological and clinical variables ($p > 0.05$) (Table S2).

About the number of MDT doses administered to Group R: two patients (5%) received six doses, 13 patients (32.5%) received 12 doses and 25 patients (62.5%) received 24 doses. The numbers of MDT doses administered to Group C were: two patients (5%) received six doses, 20 patients (50%) received 12 doses and 18 patients (45%) received 24 doses.

The mean hBI in the skin sample of patients included in Group R was 3.23 (SD $\pm$ 1.66) and the median was 3 (Min. 0 Max. 6) versus a mean in Group C of 1.8 (SD $\pm$ 1.88)

and median of 2 (Min. 0 Max. 6) ($p = 0.004$). The ROC curve was utilized to detect cutoff point values and optimize sensitivity as a function of specificity. A cutoff value for hBI $\geq 3+$ in skin biopsy resulted in a sensitivity of 72% and a specificity of 65% were found to detect LR (AUC: 0.72; cutoff $\geq 3+$; $p = 0.0002$) (Figure 3) (Table S3).

Figure 3. Sensitivity and specificity values and area under the curve as a function of the skin histological bacillary index and ELISA index at the end of treatment.



In Group R, EI results for anti-PGL-I were a mean of 5.04 ($SD \pm 4.15$) and a median of 3.45 (Min. 0.44 Max. 14.02) while Group C showed a mean of 2.44 ($SD \pm 2.30$) and a median of 1.43 (Min. 0.02 Max. 9.94) ($p = 0.0024$). The EI presented 71% sensitivity and 62% specificity to detect RH (AUC: 0.70; cutoff of ≥ 3.6 ; $p = 0.0012$) (Figure 3).

Regarding the histopathological characteristics of the skin fragments biopsied at the end of treatment (Table 1), there was an association between Group R and the

following variables: neural/perineural lymphocytic infiltrate ($p = 0.0044$), foamy granuloma ($p < 0.0001$) and hBI from skin biopsy ($p = 0.0004$). For the other characteristics analyzed, the proportions of these categories did not differ between the two Groups ($p > 0.05$).

.Table 1. Histopathological characteristics in skin samples biopsied at the end of multidrug therapy.

Variables		Relapse	Control	<i>p-value</i>
Epithelioid granuloma	Negative	36 (90)	38 (95)	0.3917
	Positive	4 (10)	2 (5)	
Neural/perineural lymphocytic infiltrate	Negative	24 (60)	35 (87.5)	0.0043
	Positive	16 (40)	5 (12.5)	
Intraepithelial lymphocytes	Negative	38 (95)	37 (92.5)	0.6431
	Positive	2 (5)	3 (7.5)	
Epidermal atrophy	Negative	14 (35)	15 (37.5)	0.8161
	Positive	26 (65)	25 (62.5)	
Perivascular and periadnexial edema	Negative	28 (70)	24 (60)	0.3478
	Positive	12 (30)	16 (40)	
Dermal fibrosis	Negative	37 (92.5)	37 (92.5)	0.9999
	Positive	3 (7.5)	3 (7.5)	
Non-cohesive lymphocytic infiltrate	Negative	18 (45)	22 (55)	0.3707
	Positive	22 (55)	18 (45)	
Foamy granulomas	Negative	2 (5)	18 (45)	<0.0001
	Positive	38 (95)	22 (55)	

The data were subjected to logistic regression analysis and the results are displayed in Table 2.

.Table 2. Statistical significance of the analyzed variables, assessed by univariate and multivariate logistic regression

		Univariate analysis				Multivariate analysis			
		<i>p-value</i>	Odds Ratio (OR)	(CI 95% for OR)		<i>p-value</i>	Odds Ratio (OR)	(CI 95% for OR)	
Sex		0.9999	1.00	0.41	2.43	-	-	-	-
Age		0.0791	0.97	0.95	1.003	-	-	-	-
Initial operational classification ^s		1.0000	1.00	0.13	7.47	-	-	-	-
Number of doses		0.1897	1.66	0.78	3.56	-	-	-	-
Operational classification de relapse ^s		0.9996	1.00	-	-	-	-	-	-
Epithelioid granuloma		0.4047	2.11	0.36	12.24	-	-	-	-
Neural/perineural lymphocytic infiltrate		0.0076	4.67	1.51	14.45	0.0350	4.58	1.11	18.87
Lymphocytes in the epidermis and/or eccrine ductal epithelium		0.6463	0.65	0.10	4.11	-	-	-	-
Epidermal atrophy		0.8161	1.11	0.45	2.77	-	-	-	-
Perivascular and/or periadnexial edema		0.3497	0.64	0.25	1.62	-	-	-	-
Dermal fibrosis		0.9999	1.00	0.19	5.28	-	-	-	-
Non-cohesive lymphocytic infiltrate		0.3719	1.49	0.62	3.60	-	-	-	-
Foamy granulomas		0.0005	15.55	3.29	73.41	0.0212	7.36	1.35	40.22
Histological bacillary index		0.0015	1.56	1.19	2.06	0.0485	1.39	1.00	1.92
Anti-PGL-I serology		0.0031	1.28	1.09	1.51	0.0628	1.19	0.99	1.44
qPCR of skin biopsy		0.0908	2.34	0.87	6.29	-	-	-	-
qPCR of intradermal smear		0.0607	2.86	0.95	8.58	-	-	-	-
Clinical form [*]	BT	1.0000	1.00	0.19	5.28	-	-	-	-
	BB	0.7240	0.78	0.19	3.14	-	-	-	-
	BL	0.2392	0.57	0.22	1.46	-	-	-	-
	LL	0.1808	1.83	0.75	4.45	-	-	-	-
Clinical form in relapse [*]	DT	0.9999	1.00	-	-	-	-	-	-
	DD	0.9996	1.00	-	-	-	-	-	-
	DV	0.9999	1.00	-	-	-	-	-	-
	VV	0.9999	1.00	-	-	-	-	-	-

^sWorld Health Organization classification; PB: paucibacillary leprosy; MB: multibacillary leprosy.

*Ridley and Jopling classification; BT: borderline-tuberculoid leprosy; DD: borderline-borderline leprosy; BL: borderline-lepromatous leprosy; LL: lepromatous leprosy.

