Neutrophil NETworking in ENL: Potential as a Putative Biomarker: Future Insights

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Erythema nodosum leprosum (ENL), also known as type 2 reaction (T2R) is an immune complex mediated (type III hypersensitivity) reactional state encountered in patients with borderline lepromatous and lepromatous leprosy (BL and LL) either before, during, or after the institution of anti-leprosy treatment (ALT). The consequences of ENL may be serious, leading to permanent nerve damage and deformities, constituting a major cause of leprosy-related morbidity. The incidence of ENL is increasing with the increasing number of multibacillary cases. Although the diagnosis of ENL is not difficult to make for physicians involved in the care of leprosy patients, its management continues to be a most challenging aspect of the leprosy eradication program: the chronic and recurrent painful skin lesions, neuritis, and organ involvement necessitates prolonged treatment with prednisolone, thalidomide, and anti-inflammatory and immunosuppressive drugs, which further adds to the existing morbidity. In addition, the use of immunosuppressants like methotrexate, azathioprine, cyclosporine, or biologics carries a risk of reactivation of persisters (Mycobacterium leprae), apart from their own end-organ toxicities. Most ENL therapeutic guidelines are primarily designed for acute episodes and there is scarcity of literature on management of patients with chronic and recurrent ENL. It is difficult to predict which patients will develop chronic or recurrent ENL and plan the treatment accordingly. We need simple point-of-care or ELISA-based tests from blood or skin biopsy samples, which can help us in identifying patients who are likely to require prolonged treatment and also inform us about the prognosis of reactions so that appropriate therapy may be started and continued for better ENL control in such patients. There is a significant unmet need for research for better understanding the immunopathogenesis of, and biomarkers for, ENL to improve clinical stratification and therapeutics. In this review we will discuss the potential of neutrophils (polymorphonuclear granulocytes) as putative diagnostic and prognostic biomarkers by virtue of their universal abundance in human blood, functional versatility, phenotypic heterogeneity, metabolic plasticity, differential hierarchical cytoplasmic granule mobilization, and their ability to form NETs (neutrophil extracellular traps). We will touch upon the various aspects of neutrophil biology relevant...
to ENL pathophysiology in a step-wise manner. We also hypothesize about an element of metabolic reprogramming of neutrophils by *M. leprae* that could be investigated and exploited for biomarker discovery. In the end, a potential role for neutrophil derived exosomes as a novel biomarker for ENL will also be explored.

**Keywords:** ENL, neutrophils, biomarker, NETs, biology, reprogramming, exosomes

**INTRODUCTION**

Leprosy is a chronic granulomatous infectious disease caused by an intracellular pathogen *Mycobacterium leprae* where the clinical spectrum of the illness is dictated by the host immune response to the pathogen (1). The clinical course of leprosy is punctuated by dynamic, unpredictable, painful immunemediated inflammatory episodes known as reactions. These leprosy reactions are a major clinical concern and up to 50% of leprosy patients experience at least one reaction during their lifetime (2). Erythema nodosum leprosum (ENL)/type 2 reaction (T2R), an acute nerve-destructive immune exacerbation, is encountered in patients with borderline lepromatous (BL) and lepromatous leprosy (LL) and can occur before, during, or after multi-drug treatment (3). It is estimated that annually over 50,000 newly diagnosed leprosy patients are at risk of developing ENL (4). These patients classically present with tender, erythematous, evanescent subcutaneous nodules along with multisystemic illness manifesting as neutrophilic leukocytosis, fever, sepsis-like malaise, irritis, iridocyclitis or conjunctivitis, arthritis or arthralgia, bone pain and tenderness especially tibial tenderness, dactylitis, lymphadenitis, oedema of extremities, orchitis, and neuritis ultimately leading to significant sequelae associated with peripheral nerve dysfunction. The clinical manifestations of ENL can occur in any of 3 patterns viz. single acute episodes, recurrent episodes, and chronic disease with varying grades of severity (5). ENLIST ENL Severity Scale (EESS) a 10-point severity scale system is a validated tool used in clinics to assess ENL severity (6). Atypical clinical manifestations like pustular, bullous, necrotic, and annular ENL have also been reported and sometimes T2Rs may present with rheumatologic complaints leading to misdiagnosis. Besides a high index of clinical suspicion, point-of-care diagnostic assays or biomarkers are needed that could be used by leprosy workers in the field to confirm the diagnosis and initiate treatment to avoid deformities and disabilities.

The incidence of this multi-system inflammatory disorder is certainly on the rise with the increasing number of multibacillary cases (7). The bactericidal effect of Multi-drug therapy (MDT) or other antibiotics on *M. leprae* results in the release of an enormous amount of bacterial breakdown products that are recognized by the innate immune system as antigens resulting in activation of pro-inflammatory cytokines (7, 8).

These reactions are often diagnosed late in the absence of reliable, early diagnostic tests. Attempts to identify general biomarker signatures (Table 1) for ENL are often hampered due to patient heterogeneity, different male/female ratios across various studies, lack of sufficient power of many studies due to low number of disease subjects, long incubation times of leprosy and lack of appropriate control groups (LL/BL patients without ENL, instead of healthy controls) (9). There is an unmet need for predictive correlates as a diagnostic tool allowing early diagnosis of ENL during MDT in high-risk leprosy patients that is simultaneously useful for discriminating patients with ENL from those with Type-1 reactions.

Neutrophils, ascribed as warriors against pathogens and critical actors in the innate immune system with high pro-inflammatory activity constitute the dominant immune cell population in human blood with ∼10^11 neutrophils generated every day in the bone marrow (24, 25). They are the first responders to infection and inflammation, providing a key pivot between resolution or propagation of collateral damage that can result in multi-system organ failure. They possess an enormous armamentarium to counter pathogens. These cells are endowed with huge microbialcidal potential by virtue of their phagocytic capacity, ability to produce reactive oxygen intermediates, a rich array of granule-derived lytic enzymes and a tendency to release neutrophil extracellular traps (NETs). A vast body of literature shows that neutrophil dysfunction can contribute significantly to sepsis and a variety of autoimmune diseases (24, 26, 27). Neutrophils can promote cancer progression by facilitating matrix remodeling, stimulating angiogenesis, and by disabling T-cell-dependent antitumor immunity (28).

