Neopterin as a Marker for Immune System Activation

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Neopterin as a Marker for Immune System Activation

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Abstract: Increased amounts of neopterin are produced by human monocytes/macrophages upon stimulation with the cytokine interferon-γ. Therefore, measurement of neopterin concentrations in body fluids like serum, cerebrospinal fluid or urine provides information about activation of T helper cell 1 derived cellular immune activation. Increased neopterin production is found in infections by viruses including human immunodeficiency virus (HIV), infections by intracellular living bacteria and parasites, autoimmune diseases, malignant tumor diseases and in allograft rejection episodes. But also in neurological and in cardiovascular diseases cellular immune activation indicated by increased neopterin production, is found.

Major diagnostic applications of neopterin measurements are, e.g. monitoring of allograft recipients to recognize immunological complications early. Neopterin production provides prognostic information in patients with malignant tumor diseases and in HIV-infected individuals, high levels being associated with poorer survival expectations. Neopterin measurements are also useful to monitor therapy in patients with autoimmune disorders and in individuals with HIV infection. Screening of neopterin concentrations in blood donations allows to detect acute infections in a non-specific way and improves safety of blood transfusions.

As high neopterin production is associated with increased production of reactive oxygen species and with low serum concentrations of antioxidants like α-tocopherol, neopterin can also be regarded as a marker of reactive oxygen species formed by the activated cellular immune system. Therefore, by neopterin measurements not only the extent of cellular immune activation but also the extent of oxidative stress can be estimated.

INTRODUCTION

Activation of the immune system plays a key role in various diseases like infections, autoimmune and malignant tumor diseases or in cases of allograft transplantation. Also in neurological and cardiovascular diseases immunological processes are discussed. For clearing out pathogenesis and finding appropriate therapy, early and sensitive monitoring of immunological changes in patients is important. With this respect, by measurements of neopterin in human body fluids monitoring of cellular immune activation can by sensitively and easily done [1-3].

BIOSYNTHESIS OF NEOPTERIN DERIVATIVES

Neopterin, 2-amino-4-hydroxy-6-(D-erythro-1´, 2´, 3´-trihydroxypropyl)-pteridine (Fig. **1**), belongs to the class of pteridines which biosynthetically derives from guanosine triphosphate (GTP). GTP cyclohydrolase I (EC 3.5.4.16) cleaves the purin to synthesize 7,8-dihydroneopterin triphosphate. This intermediate is converted by 6-pyruvoyl-tetrahydropterin synthase to form dihydrobiopterin in the biosynthetic pathway of 5,6,7,8-tetrahydrobiopterin. Tetrahydrobiopterin is an essential cofactor of several mono-oxygenases including phenylalanine-, tyrosine- and tryptophan-5-hydroxylase, and the nitric oxide synthases (for further details see Werner-Felmayer, G., *et al.* and Gorren, A.C.F., & Mayer, B., this issue).

When GTP cyclohydrolase I is activated, most cells like fibroblasts or endothelial cells of several species produce tetrahydrobiopterin and only scarce amounts of neopterin derivatives are formed. However, due to a relative deficiency of 6-pyruvoyl-tetrahydropterin synthase in human and primate monocytes/macrophages, activation of GTP cyclo-hydrolase I leads to an accumulation of 7,8-dihydroneopterin triphosphate at the expense of tetrahydrobiopterin. 7,8-Dihydroneopterin triphosphate is then converted by phos-phatases to neopterin and 7,8-dihydroneopterin (Fig. **2**) [4].

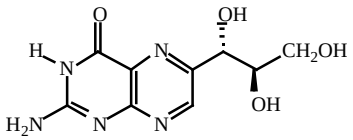

Fig. (1). Neopterin (2-amino-4-hydroxy-6-(D-erythro- 1´,2´, 3´-tryhydroxypropyl)-pteridine).