In the univariate logistic regression analysis, the associations were significant ($p < 0.05$) for the presence of lymphocytic neural/perineural infiltrate, foamy granuloma, and hBI. At the end of treatment, when the presence of neural/perineural lymphocytic infiltrate was verified, the odds for LR were increased 4.7-fold (OR=4.67; 95% CI 1.51-14.45; $p=0.0076$), compared to the same odds for Group C. The presence of foamy granuloma presented an elevated association, 15.6-fold increased odds, within the Group R (OR=15.55; 95% CI 3.29-73.41; $p=0.0005$). Concerning hBI in the skin biopsy at the end of treatment, if $\text{hBI} \geq 1+$, the odds for relapse were augmented by 1.6-fold (OR=1.56; 95%CI 1.19-2.06; $p=0.0015$) for the occurrence of LR. For each additional hBI log, the LR odds increased by 56% (up to 9.36-fold increased odds, if $\text{hBI} = 6+$). For the other histopathological characteristics between the two Groups, there were no associations. Therefore, these other histological surrogates evaluated do not seem to be associated with risk or protective factors.

Among the serological findings, the associations were significant for the IgM anti-PGL-I serology variable. According to the analysis, when at the end of treatment, $\text{EI} \geq 1$, there was a 4.7-fold increase in LR odds (OR=4.67; 95%CI 1.51-14.45; $p=0.0031$). It is important to highlight that for each additional EI unit, the odds for LR rose by 367% (11.01-fold increased odds, if $\text{EI} = 3$, for instance).

Multivariate regression analysis was employed to test whether histopathological and laboratory characteristics could predict LR. The data showed that the joint presence of the following variables: lymphocytic neural/perineural infiltrate (OR=4.58; 95% CI 1.11-18.87; $p = 0.0350$), foamy granuloma (OR=7.36; 95% CI 1.35-40.22; $p = 0.0212$)

and $\text{hBI} \geq 1$ ($\text{OR}=1.39$; $95\%\text{CI } 1-1.92$; $p = 0.0485$), significantly predicted LR. Furthermore, in a statistical model, when associating the histopathological variables: the presence of lymphocytic neural/perineural infiltrate, foamy granuloma, and $\text{hBI} \geq 3+$ from a skin biopsy and $\text{EI} \geq 3.6$ for IgM anti-PGL-I serology, a high probability (94.32%) for LR was found (Table S4).

Discussion

After the initial selection of 673 patients, this case-control study was carried out with 80 individuals distributed into two paired groups, with 40 participants in Group R and 40 individuals in Group C. This final sample was due to the application of strict criteria for inclusion in the research and careful pairing between Groups, which made it possible to exclude statistical differences between the Groups ($p > 0.05$). At the time of treatment discharge due to cure, all patients were analyzed for their response to the fixed MDT regimen with 6, 12, or 24 doses of rifampicin, dapsone, and clofazimine, with particular inference to histopathological changes in the skin sample and, EI for anti-PGL-I IgM serology.

Various definitions have been suggested for LR (12–14,36,37). According to the WHO, LR occurs when the patient who has completed the full course of MDT doses subsequently develops new signs and symptoms of leprosy during the surveillance period of 2 years for PB leprosy and 5 years for MB leprosy or later (36). It should be emphasized that the recommended MDT regimen is administered for a fixed period, regardless of the disappearance of skin lesions or the bacilliferous status of the individual (13). Other authors have listed criteria for LR that include: the emergence of new skin lesions or reactivation of previously existing ones, $\text{sBI} \geq 2+$, new peripheral neural involvement, histological evidence of LR on the skin or nerve, and ocular leprosy activity (12, 37). Yet the MS defines relapse cases as those leprosy patients treated regularly with correctly

indicated standardized official therapies, which were discharged due to cure and then returned to presenting new clinical signs and symptoms of active infectious disease over a period of five years or longer (14). For this study, we adopted the definition coined by the MS.

In this research, over a 12-year follow-up period, the average LR time was 89.2 months and the relapse rate was 10.7% (198/1,385). Previous, we analyzed 126 LR cases diagnosed within a period of six years and observed a similar LR rate, 11.89% (126/1,059), after a time mean of 10 years, which was attributed to better post-discharge surveillance and notification of these cases (20,38). The LR rate tends to increase considerably the longer patients are monitored, a process that becomes more difficult after the sixth year (39,40). The LR rate is the most important measure of the effectiveness of MDT for leprosy (17,18). A tertiary reference center for leprosy conducted a study on MB patients and found a mean time of 9.41 years between the completion of MDT and the LR, with a rate of 68.2% (15/22) after an interval ≥ 6 years, most likely on account of persisting *M. leprae* (12). Another study showed an LR rate of 4% (6/136) six years after treatment discharge, which rose to 13% (17/136) after 8 years of follow-up (39). This proves that the relapse numbers vary depending on the length of time patients are monitored.