ENL is considered a neutrophil-mediated immune-complex reactional state, histologically defined by leukocytoclastic vasculitis and prominent neutrophilic infiltrate throughout the dermis and subcutis during the acute stage that is gradually replaced by lymphocytes with the evolution of the lesion (3–6). Neutrophils are considered as the “signature cell” in T2R that contribute to ENL-associated multi-systemic inflammation in multiple ways (29). Neutrophils circulating in peripheral blood of multibacillary leprosy patients are heavily bacillated, with no apparent sign of systemic inflammation (30). These neutrophils are effectively cleared of bacilli only after some months of multidrug therapy (31). A variety of neutrophilic functional abnormalities including enhanced spontaneous defective random migration, chemotaxis and chemokinesis, and increased apoptosis associated with increased production of pro-inflammatory cytokines (32) has already been demonstrated in ENL patients suggesting the role of neutrophils as active effector players in T2R (32–36).

In the present review, novel aspects of neutrophil biology and their links to ENL pathophysiology are discussed, highlighting their clinical usefulness as a potential biomarker.
| S No | Biomarkers | Total No. of subjects | Sample type | Applied technique | Remark | Place of study | Year (References) |
|------|------------|-----------------------|-------------|------------------|--------|----------------|------------------|
| 01   | IL-7, PDGF-BB, IL-6, VEGF | ENL-10 Non-reactional LL-10 | Plasma | Luminex | Elevated plasma IL-7 and PDGF-BB levels can act as a putative biomarker for ENL when compared with Type 1 reaction leprosy patients | Goiania city, Brazil | 2009 (10) |
| 02   | Alpha 1-Acid glycoprotein (AGP) | ENL (Untreated)-6 ENL (Treated)-6 Non-reactional LL-6 RR-7 BT-5 BB-4 BL-5 HC-9 | Serum | ELISA, 2D electrophoresis MALDI-TOF | Increased serum levels of AGP in untreated ENL patients can be used as biomarker to differentiate these patients from non-reaction patients | Madurai, India | 2010 (11) |
| 03   | Alpha 1-Acid glycoprotein (AGP) | ENL-32 RR-17 Non-reactional LL-39 | Serum Skin biopsies | ELISA qPCR | The higher AGP levels as reflected in the serum profile, in active reaction patients than in controls with no reaction could be an early indication of disease progression. | Delhi, India | 2015 (12) |
| 04   | CD 64 on neutrophils | ENL (Untreated)-11 ENL (Treated)-10 LL-8 HC-10 | Whole blood and skin biopsies | Flowcytometry (Whole blood) qPCR (Skin Biopsy) | CD64 was significantly down regulated in ENL lesions after beginning thalidomide treatment. Increased CD64 expression on the surface of neutrophils in circulation as well as in biopsy can be used as biomarker for ENL. | Rio de Janeiro, Brazil | 2016 (13) |
| 05   | Pentraxin-3 (PTX-3) | ENL (MB)-27RR (MB)-11RR (PB)-9MB-19PB-15 | Serum | ELISA | PTX-3 levels were assessed as a biomarker for active ENL which was suppressed after thalidomide treatment | Rio de Janeiro, Brazil | 2017 (14) |
| 06   | LID-1 | ENL-41 RR-119 Reaction-free-292 | Serum | ELISA | Higher serum levels of LID-1 in RR patients isolates them from the non-reaction patients | Goiania city, Brazil | 2017 (15) |
| 07   | Activated B cells and tissue-like memory B (TLM-B) cells | ENL-41 Non-reactional LL-30 | Whole blood | Flowcytometry | Increase in activated B cells and reduction in tissue-like memory B cells in ENL as compared to LL patients can be used as biomarker for ENL. | Addis Ababa, Ethiopia | 2017 (16) |
| 08   | C1q (C1qA, C1qB, and C1qC) | ENL (untreated)-30 Non-reactional ENL-30 | Whole blood and skin biopsies | qPCR, ELISA | Circulating C1q in the peripheral blood of untreated ENL patients was significantly lower compared to LL patient controls. C1qA and C1qC gene expression were substantially increased in the skin biopsies of untreated ENL patients compared to LL controls. | Ethiopia (Addis Ababa) | 2018 (17) |
| 09   | Neutrophils in dermis | ENL-10 MB-23 | Skin biopsies | Histopathology | Elevated number of neutrophils can be used as a biomarker for the ENL. | Denpasar, Bali | 2018 (18) |
| 10   | IgM, IgG1, C3d | ENL-29 RR-35 MB-51 HC-15 | Serum | ELISA | Association of increased IgM, IgG1, and C3d-associated immune complexes could be used to estimate risk of developing reactions. | Natal, Brazil | 2019 (19) |
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Continued

| S No | Biomarkers | Total No. of subjects | Sample type | Applied technique | Place of study | Remark |
|------|------------|-----------------------|-------------|-------------------|----------------|--------|
| 11   | Low density neutrophils (CD4+CD15+) | NA         | Whole blood | Flowcytometry     | Rio de Janeiro, Brazil | Increased circulating activated low density neutrophils associated with ENL. | 2020 (20) |
| 12   | IL10R1 on neutrophils                   | ENL-28     | Whole blood | Flowcytometry     | Rio de Janeiro, Brazil | IL10R1 was used as a potential indicator of ENL. The levels of IL-10R1 were detected in measurable amount in ENL patients as compared to BU-L. | 2021 (21) |
| 13   | Neutrophil-lymphocyte ratio             | Non-reactional BU-L-28 | Whole blood | Automated blood counter | 2021 (20) | Neutrophil-to-lymphocyte ratio could be a potential biomarker for diagnosis of leprosy reaction and useful for discriminating patients with type 2 reaction from those with type 1 leprosy reactions. | 2021 (23) |
| 14   | IL-6                                    | ENL-56     | Whole blood | Cytokine bead array (Flowcytometry) | Rio de Janeiro, Brazil | High serum levels of interleukin-6 were observed during ENL, primarily in patients with more severe disease and IL-6 levels decreased after specific therapy. Elevated levels of IL-6 can be used as a biomarker. | 2021 (23) |
| 15   | IL-17                                   | MB without ENL-16 | Serum | Automated blood counter | 2021 (20) | Neutrophils with lower buoyancy in circulation, better defined as low density neutrophils (LDNs). Neutrophil-lymphocyte ratio could be a potential biomarker for diagnosis of leprosy reaction and useful for discriminating patients with type 2 reaction from those with type 1 leprosy reactions. IL-10R1 was used as a potential indicator of ENL. IL-17 production by lymphocytes. The short-lived CD66B+CD117+CD34− neutrophil precursors mature rapidly inside the bone marrow and are released into the peripheral blood where the expression of membrane-bound receptors such as CXCR4 and CD117 (c-kit) and integrin α4β1 (VLA4) gradually decreases while expression of toll-like receptor 4 (TLR4) and CXCR2 and other chemokine receptors progressively increases (37–39). Throughout granulopoiesis, chemokine receptors act in concert with selectins and β2 integrins in a short feedback circuit to regulate the circadian fluctuations of circulating neutrophils. | 2021 (23) |