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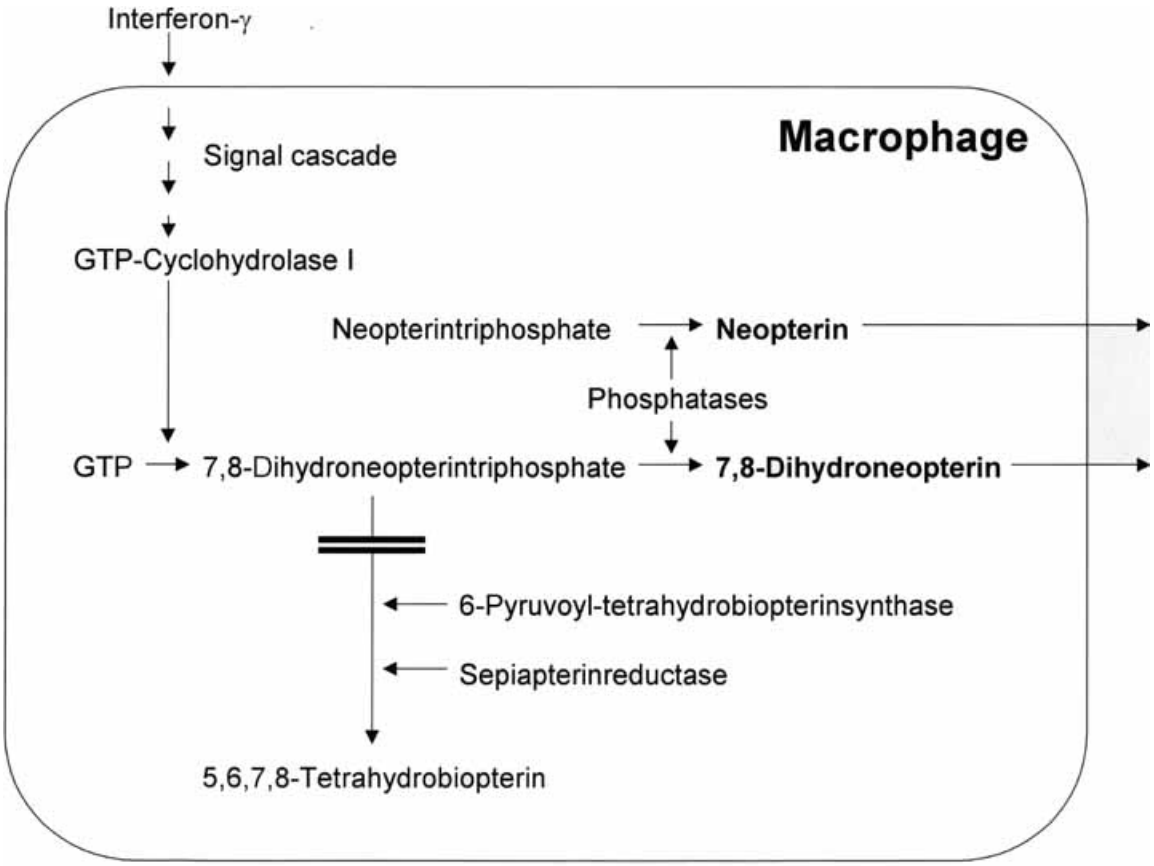

Fig. (2). Biosynthesis of neopterin derivatives in human monocytes/macrophages. Synthesis starts from GTP (guanosine triphosphate) by induction of GTP cyclohydrolase I via interferon-γ. Large amounts of neopterin derivatives are accumulating at the expense of biopterin derivatives due to a constitutive deficiency of 6-pyruvoyl tetrahydrobiopterin synthase in human macrophages.

of activated human monocytes/macrophages and in body fluids of humans and primates in a rather constant ratio of neopterin per 7,8-dihydroneopterin of about 1:3 [1,2,5]. From this background, human monocytes/macrophages appear to constitute the most relevant source of neopterin and 7,8-dihydroneopterin.

PTERIDINE PRODUCTION AND IMMUNOLOGY

Interferon-γ (IFN-γ) is the central stimulus for the activation of GTP cyclohydrolase I, and therefore, in the 6-pyruvoyl-tetrahydropterin synthase-deficient human monocytes/macrophages neopterin and 7,8-dihydronepterin accumulate [6]. *In vitro*, neopterin production can also be induced upon stimulation with interferon-α, however at least a 1000-fold higher dose is necessary to achieve a comparable neopterin level as compared with IFN-γ [6]. Other potent inducers of macrophage activity such as zymosan, phorbol ester, colony stimulating factor, granulocyte/monocyte colony-stimulating-factor or interferon-β, do not induce the release of significant amounts of neopterin [7], but lipopolysaccharide and tumor necrosis factor-α (TNF-α) superinduce IFN-γ-mediated neopterin production [8].

Also other cells, such as human umbilical vein endothelial cells [9] or cultured kidney epithelial cells [10], may produce neopterin upon stimulation with IFN-γ, but to smaller amount than macrophages [10]. This neopterin production by epithelial cells is accompanied by the production of tetrahydrobiopterin [11]. As in human diseases like, e.g., infections by human immunodeficiency virus (HIV), increased neopterin but not biopterin production can be found [12], in case of immune stimulation by IFN-γ neopterin is mainly produced by human monocytes/ macrophages and not by epithelial cells [13].

In patients several cytokines such as, e.g. interleukin-(IL)-2, which are able to induce IFN-γ -release from T cells, also provoke neopterin release [14]. Similarly administration of granulocytes/monocytes stimulating factor (GM-CSF) enhances neopterin production in patients [15] probably by increasing the number of monocytes/macrophages.

In patients, usually a significant relationship extsts between neopterin and IFN-γ concentrations [16]. However, also treatment with IFN-α may increase neopterin concentrations, and as expected, in patients with chronic hepatitis C the significant correlation between neopterin and IFN-γ concentrations which exists at baseline was completely abolished when patients were treated with high doses of IFN-α2b [17]. When treatment was stopped, the correlation between neopterin and IFN-γ reappeared. Thus, in chronic hepatitis C, treatment with IFN-α2b seemed to increase neopterin concentrations without an elevation of IFN-γ.

