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Serial measurement of serum cytokines, cytokine receptors and neopterin in leprosy patients with reversal reactions

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Summary Serum levels of cytokines (IL-4, IL-5, IFN-γ, TNF-α), cytokine receptors (TNFR I and II) and one monokine (neopterin) were estimated in seven leprosy patients to establish disease associated markers for reversal reactions (RR). Sera were collected at diagnosis of leprosy, at the onset of reversal reaction and at different time points during and at the end of prednisone treatment of reactions. It was expected that the serum cytokine and monokine profile before and at different time points during reactions would provide guidelines for the diagnosis and monitoring of reversal reactions in leprosy. The cytokines and cytokine receptors were measured by ELISA, whereas a radioimmunoassay was used for neopterin measurement. Six of the seven patients showed increased levels of neopterin either at the onset of RR or 1 month thereafter, and levels declined on prednisone treatment to that seen at the time of diagnosis without reactions. No consistent disease associated cytokine profile was observed in these patients. Interestingly, serum TNF-α levels were increased in the same patients even after completion of prednisone treatment, indicating ongoing immune activity. In conclusion, this study demonstrates that despite cytokines levels in leprosy serum being inconsistent in relation to reversal reactions, serum neopterin measurement appears to be an useful biomarker in monitoring RR patients during corticosteroid therapy.

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Introduction

The immunopathology of leprosy is primarily due to immune interaction between subsets of T cells, antigen presenting cells and *Mycobacterium leprae* antigens. Such interactions produce type 1/type 2 cytokines[1–6] and activated macrophage products (monokines),[7] which act primarily as molecular signals for communication between immune cells and target resident cells. They play a pivotal role in the dynamics of the host immune response and tissue damage. During the chronic course of leprosy, sudden increases in immune activity may occur which are called reactions. These are either type I (reversal reaction, RR), due to an acute increase in the cell mediated immune response, or type II (erythema nodosum leprosum, ENL), described as an immune complex mediated disease.[8] In respect to T cell cytokine responses in leprosy, it was demonstrated that *M. leprae* responsive T cell clones from RR lesions were polarized to a type 1-like cytokine profile.[9] Similarly, peripheral blood mononuclear cells (PBMC) from ENL patients also displayed a type 1 cytokine secretion profile.[10] These approaches have been useful in understanding the immunopathology of the different disease states of leprosy. However, they are difficult for routine monitoring of clinical states of patients and in aiding diagnosis. In this respect, measurement of serum cytokines provides a simpler and cheaper alternative. Besides T cell cytokines, detection of neopterin, a monocyte/macrophage activation product,[7,11–13] was used as a marker for cell mediated immune activity in leprosy and other diseases[7,14,15] with elevated levels detected in 75% of leprosy patients.[7] Levels of neopterin were found to be significantly elevated in reactional as compared to non-reactional leprosy patients.[11]

Prednisolone, the drug of choice in the treatment of type 1 reactions, suppresses inflammatory processes, and is of great importance in the recovery of nerve function after the reaction.[16] Corticosteroids usually influence the cytokine milieu in patients with RR, causing a decrease in the pro-inflammatory cytokines IFN-γ and TNF-α while in one study, levels of the anti-inflammatory IL-10 were increased.[6] High levels of neopterin, seen in four patients with RR and two patients with ENL in another study, were found to decline on corticosteroid therapy.[11] Hence measurement of cytokine levels along with neopterin might be useful in the monitoring of corticosteroid therapy during leprosy reactions.

The goal of the present study was to identify cytokine/monokine profiles in sera or plasma of leprosy patients associated with the onset of reversal reactions and to assess change in these profiles with corticosteroid treatment in the hopes of providing indicators for diagnosing reactions and, further, to monitor the patients during corticosteroid therapy.