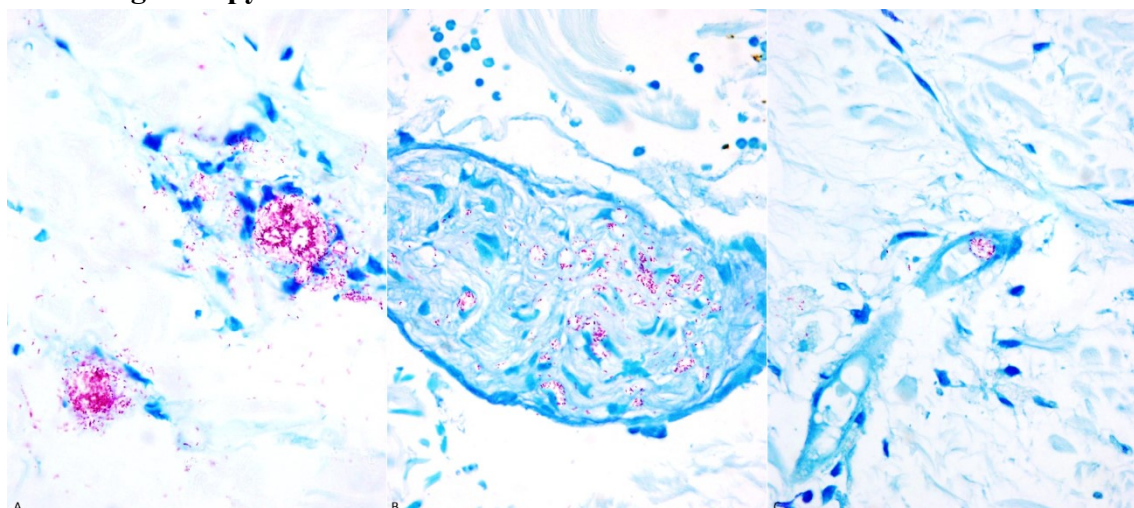
LR is influenced by factors other than follow-up time, such as leprosy classification, MDT duration, reaction episodes, and BI (41). All patients included in this research were treated with a fixed MDT/WHO course. As to the length of MDT, 62% (25/40) of those who relapsed received 24 doses. A study that monitored patients treated with 24 doses of MDT for a period of ≥ 12 years reported an LR rate of 9% (13/142) (42). In our study, 33% (13/40) of LR cases received 12 doses of MDT. However, a cohort that included 713 patients over 12 years who received 12 doses of MDT found only 1.4%

(10/713) of LR (43). When reviewing the methodology applied in detail, these LR cases were selected only through SINAN, some cases had just one-year follow-up; whereas life expectancy was used for others, which may have underestimated the true LR rate in the studied population. Another study team followed 162 MB patients treated with 12 doses of MDT for 4.08 years, of whom 8% (13/162) presented LR within two years, which was attributed to treatment insufficiency or failure (44). Another study monitored 300 patients with MB leprosy who completed one year of MDT, in which no LR was found within 5 years after the end of treatment; however, an LR rate of 3% (8/300) was detected between 6 and 9 years later and it rose to 5% (15/300) after 10 years after ending the MDT (38). While there are studies that assert LR is infrequent, others report it as common. In contemporary times, this discussion is fundamentally relevant to us, since variations in LR rate estimates are abysmal among different countries (17). Nonetheless, in individuals treated with MDT, the LR rates are certainly not low, as demonstrated not only in our experience (20) but also in the experience of others (42).

Histological bacillary skin index at treatment discharge due to cure

With the introduction of multidrug therapy, the alcohol-acid-resistant bacilli not only fragment but also decrease progressively in number, with loss of color by losing their FF staining affinity (3,29,45). In the TT and BT forms, with few lesions and a low bacillary index ($hBI \leq 2+$), the bacilli disappear in a few months or years. On the contrary, in those forms with a high bacillary index ($hIB = 5-6+$), bacillary clearance may be a later phenomenon (3) (Figure 4).

.Figure 4. Bacilloscopy on the skin sample with Faraco-Fite staining after the end of multidrug therapy.



A, numerous fragmented and granular bacilli (IB=6+) inside macrophages and in the interstitium (100x). B, high residual bacillary load within the neural branch; and, C, some fragmented bacilli in the endothelium of venules (40x).

Studies have postulated that the presence of intact bacilli or $BI \geq 2+$ confirmed by intradermal smear, skin, or nerve biopsy defines LR after discharge from treatment (3,37,46). Likewise, the appearance of new skin lesions with a BI greater than any other preexisting lesions indicates LR (46,47). In this research, we found that $hBI \geq 1+$ in the skin sample biopsied at the end of MDT increased the odds of LR by 1.56 per hBI log. By contrast, a cohort that evaluated 299 cases of MB leprosy could not find statistical significance in its data to predict the risk of LR when analyzing the sBI at the end of treatment ($p=0.158$) (48).

A recent study evaluated 36 patients who completed the fixed MDT course of 12 doses and found that 100% (36/36) of the cases maintained histologically active skin lesions with $hBI = 3-4+$ and the presence of live bacilli documented by rtPCR technique in two steps using three different rRNA genes (49). Solid bacilli, sometimes in globi, are indicative of bacillary proliferation (3,12).

Furthermore, in our study, when considering hBI values $\geq 3+$, we could predict LR with moderate sensitivity (72%) and specificity (65%) at the end of MDT. Other studies show the higher the BI at the end of MDT or the shorter the duration of treatment the greater the risk of LR (46,49,50). As to the bacillary index, 21.3% (39/184) of patients with sBI $\geq 5+$ presented disease relapse, compared with, in 13.6% (25/184) of those cases with sBI $< 5+$ and in only 7.1% (8/113) of those achieved negativity at the end of treatment (46). Furthermore, LL patients with high initial BI have a weak cellular immune response and a weak macrophage response to deal with these remaining organisms (50). A study that evaluated 555 cases of MB leprosy found a high LR rate (39%) in patients with BI $\geq 4+$ after being treated with 24 months of MDT (51). Therefore, both sBI and skin hBI should be evaluated at the end of leprosy drug therapy, as these data can predict the odds of LR. And, a negative or steeply decreasing hBI should be used as an objective criterion to define leprosy cure, and guide patients' management, and reduce LR rates.

ELISA index of IgM anti-PGL-I serology

The most widely used antigen for serological diagnosis of leprosy, patient classification, treatment monitoring, risk of relapse, and in the selection of contacts at the highest risk of becoming ill is anti-PGL-I (52–54). The detection of the IgM anti-PGL-I by ELISA is even more useful in cases of MB leprosy, which is associated with a greater quantity of bacilli in the skin (20,55,56). Levels of IgM anti-PGL-I are directly related to the antigenic load and indicative of active infection (57). This is why monitoring IgM anti-PGL-1 levels during MDT can serve as a sensitive tool for evaluating treatment effectiveness (58).

In our study, positive IgM anti-PGL-I serology ($EI \geq 1$) at the end of MDT treatment showed an increased LR odds of 1.56 per unit. Another study verified that

patients with positive PGL-I serology (*ML Flow*) after discharge from MDT were 10.40 times more likely to develop leprosy reactions (59). The humoral response assessed by anti-PGL-I serology is directly proportional to the bacillary load, measured by both the sBI and hBI (59,60). Seropositivity may be the first indicator of LR (61). This demonstrates that bacilli can remain protected from the immunological effects of treatment and subsequently proliferate in more favorable conditions (53). In our research, when using an EI cutoff ≥ 3.6 , we found moderate sensitivity (71%) and specificity (62%) to predict LR. It is believed that with simple and robust techniques, the use of PGL-I serology in public health programs is viable (54).