As IL-2 and especially IFN-γ are cytokines typically produced by T helper (Th) cells subtype 1 [18], which are promoting immune response mediated by cytotoxic T cells, increased production of neopterin in body fluids can be used to monitor activation of cell-mediated immunity. Accordingly, diseases like virus infections are accompanied by increased neopterin production, because cell-mediated immune response dominates [2]. On the other hand, in situations like, e.g., acute bacterial infections, only moderate neopterin production can be found. In this case, Th2 cell immune response dominates, characterized by the formation of IL-4, -5, -6, -9, -10 and -13, which are supporting humoral immune response [19]. A cross-regulatory influence exists between Th1 and Th2 cell mediated immune responses, down-regulating each other when activated. This is evident from *in vitro* [20] and *in vivo* [21] investigations. However, sometimes an activation of both T helper cell-compartments alternatively or a shift to the Th0 phenotype seems to exist [22]. E.g., in acute episodes of graft-versus-host disease after human allogeneic bone marrow transplantation, a significant positive correlation between plasma neopterin levels and the Th2-derived cytokine IL-10 was found [23]. As neopterin derivatives were found to be produced in large quantities by human monocytes/macro-phages upon stimulation with IFN-γ [6] and the amount of neopterin secreted correlates with the capacity of the same cells to produce reactive oxygen species (ROS) [7,24], neopterin derivatives may be regarded as an indicator for oxidative stress due to immune activation as well [25,26].

THE BIOLOGICAL IMPACT OF NEOPTERIN DERIVATIVES

A clear biological function of neopterin derivatives is not fully understood up to now, but recently neopterin derivatives were found to interfere with redox-systems. For example, neopterin enhances chloramine-T- and H_2O_2-mediated chemiluminescence *in vitro* [27]. Interestingly, effects of neopterin on H_2O_2 are enhanced at neutral and slightly alkaline pH in the presence of iron chelator complexes [28]. In other environmental conditions, e.g., in the absence of iron, neopterin is a potent scavenger of H_2O_2-induced chemiluminescence [27-29]. In addition, neopterin and 7,8-dihydroneopterin inhibit xanthine oxidase [30], and neopterin was found to suppress superoxide-generating NADPH-oxidase in macrophages stimulated with phorbolmyristate acetate [31].

Reduced pteridine derivatives like 7,8-dihydroneopterin and 5,6,7,8-tetrahydroneopterin are antioxidants and were generally described to be potent scavengers *in vitro* and *in vivo* [27-29]. Only at very high concentrations (>3mM) 7,8-dihydroneopterin acts as an enhancer, and this is true even in the absence of iron [32-33]. Neopterin is also able to enhance toxicity of peroxynitrite, which is formed upon reaction of nitric oxide with superoxide anion. Thus, tyrosine nitration by peroxynitrite is enhanced by neopterin, whereas 7,8-dihydroneopterin inhibits this process [34].

Additional findings made in bacterial cultures and in molecular biological experiments further support the view that neopterin and 7,8-dihydroneopterin are capable of modulating the effects of ROS. For example, neopterin was found to enhance H_2O_2-, hypochlorite- or chloramine-T-mediated toxicity against bacteria, whereas 7,8-dihydroneopterin suppressed these effects [27, 35, 36]. From these findings it seems to be possible that neopterin derivatives influence the effects and cytotoxicity of ROS which are produced by activated macrophages within the oxidative burst. In human monocytes/macrophages neopterin is produced at the expense of biopterin derivatives, which are utilized for nitric oxide production in other species. *In vitro* investigations with human macrophages reveal that stimulation of high-output nitric oxide production can be achieved only after rather complex pretreatment with cytokines such as IL-4 [37], and it seems for the *in vivo* situation that formation of nitric oxide by human macrophages probably will be of limited relevance. By modulating the toxicity of other ROS like hydrogen peroxide, the production of neopterin derivatives may compensate the relative deficiency to produce nitric oxide in human and primate macrophages (Fig. **3**) [38].

Neopterin and 7,8-dihydroneopterin were also found to interfere with intracellular signaling pathways known to be influenced by oxidative stress. Neopterin and 7,8-dihydo-neopterin activate the redox-sensitive transcription factor nuclear factor-κB (NF-κB) in Jurkat cells [39] and in murine vascular smooth muscle cells [40]. *In vitro*, neopterin inhibits hypoxia-induced erythropoietin gene expression and erythropoietin formation in HepG2 cell cultures [41]. In the same way neopterin was able to lower erythropoietin production in hypoxically perfused isolated rat kidneys [42]. Neopterin proved as a stimulus for cytokine-inducible nitric oxide synthase gene expression in rat vascular smooth muscle cells [43]. Both, neopterin and 7,8-dihydronepterin, proved together with c-GMP as inductors of the redox-sensitive proto-oncogene c-fos in NIH 3T3 fibroblasts [44] as well as neopterin enhanced cell damage caused by UV-A irradiation of B-16 melanoma cells [45].