**NEUTROPHIL BIOLOGY**

Neutrophil Generation and Activation: Birth and Evolution of “Forefront Warriors”

Steady-state granulopoiesis is stringently regulated by various factors including PU.1, CCAAT/enhancer binding protein α and growth factor independence 1. Normal homeostatic neutrophil counts are maintained by expression of granulocyte colony stimulating factor (G-CSF) tied to interleukin (IL)-23 and IL-17 production by lymphocytes. The short-lived CD66B+CD117+CD34− neutrophil precursors mature rapidly inside the bone marrow and are released into the peripheral blood where the expression of membrane-bound receptors such as CXCR4 and CD117 (c-kit) and integrin α4β1 (VLA4) gradually decreases while expression of toll-like receptor 4 (TLR4) and CXCR2 and other chemokine receptors progressively increases (37–39). Throughout granulopoiesis, chemokine receptors act in concert with selectins and β2 integrins in a short feedback circuit to regulate the circadian fluctuations of circulating neutrophils.

During acute or chronic infection, “emergency granulopoiesis” occurs as the earliest protective innate response, with increased production of IL-23 and IL-17 in the inflamed milieu providing increased G-CSF and IL-6 leading to appearance of immature neutrophils with lower buoyancy in circulation, better defined as Low Density Neutrophils (LDNs) (40).

Neutrophilic leukocytosis as an indicator of systemic inflammation is one of the earliest features noted in ENL patients. Among various parameters used for monitoring neutrophil counts, the neutrophil-to-lymphocyte ratio (NLR) has been recognized as a unique, stable marker reflecting underlying acute inflammatory response. NLR is considered an easy, inexpensive, and reproducible parameter associated with clinical outcome and disease severity (41–45). In recent years, its role as an independent prognostic factor for neoplasms and as an inflammatory biomarker in various acute and chronic cardiovascular/metabolic/ infectious/inflammatory diseases has been increasingly established (22, 46, 47).

Gomes et al. reported a mean NLR value of 9.7 ± 2.4 (p < 0.001) for ENL diagnosis that easily differentiated ENL patients from those with Type 1 reaction with high validity cut-off of 2.95, high diagnostic accuracy (accuracy 78.0%, sensitivity 81.0%, specificity 74.0%) and an area under curve value of 0.796 (22), higher than those observed for other infectious diseases (48–50).

Oliveira et al. have already investigated the potential of *M. leprae* to induce neutrophil-mediated secretion of pro-inflammatory cytokines subsequent to their treatment with *M. leprae ex vivo* (32). Their observation reiterated the role of neutrophils as effector cell in T2R.

The neutrophil surface expression of CD64 (FcγRI), a marker of neutrophilic activation, is upregulated by a direct effect of interferon-gamma (IFN-γ) and G-CSF on precursor cells in bone marrow. Increased CD64 expression on circulating neutrophils as well as increased mRNA expression in situ in ENL skin lesions has been correlated with severity and advocated as a prognostic biomarker for ENL (13).

Bioinformatic pathway analyses of the gene expression profiles from ENL skin lesions have observed a role of an
integrated axis comprising of TLR2/FcR activation/neutrophil migration/inflammation as a mechanism of neutrophil recruitment in ENL providing a deeper insight into neutrophil biology (51). Transcriptomic analysis has shown elevated expression of genes involved in neutrophilic degranulation and activation (52, 53). Global transcriptional profiling of peripheral blood mononuclear cells via microarray showed 517 differentially expressed genes revealing a granulocytic gene signature with significant involvement of the innate immune system (54).

Acute systemic inflammation gives rise to three different subsets of neutrophils in circulation viz., conventional nuclear-segmented neutrophils (CD16\textsuperscript{bright}/CD62L\textsuperscript{bright}), banded neutrophils (CD16\textsuperscript{dim}/CD62L\textsuperscript{bright}), and a third subset comprising of CD16\textsuperscript{bright}/CD62L\textsuperscript{dim} neutrophils exhibiting immunosuppressive properties (38).

The presence of a unique subset of IL-10R1 expressing neutrophils in peripheral circulation and in situ in skin lesions of ENL patients and its clinical usefulness indicating disease outcome has also been demonstrated (21).

Apart from these inflammatory neutrophils, varied neutrophil subsets based on survival time, density, NET-releasing capacity, receptor expression profile, and functions have been observed (55).

Defined in 1986, a subset of neutrophils was found to be co-segregated with monocytes in the density gradient isolation and termed as LDNs based on their density being <1.07 gm/ml. These LDNs are comprised of a broad cell population with both pro-inflammatory and anti-inflammatory properties displaying mature hyper-segmented, as well as immature banded, nuclei. Hassani et al. have defined these LDNs as CD16\textsuperscript{dim}/CD62L\textsuperscript{high} banded neutrophils exhibiting enhanced bacterial containment, spontaneous NETs generating ability, and the capacity to supress T-cell proliferation (56). LDNs tend to express increased levels of CD66b, CD11b, and CD35 in stimulated state in comparison to normal density neutrophils (57, 58). Studies in Systemic Lupus Erythematosus have suggested LDNs to be a distinct neutrophil subset with an increased level of copy number alterations, losses of heterozygosity and microsatellite instability, distinct proteome, and biomechanical properties impacting their ability to travel through the vasculature (56, 59–62).

It is tempting to speculate that M. leprae has the potential to alter the immunomodulatory capacity of neutrophils resulting in the shift to lower buoyancy that could be related to maturation stage/degranulation status/activation level of neutrophils during ENL. Elucidation of the molecular underpinnings of the role played by LDNs in ENL pathophysiology could provide better insight into their use as biomarkers in clinical practice.

Thus, studying and monitoring neutrophil phenotypic, density, and functional heterogeneity for better assessment of immune response and severity of ENL pathology holds promise in their exploration as ENL biomarkers.