A retrospective cohort analyzed 201 cases of leprosy reaction and found respective median survivals of 5 and 9 months for cases with negative EI (26/51) and positive EI (75/150), demonstrating that anti-PGL-I serology, at the time of diagnosis, is the most important prognostic factor for leprosy reactions after the initiation of MDT (62). However, few studies have evaluated the usefulness of serology to predict which patients may have LR (54,63). We defend the use of negative or steeply decreasing EI in the role of objective criteria for defining leprosy cure.

Histopathological findings in post-treatment skin biopsies

This is the first study that establishes the odds of LR using histopathological parameters in skin biopsy at the end of MDT. The neurological and cutaneous manifestations of leprosy are polymorphic and are strongly correlated with the host's immune response, in which the tuberculoid pole with Th1 activity corresponds to the more limited presentations and the lepromatous pole with Th2 activity to the MB forms (64). The WHO recommends fixed-duration MDT, regardless of whether the lesions disappear or persist after treatment (13,65). Although some studies show that in most

individuals, at the end of MDT, healing occurs over time due to the resolution process (13), others indicate that the lesions can remain active (66,67).

In our current work, we categorized patients by R&J clinical form at the time of LR, we observed that 40.5% had the BT form (51/126) while 37.3% had LL (47/126), and 96% of cases (121/126) were considered MB/WHO leprosy (20). A comparative study that evaluated the histopathological response of 44 PB patients to treatment with MDT, diagnosed as residual cutaneous lesions, in resolution and active in 30% (13/44), 9% (4/44), and 61% (27/44), respectively (65). Another study that assessed 30 PB patients who also received a six-month MDT regimen and were examined clinically and histopathologically before instituting the medication and at six months and 12 months after MDT found histopathological activity in 50% and 25% of patients after six months and 12 months, respectively (66). However, in a study with a similar design, with 43 PB patients who completed the MDT protocol, microscopic findings of persistent disease activity were present in only 7% of cases (3/43) (13). It should be noted that these data do not document LR cases, but rather treatment failure, which tends to occur more closely to the end of MDT.

Histomorphological alterations at the end of treatment that reflect disease activity include the persistence of well-formed granulomas and epithelioid cells, neural/perineural lymphocytic infiltrate, presence of bacilli within the endothelium and periadnexal lymphohistiocytic infiltrate (3,13,65,68). The reason for the greater attention so far given to macrophages and lymphocytes is due to their predominance in the histology of leprosy lesions (69).

It is believed that, in active disease, there is rapid cell renewal and macrophages have abundant eosinophilic cytoplasm (epithelioid macrophages), which are also frequently observed in LR (70). However, in our research, this finding was present in only

10% (4/40) of individuals with LR, all MB cases, with no statistical significance ($p = 0.3917$) when compared to Group C. In the TT and BT forms, epithelioid granulomas disappear progressively, with gradual loss of epithelioid cells, without fibrosis, and progressively take on the clinical picture of a superficial perivascular lymphocytic infiltrate over 1 to 3 years after MDT (29). Also considering the presence of epithelioid granulomas as a marker of disease activity, an already-cited study that analyzed the histopathological response to MDT in PB leprosy found this finding in a minority of research participants (13).

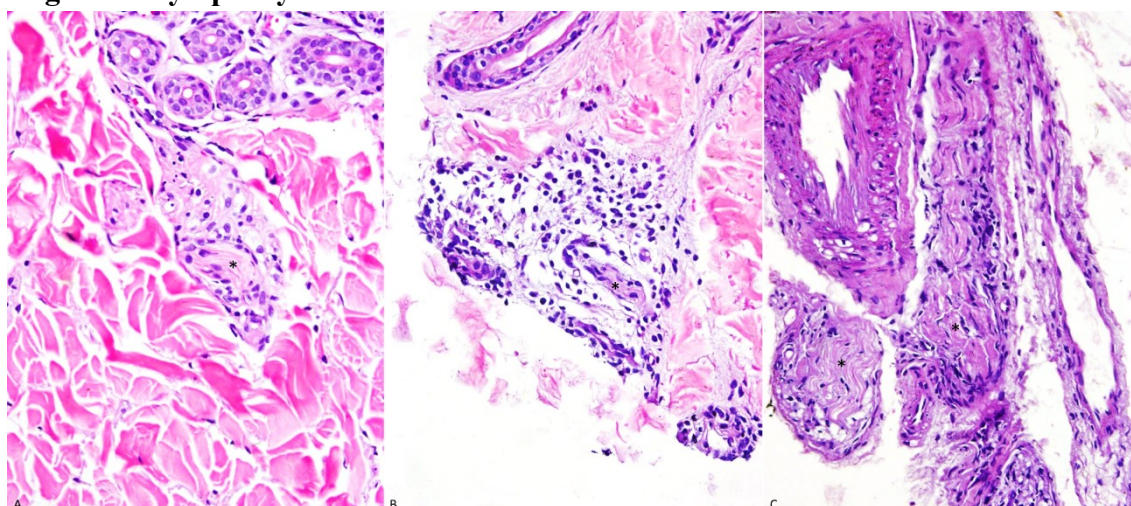
In skin biopsy, recognizing the histopathological characteristics of regressive phenomena is important in evaluating the effectiveness of treatment (3). Various histopathological findings – namely epidermal atrophy and hyperpigmentation, reduction or absence of dermal lymphocytic infiltrate, superficial perivascular edema, fibromyxoid alterations in the dermis, loosening of granulomas, reduction in the absolute number and cytoplasmic vacuolization of epithelioid macrophages, disappearance of multinucleated giant cells, foci of lymphocytes intraepithelial lesions in the epidermis or eccrine duct and foamy granulomas – are described as indicators of leprosy regression in those patients who completed the MDT regimen (13,65,68).