Neopterin and 7,8-dihydroneopterin can induce apoptosis in rat alveolar epithelial cell line L2 [46]. In human monocytic U937 cells 7,8-dihydroneopterin shows a biphasic effect: lower concentrations of it (<300μM) decrease TNF-α-induced apoptosis, whereas higher concentrations (5mM) superinduce apoptosis [32]. The high dose of 7,8-dihydroneopterin, in combination with TNF-α, apparently leads to increased formation of ROS, since apoptosis could be suppressed by antioxidants. This observation is in accordance with data from the literature, showing reduced pteridines to react with O_2 under formation of free radicals [47]. This fact is strengthened by the observation that 7,8-

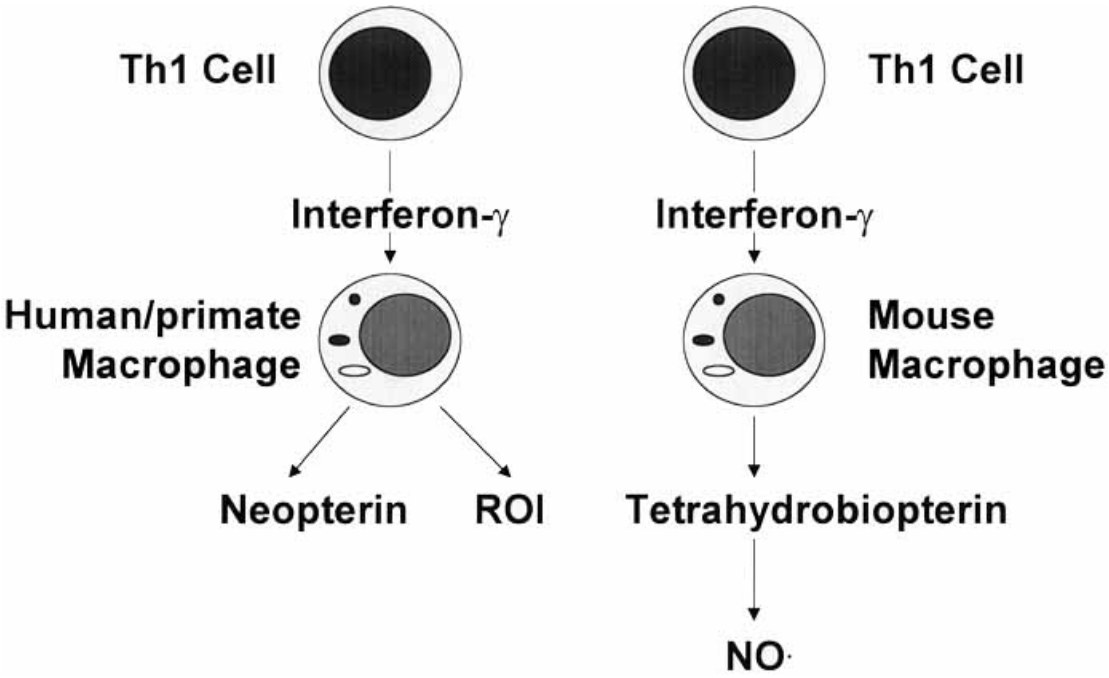

Fig. (3). Upon stimulation with interferon-γ, only human and primate macrophages produce large amounts of neopterin derivatives, whereas, e.g., murine macrophages produce tetrahydrobiopterin as cofactor of nitric oxide (NO) synthase (ROI = reactive oxygen species).

dihydroneopterin catalyzes the formation of hydroxyl radical from molecular oxygen as was shown by the hydroxylation of salicylic acid [48] (for further details see also Oettl, K., & Reibnegger, G., this issue).

In addition, neopterin derivatives increase intracellular Ca^{++} levels in human monocytes [49] and 7,8-dihydroneopterin together with H_2O_2 up-regulates the production of IFN-γ by T-cells, thereby establishing an autocrine feed-back loop [50]. Based on these observations, we hypothesize that neopterin and 7,8-dihydroneopterin may act as endogenous agents influencing redox balances in biological systems [25]. As neopterin and 7,8-dihydroneopterin are concomitantly produced, intracellular and environmental conditions, such as pH value or iron availability, may direct whether the enhancing or scavenging properties of the neopterin and 7,8-dihydroneopterin mixture become relevant. Recently it was demonstrated *in vitro* that hypochlorous acid oxidizes 7,8-dihydroneopterin to form neopterin, thereby increasing the neopterin/7,8-dihydroneopterin ratio towards neopterin accompanied by an increased oxidative potential in the microenvironment [51].