**Neutrophil Cytoplasmic Granules and Their Contribution Toward Phenotypic Heterogeneity and Functional Versatility: The Armamentarium**

After stimulation by diverse stimuli, neutrophils mobilize different granules including primary/azurophilic, secondary/specific, and tertiary/gelatinase granules that subsequently degranulate releasing various compounds that modulate neutrophil trafficking and migration, and also facilitate their interaction with other innate and adaptive immune cells, thus playing an important role in inflicting collateral tissue damage. These granules are acquired sequentially during neutrophil maturation in bone marrow. The release of granular protein is a tightly regulated receptor-coupled process, mediated by distinct signaling events for each granule type, allowing the selective, differential, hierarchical mobilization of distinct subsets with the tertiary granules being the most readily mobilizable upon activation followed by specific granules, the primary ones bringing up the rear. This distinct readiness of different granule subsets toward degranulation modulates neutrophil heterogeneity and functional versatility by altering the neutrophil cell-surface protein composition that generates different cellular phenotypes underlying remarkable neutrophil plasticity (28, 53, 63–65).

These released granule matrix proteins act as mediators of neutrophil orchestration of innate and adaptive immunity. Teles et al. have demonstrated increased expression MMP2 and MMP9 mRNA expression in skin biopsy specimens as well as their increased serum levels in ENL patients. MMP3 has already been implicated in mediating vasculitic ENL processes (66, 67). Pentraxin-3, stored in specific granules rises quickly after degranulation and has already been shown to be associated with the exacerbation of inflammation in ENL (14).

These released contents of neutrophil granules and surface expression level of various markers of neutrophil activity i.e., CD66b (specific granule) and CD11b (tertiary granules) could be explored for their role in ENL progression.

**Neutrophil Extracellular Traps: Extra Armor**

After encountering micro-organisms, neutrophils destroy them intracellularly as well as extracellularly: in their fight with microbes, dead neutrophils provide chromatin and proteins to form NETs and, live neutrophils create a cytokine network (68).

NETs play a role in autoimmunity and thrombosis by accelerating the inflammatory response either as danger-associated molecular patterns or complement activators (69–76). They have not only been implicated as drivers of inflammation, but are also linked to resolution of inflammation, thus emerging as a double-edged sword and making them promising targets for future biomarker discovery.

Notably, NETs have been reported to be associated with disease-specific bioactive proteins loaded onto them (70). Intriguingly, emerging clinical and experimental studies indicate that neutrophils are able to release intrinsically and qualitatively
different extracellular NETs decorated with disease-specific bioactive proteins dictated by the diseased inflammatory environment containing tissue factor, IL-1β, IL-17, and LL-37, suggesting systemic inflammation driven transcriptional-reprogramming in circulating neutrophils, which triggers de novo expression of disease-specific protein fingerprints which could accelerate the inflammatory response (77).

The potential of M. leprae to induce NETs formation in vitro and the contribution of these NETs in triggering ENL has already been investigated. da Silva et al. have clearly demonstrated NETs abundance in skin lesions and significantly increased DNA-histone/DNA-MPO complexes in the serum of ENL patients in addition to an increased tendency of peripheral blood neutrophils from ENL patients toward spontaneous NETs formation that showed marked reduction in all evaluated in vivo and ex vivo NETosis parameters in response to thalidomide treatment (78).

Investigating ENL-specific bioactive proteins loaded on NETs could offer insight into the pathogenesis of ENL and provide promise in developing disease-specific biomarkers therefore we advocate that the prognostic values for increased serum levels of NETs/NETs-related markers should be explored in future prospective large cohorts of multibacillary leprosy patients.

**Metabolic Reconfiguration of Neutrophils During M leprae Infection: Exploitation of Warriors by an External Agency**

Over the last decade, a new concept of “immunometabolism” has emerged, which describes the changes that occur in the intracellular metabolic pathways in host immune cells during proliferation, differentiation, activation, and execution of effector function thereby maintaining body homeostasis (79–82). There is a vast body of literature indicating crosstalk between cellular metabolism and inflammatory/immune responses and how these two influences each other. This metabolic reprogramming of immune cells also has implications in the regulation of antitumor immune response as immune cells become tolerogenic and inefficient in cancer cell eradication (83). Tumor-elicited c-kit signaling triggers metabolic neutrophil modification leading to sustained levels of reactive oxygen species that suppress the functions of anti-tumor CD8+ T cells (84).

As recently as 2019, several investigators started studying the relationship between metabolism and the immune response at the cellular and organismal levels in infectious diseases and investigating how the pathogen-induced remodeling in the host cell metabolism influences the infectious disease pathogenesis and host physiology (85–87).

Our understanding of how host macrophage and Schwann cell metabolism can be altered by M. leprae and how these metabolic alterations can influence the outcome of infection has grown considerably over the past few years (88, 89). M. leprae chemically and metabolically impacts the cytosolic environment of the host cell, facilitating its persistence, proliferation, dissemination, and continued transmission (90–92). Histochemical, metabolomic, and transcriptional analyses have already confirmed that many biosynthetic pathways, such as those of cholesterol, phospholipids, and fatty acid biosynthesis are upregulated, underscoring the metabolic interface in the molecular pathogenesis of leprosy (93, 94). In Schwann cells, host cell energy metabolism is subverted, culminating in reduced mitochondrial action potential and reduced generation of reactive oxygen species resulting in allocation of as much carbon and reducing power as possible to lipid synthesis (95–97). Furthermore, pathways regulating tryptophan and iron metabolism have also been found to be defective in leprosy patients, favoring pathogen survival with high bacterial loads (97–99). Upregulation of omega-3 and omega-6 PUFAs metabolism and the presence of higher levels of omega-6-derived prostaglandin E2, lipoxin A4, and omega-3-derived lipid mediators have been reported in LL patients with high bacterial index (94). Lipid droplet accumulation represents a link between innate immune response and energy metabolism, making it worthwhile to study immunometabolic associations in leprosy (92).

Neutrophils exhibit a remarkable metabolic plasticity allowing them to survive in extremes of metabolite availability and contributing toward neutrophil population heterogeneity described in cancer-associated neutrophils and in density-gradient isolated low and high buoyancy neutrophils seen in auto-immune diseases (100). The metabolic reconfiguration of neutrophils effected by mediators released during chronic inflammatory states and carcinogenesis is an area of intense investigation and research (101).

However, the potential of M. leprae to induce alterations in biosynthetic pathways associated with neutrophil maturation, activation, and degranulation by virtue of its metabolic reprogramming ability remains unexplored. It is interesting to speculate that metabolic reconfiguration of neutrophils during M. leprae infection might differentially regulate various cytokines and chemokines as well as antimicrobial responses, resulting in detrimental overt inflammation that could ultimately determine the outcome of M. leprae-host interactions.