In the BL and LL forms, fatty degeneration of macrophages, with the appearance of cytoplasmic vacuoles, characterizes the regressive lesions, which disappear slowly over 2 to 5 years or more (3,29,45). However, it is known that in some patients, some foam cells can persist for decades (3,74). While some authors argue that granulomas made up of uniformly foamy macrophages and containing fragmented and granular bacilli debris are morphological alterations observed in treated leprosy and denote regression (68), other studies have focused on lipid homeostasis and its role in the physiopathology of the infection; homeostatic imbalance can lead to the formation of foam cells (75).

Lymphocytic infiltrate of nerves

We consider neural/perineural lymphocytic infiltrate as variable quantities of lymphocytes around or inner side of the cutaneous neural branches (Figure 5). In this analysis, the presence of neural/perineural lymphocytic infiltrate was found in 40% (16/40) of patients who had disease relapse, which implied 4.67-fold greater for LR (Figure 6). We were not able to find other studies that attributed predictive value to lymphocytic infiltrate in nerves for LR in our literature review.

.Figure 5. Lymphocytic infiltration of nerves

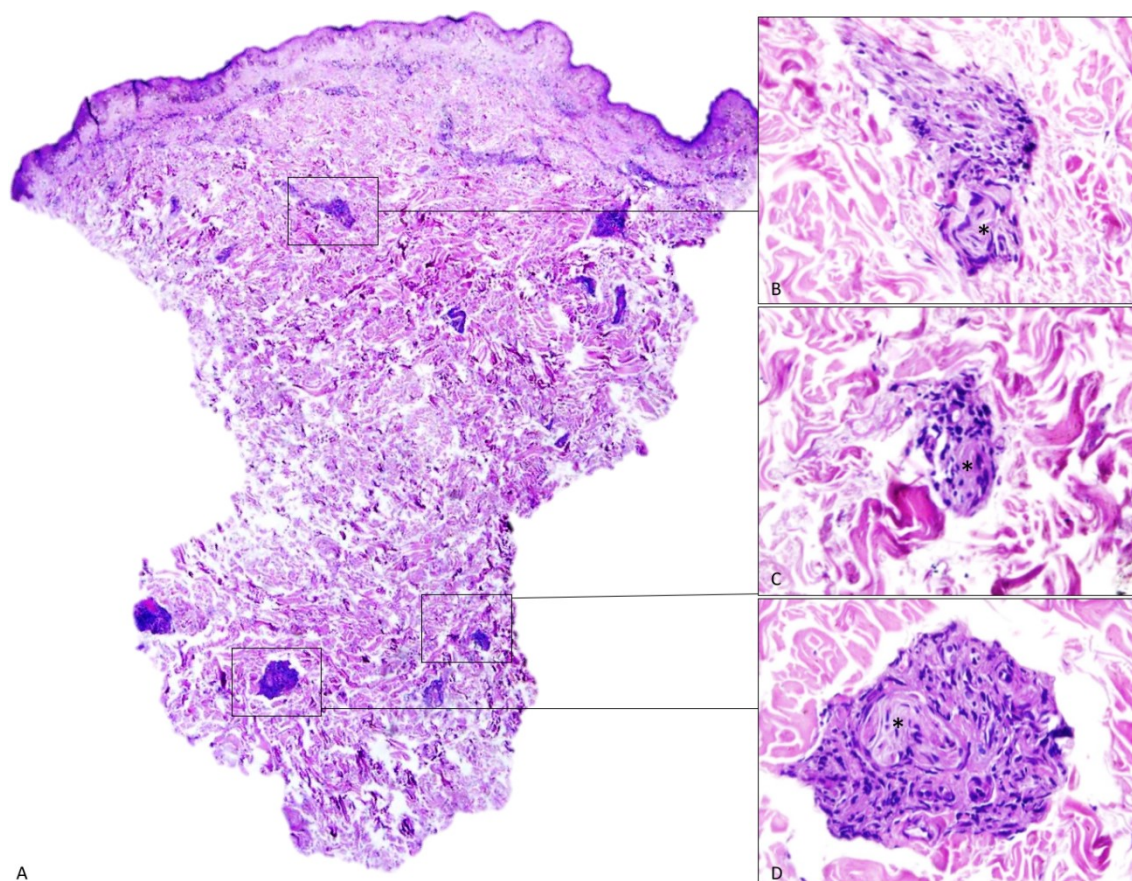


In skin biopsies at the end of multidrug therapy, different degrees of lymphocytic infiltrate were found in the neural branches (*). In A, few lymphocytes permeate the Schwann cells; in B, lymphocytes predominate around the fillet; and, in C, the lymphocytic infiltrate is moderate and located in the endoneurium (HE, 40x).

It should be noted that leprosy is a primarily neural disease that can manifest itself with an intradermal pattern, as documented in a study that analyzed 164 cases of pure neural leprosy (PNH) and found 9 the intradermal pattern in 9.6% (7/73) and 8.8% (8/91) of patients with short (≤ 12 months) and long (> 12 months) disease duration, respectively (71). It is understood that as a consequence of the lymphocyte response, the neural

branches of the skin suffer perineural and intraneural fibrosis that “bury” the live *M. leprae* in these nerves, thus serving as a focus for LR (17,72). Another study evaluated 93 cases of PNH and found 29% (27/93) to be RH, with a mean time of 11.8 years since discharge from treatment, and found histopathological changes such as lymphocytic infiltrate, and fibrosis in all of them (73). Therefore, we recognize the importance of neural/perineural lymphocytic infiltrate as a sign of persistence of disease activity and a predictor of LR as demonstrated by our data (Figure 6).