In vivo observations are able to confirm the *in vitro* experiments: In older-aged people [52] and in elderly demented patients [53] an inverse correlation between increased neopterin levels and decreased levels of the antioxidant α-tocopherol was demonstrated. In patients with diabetic nephropathy a correlation exists between neopterin and advanced oxidation protein products [24], the latter compounds represent a reliable indicator for the degree of oxidant-mediated protein damage. Therefore, the result suggests a relation between oxidative stress and monocyte activation as monitored by neopterin concentrations. The data agree well with the conclusion that neopterin is not only an indicator for oxidative stress resulting from immune activation, but neopterin itself contributes to oxidative stress by modulating the effects of ROS [25].

MEASUREMENT OF NEOPTERIN CONCENTRATIONS IN BIOLOGICAL FLUIDS

Neopterin and 7,8-dihydroneopterin are small molecular mass molecules (253 and 255 D), which are produced and released in a remarkably constant proportion with a ratio of aromatic neopterin to total (aromatic plus acid-oxidizable 7,8-dihydroneopterin) neopterin of 1:3 for urine and arterial blood [5] and 1:2 for serum obtained from venous blood samples. Since dihydroforms of pteridines are labile, collection and storage of samples is critical and problematic for large scale clinical handling. In daily clinical routine, advantageously only the more stable neopterin is being quantified. Serum neopterin may be determined by immunoassays in an easy way, neopterin concentrations averaging 5.3 ± 2.7 nM in healthy adults [1, 5]. Since neopterin is constantly distributed in body fluids, alternative or additional measurement of neopterin concentrations in urine specimens can be performed. To take variations of urine densities into account, urinary neopterin concentrations

are expressed in µmol/mol creatinine which can advantageously be done by examining samples by high-pressure liquid chromatography [54]. For reference values see Table **1**.

NEOPTERIN IN INFECTIOUS DISEASES

During acute viral infections strongly increased neopterin production can be observed, which correlates with the activity of the disease. This was shown in, e.g., acute viral hepatitis, Epstein-Barr-virus- and cytomegalovirus-infections, measles, mumps, chickenbox, rubella and influenza [55-61]. Thereby elevated neopterin levels in body fluids may be already found at the end of the incubation period before onset of clinical symptoms. The highest neopterin levels occur just before specific antibodies against the virus become detectable, which occurs up to about two to four weeks after onset of increased neopterin production. On seroconversion, neopterin concentrations decline and normalize, if the immune system successfully competes the infecting agent (Fig. **4**).

In case of chronic viral infections such as those by HIV, neopterin production declines after seroconversion, but does not normalize. Thus, more than three quarters of the HIV-infected show still elevated neopterin production although being without symptoms. Already in this early phase of the disease, the amount of neopterin production is of prognostic value: The higher the neopterin concentration in serum or urine, the more rapid disease progression and development of the acquired immunodeficiency syndrome (AIDS) will happen. With disease progression neopterin production increases again with the highest values when full-blown AIDS develops, paralleled by decreasing CD4+ T-lymphocyte counts [62-68]. In general, neopterin

Table 1. Reference Values of Neopterin in Urine and Serum

Urine (µmol neopterin/mol creatinine)				
	Children			
Age (years)	**Mean ± SD**	**97.5th percentile**		
1-3	267 ± 94	432		
3-7	226 ± 76	405		
7-11	181 ± 73	374		
12-14	171 ± 73	343		
15-18	144 ± 65	320		
	Men		**Women**	
Age (years)	**Mean ± SD**	**97.5th percentile**	**Mean ± SD**	**97.5th percentile**
19-25	123 ± 30	195	128 ± 33	208
26-35	101 ± 33	182	124 ± 33	209
36-45	109 ± 28	176	140 ± 39	239
46-55	105 ± 36	197	147 ± 32	229
56-65	119 ± 39	218	156 ± 35	249
>65	133 ± 38	229	151 ± 40	251
Serum (nmol/l)				
	Serum			
Age (years)	**Mean ± SD**	**95th percentile**		
<18	6.8 ± 3.6	13.5		
19-75	5.3 ± 2.7	8.7		
>75	9.7 ± 5.0	19.0		

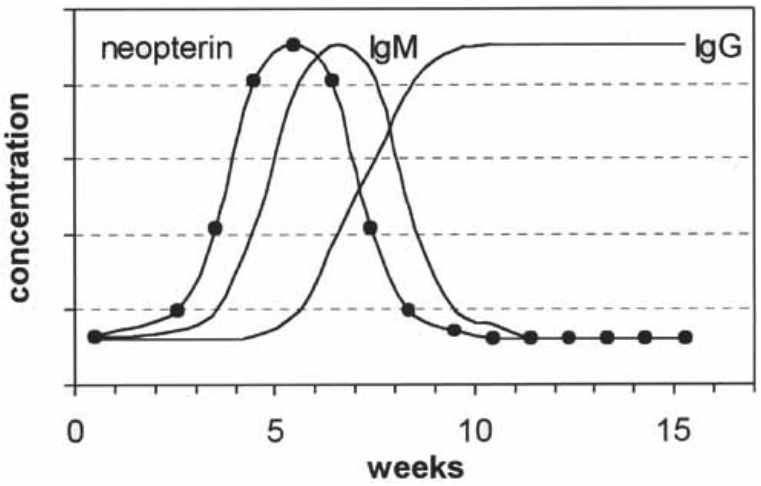

Fig. (4). Schematic course of neopterin (filled circles) concentrations in serum or urine and immunoglobulin (Ig) levels in humans during acute virus infection.

