Fig. 1: Neopterin (D-erythro-1', 2', 3'-trihydroxypropylpterin)

Determinations of neopterin levels reflect the stage of activation of the cellular immune system which is of importance in the pathogenesis and progression of various diseases, e.g., in viral infections, in autoimmune or inflammatory diseases, rejection episodes following allograft transplantation and in several malignant diseases. Besides the importance of neopterin as a diagnostic marker new aspects point to a possible biological role of neopterin derivatives
(hard copies of this monograph are available at www.brahms.de).

1. Introduction

Increased neopterin concentrations in body-fluids, such as serum or urine, are connected with diseases linked with cellular immune reaction [1-6], e.g. viral infections, including HIV infection and infections by intracellularly living bacteria or parasites, autoimmune diseases, inflammatory diseases, rejection episodes following organ transplantation and certain malignant diseases. In all these different diseases the cellular immune system is involved in the pathogenesis and/or affected by the underlying disease process, and neopterin concentrations are very closely linked with the progression of these diseases. Therefore it is of interest for laboratory diagnosis to measure the degree of activation of the human immune system. This is possible in an easy but specific way by the determination of neopterin concentrations.

2. Relationship between immune activation, release of cytokines and formation of neopterin

2.1. Immunological and biological background of neopterin formation

If T-lymphocytes recognize foreign or modified-self cell structures they start producing different mediators, so-called lymphokines, such as interferon-γ. In a next step the formed interferon stimulates human monocytes/macrophages for the production and release of neopterin (Fig.2) [7]. Neopterin, 6-D-erythro-trihydroxypropyl-pterin, is a substance of low molecular mass, that becomes biosynthesized from guanosine-triphosphate (GTP) by the key-enzyme of pteridine-biosynthesis: GTP-cyclohydrolase I.
Thereby, GTP-cyclohydrolase cleaves guanosine triphosphate to form 7,8-dihydroneopterin-triphosphate as an intermediate. Unlike other cells and species human monocytes/ macrophages only have a small constitutive activity of the biopterin-forming enzyme pyruvoyl-tetrahydropterin synthase (PTPS), so that almost exclusively neopterin and 7,8-dihydroneopterin become synthesized and released [8]. Because of this considerable amounts of neopterin are only found in body-fluids of humans and primates. Accordingly, in supernatants of cultured human cells other than monocytes/macrophages or cells of other species almost exclusively biopterin derivates are detected and only in cell cultures of human monocytes/macrophages or of the myelomonocytic cell line THP-1 relevant amounts of neopterin are released. Interferon-γ was identified as the only cytokine that induces significant production of neopterin [9]. Other cytokines like different interleukins as well as factors inducing phagocytosis, e.g. zymosane, are unable to operate in this way. Also tumor necrosis factor-α cannot induce the formation of neopterin directly, it however costimulates the release of neopterin triggered by interferon-γ [10, 11]. There is reason to believe that the extent of neopterin formation reflects the combined effect of positively- and negatively-regulating influences on the population of monocytes/macrophages activated by interferon-γ. Interferon-γ is mainly formed by activated T-lymphocytes
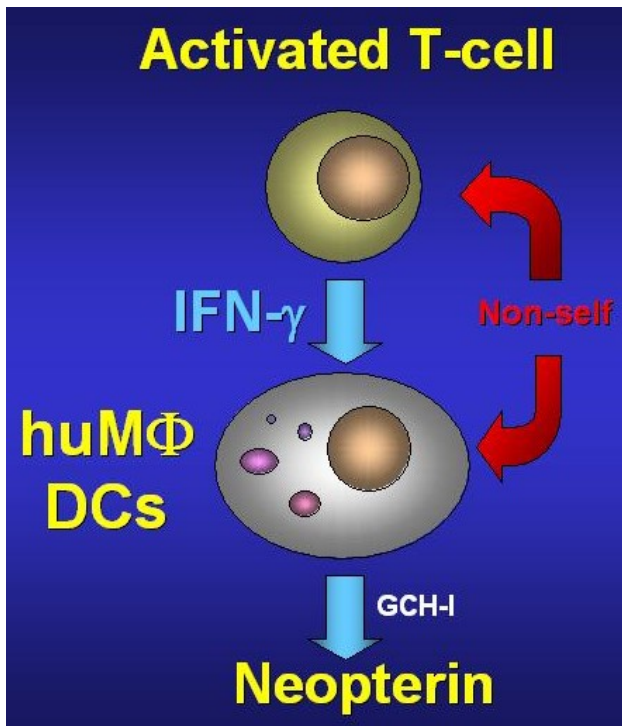
Figure 2: During cellular immune response, activated T-lymphocytes of the TH1-subtype release interferon-γ which in human macrophages (MΦ) and dendritic cells (DC) stimulates the enzyme GTP-cylohydrolase (GCH) and thus neopterin production.