.Figure 6. Involvement of nerves by lymphocytes after leprosy treatment



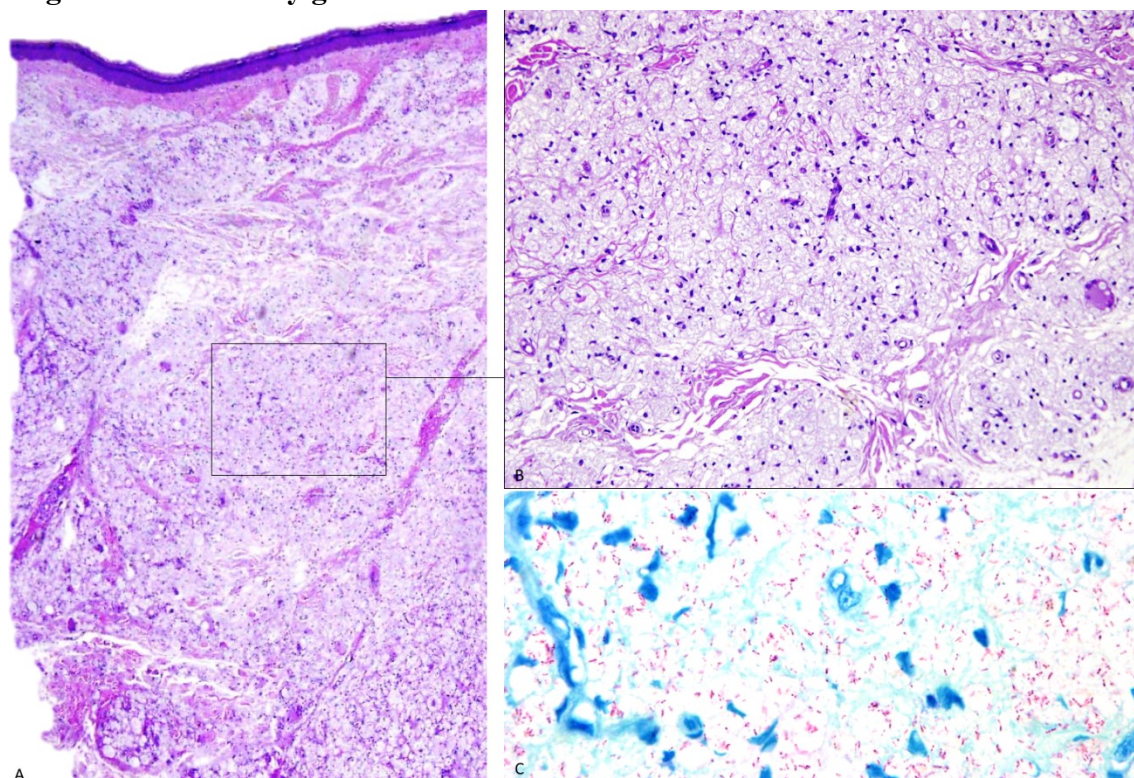
Man, 33 years old, BL, treated with 12 doses, relapsed in 5 years and 10 months in the BT form. A, Fragment of skin with numerous neural branches infiltrated by lymphocytes (H&E, 4x). B, C, and D, in detail, the neural branches (*) of different thicknesses and intensities of active inflammation (HE, 40x).

Foamy Granulomas

Foamy granulomas, in our study, are characterized by large collections or sheets of xanthomatized macrophages in the skin, distributed diffusely or in clusters where epithelioid cells are absent (Figure 7). These large collections of foamy macrophages were present in almost all LR cases (38/40) which yielded, in this analysis, highly elevated odds (> 15.5) for LR. Therefore, the presence of foamy granulomas should not be interpreted merely as post-treatment regressive alterations. The foamy aspect of M2 macrophages infected by *M. leprae* is an excellent example of the impact of metabolic modulation on the immunological control of leprosy (76). We reinforce that this finding, when present after MDT, should be signed out in the pathology report as a warning for LR.

It is known that bacterial multiplication stimulates an influx of macrophages, which in LL leprosy are probably the ideal host cells (70). Foamy degeneration of nerves in LL leprosy was observed in a study that analyzed nerve biopsies and *in vitro* cultures of Schwann cells, documenting cholesterol biogenesis induced by *M. leprae* and recognizing that this process plays a central role in bacterial survival (77).

.Figure 7. Skin foamy granuloma after fixed duration treatment



Woman, 42 years old, VV, treated with 24 doses, relapsed in 7 years and 1 month in the VV form. A, fragment of skin with marked and diffuse histiocytic infiltrate (H&E, 4x). B, Foamy macrophage sheets (HE, 20x). C, several fragmented and granular bacilli inside macrophages (Faraco-Fite, 40x).

The electron microscopy examination of the foamy macrophage made it possible to observe that *M. leprae* lives and multiplies within xanthotomized, lipid-rich phagosomes (78). Current studies confirm that *M. leprae* alters the lipid composition of the host macrophage through the induction of *de novo* cholesterol biosynthesis, stimulation of LDL receptor expression, and increased LDL-cholesterol absorption, which has pathophysiological consequences for bacterial persistence in the host (79).

In lipid and energy homeostasis, lipid droplets (LD) play a central role. LD are storage organelles that originate from the endoplasmic reticulum and can associate with most other cellular organelles through membrane contact sites (80). Recent discoveries

have demonstrated that LD are highly induced in a regulated manner during *M. leprae* infection, with important functions in the pathogenesis of the disease, including strategic roles in pathogen evasion and as a rich source of nutrients that enable *M. leprae* persistence (81).

Finally, we found an LR probability of up to 94.32% when combining histopathological findings (neural/perineural lymphocytic infiltrate, foamy granulomas, and BI $\geq 3+$) and serological findings (EI ≥ 3.6). Thus, we highly emphasize an urgent need to establish objective criteria for leprosy cure beyond simply the discharge from MDT. We encourage the use of auxiliary diagnostic tools and all available technology such as: surgical pathology, serology, molecular tests, ultrasonography of peripheral nerves, electroneuromyography, etc. (Table 3) (20,82,83).

.Table 3. Proposal for an objective monitoring therapy response at the beginning, middle, and end of treatment.

Complementary exam	Criteria for discharge due to cure
Histopathology of the skin	BI = 0 or falling Absence of globi Absence of solid bacilli Absence of selective lymphocytic infiltrate of nerves
Intradermic smear	BI = 0 or falling Absence of globi Absence of intact bacilli
Anti-PGL-I IgM serology	EI < 1 (negative) or falling
qPCR for <i>M. leprae</i> (RLEP)*	Increase of $CT^{\S} > 35$, in both samples (smear and biopsy) and DNA load (copies of DNA) decreasing
Electroneuromyography	Absence of activity from neural damage
Ultrasonography of peripheral nerves	Absence of activity from neural damage

*Intradermic smear and/or fragments of biopsied skin.