Materials and methods

PATIENTS

Seven leprosy patients, classified clinically and histologically according to Ridley–Jopling criteria[17] at the Leonard Wood Memorial Centre for Leprosy Research, Cebu, Philippines were studied. Six of the patients were classified clinically as BL and one as LL. Histologically five patients were classified as BL and two as LL subpolar. The BI of the patients at diagnosis varied between 2·8 and 4. Details of the patients are given in Table 1. The age of the patients ranged between 20 and 56 years. These patients, who developed reversal reactions, were studied serially. Reversal reactions were diagnosed clinically and graded as mild, moderate or severe according to the following criteria: mild (1+), slight erythema, slight swelling of

Table 1. Patients included in the study. ROM = rifampicin (600 mg) + ofloxacin (400 mg) + minocycline (100 mg) monthly, MDT = WHO multibacillary MDT,[35] BI = Bacterial Index,[18] LLs = LL subpolar

Patient no.	Diagnosis			Therapy	Onset of RR	Severity of RR
	Clinical	Histological	BI			
3	BL	BL	3·6	MDT	6 months	MODERATE
4	BL	BL	3·1	ROM	2 weeks	MODERATE
5	BL	BL	3·5	MDT	3 months	MILD
10	BL	BL	3·1	MDT	1 month	MODERATE
11	BL	BL	4·0	MDT	10 months	MODERATE
17	BL-LL	LLs	2·8	MDT	4 months	MODERATE
19	LL	LLs	3·8	MDT	5 months	MODERATE

existing lesions, may or may not have slight nerve tenderness; moderate (2+), erythema, oedema of existing lesions with development of new oedematous/erythematous lesions associated with oedema of hands and feet and definite nerve tenderness; severe (3+), marked erythema and oedema of existing lesions, some ulcerating, new oedematous/erythematous skin lesions, some erythematous, definite pain and tenderness of peripheral nerve trunks with fever of 39°C and above, joint pains and muscle pain. Then, six of the patients had reactions of moderate severity and 1 had a reaction of mild severity. Reactions were treated using prednisone, at a dose of 25–30 mg per day, in divided doses. The treatment was gradually tapered off and stopped when the reaction subsided. All patients showed marked improvement on prednisone treatment with complete subsidence of reversal lesions.

Approximately 20 ml of blood was obtained from the patients, with informed consent, under the supervision of one of the investigators (TTF). Serial samples were obtained from the patients at diagnosis and subsequently on manifestation of RR, 1 month, 2 months at the end of steroid treatment and then again 1–2 months after steroid treatment. Collected sera or plasma were shipped in dry ice to the Academic Medical Centre, Amsterdam and stored at −80°C until further analysis.

CYTOKINE/MONOKINE MEASUREMENTS

Cytokines

The assayed cytokines included IFN-γ, TNF-α, IL-4 and IL-5 and cytokine receptors TNF-αR (p55 and p75). The cytokine levels were measured using commercially available kits (Pelikine, CLB, The Netherlands) according to manufacturer's instructions.

Neopterin

Plasma neopterin levels were measured using a commercially available RIA kit as described previously.[11] The assay is based on the competition of unlabelled neopterin of the plasma or standards and radiolabelled neopterin for the binding sites of a neopterin specific antibody. The concentration of unlabelled neopterin in serum is inversely proportional to the amount of radioactivity in the neopterin-antibody complex. The upper limit of the normal range is approximately 10 nmol/l in serum.[18]

STATISTICAL ANALYSIS

The Wilcoxon rank test for paired samples was used to analyse the differences in cytokine/monokine levels in the patients at the different disease stages.

Results

Sera of each study patient before the onset of reactions served as controls. The levels of cytokines/monokine in the sera were also compared with levels of these cytokines/monokine in normal healthy individuals irrespective of ethnic origin as reported in literature. The results of the cytokine/monokine assays are shown below.

CYTOKINES

Assessment of inflammatory cytokines TNF-α and IFN-γ showed that four of seven patients had TNF-α concentrations between 80 and 130 pg/ml at diagnosis. However, only two of these patients showed sustained high levels when developing RR. Conversely, two patients who did not show detectable TNF-α at diagnosis, had increased levels at onset of RR. No significant difference was found in TNF-α levels before and at onset of RR or during corticosteroid treatment of the patients since higher TNF-α levels were seen at different time points during treatment of reactions and even after completion of treatment in four of the patients (Figure 1). IFN-γ was below the detection limits of the ELISA in most sera and plasma samples tested (data not shown). IL-4 levels varied between 0 and 200 pg/ml at the onset of the disease but no significant difference was noted with the onset of reactions or with the institution of corticosteroid treatment. IL-5 levels were low in all the sera tested (data not shown). TNF-α receptor levels (p55 and p75) showed minor increases at onset of RR though the differences were not statistically significant (data not shown). To sum up, no consistent