and it is the definite trigger for neopterin formation. That is why the degree of activation of the T-lymphocytes, especially of so-called TH-1-type cells, which are responsible for the production of interferon-γ and interleukin-2 [Fig. 2], is of great importance for neopterin production [12].

Therefore substances that may influence the activity of this T-cell population are able to modify the degree of the activation of monocytes/macrophages indirectly and as a consequence the formation of neopterin. Exogenous addition of interleukin-2 to peripheral mononuclear cells but also in vivo leads to an increase of neopterin expression by the way of activation and expansion of T-cells, although there is no direct influence of this cytokine on neopterin production by monocytes/macrophages. Similarly, interleukin-12 is also able to amplify neopterin formation. On the opposit, immunsupressants such as cyclosporin-A, which inhibits formation of cytokines by Tcells, also reduce neopterin production.
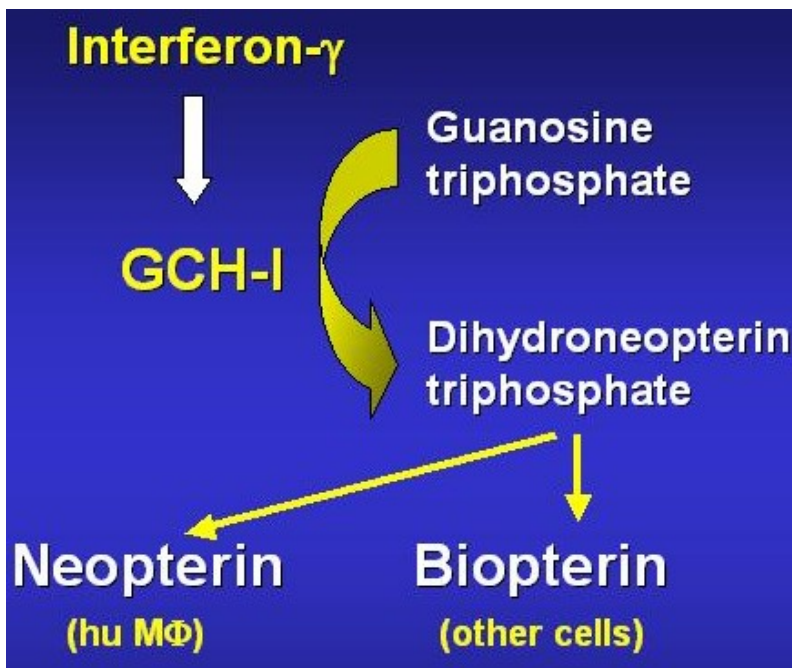

Figure 3: Formation of neopterin from guanosine triphosphate (GTP): cyclohydrolase 1 (GCH-1) cleaves GTP to form 7,8-dihydroneopterin-triphosphate. Because of a relative deficiency of the 6-pyruvoyl-tetrahydropterin synthase (PTPS) in human monocytes/macrophages neopterin and 7,8-dihydroneopterin are formed after dephosphorylation and oxidation at the expense of biopterin derivatives.

The value of neopterin monitoring is superior compared to the direct analysis of interferon-γ. Neopterin is biochemically inert and its half-life in the human organism is only due to renal excretion. The biological half-life and as a consequence the diagnostic accessibility of cytokines are limited by several circumstances: for instance released interferon-γ is bound very quickly to target structures or becomes neutralized by soluble

receptors. Because of this, locally formed cytokines often are unable to reach the blood circulation and are no good targets for routine laboratory diagnosis [13]. Beyond that, measurement of neopterin levels does not solely reflect the effect of one single cytokine, rather it allows to determine the total effect of the immunological network and interactions on the population of monocytes/ macrophages. The reflection of the multiple cooperations between immunocompetent cells seems to be the basis for the remarkable value of neopterin analyses as an immunodiagnostic tool.

