Interferon-\(\gamma\) and urine neopterin in attacks of the hyperimmunoglobulinaemia D and periodic fever syndrome

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Abstract. The hyperimmunoglobulinaemia D and periodic fever (hyper-IgD) syndrome is typified by recurrent unpredictable febrile attacks with abdominal pain, joint involvement (arthralgias/arthritis), headache, skin lesions and a polyclonal elevation of serum IgD (> 100 \(\mu\text{M L}^{-1}\)). Interferon-gamma (IFN-\(\gamma\)) is a major proinflammatory cytokine which could play a role in the pathogenesis of the attacks. There is a need for parameters (if possible non-invasive) to monitor disease activity. A potential candidate is neopterin which is released by monocytes/macrophages when stimulated with IFN-\(\gamma\), excreted unchanged in urine, and appears to be an early and sensitive marker for activation of the immune system. We measured rectal body temperature, serum IFN-\(\gamma\), and urine neopterin in 10 hyper-IgD patients both during and between attacks. The body temperature rose to a mean of 38.9°C on the first day of the attack and normalized within 5 days. Serum IFN-\(\gamma\) during the first day of the attack was 2.98 \(\mu\text{M L}^{-1}\) and was significantly lower during remissions. The urine neopterin excretion was 268 ± 170 \(\mu\text{mol mol}^{-1}\) creatinine between attacks and was significantly increased to 638 ± 275 \(\mu\text{mol mol}^{-1}\) creatinine on the first day of symptoms. Maximal urine neopterin values were reached on the fourth day of the attack (1051 ± 387 \(\mu\text{mol mol}^{-1}\) creatinine) and excretion gradually declined and attained values below 400 \(\mu\text{mol mol}^{-1}\) creatinine after 9 days. There was a good correlation between serum IFN-\(\gamma\) and urine neopterin. The increases in serum IFN-\(\gamma\) and urine neopterin suggest activation of the cellular immunity during the febrile attacks of the hyper-IgD syndrome. Furthermore, the activation of the cellular immune system appears to persist several days after normalization of the body temperature. The significant correlation between IFN-\(\gamma\) and urine neopterin in the hyper-IgD syndrome accords with experimental data suggesting that IFN-\(\gamma\) is the dominant factor in the release of neopterin. Our study shows that urinary neopterin is a good quantitative and qualitative parameter to monitor disease activity in patients with the hyper-IgD syndrome.

Keywords. Hyper-IgD syndrome, interferon-gamma, urine neopterin.

Introduction

The hyperimmunoglobulinaemia D and periodic fever syndrome (hyper-IgD) is characterized by recurrent attacks of fever associated with persistently elevated polyclonal serum IgD levels (> 100 \(\mu\text{M L}^{-1}\)). [1] An autosomal recessive inheritance is typical [2]. Symptoms commence at an early age and persist throughout life and the febrile attacks occur every 4–8 weeks, lasting 3–7 days. During these episodes the patients may suffer from abdominal distress (vomiting, diarrhoea and pain), skin lesions, joint involvement, headache and lymphadenopathy [3,4]. The cause of the syndrome is unknown but since attacks are featured by an important inflammatory reaction, IFN-\(\gamma\) as a major proinflammatory cytokine could play a role in the pathogenesis. Therefore, we measured IFN-\(\gamma\) as well as neopterin, a pteridine derivative, which is released by monocytes/macrophages when stimulated with IFN-\(\gamma\) [5]. Neopterin, is conveniently excreted in urine and can be measured easily. It appears to be an early and sensitive marker of activation of the immune system [6]. In inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus urinary neopterin levels correlate closely with the disease activity [7,8]. The present study investigates whether IFN-\(\gamma\) and urine neopterin are increased during the febrile attacks of the hyper-IgD syndrome and assesses the clinical
utility of urinary neopterin as marker of disease activity in these patients.

