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Urinary Neopterin, Antigenaemia and CD4/CD8 Ratio in HIV Positive Children under AZT Therapy

Gian Vincenzo Zuccotti¹⁾, Giacomo Biasucci, Manuela Brioschi, Vittoria Locatelli, Angela Antonucci, Enrica Riva and Marcello Giovannini

Clinica Pediatrica V — Università di Milano, Ospedale San Paolo, Via A. Di Rudinì 8, 20142 Milano, Italy

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Summary

We evaluated urinary neopterin in seven HIV positive children, aged 4 months to 4 years, 11 months, under Zidovudine (AZT) treatment, 300 mg/mq/12 hourly per os.

The results were compared with HIV antigenaemia (HIV Ag) and CD4/CD8 ratios.

Neopterin ($\mu\text{mol/mol}$ creatinine) was determined by HPLC; HIV Ag assessed by enzyme-immunoassay and T-cell subsets by flow-citometry.

Blood and urine samples were obtained before and at 3 and 6 months after starting therapy.

There was no significant trend in CD4/CD8 ratios during AZT treatment. Neopterin and HIV Ag showed a statistically significant decrease (median values 3239, 1679, 2062 $\mu\text{mol/mol}$ creatinine; 2.7, 1.3, 1.2 sample optic density/cut off optic density respectively; $p < 0.05$ by Friedman's test) and were also significantly correlated ($p = 0.01$ by Spearman's ranks correlation test).

We conclude that urinary neopterin is useful in monitoring AZT therapy in HIV positive children.

Introduction

During the last 10 years, several studies demonstrated high amounts of neopterin (N) in the urine of HIV positive adults, and have shown that N concentrations directly correlated with progression of the disease and with CD4/CD8 ratio inversion (1, 2).

In the wake of these studies, we studied urinary excretion of N in HIV positive children (3, 4). Our studies confirmed that urinary N is a useful and reliable prognostic marker in HIV infection in children as well as in adults; it did not seem to be useful for early diagnosis since maternal activated T lymphocytes, still circulating in the blood stream during the first 3 months of age, might be responsible for false positive results.

In the present paper we assess the value of urinary N in the follow-up of symptomatic HIV positive children receiving Zidovudine (AZT) treatment.

Patients and Methods

Seven children, 4 males and 3 females, ranging in age from 4 months to 4 years, 11 months, were enrolled in this study.

They all attended the Regional Referral Centre for the Diagnosis, Follow-up and Therapy of HIV Positive Children, at the 5th Department of Pediatrics, University of Milan.

Four had non-specific symptoms (fever, diarrhoea, hepatosplenomegaly, generalized lymphadenopathy, weight loss), one a lymphoid interstitial pneumonia (LIP) and two were more severally affected with a wide range of problems (encephalopathy, P. pneumo-

¹⁾ Author to whom correspondence should be addressed.

cystis carinii pneumonia, oral candidosis, meningitis, hepatitis) (Atlanta CDC classification: 4 patients in P2A, 1 in P2AC and 2 in P2ABD1D2D3F classes). All the children underwent AZT treatment, 300 mg/mq/twice daily per os.

Urine samples for N determination were collected before starting treatment and 3 and 6 months.

Urinary N determination was performed by high performance liquid chromatography and values were expressed as $\mu\text{mol/mol}$ urinary creatinine. Urinary samples were protected from light and stored at -80°C until analysis. Just before the analysis 100 μl of urine were mixed with 1 ml of a solution containing phosphate buffer 15 mmol/l and EDTA 5.4 mmol/l, pH 6.4. Only 10 μl of this solution were injected.

Neopterin used for standard solution was purchased from Dr. Schircks (Jona-Switzerland).

A 15 mmol/l potassium phosphate buffer solution, pH 6.4 was utilized as mobile phase at flow rate 0.8 ml/min.

HIV Ag was determined by an immunoenzymatic test (f: sample optic density/cut off optic density) while T cell subsets by flow cytometry.

Neopterin values at the different collection times were compared each other and to the corresponding values of CD4/CD8 ratio and antigenaemia (HIV Ag).

Statistical analysis were performed by Friedman's test and by Spearman's ranks correlation test.

Results

Urinary N and HIV Ag values (Fig. 1) showed a statistically significant decrease following treatment. There was no obvious trend in CD4/CD8 ratios (Table 1).

Table 1. CD4/CD8 ratio during AZT treatment

	Median	Range
Before therapy	0.56	0.10–1.00
3 months later	0.40	0.20–0.75
6 months later	0.42	0.18–0.65

Figure 1. HIV Ag and Urinary Neopterin during AZT treatment.

N and HIV Ag values were significantly correlated (Spearman's ranks correlation test $r = 0.593$, $p = 0.01$) but no correlation was found between the CD4/CD8 ratio and the response to the AZT treatment.

After 3 months treatment there was a sharp improvement in the clinical picture in all seven children. In particular four children with non specific symptoms (P2A class) showed improvement in their hepatosplenomegaly and lymphadenopathy with a decreased number of febrile days. The child with LIP (P2AC class) showed resolution and even the two patients in a more advanced stage of the disease (P2A 3D1D2D3F class) showed reduced fever, fewer infections, improved weight gain and better neuromotor progress.

Discussion

These preliminary data suggest that urinary N determination may be useful in the monitoring of AZT therapy. As a measure of response it appeared to be at least as sensitive as HIV Ag.

It may also be more reliable since in 2 out of 7 patients at 3 months HIV Ag levels were equal or even higher than before treatment whereas urinary N levels were, without exception, lower than before treatment.

Urinary N measurements are non invasive and well detect infection at an early stage, thus sparing children frequent hospitalizations and blood drawings, and enabling the physician to achieve a better compliance.

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