Beside the processes that are connected with an activated cellular immune system, increased neopterin levels can be found in a rare inborn metabolic defect of biopterin biosynthesis, namely atypical phenylketonuria (PKU), in which detection of neopterin is also used for differential diagnosis [14]. Atypical PKU is linked with increased blood levels of phenylalanine in blood as it is the case in classical PKU and it is diagnosed in the first months of life. The incidence of this inborn defect which leads to an increase of neopterin in body-fluids is lower than 1 out of 1 million newborns.

2.2. Neopterin derivates and redox systems

Recent data indicate a possible participation of neopterin derivates within cytotoxic mechanism of macrophages [15]. On the one hand, a strong correlation between detection of neopterin and ability of monocytes/ macrophages to set free reactive oxygen species [16] was found. Thus, neopterin determination can be considered as an indirect marker for the amount of immunologically induced oxidative stress. On the other hand, neopterin derivates are able to influence the effects of reactive oxygen, nitrogen and clorine species. Investigating luminol chemiluminescence it was demonstrated that neopterin is a potent enhancer of various reactive substances, such as H_2O_2, HOCl, chloramine and peroxynitrite ($ONOO^-$, an effector molecule of nitric oxide radical, NO), while 7,8-dihydroneopterin is mainly acting as a scavanger. These results suggest a new physiological role of the neopterin formation, namely as an endogenous regulator of cytotoxic effector functions of activated macrophages [15].

Since neopterin production is a specific peculiarity for macrophages of primates it is of interest to clarify for what purpose these cells have obtained this additional ability. One of the most important cytotoxic reactions of macrophages stimulated by interferon-γ is the production of NO from arginine by the so-called inducible NO synthase (iNOS). This mechanism is known for various species, but until now it was detectable in humans only very limited [19]. iNOS requires 5,6,7,8-tetrahydrobiopterin as a cofactor for its function which is available only in a very low concentration in activated human monocytes/macrophages because of the excess production of neopterin. Therefore, it seems reasonable that this in the human monocytes/ macrophages deficient cytotoxic mechanism should be compensated by enhanced production of neopterin [20]. Besides it was found that neopterin is also able to enhance the effects of $ONOO^-$ [18, 21].

Neopterin derivates seem to be able to interfere also with molecular biological pathways that are regulated by redox-balances in general. In vitro, derivatives of neopterin induce expression of the nuclear factor-κB [22,23] and of the iNOS-gene [24]. Similar to this, programmed cell death, apoptosis, is induced or intensified by neopterin derivates [25,26] and for example formation of erythropoetin is inhibited [27]. Recent reports show that neopterin and 7,8-dihydroneopterin upregulate expression of proto-oncogene c-fos [28], so these substances may be even involved in malignant transformation of cells.

3. Neopterin detection in body fluids

Neopterin is a low molecular weight substance which is biologically and chemically stable in body fluids, and it can be applied without difficulty for routine measurements in laboratory diagnosis. Neopterin is eliminated by the kidney, and changes of neopterin concentrations in serum are reflected by urine levels [30] as long as renal function is not impaired. In fact, there is an equal sensitivity of neopterin measurements either in serum or urine. Measurement of 7,8-dihydroneopterin is not suitable for routine application in laboratory diagnosis [29]. This derivative is chemically unstable and easily decomposes due to oxidation, so that more stringent criteria for sample collection and handling restrict its usefulness as a clinical diagnostic. Because also neopterin is slightly sensitive to direct sun light irradiation, samples must be protected from light during transport and storage. In general enveloping of the samples, e.g. in aluminum foil, is sufficient, alternatively dark tubes may be used for collecting samples.

3.1. Detection in protein containing body fluids

Favourably neopterin levels in serum, plasma and other protein-containing body fluids, e.g. cerebrospinal fluid, pancreatic juice or ascites are determined by immunoassays (ELISA or radioimmunoassay) [29,30-32]. Neopterin concentrations in serum or plasma do not differ. For a single measurement of neopterin by immunoassay 20-100μl of serum, plasma or cerebrospinal fluid are required.

The content of neopterin in serum or plasma is stable for 3 days at room temperature, this allows mailing of samples without special cooling. For storage until one week cooling at 4°C is adequate, for a longer period of

time the samples must be kept frozen (-20°C till three months). Repeated thawing-freezing cycles must be avoided, it may decrease the content of neopterin. For measurements of neopterin concentrations in bile fluid [33] it is useful to dilute the samples with physiologic NaCl solution (=0.9% acqueous NaCl), e.g. 100µl bile fluid plus 1000µl NaCl solution.