More research is needed to identify metabolic adaptations occurring in neutrophils and to link them to the corresponding exaggerated immune responses in ENL. Unraveling the cellular and molecular mechanisms involved in the regulation of metabolism in different neutrophil populations can lead to the discovery of biomarkers useful for investigating susceptibility to this life-threatening complication of leprosy, facilitating its early diagnosis and monitoring disease progression.

**Neutrophil Derived Exosomes**

Exosomes are native nanovesicles that can regulate pathological processes including cancer cell immunity, immune regulation, inflammation, angiogenesis, fibrosis, and cell proliferation/differentiation/death. Emerging studies project exosomes as “high-resolution snapshots” that can efficiently and dynamically capture the complexity of cancer disease progression. However, for chronic inflammatory, autoimmune, infectious, metabolic, and fibrotic diseases exosome research is yet to mechanistically and technologically advance beyond “photograph negatives” that require extended processing to extract meaningful clinical information and benefits (85, 102–104). Diverse exosome cargo comprising of nucleic acids (DNA,
RNA, miRNA), proteins, and microbial-derived components mediating cell-cell communication can potentially capture altered cell physiology, reflect disease progression/severity, stratify risks for therapeutic consideration (as in tumor patients), and inform decision-making between active surveillance or further clinical work-up.

Studies conducted so far have demonstrated the release of exosomes during extracellular and intracellular bacterial infections. The significance of these cargos in host-pathogen interactions is at a preliminary stage of investigation, and the role of exosomes in host defense and in determining disease outcomes remains unexplored (86, 87, 105–108). Identification of neutrophil-derived exosomes as a new subcellular entity could result in elucidation of a molecular player providing a fundamental link between neutrophil-driven inflammation and multi-organ tissue damage observed in ENL with far-reaching implications for future research. Neutrophil-derived exosomes have already been demonstrated as a new means of intercellular communication promoting extracellular matrix destruction in an array of chronic inflammatory lung disease and have key immunomodulatory roles in a variety of inflammatory dermatological disorders, justifying their role as biomarkers indicating a variety of pathophysiological states (109–111).

Neutrophil-derived exosomes could be candidates for various clinical applications in leprosy. Analysis of exosomal content can reveal signature molecules including proteins and miRNAs, which might be relevant in detecting ENL susceptibility in LL patients, identifying patients who require prolonged treatment, and indicating the prognosis of immune reactions. In future endeavors, the functional and diagnostic potential of neutrophil-derived exosomal protein and/or miRNAs in leprosy could be investigated.

**FUTURE INSIGHTS**

- The utility of NLR in ENL needs to be tested in large prospective cohorts of multibacillary patients: the identification of adequate cut-off values or longitudinal evaluations over a prolonged treatment period is mandatory to determine its clinical usefulness.
- The phenotypical and functional divergence of neutrophil subpopulations in ENL needs further evaluation. Identifying subpopulations and related inflammatory mediators is of utmost significance within the context of applied immunology, as this allows for their application in clinical practice to assess disease activity, severity and the course of the reaction (acute vs. chronic).
- Investigations are required to elucidate the frequency and clinical significance of LDNs as well as expression of various activation surface markers to test their potential as a biomarker for ENL.
- The potential prognostic serum levels of NETs/NETs associated proteins should be evaluated in prospective follow up studies of ENL patients and its correlation with disease activity tested in larger cohorts. This could prove helpful in determination of the duration of therapy.
- How *M. leprae* interferes with metabolic pathways in neutrophils to precipitate ENL in LL patients merits further exploration.
- We support investigations into the concept of “*M leprae*-specific transcriptional-reprogramming” in neutrophils/NETs. Subsequent translation can yield altered molecular configurations that can be packaged into exosome vesicles and released out of NETs; such NET-associated exosomes may serve as potential “messengers/transmitters” that may cross talk with other active immune cells/tissue resident cells/Schwann cells resulting in the progressive fulminant multi-organ pathology seen in ENL.

**OPEN QUESTIONS**

- What are the signals that cause neutrophil activation and subsequent development into LDNs following *M. leprae* infection?
- How do the newly recognized neutrophil subsets/subtypes/subpopulations (identified by phenotypic markers and segregated on basis of density centrifugation) contribute to ENL pathophysiology?
- Do these neutrophil subpopulations exhibit any differences in granule protein content and secretion in the context of ENL susceptibility?
- Does *M. leprae* alter metabolic configuration of neutrophils? How does it alter its metabolic configuration to precipitate immunological exacerbation in LL patients?
- How does *M. leprae* use its weaponry (lipid virulence factors) to exploit various host biological pathways to rewire the host innate immune/cytokine responses in ENL?

Many aspects related to ENL pathophysiology remain enigmatic. Answers to these questions have the potential to facilitate discovery of clinical useful biomarkers in the near future.

**SUMMARY AND CONCLUSION**

Profound metabolic changes are posited to occur in neutrophils during normal physiological processes and under various pathophysiological conditions including *M. leprae* infection, more research is required to shed light on the signaling pathways governing these perturbations and to elucidate the intricate interactions among various metabolic programming pathways in neutrophils, with the hope that this basic research can translate into the development of biomarker discovery and novel therapeutics and diagnostic strategies. This might help to identify patients who are prone to develop recurrent or chronic/recalcitrant T2R and initiate them on to targeted therapy.

Neutrophils are the key players in ENL and have a potential role as biomarkers of disease outcome or as therapeutic targets. However, there is still much work to be done before they...
might be used as validated prognostic markers. Moreover, newer therapeutic agents targeting particularly neutrophil migration and mobilization without causing global immunosuppression could be designed.

Exploiting various aspects of neutrophil biology (Figure 1) can lead us to discover signature molecules that can be investigated as biomarkers for ENL. These biomarkers could also help us in diagnosis as well as prognostication of ENL patients.

We envision that liquid-biopsy based exosomal biomarkers for ENL can be developed and validated in the near future with the potential for enhanced early detection, improved diagnosis, disease outcome predictability, and therapeutic window determination.

Moreover, an integration of information from different “-omics” studies could provide scientists and clinicians with a powerful tool to understand the various aspects of neutrophil biology involved in development and progression of ENL. It will provide a great opportunity and hope to identify biomarkers specific for different cellular processes/states along with those indicating efficacious therapeutic intervention.