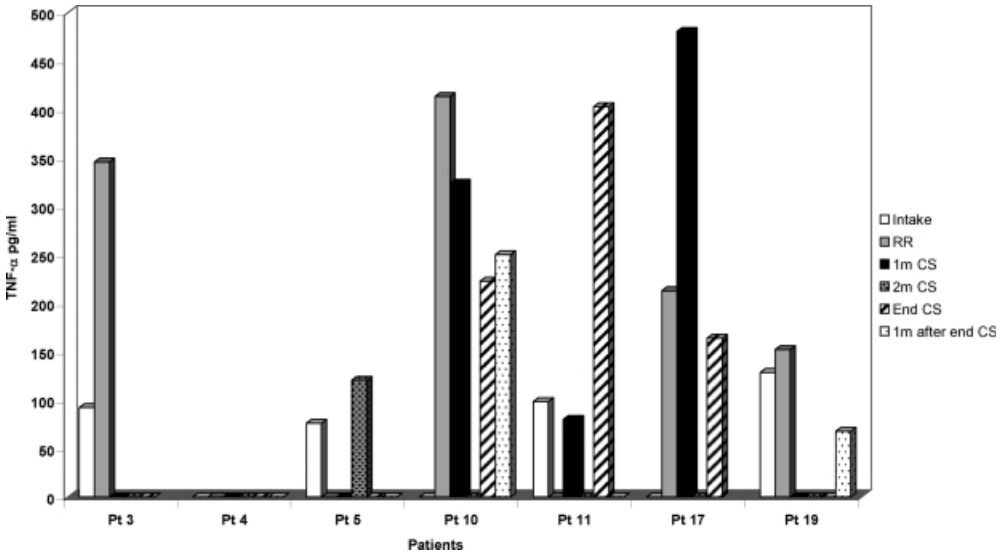

Figure 1. Trends in serum TNF-α concentrations in individual patients. CS = corticosteroid (prednisolone).

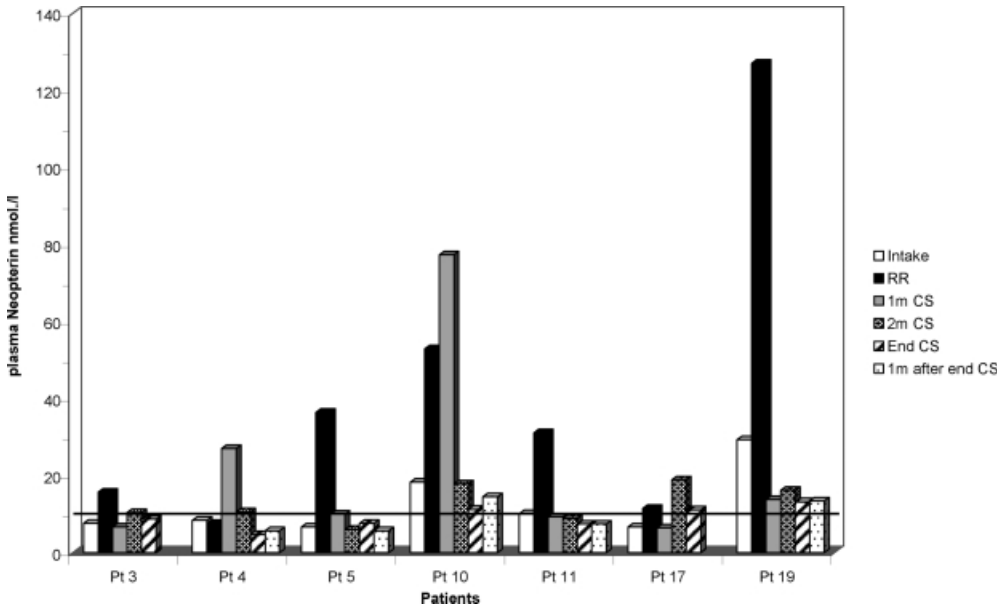

Figure 2. Trends in serum neopterin concentrations in individual patients CS = corticosteroid (prednisolone). Line indicates cut-off based on a previous study.[11]

cytokine profile within patient serum could be associated with development of RR or response to corticosteroid treatment of these patients.

