Serum neopterin as an immunological marker of disease activity in inflammatory diseases
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Chapter 8

Summary and Conclusions

Neopterin was first identified from serum of leukaemia patients, and from royal jelly (3) in 1981. Originally H. Rembret intended to name this compound "pyronuric acid", but in order to indicate that it is a new metabolite, derived from nucleic acid, he suggested the name "neopterin" (2) to denote that it might start a new (nucleic acid) metabolism in the body. It was found that neopterin is present in the urine of healthy persons and in a wide variety of healthy and pathological conditions.

In recent years, new pathological metastases of neopterin have been described such as hepatitis, vaginal infection, and rheumatic fever. In pathological conditions, neopterin is present in the urine of patients with malignant diseases, as well as in patients with inflammatory diseases (3). In vitro studies (4, 5) revealed that human monocyte-macrophages produce neopterin when stimulated by interferon. This neopterin is released from activated cells. Other cell types do not produce measurable amounts of neopterin following various stimuli. Therefore, neopterin producing appears to be closely associated with proliferation of the cellular immune system. These in vitro experiments are consistent with the results of numerous clinical studies.

High neopterin levels are found in different inflammatory diseases and certain malignant diseases and can be measured in serum and urine. Determination of the neopterin level in serum and urine from the patients have been demonstrated to be predictive for the course and progression of the disease and the response to therapy. In serum, neopterin level rapidly declines in most cases (4), probably due to the induction of immunoreactive proteins, which stimulate release of neopterin. Thiosulfate has been observed during immune-suppressive therapy. In recent years, new pathological metastases of neopterin have been observed such as hepatitis, vaginal infection, and rheumatic fever. In pathological conditions, neopterin is present in the urine of patients with malignant diseases, as well as in patients with inflammatory diseases (3). In vitro studies (4, 5) revealed that human monocyte-macrophages produce neopterin when stimulated by interferon. This neopterin is released from activated cells. Other cell types do not produce measurable amounts of neopterin following various stimuli. Therefore, neopterin producing appears to be closely associated with proliferation of the cellular immune system. These in vitro experiments are consistent with the results of numerous clinical studies.

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SUMMARY AND CONCLUSIONS
Chapter 1
Neopterin was isolated from larvae of bee (1), from worker bees, and from royal jelly (2) in 1963. Originally, H. Rembold intended to term the new compound, 2-amino-4-hydroxy-(erythro-1',2',3'-tri hydroxypropyl)—pteridine, “novapterin,” to indicate that it was a new (from Latin, novum) molecule isolated from honey bees (Latin, Apis) and with a pterin structure. The compound finally was termed “neopterin” to denote that it might start a new (Greek, neo) epoch in pteridine research. Following the identification of a pteridine as the fluorescent component that was elevated in the urine of mice with Ehrlich ascites tumor, compared to healthy mice, the corresponding substance from human urine was isolated and characterized. It was found that the fluorescent component previously observed in urine of patients with malignant diseases was neopterin. Wachter and co-workers found elevated rates of neopterin excretion in a group of patients with various malignant disorders, as well as in patients with viral diseases (3). In vitro studies (4,5,6) revealed that human monocytes / macrophages produce neopterin when stimulated by interferon-γ. This lymphokine is released from activated T cells. Other cell types do not produce measurable amounts of neopterin following various stimuli. Therefore, neopterin production appears to be closely associated with activation of the cellular immune system. These in vitro experiments are consistent with the results of numerous clinical studies. High neopterin levels are found in different inflammatory diseases and certain malignancies and can be measured in serum and urine. Determination of the neopterin level in serum and urine from these patients have been demonstrated to be predictive for the course and progression of the disease and the response to therapy as its level rapidly declines and normalizes (7). Patients undergoing immunostimulatory treatment show increased levels of neopterin, probably due to the induction of immunoregulatory cascades which stimulate release of interferon-γ. The opposite has been observed during immunosuppressive therapy. In recent years new physiological functions of neopterin have been discovered such as inducing or enhancing cytotoxicity, inducing apoptosis and the role of a chain breaking antioxidant (8).

Chapter 2
Ample evidence has been presented that in psoriatic skin immune activation takes place thus leading to interferon gamma production by T lymphocytes (9). Fuchs and co-workers reported results (serum and urine) in seven psoriasis
patients (10). They found elevated neopterin levels and significant correlation with the psoriasis area and severity index (PASI). Our data do not support these findings. We hypothesize that in a local skin lesion the production and release of neopterin by monocytes/macrophages in patients suffering from mild to severe psoriasis is not always sufficient to induce detectable neopterin production.

Chapter 3
It has been suggested that activated macrophages even promote tumor growth. In several malignant diseases, elevated levels of neopterin in urine and serum were observed (11). We measured by Radio-Immuno-Assay in patients with mycosis fungoides and Sézary syndrome. Results were compared with those of patients with psoriasis, atopic dermatitis and healthy controls. Neopterin levels were significantly elevated in patients with mycosis fungoides compared with patients with psoriasis vulgaris, atopic dermatitis and healthy controls (P<0.05). There was no significant difference between Sézary syndrome and psoriasis vulgaris, atopic dermatitis or healthy controls (P>0.05). High levels of serum neopterin in this study demonstrate the presence of activated T lymphocytes in patients with mycosis fungoides and support the view that longitudinal studies could be of help in determining the utility of neopterin concentrations during therapy, for the identification of relapses and the effect of the therapy. In case of Sézary syndrome, more patients should be evaluated.