**AUTHOR CONTRIBUTIONS**

SS and KS: literature search, manuscript preparation, and experimental studies. MS: literature search and manuscript review. TN and SD: definition of intellectual content, manuscript editing, manuscript review, and clinical studies. RM: definition of intellectual content and manuscript review. SC: concepts, design, literature search, manuscript preparation, manuscript editing, and guarantor. All authors contributed to the article and approved the submitted version.
REFERENCES

1. Lockwood DNJ. Chronic aspects of leprosy-neglected but important. Trans R Soc Trop Med Hyg. (2019) 113:812–16. doi: 10.1093/trstmh/try131

2. Walker SL, Lockwood DNJ. Leprosy type 1 reactions and erythema nodosum leprosum. Artig Revisao. (2008) 83:75–82. doi: 10.1590/S0365-05962008000100010

3. Bhusan K, Sunil D, Inderjeet K. Epidemiological characteristics of leprosy reactions: 15 years experience from North India. Int J Lepother Mycobact Dis. (2004) 72:491. doi: 10.1489/1544-581X(2004)072<0125:ECOLORY>2.0.CO;2

4. Walker SL, Lebas E, Butlin CR, Shah M, Maghanoy A, Lambert SM, et al. Leprosy clinical severity scale for erythema nodosum leprosum: an international, multicentre validation study of the ENLIST ENL severity scale. PLoS Negl Trop Dis. (2017) 11:e0005716. doi: 10.1371/journal.pntd.0005716

5. Bhat RM, Vaidya TP. What is new in the pathogenesis and management of erythema nodosum leprosum. Indian Dermatol Online J. (2020) 11:482–92. doi: 10.4103/iodj.IDOJ_561_19

6. Walker SL, Sales AM, Butlin CR, Shah M, Maghanoy A, Lambert SM, et al. Leprosy: new insights and open questions. Front Physiol. (2018) 9:311. doi: 10.3389/fphys.2018.00313

7. Schmitz V, Tavares IF, dos Santos Pacheco F, Gandini M, Meyer RM, Schmitz V. Neutrophils in leprosy. J Leukoc Biol. (2019) 106:2921. doi: 10.1038/s41400-019-0864-3

8. Chatterjee G, Kaur S, Sharma VK, Vaishnavi C, Ganguly NK. Bacterialia in leprosy and effect of multidrug therapy. Lepr Rev. (1989) 60:197–201. doi: 10.5935/0305-7518.19890025

9. Oliveire RB, Moraes MO, Oliveira EB, Sarno EN, Nery JC, Sampaio EP. Neutrophils isolated from leprosy patients release TNF-α and exhibit accelerated apoptosis in vitro. J Leukoc Biol. (1999) 65:364–71. doi: 10.1002/jlb.65.3.364

10. Goihman-Yahr M, Rodriguez-Ochoa G, Aranzazu N, Pinardi ME, de Gomez ME, Ocanto A, et al. In vitro activation of neutrophils by suspensions of Mycobacterium leprae. J Infect Dis. (1979) 139:570–4.

11. Goihman-Yahr M, Convit J. Dermal and in vitro specific serology evaluated in a Brazilian cohort of leprosy patients (U-MDT/CT-BR). PLoS Negl Trop Dis. (2012) 6:e000369. doi: 10.1371/journal.pntd.000369

12. Schmitz V, Tavares IF, Pignataro P, Machado ADM, Pacheco S, Schmitz V. Neutrophils in leprosy. Front Immunol. (2019) 10:495. doi: 10.3389/fimmu.2019.00495

13. Negera E, Walker SL, Bekele Y, Dockrell HM, Lockwood N. Increased activated memory B-cells in the peripheral blood of patients with erythema nodosum leprosum reactions. PLoS Negl Trop Dis. (2017) 11:e0006121. doi: 10.1371/journal.pntd.0006121

14. Negera E, Walker SL, Bekele Y, Dockrell HM, Lockwood N. Increased activated memory B-cells in the peripheral blood of patients with erythema nodosum leprosum reactions. PLoS Negl Trop Dis. (2017) 11:e0006121. doi: 10.1371/journal.pntd.0006121

15. Negera E, Walker SL, Bekele Y, Dockrell HM. Complement C1q expression in Erythema nodosum leprosum. PLoS Negl Trop Dis. (2018) 12:e0006321. doi: 10.1371/journal.pntd.0006321

16. Darmaputra IGN, Herwanto N, Rusyati LM, Riaaw W, Endaryanto A, Prakoeowa CRS. Distribution of iNOS expressions and TNF neutrophil-cells as well as PGE2 and S100 Schwann cell dermal nerves in the erythema nodosum leprosum patients. Balt Med J. (2018) 7:262. doi: 10.15562/bmj/v171879

17. Noger L, Nascimento LS, Amorim FM, Monteiro RG, Freire-neto FP, Carmo M, et al. Differential immunoglobulin and complement levels in leprosy prior to development of reversal reaction and erythema nodosum leprosum. PLoS Negl Trop Dis. (2019) 13:e0007089. doi: 10.1371/journal.pntd.0007089

18. Negera E, Walker SL, Brandão SS, Ferreira H, Rodrigues F, Brandão J, et al. Erythema nodosum leprosum neutrophil subset expressing IL-10R1 transmigrates into skin lesions and responds to IL-10. ImmunoHorizons. (2021) 4:47–56. doi: 10.4094/immunohorizons.1900088

19. Sahu et al. Neutrophils in ENL.
Neutrophils in ENL

after curative resection for hepatocellular carcinoma. World J Surg. (2008) 32:1757–62. doi: 10.1007/s00268-008-9552-6

Arab B, Bhatt VR, Phoakkan J, Murakuta S, Kohn N, Terjanian T, et al. Usefulness of the neutrophil-to-lymphocyte ratio in predicting short- and long-term mortality in breast cancer patients. Ann Surg Oncol. (2012) 19:2127–40. doi: 10.1245/s10434-011-2814-0

Cedrés S, Torrejon D, Martinez A, Martinez P, Navarro A, Zamora E, et al. Neutrophil to lymphocyte ratio (NLR) as an indicator of poor prognosis in stage IV non-small cell lung cancer. Clin Transl Oncol. (2012) 14:846–69. doi: 10.1007/s12099-012-0872-5