Patients and methods

Patients

Ten patients, six female and four male, with the hyper-IgD syndrome were included in the study and the diagnosis of hyper-IgD syndrome was made according to standard criteria [3]. The mean age at time of sampling was 28.8 years (range 8–61 years). Patients were instructed to commence sampling on the first day of the attack and to continue until the fever had subsided, with a maximum of 10 days sampling. Each day the first morning urine specimen was sampled in a special container, shielded from light, and frozen at -20°C until analysis. The rectal body temperature was measured daily, using a digital thermometer. Samples for urine neopterin concentrations were obtained in each patient between attacks to give baseline values. Sampling of serum for IFN-γ measurements was performed on the first day of the attack and during remission.

Laboratory measurements

The IFN-γ concentration was determined by a commercially available immunoradiometric assay. (Medgenix, Amersfoort, the Netherlands). The microtitre plate wells are coated with monoclonal anti IFN-γ. After washing, radiolabelled (¹²⁵I) anti-IFN-γ was added. Bound radioactivity was determined in a gamma counter and the IFN-γ concentrations were calculated from standard curves. The lower limit of detection of the assay was 0.2 IU mL⁻¹. The mean value obtained in healthy controls is 0.55 ± 0.14 IU mL⁻¹ (manufacturer’s information).

Determination of neopterin was by reversed-phase high-performance liquid chromatography with minor modifications [9]. Briefly, urine samples were centrifuged to remove debris, diluted in 1 to 10 with water containing dimethylpterine as an internal standard and injected directly onto a Techsphere 5 ODS column (HPLC Technology Ltd). A binary gradient elution was used with an initial mobile phase of 2% methanol in 15 mmol L⁻¹ phosphate buffer, pH 6.4, increasing to 25% methanol after 12 min, and creatinine was detected separately using a kinetic alkaline picrate (Jaffe) method. The ratio of neopterin to creatinine was calculated to compensate for variations of urine density. The median urine neopterin value for a group of 65 healthy controls was 149 µmol mol⁻¹ creatinine (range 62–273) [8].

Statistical analysis

The paired non-parametric Wilcoxon test was used for statistical comparison of values obtained during active disease compared to remission values. Probability (P) values were calculated on the basis of two-tailed tests. A correlation coefficient was calculated with the Pearson’s correlation test. A P value of <0.05 considered to be the lowest level of significance. Data are given in mean ± standard deviation.

Results

The attacks in the hyper-IgD were featured by an increase of the mean body temperature to 38.9°C on the first day of the attack which returned to normal by day 5 (Fig. 1).

Serum for IFN-γ measurements was obtained from nine patients during an attack. The IFN-γ concentration during the first day of the attack was 2.98 ± 3.55 (median 1.33) IU mL⁻¹ and was significantly lower, 0.59 ± 0.39 (median 0.44) IU mL⁻¹ during remissions (Fig. 2). For 10 hyper-IgD patients a total 69 urine
samples (mean 6.9 per patient) were collected during attacks. Three patients collected the complete set of urine samples for the 10 days. The control urine neopterin excretion for the 10 patients was 268 ± 170 (median 199) μmol mol⁻¹ creatinine, significantly lower than values obtained on the first day of symptoms: 638 ± 275 (median 604) μmol mol⁻¹ creatinine (Fig. 2) (P = 0.002). All patients had higher total urine neopterin during attacks compared to between attacks. The mean urine neopterin increased in the first days of the attack and maximal values were reached on the fourth day of the attack (1051 ± 387 μmol mol⁻¹ creatinine). The urine neopterin gradually declined and attained values below 400 μmol mol⁻¹ creatinine after 9 days (Fig. 3). One patient started sampling 2 days before the onset of the febrile attack. In the days preceding the attack neopterin excretion values were 245 and 280 μmol mol⁻¹ creatinine. The excretion increased respectively to 345 and 780 μmol mol⁻¹ creatinine on the first 2 days of the febrile attack. There was a good correlation between serum IFN-γ and urine neopterin excretion: \( r = 0.6675 \) (P < 0.05). The decrease in body temperature and thus the end of an attack, anticipated the decrease of urine neopterin by 4–5 days. In five patients the activation of the cellular immune system (urine neopterin > 200 μmol mol⁻¹ creatinine) persisted during remission despite apparent clinical well-being.