3.2. Neopterin measurements in urine

Usually for the determination of neopterin in urine samples high pressure liquid chromatography (HPLC) on reversed phase (e.g., C18) with Sörensen phosphate buffer (e.g., 0.015 M, pH = 6.4) as eluent (flow rate = 1.0 ml/min) is applied. It allows a rapid, sensitive and precise detection and meets the requirements of quality in clinical chemical investigations [29]. 100 µl urine are mixed with 1000 µl elution buffer containing 2g/l EDTA to dissolve eventual urinary sediments. Neopterin is detected by measurement of its natural fluorescence (excitation wavelength 353 nm, emission wavelength 438 nm). After each day (= up to 100 samples), cleaning of the column is performed with a 20 min. gradient from buffer via water to 100% methanol, remaining on methanol for 20 min., then gradient via water goes back to buffer (within 20 min.).
Immunological methods are identical to the HPLC measurement. However, it has to be considered that neopterin concentrations in urine specimens spread within a greater range, because physiological dilution of urine adds to immunological changes of concentrations. Thus, it may become necessary to repeat measurements of the urine samples with concentrations beyond the range of linearity after an additional dilution step.
It is appropriate to use the first morning urine instead of the 24 hours urine to avoid laborious collection of 24 hours urine. Physiological differences in urine densities are taken into account by calculating the ratio of neopterin concentrations versus urinary creatinine which can be used as an internal standard since creatinine is excreted in relatively constant amounts during the day. When using HPLC for neopterin measurements, creatinine concentrations can be measured in parallel in the same chromatographic run by detection of its UV-absorption at 235 nm wavelength. The quotients neopterin/creatinine are usually expressed in µmol neopterin/mol creatinine. There exists a diurnal variation of the neopterin/creatinine ratios: in first morning urine specimens higher ratios (about +20%) are found than during the day (Table 1).
For a single detection by HPLC 100 µl of urine are necessary. Neopterin concentrations in urine specimens are stable for at least 3 days at room temperature, for storage till one week cooling at 4°C is sufficient, for a longer period of time samples must be kept frozen. Repeated thawing-freezing cycles have to be avoided.

3.3. Normal values

There is no difference between neopterin values detected in serum or plasma. On average the concentrations are 5.2+2.5 nmol/l neopterin (Table 1). Normal values and upper limits of tolerance (95th percentiles) depend on age. Therefore it is necessary to classify neopterin concentrations according to three age groups (Table 1). Neopterin concentrations in cerebrospinal fluid are somewhat lower than those in serum or plasma [34].

Tabelle 1: Neopterin concentrations (mean + S.D. and 97.5th percentiles) in various body fluids of healthy individuals

Neopterin in urine (µmol/mol creatinine):

Age	Males	97.5th	Females	97.5th
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

Neopterin in serum (nmol/l):

Age	Males and females	
19-75	5.3+2.7	8.7
>75	9.7+5.0	19.0

Neopterin in cerebrospinal fluid (nmol/l):

Age	Males and females	
19-75	4.2+1.0	5.5

Neopterin concentrations detected in the first morning urine are about 1500 nmol/l, concentrations of creatinine about 12 mmol/l; thus the normal value of healthy persons is calculated as about 125±5 µmol neopterin/mol creatinine. Normal values and upper limits of tolerance of urinary neopterin concentrations in healthy persons slightly depend on age and sex, which is mainly due to variations of urinary creatinine concentrations (Table 1).

Renal clearence of neopterin is similar to that of creatinine. Therefore neopterin per creatinine ratios in urine are not influenced by renal impairment [30]. Unlike to that blood neopterin concentrations depend on renal function, reduced renal excretion causes accumulation of neopterin in blood and one may find extremly high values in serum or plasma (200 nmol/l and more) in patients wirth uremia [35]. Thus, in patients with impaired renal function accumulation of neopterin may occur in the blood which is in addition to the enhanced formation of neopterin by immune system activation. Calculating the neopterin per creatinine ratio is suitable to at least partly account for the accumulation due to renal retardation.

4. Clinical application of neopterin measurements

In Table 2 useful applications of neopterin measurements in clinical laboratory diagnosis are listed. The following chapters provide a more detailed description of the value of neopterin measurements in these clinical situations.