Torun S, Tunc BD, Svukh B, Yildiz H, Tas A, Sayilir A, et al. Assessment of neutrophil-lymphocyte ratio in ulcerative colitis: a promising marker in predicting disease severity. Clin Res Hepatol Gastroenterol. (2012) 36:491–97. doi: 10.1016/j.cjemre.2012.06.004

Loonen AJM, De Jager CPC, Tosserams J, Kusters R, Hilbink M, Verhe J, et al. Transcriptional changes that characterize the immune reactions of leprosy. Semin Immunopathol. (2013) 35:455–63. doi: 10.1007/s00281-013-0375-7

van der Linden M, van den Hoogen LL, Westerlaken GHA, Fritsch-Stork J, van Roon JAG, Radstake TRD, et al. Neutrophil extracellular trap release is associated with antinuclear antibodies in systemic lupus erythematosus and anti-phospholipid syndrome. Rheumatol (United Kingdom). (2018) 57:1228–34. doi: 10.1093/rheumatology/key067

Denny MF, Yalvarthi S, Zhao W, Thacker SG, Anderson M, Sandy AR, et al. A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs. J Immunol. (2010) 184:3284–97. doi: 10.4049/jimmunol.0902199

Bashant KR, Aponte AM, Randazzo D, Rezvan Sangsari P, Wood AJT, Bibby JA, et al. Proteomic, biomechanical and functional analyses define neutrophil heterogeneity in systemic lupus erythematosus. Ann Rheum Dis. (2021) 80:209–18. doi: 10.1136/annrheumdis-2021-218338

Hidalgo A, Chilvers ER, Summers C, Koenderman L. The neutrophil life cycle. Trends Immunol. (2018) 40:584–97. doi: 10.1016/j.it.2019.04.013

Rosaules C. Neutrophils at the crossroads of innate and adaptive immunity. J Leukoc Biol. (2020) 108:377–96. doi: 10.1002/jlb.4MIR0220-574RR

Othman A, Sakehri M, Filip JG. Roles of neutrophil granule proteins in orchestrating inflammation and immune. FEBS J. (2021). doi: 10.1111/febs.15803. [Preprint ahead of print].

Teles RMB, Teles RB, Amaeut TP, Moura DF, Mendonça-Lima L, Ferreira H, et al. High metal matrix metalloproteinase production correlates with immune activation and leukocyte migration in leprosy reactional lesions. Infect Immun. (2010) 78:1012–21. doi: 10.1128/IAI.00896-09

Youssef SS, Atia EAS, Awad NM, Mohamed GF. Expression of matrix metalloproteinases (MMP-3 and MMP-9) in skin lesions of leprosy patients. J Egypt Women Dermatol Soc. (2009) 6:80–7. doi: 10.1186/1749-8099-6-8

Gallina S, Fedorova NV, Golenkina EA, Stadnichuk VI, Sudina GF. Cytofomes versus neutrophil extracellular traps in the fight of neutrophils with microbes. Int J Mol Sci. (2020) 21:1–22. doi: 10.3390/ijms21020586

Zhao X, Yang L, Chang N, Hou L, Zhou X, Yang L, et al. Neutrophils undergo switch of apoptosis to NETosis during murine fatty liver injury via SIP receptor 2 signaling. Cell Death Dis. (2020) 11:379. doi: 10.1038/s41419-020-2582-1

Sollberger G, Tilley DO, Zychlinsky A. Neutrophil extracellular traps: the Biology of chromatin externalization. Dev Cell. (2018) 44:542–53. doi: 10.1016/j.devcel.2018.01.019

Neubert E, Meyer D, Kruss S, Erpenbeck L. The power from within - disease: potential anti-NETs therapeutics. Clin Rev Allergy Immunol. (2020) 1:1–8. doi: 10.1007/s12016-020-08804-7

Semenza LG, Wang J, Zhu Y, Ojeda JG, Buvoli SL, et al. REDD1/autophagy pathway promotes thromboinflammation and fibrosis in human systemic lupus erythematosus (SLE) through NETs decorated with tissue factor (TF) and interleukin-17A (IL-17A). Ann Rheum Dis. (2019) 78:238–48. doi: 10.1136/annrheumdis-2018-213181

Mutua V, Gershwin LJ. A review of neutrophil extracellular traps (NETs) in disease: potential anti-NETs therapeutics. Cell Death Dis. (2020) 11:e040915. doi: 10.1038/s41419-020-0958-z

Fouesert E, Toes R, Desai J. Neutrophil extracellular traps (NETs) take the central stage in driving autoimmune responses. Cells. (2020) 9:40915. doi: 10.3390/cells9040915

Stakos D, Skendros P, Konstantinides S, Risit K. Traps N’ Clots: NET-mediated thrombosis and related diseases. Thromb Haemost. (2020) 120:373–83. doi: 10.1055/s-0039-3402731

Da Silva CO, Dias AA, Da Costa Nery JA, De Miranda MacHado A, Gomes-de-Vale O, et al. High expression of neutrophil granule proteins in the serum of patients with hepatocellular carcinoma after curative resection. J Gastrointest Oncol. (2018) 9:40915. doi: 10.3390/cells9040915

Kumar R, Singh P, Kolloli A, Shi L, Bushkin Y, Yagi T, et al. Immunometabolism of phagocytes during mycobacterium tuberculosis infection. Front Mol Biosci. (2019) 6:1–20. doi: 10.3389/fmbiol.2019.00105

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81. Shuvalov O, Daks A, Fedorova O, Petukhov A, Barlev N. Linking metabolic reprogramming, plasticity and tumor progression. Cancers (Basel). (2021) 13:1–25. doi: 10.3390/cancers13040762

82. Xia L, Oyang L, Lin J, Tan S, Han Y, Wu N, et al. The cancer metabolic reprogramming and immune response. Mol Cancer. (2021) 20:1–21. doi: 10.1186/s12943-021-01316-8

83. Guerra L, Bonetti L, Brenner D. Metabolic modulation of immunity: a new concept in cancer immunotherapy. Cell Rep. (2020) 32:107848. doi: 10.1016/j.celrep.2020.107848

84. Rice CM, Davies LC, Subleski JJ, Maio N, Gonzalez-Cotto M, Andrews RA, et al. Metabolic modulation of immunity: a new concept in cancer immunotherapy. Cell Rep. (2020) 32:107848. doi: 10.1016/j.celrep.2020.107848

85. Rice CM, Davies LC, Subleski JJ, Maio N, Gonzalez-Cotto M, Andrews RA, et al. Metabolic modulation of immunity: a new concept in cancer immunotherapy. Cell Rep. (2020) 32:107848. doi: 10.1016/j.celrep.2020.107848