**Discussion**

Serum concentrations of IFN-γ and urine excretion of neopterin correlated well with the clinical condition of the hyper-IgD patients. IFN-γ increased fivefold over remission values during active disease. IFN-γ participates in the human inflammatory response, partly through its ability to induce production of TNF-α and other cytokines [10]. IFN-γ itself may be a pyrogenic compound, but its presence does not explain all signs and symptoms in the hyper-IgD syndrome [11]. Other proinflammatory cytokines possibly involved in the pathogenesis are currently being studied by us. We found that urine neopterin values are low between attacks of the hyper-IgD syndrome but increase significantly during attacks. Neopterin is

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**Figure 2.** The left panel refers to the individual concentrations of circulating IFN-γ in nine patients during and in between attacks of the hyper-IgD syndrome. The mean and standard error of the mean are displayed at either side. IFN-γ concentrations were significantly higher during active disease compared to remission (P < 0.05). The right panel indicates the individual urine neopterin values for 10 patients between and during attacks of the hyper-IgD syndrome. The mean and standard error of the mean are displayed at either side. Note the significant elevation of urine neopterin during attacks (P = 0.002).

**Figure 3.** Urine neopterin in μmol mol⁻¹ creatinine ratios during the first 10 days of an attack in 10 patients with the hyper-IgD syndrome. Day 1 denotes the first day of the attack as defined by the symptoms. Data are expressed as mean and standard deviation.
a non-specific marker of cellular activation being produced by human macrophages in response to IFN-γ [5]. Neopterin is a stable compound, readily detectable and easy to measure. Since neopterin is not metabolized, the urine excretion reflects the integrated release of neopterin [12]. Furthermore, serum IFN-γ and urine neopterin correlate significantly in the hyper-IgD syndrome which is in line with these experimental data in vitro suggesting that IFN-γ is the dominant factor involved in the release of neopterin [5]. The course of the urine neopterin excretion during the attacks demonstrates that maximal values are attained within 4 days. Data from one patient indicate that neopterin excretion values are normal in the days immediately before an attack. This may suggest that the value of neopterin as a predictor of attacks is rather limited. The end of the attack, as indicated by the normalization of the rectal body temperature and disappearance of the symptoms occurs in 5 days, but at the same time urine neopterin still continued to be elevated above individual reference values. Thus, the activation of the cellular immune system appears to persist after normalization of the temperature.

Our study shows that urinary neopterin is a good quantitative and qualitative parameter to monitor disease activity in patients with the hyper-IgD syndrome. A reliable non-invasive parameter is needed in the management of the hyper-IgD syndrome because many patients are young (< 10 years), and because of the outpatient basis of the treatment. Moreover, evaluation of drugs in a disorder characterized by unpredictable febrile attacks requires a dependable parameter. In studies evaluating the effect of colchicine in familial Mediterranean fever, another periodic fever syndrome, patients were requested to keep records of their attacks with symptom diaries and/or postcards (to be sent to the investigators when having an attack) [13-15]. Symptom diaries depend to a large extent on self-interpretation by the patient and consequently more objective and accurate parameters are clearly needed. The use of urine neopterin to monitor disease activity has great advantages; the patients can sample and store urine at home in their home freezer for up to 6 months contrasting with serum that requires processing.

In conclusion, urine neopterin appears to be a good monitor of disease activity in the hyper-IgD syndrome. Neopterin measurements can be useful when evaluating the effect of therapy for the hyper-IgD syndrome. Our data suggest that attacks of the hyper-IgD syndrome are associated with macrophage activation as evidenced by increased serum IFN-γ and urine neopterin excretion.

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