Short Report: Differentiation of Patients with Leprosy from Non-Infected Individuals by the Chemokine Eotaxin/CCL11

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Abstract. Diagnosis of leprosy is usually made clinically and there are no tests available for the routine laboratory diagnosis of the disease. The aim of this study was to investigate the potential role of chemokines as biologic markers of disease activity. We used an enzyme-linked immunosorbent assay to measure chemokines in plasma of patients with leprosy (LE) and non-infected (NI) individuals. There were significantly greater concentrations of the chemokines CCL3 and CCL11 in plasma of LE patients than in NI individuals. When the use of CCL11 to differentiate LE patients versus NI individuals was evaluated, the area under the receiver-operator-characteristic curve was 0.95 ± 0.03 (P < 0.0001). In a group of selected individuals, CCL11 was useful in diagnosis of leprosy, thereby suggesting that measurement of this chemokine may be useful as an aid in diagnosing leprosis.

Leprosy is a chronic infectious disease caused by Mycobacterium leprae. This infection is considered a challenging public health problem in developing countries and affects more than one million persons in Africa, Asia and South America.\(^1\)\(^2\) Leprosy has a clinical spectrum that includes dermatologic lesions and peripheral neuropathy.\(^3\) The gold standard for diagnosis of leprosy is a full-thickness skin biopsy sample obtained from the margin of an active lesion, which is fixed in formalin, embedded in paraffin, and examined by an experienced pathologist.\(^4\) However, because this method is not available in most health services, the current diagnosis is mainly clinical. This diagnosis is based on the presence of hypopigmented or reddish patches with loss of sensation, thickened peripheral nerves, and/or the identification of acid-fast bacilli on a slit skin-smear.\(^2\) Skin smears have high specificity but low sensitivity, and are largely dependent on the expertise of a technician.\(^5\)

No serologic tests are available for the routine laboratory diagnosis of leprosy.\(^4\) The detection of antibodies to phenolic glycolipid I is promising but appears more suitable for the classification of leprosy patients into multibacillary (MB) or paucibacillary (PB) forms.\(^6\)\(^7\) Thus, development of alternative diagnostic strategies is necessary. Chemokines are potent chemoattractants of specific leukocyte subsets and are involved in several chronic inflammatory conditions.\(^8\)\(^9\)\(^10\)\(^11\)\(^12\)\(^13\) For instance, monocyte chemotactic proteins (MCP-1/CCL2), RANTES/CCL5, and interleukin-8 (IL-8)/CXCL8 expression is elevated in bronchoalveolar lavage fluid and plasma of patients with active pulmonary tuberculosis.\(^11\)\(^12\)\(^13\) There are few studies evaluating the expression and role of chemokines in the pathogenesis of M. leprae-infected individuals. In the present study, we used an enzyme-linked immunosorbent assay (ELISA) to measure CC and CXC chemokines in plasma of leprosy patients and non-infected individuals. We sought to evaluate their potential as biologic markers of disease activity.

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Fifty-nine patients (mean ± SD age = 38.3 ± 17.3 years, 35 males and 24 females) with a diagnosis of leprosy were recruited for this study before treatment. Diagnosis and classification of leprosy were based on clinical assessment and detection of acid-fast bacilli in skin slit smears by an experienced technician. The leprosy patients (LE) were classified as PB (n = 36), i.e., less than five skin lesions without a peripheral nerve affected and/or just one nerve trunk affected, or as MB (n = 23), i.e., more than five skin lesions and/or more than one nerve trunk affected, according to the operational criteria of the World Health Organization.\(^14\)\(^15\) For comparison 20 age- and sex-matched subjects (NI) (mean ± SD age = 35.7 ± 5.9 years, 11 males and 9 females) were assessed. The control group was composed of healthy members of the community recruited by means of announcements. All patients and controls were from Governador Valadares, Minas Gerais, Brazil, where leprosy is endemic.