concentrations provide similar prognostic values for disease progression as other predictive markers like CD4+ T-cell counts or quantitative polymerase chain reaction (PCR) for HIV-1 RNA. Neopterin values also correlate with viral load [69,70]. Antiretroviral therapy is reflected in a decrease of neopterin production [71,72].

In acute bacterial infections neopterin production is usually low, as humoral immune response dominates. Therefore neopterin measurements could be an additional tool to support differential diagnosis between viral and bacterial infection in special clinical situations. This was reported in patients with pneumonia, where neopterin discriminated better than blood sedimentation rate or leucocyte counts between viral and bacterial infection [19,73]. In protracted bacterial infection neopterin production increases. Highest neopterin concentrations in body fluids are found in case of septic complications [74] and due to lipopolysaccharides of Gram-negative bacteria like, e.g., *Pseudomonas pseudomallei* [75] or *Brucella melitensis* [76]. On the other hand, bacteria like *Streptococcus pyogenes* produce exotoxins with superantigen character [77], which are supposed to play a key role in the so called "streptococcal toxic shock-like syndrome" which is associated with high neopterin production as well [78].

In infections by *Mycobacterium tuberculosis*, a facultative intracellular bacterium, cellular immunity plays a central role in immune defense. In agreement, in lung tuberculosis neopterin production correlates with the extent and activity of the disease [79] and provides useful information in therapy control [80] even if their is an HIV-1 coinfection [81]. Also in leprosy, which is caused by *Mycobacterium leprae*, activated cellular immune response is indicated by increased neopterin production and, 75% of patients with tuberculoid and lepromatous leprosy present with elevated urinary neopterin excretion [82].

In infections by parasites, like acute malaria high neopterin formation was found [83,84]. After effective chemotherapy elevated concentrations in serum or urine rapidly decrease to normal values within several days [85]. An inverse association between the duration of parasitic load and neopterin production was found, indicating that with duration of the disease humoral immunity develops while severity of the disease and cellular immune activation decline [86].

In Lyme neuroborreliosis, a late complication of infections by tick-born spirochete *Borrelia burgdorferi*, high neopterin concentrations are found in cerebrospinal fluid of patients, whereas serum neopterin levels are not markedly increased, confirming intrathecal neopterin production [87,88].

NEOPTERIN IN MALIGNANT TUMOR DISEASES

As malignant tumor cells exhibit with altered cell surface compared to non-malignant cells, they may cause reactions of the specific cellular immune system and thereby also neopterin production. The frequency of elevated neopterin concentrations in serum or urine of patients with malignant diseases at the moment of diagnosis depends on the tumor type and varies from about 90% in hematological neoplasias to about 20% in tumors like breast cancer or malignant melanoma [89]. Intermediate frequencies of elevated neopterin concentrations are found in ovarian cancer (about 80%), pancreatic carcinoma (about 70%), lung cancer (about 58%) and cervical carcinoma (about 55%). Therefore neopterin measurements are certainly insufficient for screening or diagnostic purposes (Table **2**).

Not only tumor type, but also tumor stage influences the extent of neopterin elevation. In general, advanced stages show higher neopterin values than earlier ones. On the other hand, successful treatment, indicated by remission in hematological neoplasias, is associated with decline or even normalization of neopterin values in most cases [89,1]. Also a correlation between urinary neopterin levels and the estimated total mass of tumor cells was shown [90].

The most striking observation from several studies on malignant tumor diseases is that neopterin concentrations

Table 2. Increased Neopterin Concentrations in Body Fluids and their Prognostic Impact in Patients with Cancer at the Moment of Diagnosis of the Disease

Tumor	Frequency with increased neopterin	Prognostic value
Hodgkin`s disease (stage I-IV)	70-100	yes
CML	95	no
non-*Hodgkin*`s disease, CLL	92	yes
ovarian carcinoma	82	yes
uterine sarcoma	78	not done
multiple myeloma (stage I-III)	30-70	yes
pancreas carcinoma	69	not done
lung cancer	58	yes
cervical carcinoma	55	yes
colon carcinoma	48	yes
squamous cell carinoma of the oral cavity	43	yes
stomach carcinoma	42	not done
prostatic cancer	25	yes
head & neck carcinoma	23	not done
malignant melanoma	below 25	not done
breast carcinoma	18	yes
hepatocellular carcinoma	not done	yes