Table 2.: List of applications for neopterin measurements in clinical diagnosis

Infections
virus infections and infections with intracellularly living bacteria and parasites
special importance for predicting prognosis in HIV infected persons
help for differential diagnosis between infections with bacteria versus viruses

Autoimmune diseases and other inflammatory diseases
rheumatoid arthritis, systemic lupus erythematosus, Wegener's granulomatosis, Crohn's disease, ulcerative colitis
help for differential diagnosis, e.g., rheumatoid arthritis versus osteoarthritis

Malignant diseases
predicting prognosis and follow-up in gynecological and hematological neoplasias, bronchus carcinoma and cancer of the prostate, gastrointestinal cancer

Allograft transplantation
Recognition of immunological complications like rejection or infection

Monitoring of treatment
immunmodulating therapy with cytokines
in diseases leading to increased neopterin levels (e.g., antiretroviral therapy of HIV-infection, antibacterial therapy of tuberculosis)
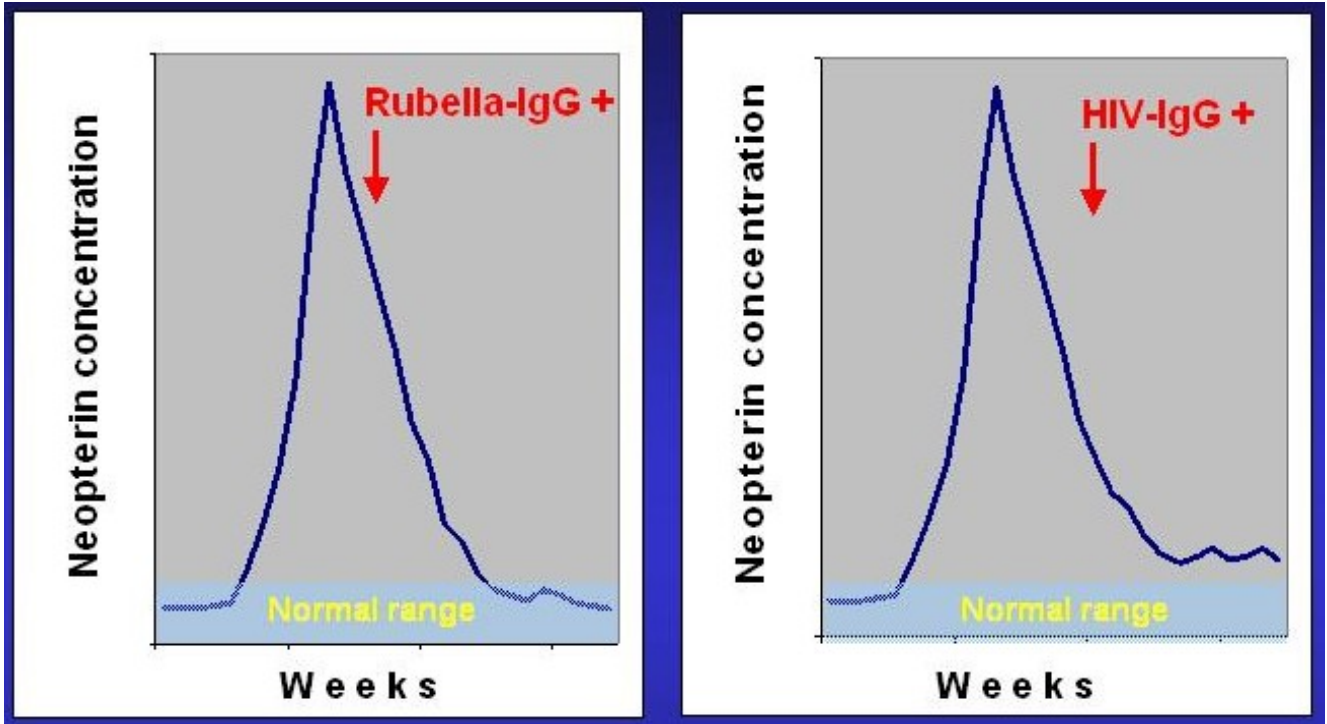
4.1. Infections

When the organism is challenged by infections with viruses, intracellularly living bacteria, parasites or fungi the cellular immune system becomes activated. In addition, immunological processes can be initiated by endotoxins produced by gram-negative bacteria which leads to an activation of T-lymphocytes and formation of interferon-γ and thus may lead to an increase of neopterin concentrations in body fluids.