The concentration of chemokines in plasma from LE patients and NI individuals was measured using sandwich ELISA kits for MCP-1/CCL2, macrophage inflammatory protein-1α (CCL3/MIF-1α), CCL11/eotaxin, monokine induced by interferon-γ (CXCL9/MIG), and interferon-γ-inducible 10-kD protein (CXCL10/IP-10) (DuoSet; R&D Systems, Minneapolis, MN). All samples were assayed in duplicate and on the same day. The detection limits for these assays were 5 pg/mL for CCL2 and CCL3 and 20 pg/mL for CCL11, CXCL9, and CXCL10.

Differences between two groups were evaluated using Student's t-test or the Mann-Whitney U test on normally or non-normally distributed data, respectively. Differences among three groups were evaluated using analysis of variance or the Kruskal-Wallis test in normally or non-normally distributed data, respectively. For categorical data (e.g., sex), Fisher's exact test was used. The diagnostic performance of CCL3 and CCL11 values in the diagnosis of LE was evaluated using the receiver-operator-characteristic (ROC) curve. Sensitivity and specificity were calculated considering the cut-off point that provided the highest accuracy. The Hanley and McNeil method was used for comparing ROC curves.\(^16\) Statistical significance was set at P < 0.05.

There was no significant difference in the concentration of CXCL9 (mean ± SE = 3,302.0 ± 1,104.0 pg/mL for LE pa-
tients and 1,871.0 ± 547.4 pg/mL for NI individuals; \( P = 0.276 \) and CXCL10 (mean ± SE = 4,789.0 ± 850.4 pg/mL for LE and 2,217.0 ± 616.6 pg/mL for NI; \( P = 0.852 \)) in plasma of LE patients and NI individuals. There was a trend for increased CCL2 plasma levels in LE patients (mean ± SE = 236.3 ± 33.13 pg/mL for LE patients and 141.0 ± 17.81 pg/mL for NI individuals; \( P = 0.060 \)).

Figure 1 shows the plasma levels of CCL3 and CCL11 chemokines in LE patients and NI individuals. There were significantly greater concentrations of CCL3 (mean ± SE = 150.3 ± 93.8 pg/mL for LE patients and 32.4 ± 23.3 pg/mL for NI individuals; \( P < 0.0001 \)) and CCL11 (mean ± SE = 850.4 ± 73.8 pg/mL for LE patients and 136.9 ± 39.4 pg/mL for NI individuals; \( P < 0.0001 \)) in plasma of LE patients than in plasma of NI individuals (Figure 1).

The concentration of CCL3 was significantly increased in plasma of PB leprosy patients (mean ± SE = 192.1 ± 152.4 pg/mL) and MB leprosy patients (mean ± SE = 84.7 ± 37.6 pg/mL) patients than in plasma of NI individuals (mean ± SE = 32.4 ± 23.3 pg/mL; \( P < 0.001 \)) (Figure 1). The concentrations of CCL11 chemokine were also significantly greater in plasma of PB leprosy patients (mean ± SE = 659.2 ± 71.0 pg/mL) and MB leprosy patients (mean ± SE = 1,158.0 ± 132.8 pg/mL) than in plasma of NI individuals (mean ± SE = 136.9 ± 39.4 pg/mL; \( P < 0.001 \)) (Figure 1). The concentrations of CCL11 were significantly greater in MB leprosy patients than in PB leprosy patients (\( P < 0.01 \)).

The ROC curves were plotted to investigate the diagnostic potential of CCL3 and CCL11. As shown in Figure 2a, the diagnostic performance of the measurement of CCL3 was moderate, with an area under the ROC curve of 0.73 ± 0.08 (\( P < 0.003 \)), which suggested that measuring this chemokine could differentiate LE patients from NI individuals, although not perfectly. In contrast, the performance of CCL11 in diagnosis of LE was excellent (Figure 2b). The area under the ROC curve was 0.95 ± 0.03 (\( P < 0.0001 \)), which indicated a good diagnostic performance of CCL11, which was significantly better than the diagnostic performance of CCL3 (\( P = 0.005 \)). A level of CCL11 > 275 pg/mL had a sensitivity of 90% and a specificity of 95% in detecting LE. When the ability of CCL11 to differentiate between LE patients with MB forms and PB forms was assessed, the area under the ROC curve was 0.82 ± 0.05 (\( P < 0.0001 \)). A level of CCL11 > 472 pg/mL had a sensitivity of 82% and a specificity of 63% in differentiating MB forms from PB forms of the disease.