86. Biadglegne F, König B, Rodloff AC, Dorhoi A, Sack U. Composition and function of neutrophils in ENL. J Clin Med. (2021) 10:1445. doi: 10.3390/jcm10010145

87. Spencer N, Yeruva L. Role of bacterial infections in extracellular vesicles release and impact on immune response. Biomed J. (2021) 44:157–64. doi: 10.1016/j.bj.2020.05.006

88. de Macedo CS, Lara FA, Pinheiro RO, Schmitz V, de Berrêdo-Pinho M, Oliveira VCG, et al. Emerging function and clinical values of exosomal miRNAs in cancer. Mol Ther - Nucleic Acids. (2019) 20:1–8. doi: 10.1016/j.omtn.2019.04.027

89. Mattos KA, Oliveira VCG, Berrêdo-Pinho M, Amaral JJ, Antunes LCM, de Macedo CS, et al. Proteomic analysis of plasma exosomes from cystic echinococcosis patients provides in vivo support for distinct immune response profiles in active vs inactive infection and suggests potential biomarkers. (2020) 14:e1008586. doi: 10.1371/journal.pntd.0008586

90. Wang J, Wang Y, Tang L, Garcia RC. Extracellular vesicles in mycobacterial infections: their potential as molecule transfer vectors. Front Immunol. (2019) 10:2099. doi: 10.3389/fimmu.2019.02099

91. De Macedo CS, Anderson DM, Pascarelli BM, Spraggins JM, Sarno EN, Schey KL, et al. MALDI imaging reveals lipid changes in the skin of leprosy patients before and after multidrug therapy (MDT). J Mass Spectrom. (2015) 50:1374–85. doi: 10.1002/jms.3708

92. Valorachi AL, Teixeira L, Oliveira K da S, Maya-Monteiro CM, Bozza PT. Lipid droplet, a key player in host-parasite interactions. Front Immunol. (2018) 9:1022. doi: 10.3389/fimmu.2018.01022

93. Amaral JJ, Antunes LCM, de Macedo CS, Mattos KA, Han J, Pan J, et al. Metabonomics reveals drastic changes in anti-inflammatory/pro-resolving polyunsaturated fatty acids-derived lipid mediators in leprosy disease. PLoS Negl Trop Dis. (2013) 7:e2381. doi: 10.1371/journal.pntd.0002381

94. Al-Mubarak R, Vander Heiden J, Balganon M, Amaral JJ, Antunes LCM, de Macedo CS, et al. The cancer metabolic reprogramming, plasticity and tumor progression. Clin Exp Immunol. (2011) 165:251–63. doi: 10.1111/j.1365-2249.2011.04412.x

95. Mellor AL, Lemos H, Huang L. Indoleamine 2,3-Dioxygenase and tolerance: where are we now? Front Immunol. (2017) 8:3160. doi: 10.3389/fimmu.2017.03160

96. de Mattos Barbosa MG, da Silva Prata RB, Andrade PR, Ferreira H, de Andrade Silva BJ, da Paisão de Oliveira JA, et al. Indoleamine 2,3-dioxygenase and iron are required for Mycobacterium leprae leprosy survival. Microbes Infect. (2017) 19:505–14. doi: 10.1016/j.micinf.2017.06.006

97. Curi R, Leveda-Pires AC, Silva EB Da, Poma SDO, Zambonatto RF, Domeneph P, et al. The critical role of cell metabolism for essential neutralophil functions. Cell Physiol Biochem. (2020) 54:629–47. doi: 10.33594/000000245

98. Wang M, Yu F, Ding H, Wang Y, Li P, Wang K. Emerging function and clinical values of exosomal microRNAs in cancer. Mol Ther - Nucleic Acids. (2019) 16:791–804. doi: 10.1016/j.omtn.2019.04.027

99. Gkirtzimanaki K, et al. Tissue-infiltrating macrophages mediate an exosome-based metabolic reprogramming upon DNA damage. Nat Commun. (2020) 11:42. doi: 10.1038/s41467-019-13894-9

100. Jungin P, Chaze T, Giai Gianetto Q, Matondo M, Gout O, Gessain A, et al. Proteomic analysis of plasma extracellular vesicles reveals mitochondrial stress upon HTLV-1 infection. Sci Rep. (2018) 8:1–7. doi: 10.1038/s41598-018-23505-0

101. Fratini F, Tamarozzi F, Macchia G, Bertucelli L, Mariconti M, Birago C, et al. Proteomic analysis of plasma exosomes from cystic echinococcosis patients provides in vivo support for distinct immune response profiles in active vs inactive infection and suggests potential biomarkers. (2020) 14:e1008586. doi: 10.1371/journal.pntd.0008586

102. Vargas A, Roux-Dalvai F, Droit A, Lavoie JP. Neutrophil-derived exosomes: A new mechanism contributing to airway smooth muscle remodeling. Am J Respir Cell Mol Biol. (2016) 55:450–61. doi: 10.1165/rcmb.2016-033OC

103. Ziegenbalg A, Prados-Rosales R, Jenny-Avital ER, Kim RS, Casadevall A, Ackhar M. Immunogenicity of mycobacterial vesicles in humans: identification of a new tuberculosis antibody biomarker. Tuberculosis. (2013) 93:448–55. doi: 10.1016/j.tube.2013.03.001

104. Medeiros R, de Vasconcelos Girardi KDC, Cardoso FK, De Siqueira Mietto B, De Toledo Pinto TG, Gomeez LS, et al. Subversion of schwann cell glucose metabolism by Mycobacterium leprae. J Biol Chem. (2016) 291:21375–87. doi: 10.1074/jbc.M116.725283

105. Mattos KA, Lara FA, Oliveira VGC, Rodrigues LS, D’Avila H, Melo RCN, et al. Metabonomics reveals drastic changes in anti-inflammatory/pro-resolving polyunsaturated fatty acids-derived lipid mediators in leprosy disease. PLoS Negl Trop Dis. (2013) 7:e2381. doi: 10.1371/journal.pntd.0002381

106. Medeiros R, de Vasconcelos Girardi KDC, Cardoso FK, De Siqueira Mietto B, De Toledo Pinto TG, Gomeez LS, et al. Subversion of schwann cell glucose metabolism by Mycobacterium leprae. J Biol Chem. (2016) 291:21375–87. doi: 10.1074/jbc.M116.725283

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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