NEOPTERIN

The levels of plasma neopterin were elevated in six of seven patients at onset of RR as compared to the levels at disease diagnosis ($P = 0{\cdot}028$), although the absolute amounts of neopterin detected varied among the patients (Figure 2). These elevated levels dropped significantly at the end of prednisolone treatment to levels found at disease diagnosis in 5/6 patients ($P = 0{\cdot}018$) (Figure 2). Two patients showed increased neopterin levels after 1 month of corticosteroid treatment. However, this decreased to levels seen at disease diagnosis in both patients after 2 months of corticosteroid treatment. Thus a general trend could be observed, whereby appearance of RR correlated with elevation of neopterin levels, which declined with the institution of corticosteroid treatment. No correlation was observed between levels of neopterin at diagnosis and the duration to manifestation of RR.

Discussion

A panel of cytokines, a monokine and cytokine receptors was assessed in leprosy patients to identify patterns of expression that might be associated with detection and/or monitoring of reversal reactions. The cytokines and receptors were selected to represent commonly known type-1 (IFN-γ, TNF-α) or type-2 T cell cytokines (IL-4, IL-5), cytokine receptors (TNF-R I and II) and a macrophage activation product (neopterin) known to be involved in the immunopathology of leprosy. In the present study, IFN-γ was undetectable in most of the sera

in contrast to a previous study,[3] which reported high serum IFN-γ levels on manifestation of RR. Moderate levels of IL-4, a predominantly type-2 T cell dependent cytokine, were observed during the course of reversal reaction. The presence of IL-4 in the sera of RR patients can be justified on the basis of findings in literature.[9,19–21] Although it was difficult to establish a clear pattern, TNF-α concentrations tended to be elevated at different time points during treatment of RR and even at the end of corticosteroid treatment in four of the cases (Figure 1). This probably indicates ongoing immune activity in the patients, though clinically these patients showed complete subsidence of RR with the administration of prednisone.[22]

Nevertheless, our results show that no consistent cytokine profile within individual patient's serum could be associated with manifestation of RR or response to corticosteroid treatment. In this respect, previous studies on serum cytokine detection in leprosy reactions have often shown contradictory results. Elevated levels of TNF-α were observed in type I reactions[23,24] and found to decline with corticosteroid treatment,[24] which was contradicted by a subsequent study.[25] A very high concentration of circulating IL-2 receptor (IL-2R) was reported in RR patients and marked reduction of this level was observed with corticosteroid treatment.[26] Although elevated IL-2R was also reported in a subsequent study, the level appeared unaffected by corticosteroid treatment.[27] Our study along with that of Moubasher and co-workers[3,4] are among a few to have assessed a panel of cytokines for obtaining a broader view of the immunological responses associated with reactions. Furthermore, in contrast to most other studies, our study involved longitudinal sampling of the patients before and at fixed time points during the course of RR.

In this study, neopterin showed increasing trend with manifestation of RR and declined with prednisone treatment. A similar trend was reported previously in a retrospective study.[11] The present study thus showed the utility of plasma neopterin assay in the monitoring of RR patients, though not as a predictive parameter. However elevated serum or plasma neopterin levels need to be interpreted with caution especially in view of its reported association with a variety of conditions involving activated cell mediated immunity.[14,15,28–32] Increased neopterin concentrations are prevalent in asymptomatic HIV antibody seropositive individuals[7,33] and even in apparently healthy subjects from rural Africa showing evidences of subclinical parasitic infections.[34]

In conclusion, it appears from our results that measurement of cytokine profiles within serum/plasma of patients is of limited value in monitoring disease progression or prediction of reactions in leprosy. On the other hand, measurement of macrophage activation markers like neopterin would provide useful supportive data for diagnosis and to monitor response to steroid treatment in reversal reactions.

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