proved to be a significant and independent predictor of patients' survival. Thereby higher urinary or serum neopterin concentrations were associated with a worse outcome. This was shown, e.g., in hematological neoplasias [91-92], in carcinoma of the uterine cervix [93] or of the ovaries [94], in colon carcinoma [95], lung cancer [96,97], prostate cancer [98], hepatocellular cancer [99], squamous cell carcinoma of the oral cavity [100] and female breast cancer [101]. Furthermore, neopterin monitoring in the post-therapeutic phase can help to detect tumor relapse earlier and may provide an indication for adjuvant therapeutic measures [94]. Nevertheless, it is important to stress that in most cases conventional tumor markers, which are a product of the tumor, correlate better with tumor growth than neopterin, whereas neopterin values being a better predictor of patients` survival [94].

The worse prognosis of those with higher neopterin production might be explained by a cellular immune response against the tumor which might be stronger in patients with more aggressive tumors. However, the immune system seems nevertheless unable to eradicate the malignant process, and a kind of chronic immune activation has already been entered. From additional investigations it appears that phenomena like tumor anemia or tumor cachexia, which are often associated with malignant disease, might be a consequence of this immune stimulation. In hematological neoplasias increased neopterin showed a significant inverse correlation with blood hemoglobin [16], which might be due to an inhibition of erythropoietin formation by neopterin [41,42]. TNF-α, produced by activated monocytes/ macrophages [102], might contribute to cachexia. Thus, high

Table 3. Diagnostic Impact of Neopterin Measurements in Diseases with Increased Neopterin Formation

Infections by viruses, parasites or intracellular bacteria	Correlation with disease activity-Neopterin before seroconversion detectable-Control of therapy
Malignant tumor disease	Prognostic impact
Autoimmune diseases	Correlation with extent of the disease-Control of therapy
Organ transplantation	Monitoring for detection of immunological complications such as allograft rejection or infection
Blood transfusions	Screening test for detection of acute infections and other inflammatory diseases

levels of soluble tumor necrosis factor receptors were reported in hematological neoplasias, the concentrations of which correlated with weight loss as well as with increased neopterin and decreased hemoglobin levels [103].

NEOPTERIN IN AUTOIMMUNE DISEASES AND RELATED INFLAMMATORY DISORDERS

In autoimmune diseases an attack of the immune system against autologous structures of the organism occurs. When cellular immunity is involved, neopterin elevations should be expected. Indeed, in rheumatoid arthritis elevated neopterin concentrations are found in blood and urine correlating with the disease activity [104]. Highest neopterin levels are seen in synovial fluids of patients during the acute exacerbation of the disease [105]. Neopterin measurements can also be applied as an additional criterion to discriminate rheumatoid arthritis from osteoarthritis, because in the latter case neopterin production is usually within the normal range [104]. In patients with systemic lupus erythematosus neopterin production correlates with disease activity, as well, and in a multivariate analysis, neopterin proved as one of the most representative immune activation markers, correlating with disease activity better than, e.g., 55kD-soluble tumor necrosis factor receptor, soluble interleukin-2 receptor or beta-2-microglobulin [106]. Increased neopterin production in accordance with disease activity was also demonstrated in Wegener's granulomatosis [107], dermatomyositis [108], and inflammatory bowel diseases, such as Crohn's disease [109,110] and ulcerative colitis [111].

NEOPTERIN IN CARDIOVASCULAR DISEASES

Increased neopterin production was found in atherosclerosis of the coronary [112] and carotid arteries [113], and in acute and chronic coronary syndromes [114, 115]. Neopterin was significantly increased in patients with chronic coronary artery disease and more pronounced in patients with acute myocardial infarction [116]. An association of serum neopterin concentrations with the presence of angiographically demonstrated complex lesions in patients with unstable angina could be shown, neopterin representing as a marker for coronary disease activity [117]. Besides neopterin, other markers of inflammation like C-reactive protein or serum amyloid A are increased in patients with atherosclerosis indicating the inflammatory nature of atherosclerotic disease [112,118].

Increased neopterin production is also found in patients with dilated cardiomyopathy or chronic myocarditis, correlating with the cardiac functional class according to the New York Heart Association [119]. About 80% of patients with acute rheumatic fever present with increased neopterin production at the onset of the disease and immune activation seems to play an important role in the pathogenesis of complications, because still higher neopterin concentrations are found in patients developing a combined aortic and mitral insufficiency. Thus, neopterin measurements would allow easy assessment of the severity of cardiac involvement in acute rheumatic fever [120].

NEOPTERIN IN AGING AND NEURODE-GENERATION

In healthy individuals an increase of neopterin production with increasing age was found [121-124], which is also evident from the age dependency of the reference values of neopterin concentrations in different body fluids (see Table **1**). The reason for this phenomenon is still a point of discussions. Possibly the higher incidence of diseases associated with immune activation in the elderly such as, e.g., artherosclerosis or dementia would contribute to higher reference values. Thereby it is assumed that in some of the individuals of the reference population the pathological process has already started but is clinically not yet detectable.