4.1.1. Viral infections

In almost all patients with acute viral infections neopterin levels are increased, this was demonstrated in patients with acute hepatitis A or B [36], patients with Epstein-Barr-virus infection (infectious mononucleosis) and cytomegalovirus (CMV) infection [37] but also in patients with measles. Usually neopterin concenrations closely reflect the extent and the activity of the disease. Neopterin determinations may also be used as an

additional parameter for differential diagnosis, e.g. patients with chronic non A/non B-hepatitis show significantly higher neopterin levels than patients with non-infectious fatty liver [39].

During the course of viral infections neopterin levels usually increase already before first symptoms appear and before formation of specific antibodies becomes detectable [1]. After antibody seroconversion neopterin levels decrease and return into the normal range during the period of reconvalescence within a few days (Fig.5). The course of neopterin concentrations does not differ essentially between various viral infections, e.g. neopterin concentrations behave similar during infections with CMV, a DNA-viruses, and rubella, which is caused by an RNA-virus [40,41].

Figure 4: Schematic course of neopterin levels in a patient during an acute rubella virus infection (left, [41]) and during an acute HIV-infection (right, [42]): In both events the increase of neopterin levels begins before seroconversion (IgG+). In contrast to the rubella virus infection in which the neopterin levels return to baseline after seroconversion, they usually remain elevated following acute HIV-infection (right).

4.1.1.1. Infections with human immunodeficiency virus (HIV)

Increased neopterin levels in serum and urine can be found in almost 100% of patients with AIDS. But already in early stages of HIV-infection increased neopterin levels are frequently found [1]. The range of neopterin levels in the acute stage of the HIV infection is similar to that of other virus infections especially in the period before seroconversion, but unlike to other infections after seroconversion neopterin levels do not normalize completely in HIV infected individuals [42]. In contrast, after seroconversion neopterin levels remain outside the normal range [43,44] in about 80% of HIV infected individuals. Thus, the subsequent asymptomatic stage of HIV infection is associated with chronically increased neopterin levels in almost every individual. With the progression of the disease neopterin levels increase more and more, and highest values of neopterin in serum and urine are detected in patients with AIDS (Fig.5).

This development of neopterin concentrations was confirmed in rhesus macaques experimentally infected with simian immundeficiency virus, SIV, a simian analog of HIV [45]. In this case neopterin levels also increase after inoculation of SIV within about 5 days, again before seroconversion takes place. Afterwards neopterin levels decline but remain outside the normal range, then correlating with the progression of the infection.

Neopterin has become a well accepted parameter for the prediction of the future course of the HIV infection [43,44,46]. More rapid disease progrssion is early indicated by higher and further increasing neopterin levels. Already at an early stage neopterin values provide a significant prognostic information for the future course of the infection and for the risk of developing AIDS or for death (Fig.6). The predictive value of neopterin concentrations is at least comparable to that of the number of CD4+ T-cells [43]. More recent studies show that neopterin monitoring is also useful in addition to quantitative measurements of virus load such as
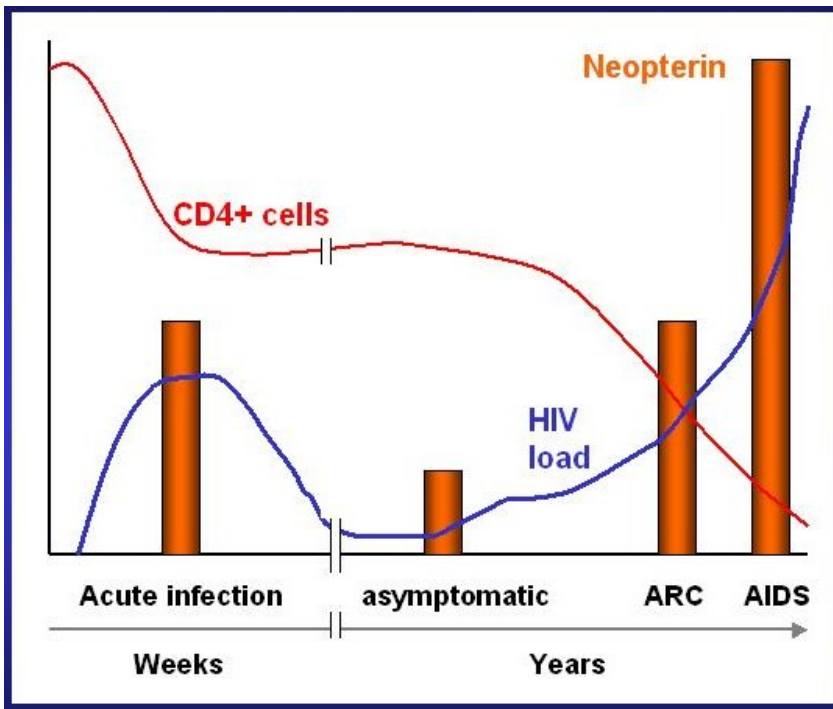
Figure 5: Schematic course of neopterin levels (yellow bars) in HIV infected persons: After the acute infection in approximately three-fourth of the HIV antibody seropositive individuals the neopterin levels remain increased also during the asymptomatic period of the infection; when progression of the disease takes place (AIDS related-complex = ARC, AIDS) neopterin levels further increase. The number of CD4+ T-cells (red) correlates usually inversely to the neopterin levels, a direct correlation exists between neopterin concentrations and quantitative measurements of virus load (blue) such as HIV mRNA measured by polymerase chain reaction.