§ CT: *cycle threshold*: point at which the *threshold* (minimum number of cycles for amplification) crosses the sample amplification line (determines the number of cycles necessary to begin amplification of the target gene sequence present in the DNA and the relative quantification of the DNA of each sample, after being corrected for the CT of endogenous control genes and control samples).

Likewise, there is a need for worldwide standardization of objective criteria for relapse, treatment insufficiency and failure in leprosy. If these efforts are neglected, the WHO goal of eliminating leprosy by 2030 (15) will not be achieved.

Conclusion

This work has produced unprecedented information on the odds for LR, which will be used for surveillance and follow-up of the population treated with the MDT recommended by the WHO. LR is a late phenomenon and occurs even after 24 doses of MDT, and thus requires prolonged follow-up.

At the time of discharge upon completion of the MDT course, histopathological findings, neural/perineural lymphocytic infiltrate, foamy granulomas, and hBI $\geq 1+$, increase the odds for LR in patients regularly treated with MDT. Likewise, seropositivity for IgM anti-PGL-I (EI ≥ 1), at the end of treatment, is also a predictive factor for LR. The association of these parameters significantly increases the probability of LR. This, in our opinion, is a favorable path for more in-depth research with a greater number of participants.

Currently, it remains necessary to establish objective criteria for real discharge due to leprosy cure. To this end, the utilization of technological and laboratory resources is useful. Active surveillance in patients with a high LR probability after discharge should be adopted to reduce the risk of neurological morbidity and deformity in this patient population and, ultimately, to control leprosy.

Study limitations

A possible limitation of this research study is its relatively small sample size. However, this sample reflects the occurrence of LR in seven years in a national reference center for leprosy. New studies may corroborate the conclusions stated herein.

Concerning qPCR, the associations could only be made quantitatively and were not significant. This is because, over the 12 years studied, the values of the results were expressed in different units.

Authors' Contributions

BCD, DFS, and IMBG elaborated the conceptualization. BCD, WVTC, and IMBG collected the data. FAR, and DROC executed the histological techniques. BCD, WVTC, JPFA, and JSD analyzed the histological sections. BCD, LBA, DEA, and IMBG evaluated the data. BCD, DEA, DFS, and IMBG helped in the design and methodology of the study. BCD, and IMBG wrote the manuscript. IMBG, and CTS revised the manuscript. IMBG obtained resources and funding. BCD, WVTC, FAR, JPFA, JSD, DFS, and IMBG executed the investigation. IMBG administered and supervised the project. BCD, WVTC, FAR, DROC, JPFA, JSD, LBA, DEA, and IMBG validated the work.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical considerations

Ethical issues were fully addressed by the authors.

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List of abbreviations

Anti-PGL-I: phenolic glycolipid I antibody

BB: borderline-borderline leprosy

BI: bacillary index

C: control

DL: borderline-lepromatous leprosy

DT: borderline-tuberculoid leprosy

EI: ELISA index

hBI: histological bacillary index

LL: lepromatous leprosy

MB: multibacillary

MDT: multidrug therapy

PB: paucibacillary

qPCR: real time quantitative polymerase chain reaction

R: relapse group

RH: leprosy recurrence

sBI: bacillary index of intradermal smear

TT: tuberculoid leprosy

WHO: World Health Organization

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Supplementary Supporting Material

Table S1. Numerical description of the variables.

	Relapse Group								Control Group							
	n	Mean	Standard deviation	Median	Q1	Q3	Minimum	Maximum	n	Mean	Standard deviation	Median	Q1	Q3	Minimum	Maximum
Age	40	48.25	13.3757	47	38.75	57	22	73	40	54.45	17.2314	58	45.75	68	13	85
Number of doses*	40	19.2	6.4019	24	12	24	6	24	40	17.1	6.4522	12	12	24	6	24
Time^{&}	40	89.18	27.8152	80.5	63.5	115.25	60	152	40	113.1	14.1436	113.5	103	125	83	158
Bacillary index from skin biopsy¹	40	2.675	1.6233	2	2	4	0	6	40	1.875	1.9636	2	0	3	0	6
Anti-PGL-I²	40	5.037	4.1497	3.45	1.3075	7.3225	0.44	14.02	40	2.4415	2.2973	1.43	0.505	3.765	0.02	9.94
Bacillary index from intradermic smear¹	40	2.865	1.6522	3.21	1.7075	4.3525	0	5	40	1.7048	1.4442	1.85	0	2.78	0	4.42

*Number of doses of multidrug therapy administered throughout the treatment.

[&]Time. For the Relapse Group, it signifies the number of months between treatment discharge due to cure and disease relapse. For the Control Group, it is the follow-up period in months from discharge due to cure until this work is carried out.

¹Bacillary index using the logarithmic scale of Ridley & Hilson.

²Anti-PGL-I Serology (IgM) using the ELISA method, considered positive if ≥ 1 .

.Table S2. Clinical and laboratory characteristics of the patients.

Clinical characteristics		Relapse Group	Control Group	G Test (likelihood ratio)
Sex	Male	23 (57.5)	23 (57.5)	0.9999
	Female	17 (42.5)	17 (42.5)	
Initial clinical form [*]	BT	3 (7.5)	3 (7.5)	0.5736
	BB	4 (10)	5 (12.5)	
	BL	11 (27.5)	16 (40)	
	LL	22 (55)	16 (40)	
Initial operational classification [§]	PB	2 (5)	2 (5)	0.9999
	MB	38 (95)	38 (95)	
Clinical form of relapse [*]	BT	11 (27.5)	-	-
	BB	0 (0)	-	
	BL	8 (20)	-	
	LL	21 (52.5)	-	
Operational classification of relapse [§]	MB	40 (100)	-	-
Laboratorial characteristics				
Qualitative anti-PGL-I ^{&}	-	7 (17.5)	14 (35.9)	0.06238
	+	33 (82.5)	25 (64.1)	
qPCR of skin biopsy	-	9 (23.7)	16 (42.1)	0,0859
	+	29 (76.3)	22 (57.9)	

^{*} Ridley and Jopling classification; BT: borderline-tuberculoid leprosy; DD: borderline-borderline leprosy; BL: borderline-lepromatous leprosy; LL: lepromatous leprosy.