In neurodegenerative diseases signs of immune activation and increased oxidative stress are evident, and increased neopterin production is found in serum and cerebrospinal fluid of patients with Alzheimer's dementia and neopterin concentrations correlate with the cognitive decline in patients [125]. Similar findings were made in patients suffering from Huntington's disease, which is characterized by progressive neuronal loss in the striatum and cortex leading to choreiform movements and dementia. Thereby neopterin production is again associated with the loss of cognitive function and higher neopterin concentrations are predictive for shorter survival expectations [126]. It is still unclear, if such chronic immune activation, indicated by increased neopterin production has any relevance for pathogenesis of the disease, but monitoring of immune activation markers like neopterin might lead to better understanding of pathology of such diseases.

NEOPTERIN IN ORGAN TRANSPLANTATION

Monitoring of solid allograft (kidney, heart, liver, pancreas) recipients by neopterin measurements in morning urine or serum/plasma has become a useful tool in surveillance after transplantation. In uncomplicated courses neopterin production remains stable or decreases to normal values after surgery, whereas sustaining high concentrations indicate an immunological complication such as organ rejection or infection. In recipients of renal allografts it was found that an increase of neopterin production precedes clinical rejection diagnosis up to four days and that high neopterin values during the initial posttransplant period are associated with poorer long-term graft survival [57,127]. Neopterin measurements support differential diagnosis of rejection and infection, the sensitivity and specificity can be significantly improved in combination with other parameters of inflammation [128) (for details see also Grebe, S.O., *et al.*, this issue). In liver transplantation, parallel measurement of neopterin concentrations in urine and bile allows discrimination between ongoing rejection and infection, because excessive bilary neopterin excretion is restricted to rejection episodes but is less frequent in, e.g., cytomegalovirus infection or hepatitis [129]. Also in pancreas transplantation, measurement of neopterin excretion in the pancreatic juice provides additional information about the origin of immune activation [130]. In bone marrow transplantation, neopterin measurement allows

to monitor the course of immune system destruction and hemopoietic reconstitution. After hemopoietic reconstitution it enables to discriminate between patients with or without increased risk of developing graft versus host disease and viral infection, respectively [131].

NEOPTERIN IN BLOOD TRANSFUSION

During the last years considerable progress regarding safety of blood transfusion was achieved either by serologic or molecular biology techniques like polymerase chain reaction (PCR) supporting blood donor screening. Nevertheless, residual infectious risk still remains in blood transfusion. Potential hazardous pathogens may remain undetected either because they are known but not screened or they are unknown so that usually performed screening technologies are ineffective. In addition, it is possible that blood is donated during the diagnostic window, when the donor is already productively infected but antibody production is not yet detectable. During acute infections neopterin concentrations in serum generally reach rather high values. This enhanced neopterin production is not specific for a certain infectious disease, but indicates that an immunological process is going on. Because various virus infections cause neopterin elevation, neopterin screening can serve as a non-specific umbrella against several known or even hitherto unknown viral pathogens. Neopterin screening of blood donations allows to detect and exclude virus infections during the acute phase when virus load is highest and allows to further shorten the diagnostic window in addition to specific serologic screening methods [132-133]. In 1994 neopterin screening of blood donations became compulsory in Austria. In this way subclinical infections or silent systemic disorders may be detected in a higher frequency by increased neopterin concentrations and suspicious blood units are discarded to increase the security of transfusion [134]. It was shown in blood donors with neopterin concentrations above 10 nmol/l that serologically verified acute cytomegalovirus infections were about 19 times more frequent than in individuals with neopterin below 10 nmol/l. Similarly, the incidence of acute Epstein-Barr virus and parvovirus B19 infections was accompanied by a about three times higher frequency in individuals with neopterin above 10 nmol/l. Also in chronic and clinically unsuspicious hepatitis C infections PCR-positively tested donations were about seven times more frequent when neopterin was above 10 nmol/l [135].

CONCLUSION

Neopterin is produced by human monocytes/macrophages upon stimulation with the cytokine interferon-γ. Therefore, measurement of neopterin concentrations in body fluids like serum, cerebrospinal fluid or urine provides information about immune activation controlled by T helper cells type 1. Major diagnostic applications of neopterin measurements are (Table **3**) monitoring of the immune status of allograft recipients, and prognostic evaluation and monitoring treatment in HIV-infected individuals and in patients with malignant diseases. Also in some neurological and in cardiovascular diseases increased neopterin production is found. Monitoring of neopterin production might lead to a better understanding of pathogenesis of these diseases. Finally, the possible detection of unknown infections by screening of blood donations is of particular relevance.

As high neopterin production is associated with increased production of reactive oxygen species by stimulated immunocompetent cells, by neopterin measurements not only the extent of cellular immune activation, but also the extent of oxidative stress due to immune system activation may be estimated.

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