A previous study quantified tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) and CCL3 mRNA by real-time, reverse transcription-polymerase chain reaction in skin biopsy samples of patients with PB leprosy. A positive correlation between TNF-\( \alpha \) and CCL3 was found. This suggests that a TNF-\( \alpha \)-induced increase in CCL3 levels could be an important step in granuloma formation and anti-mycobacterial defense.\textsuperscript{17,18} Thus, elevated levels of CCL3 in plasma of patients with LE is consistent with expression of this chemokine in skin lesions and its putative role in granulomatous inflammation.\textsuperscript{18,19}

To our knowledge, no study has evaluated the concentration of CCL11 in plasma of LE patients. In our study, there was an increased level of CCL11 in plasma of LE patients that was more significant than the level in MB leprosy patients. These findings suggest that a greater expression of CCL11 could disfavor an adequate immune response and favor parasite replication. CCL11 is a potent chemoattractant for eosinophils and may also be associated with the activation of other cell types, including mast cells and Th2 lymphocytes, within inflammatory sites.\textsuperscript{20,21} Although the present study was not designed to investigate mechanisms, CCL11 expression could potentially be a molecular signal associated with enhanced trafficking of Th2 lymphocytes and decreased protection against the parasite.\textsuperscript{20,21} Further studies using the Ridley-Jopling classification and detection of the expression of CCL11 in lymphoid organs and affected tissues are clearly necessary.

![Figure 1](image_url)

**Figure 1.** Levels of CCL3 (a) and CCL11 (c) (pg/mL) in plasma of leprosy patients (LE = 59) and non-infected individuals (NI = 20). The plasma levels of CCL3 (b) and CCL11 (d) (pg/mL) of NI and leprosy patients with the paucibacillary (PB = 36), multibacillary (MB = 23) forms of the disease are also shown. Horizontal lines represent median values.
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**FIGURE 2.** Receiver-operator-characteristic (ROC) curves for a, CCL3 (area under the ROC curve = 0.73 ± 0.08) and b, CCL11 (area under the ROC curve = 0.95 ± 0.03) in the diagnosis of leprosy patients.

needed to understand the precise pathopysiologic role of this chemokine in LE patients.

Because CCL3 and CCL11 levels were elevated in patients with LE compared with NI individual, we assessed the diagnostic performance of measuring these chemokines. It was clear that despite differences in LE patients compared with NI individuals, the measurement of CCL3 in plasma has limitations in discriminating between the two groups. In contrast, measurement of CCL11 in plasma was a good diagnostic test for differentiating LE patients from NI individuals. As assessed by the ROC curve, measurement of CCL11 was significantly more useful in diagnosis of LE patients than measurement of CCL3. A level of CCL11 > 275 pg/mL had a sensitivity 90% and a specificity of 95% in detecting disease in our sample population.

One limitation of the present study is classification of patients on the basis of only clinical parameters.14,15 The classification of patients according to the Ridley-Jopling criteria was not possible because biopsy specimens are not routinely obtained in Brazil and in most places where the disease is endemic. Moreover, it is uncertain whether CCL11 will have similar diagnostic use in differentiating LE patients from patients with diseases that are clinically similar to LE. It is important to point out, however, that some diseases, such as pulmonary tuberculosis, may have increased plasma levels of CCL11, which reinforces the view that this chemokine is not specific for leprosy, but is an inflammatory marker.13 Despite these limitations, our results clearly demonstrated that patients with leprosy had elevated levels of CCL11 and that measuring CCL11 may be useful in the diagnosis of leprosies.

In conclusion, our studies demonstrate that levels of CCL3 and CCL11 are elevated in plasma of patients with LE, which suggests that these chemokines may play a relevant role in disease pathogenesis. Moreover and more importantly, our results demonstrate that measurement of CCL11 in plasma from LE patients may be used as a biologic marker to help differentiate LE patients from NI individuals. This tenet needs to be addressed in further studies and in the general population.

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