Chapter 4
Neopterin, a product of interferon-γ-activated macrophages, is a marker for CMI activation and may be useful to detect reactional states in leprosy. Here, we compared neopterin levels in single serum samples from leprosy patients with and without reaction, with controls, and when available, serial samples among patients with and without reaction. Levels in the single sample measurements, conducted in patients with reversal reaction (RR) and with erythema nodosum leprosum (ENL) were significantly higher than levels in untreated patients. Serial serum samples, obtained from patients that developed reactions and that remained free of reaction, indicated that RR or ENL paralleled a concomitant increase in the serum neopterin level. Neopterin levels generally declined upon corticosteroid therapy. Neopterin may be a useful marker for reactional states in leprosy by providing a laboratory parameter to assess onset, progression,
response to therapy, and resolution. Longitudinal measurements in patients with and without reactions provided further insight into the value of neopterin levels. Neopterin levels clearly paralleled the occurrence of RR and ENL. However, neopterin levels in patients already receiving Prednison therapy were, not unexpectedly, relatively low. In patients not developing a reactional state, the neopterin levels did not increase above the upper limit of 10 nmol/L. This study showed that serum neopterin levels are generally increased during the development of reactional states and decline during immunosuppressive treatment. However, elevated neopterin levels in a few patients not in reaction illustrate heterogeneity in neopterin production, emphasizing the importance of clinical observations. With these baseline data, we believe that a prospective study in which neopterin levels, alone or in combination with other immunologic markers, should be evaluated as a potential tool for the early detection of reactional states. Such a study might also be useful to determine whether neopterin levels discriminate between RR and a relapse, a distinction that is sometimes difficult.

Chapter 5
Sarcoidosis is an inflammatory multiorgan disorder of unknown origin, characterized by the infiltration of T lymphocytes and mononuclear phagocytes and by the formation of noncaseating granulomas in the affected organs (12). So far, prognostic parameters predicting deterioration are missing in untreated pulmonary sarcoidosis. Assessment of serum neopterin clearly demonstrated higher levels in patients with untreated acute form of pulmonary sarcoidosis than in normal subjects. Measurement of serum neopterin, can take a place in diagnostic strategies for dealing with pulmonary sarcoidosis because it generally reflects the presence or absence of a granulomatous process (13). In patients with pulmonary sarcoidosis and pulmonary tuberculosis we compared the serum neopterin level with two other biological markers: serum angiotensin-converting enzyme (ACE) and lysozyme (LZM). We found a clear difference with the three serum markers between acute and chronic sarcoidosis and determination of serum neopterin and ACE levels could be of help in differentiating between the two disease states. Elevated serum ACE levels were found in acute as well as in chronic pulmonary sarcoidosis. Elevated serum neopterin levels were only found in the acute state of the disease. Serum neopterin concentration seems to be the most important marker in the follow up during treatment of patients with chronic
sarcoidosis or to prevent a relapse. There were no significant elevated serum levels found in the acute nor in the chronic form of pulmonary sarcoidosis for LZM. As we compared our results found in pulmonary sarcoidosis with pulmonary tuberculosis the same high concentrations for serum neopterin were found in the acute form of tuberculosis, demonstrating the activity of the macrophage in both disease states.

Chapter 6
The leishmaniases are a group of diseases caused by *Leishmania* species. Since leishmania is an obligatory intracellular parasite, host defenses are dependent on T lymphocyte activity. T cells exert an anti-leishmania role by production of lymphokines such as TNF-α and interferon-γ (14,15). In response to signals initiated by these activating factors, infected cells produce microbicidal molecules, such as reactive oxygen intermediates (ROI) and nitric oxide (NO) (16). In the immune response to *Leishmaniae*, macrophages play an important role. Macrophages, when exposed to interferon-γ release large amounts of neopterin. Neopterin, in turn, is a good indicator of cell-mediated immunity (17). Serological tests for VL respond slowly to treatment and tests for the demonstration of parasites such as lymph node-, bone-marrow- or spleen aspiration are not practical for follow up. All patients with cutaneous leishmaniasis had normal levels of neopterin before treatment; this has not been observed before, and also the DAT-titer was normal. All patients with VL had elevated serum neopterin concentrations and the DAT-titer was positive. In patients with visceral leishmaniasis followed during treatment neopterin levels decreased to values below the upper limit of the normal range (10 nmol/L). Our study suggests that sequential measurements of serum neopterin concentrations during the treatment of VL can be useful for monitoring therapeutic efficacy in patients with visceral leishmaniasis but possibly not in HIV infection. Further study of the potential of neopterin as a marker of cure seems warranted.

Chapter 7
Neopterin levels provide an especially valuable picture concerning clinical activity in diseases which show rapid and acute changes in severity of the disease. The extent and activity of infections with intracellular bacterial infections e.g. tuberculosis, leprosy and leishmaniasis correlate significantly with elevated
neopterin levels (18,19). Although serum neopterin is not disease specific, an elevated serum neopterin concentration above the upper-limit of the normal range (10 nmol/L) gives an indication of the activation of cell mediated immunity (20). A follow-up of serum neopterin concentrations during the course of an infectious disease could be useful to measure the activity of the disease and the influence of the treatment (21). The dipstick assay described here is an easy-to-perform method for the semi-quantitative measurement of serum neopterin concentrations in patients with inflammatory diseases. An internal control validates the performance of the assay. Due to its robustness and simplicity the dipstick assay seems to be highly suitable for application under field conditions.

REFERENCES