[§]Classification of the World Health Organization; PB: paucibacillary leprosy; MB: multibacillary leprosy.

[&]Considered positive if ELISA index (EI) ≥ 1 .

.Table S3. Performance of diagnostic tests.

	Skin biopsy IB¹	Anti-PGL-I serology
<i>Cutoff</i>	3	3.60
AUC	0.72	0.70
Sensitivity	0.65	0.71
Specificity	0.72	0.62
Accuracy	0.69	0.61
Positive predictive value	0.70	0.58
Negative predictive value	0.67	0.65

¹Bacillary index using the logarithmic scale of Ridley & Hilson.

.Table S4. Exposition of multivariate regression variables.

Neural/perineural lymphocytic infiltrate	Foamy granulomas	IB¹ skin biopsy	Anti-PGL-I	Probability of Relapse (%)
+	+	≥3	≥3.6	94.32
+	+	≥3	<3.6	91.13
+	+	<3	≥3.6	80.75
-	+	≥3	≥3.6	74.51
+	+	<3	<3.6	72.19
+	-	≥3	≥3.6	65.29
-	+	≥3	<3.6	64.39
+	-	≥3	<3.6	53.78
-	+	<3	≥3.6	42.47
+	-	<3	≥3.6	32.21
-	+	<3	<3.6	31.35
-	-	≥3	≥3.6	24.86
+	-	<3	<3.6	22.71
-	-	≥3	<3.6	16.99
-	-	<3	≥3.6	7.71
-	-	<3	<3.6	4.92

¹Bacillary index using the logarithmic scale of Ridley & Hilson.

5 CONSIDERAÇÕES FINAIS

- As taxas de falência terapêutica (6,7%) e de recidiva na hanseníase (10,7%) não são baixas;
- A avaliação histopatológica da biópsia de pele ao término da PQT consiste numa ferramenta útil no diagnóstico precoce da recidiva e falência terapêutica na hanseníase;
- Presença de infiltrado linfocitário neural/perineural acarreta uma chance 367% maior para recidiva na hanseníase;
- Presença de granuloma espumoso implicou numa chance 1.455% e 636% maior para recidiva na hanseníase e falência terapêutica, respectivamente;
- Índice baciloscópico positivo atribuiu, a cada unidade logarítmica, chance 55% maior para a falência terapêutica;
- Índice baciloscópico maior ou igual a 3+ acarretou sensibilidade de 73% e 72% e especificidade de 78% e 65% para a falência terapêutica e recidiva na hanseníase;
- Índice ELISA maior ou igual a 1 mostrou, a cada unidade, chance 40% e 367% maior para a falência terapêutica e recidiva na hanseníase, respectivamente;
- Índice ELISA maior ou igual a 3,9 ou a 3,6 encontrou sensibilidade de 79% e 71% e especificidade de 59% e 62% para a falência terapêutica e recidiva, respectivamente;
- A combinação de histopatologia, sorologia e testes moleculares aumentou a probabilidade de falência terapêutica e recidiva na hanseníase em até 95,41% e 94,32%, respectivamente;
- A forma clínica virchowiana se associa com falência terapêutica;
- Há a necessidade do emprego de recursos tecnológicos e objetivos para estabelecer critérios de cura real para a hanseníase;
- A combinação de métodos complementares histopatológicos, sorológicos e moleculares é essencial para o diagnóstico precoce falência terapêutica e recidiva na hanseníase, visando minimizar as incapacidades e deformidades relacionadas à doença.

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ANEXO A – FICHA DE NOTIFICAÇÃO/INVESTIGAÇÃO DE HANSENÍASE

República Federativa do Brasil Ministério da Saúde		SINAN SISTEMA DE INFORMAÇÃO DE AGRAVOS DE NOTIFICAÇÃO		Nº	
		FICHA DE NOTIFICAÇÃO/ INVESTIGAÇÃO		HANSENÍASE	
Caso confirmado de Hanseníase: pessoa que apresenta uma ou mais das seguintes características e que requer poliquimioterapia: - lesão (ões) de pele com alteração de sensibilidade; acometimento de nervo (s) com espessamento neural; baciloscopia positiva.					
Dados Gerais	1	Tipo de Notificação		2 - Individual	
	2	Agravado/doença		Código (CID10)	3 Data da Notificação
	4 UF	5	Município de Notificação	Código (IBGE)	
Notificação Individual	6	Unidade de Saúde (ou outra fonte notificadora)		Código	7 Data do Diagnóstico
	8	Nome do Paciente		9 Data de Nascimento	
	10 (ou) Idade	11 Sexo	12 Gestante	13 Raça/Cor	
Dados de Residência	14	Escolaridade			
	15	Número do Cartão SUS		16 Nome da mãe	
	17 UF	18	Município de Residência	Código (IBGE)	19 Distrito
Dados Complementares do Caso	20	Bairro		21 Logradouro (rua, avenida,...)	
	22	Número		23 Complemento (apto., casa, ...)	
	24	Geo campo 1		25 Geo campo 2	
Dados Clínicos	26	Ponto de Referência		27 CEP	
	28	(DDD) Telefone		29 Zona	
	30	Pais (se residente fora do Brasil)			
Atendimento	31	Nº do Prontuário		32 Ocupação	
	33	Nº de Lesões Cutâneas		34 Forma Clínica	
	35	Classificação Operacional		36 Nº de Nervos afetados	
Dados Lab.	37	Avaliação do Grau de Incapacidade Física no Diagnóstico		38 Modo de Entrada	
	39	Modo de Detecção do Caso Novo		40 Baciloscopia	
	41	Data do Início do Tratamento		42 Esquema Terapêutico Inicial	
Tratamento	43	Número de Contatos Registrados			
	Observações adicionais:				
Investigador	Município/Unidade de Saúde		Código da Unid. de Saúde		
	Nome		Função		Assinatura
	Hanseníase		Sinan NET		SVS 30/10/2007