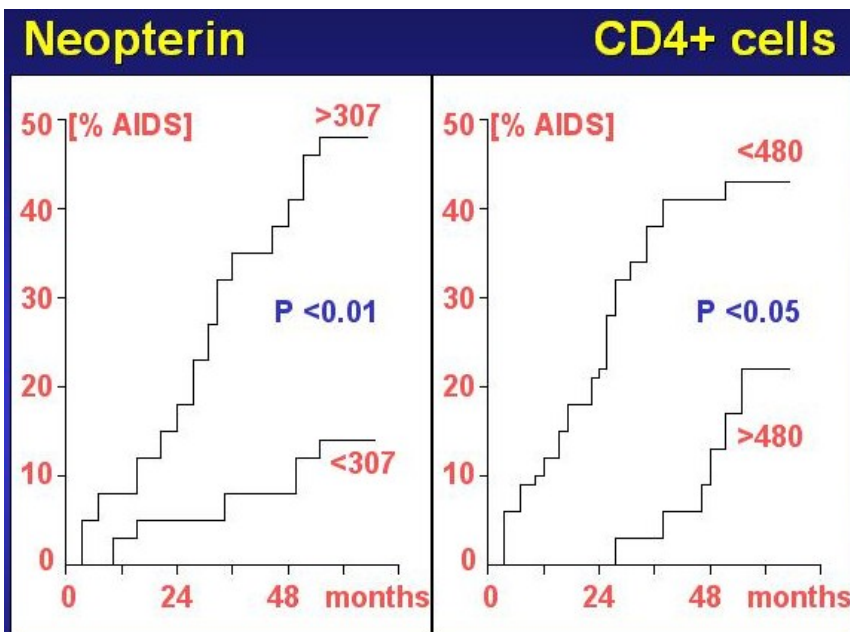dissociated HIV antigen and HIV-RNA by quantitative polymerase chain reaction [47].

Figure 6: Prognostic value of neopterin levels in HIV-infected persons: within the observation period of 72months individuals with higher neopterin levels at study entry (>307 µmol neopterin/mol creatinine in urine) developed AIDS more rapidly than individuals with higher neopterin levels (keft) [43]. In the same patients, lower CD4+ cell counts (<480/mm^3) indicated more rapid disease progression (right).

Successful treatment of HIV infection by antiretroviral therapy is associated with a decline of neopterin levels [48] within a few days. E.g., after one week HIV infected persons treated with zidovudine (azidothymidine) reached a plateau corresponding on average to 50% from the baseline value measured before treatment (Fig.7). Interestingly differences between the various reverse transcriptase inhibitors to influence neopterin levels could be found [49].

Increased neopterin levels in patients with HIV infection point to an increased activity of the cellular immune system which is caused by the reproduction of the virus in the organism. A connection was found between neopterin levels and distinct properties of the virus: HIV can be isolated much easier from patients with higher neopterin levels, and the virus isolates from individuals with higher neopterin levels tend to an accelerated replication rate with higher reverse transcriptase activity [50]. All together neopterin values emphazise the importance of immunological activation processes and cytokine cascades for the progression of HIV infection. Associations between neopterin levels in patients and the development of anemia [51] and cachexia [52] show the significance of immune activation for the clinical course of HIV infection. Moreover, neopterin derivatives themselves seem to have potency to accelerate the replication rate of HIV via an enhancment of oxidative stress [53,54].
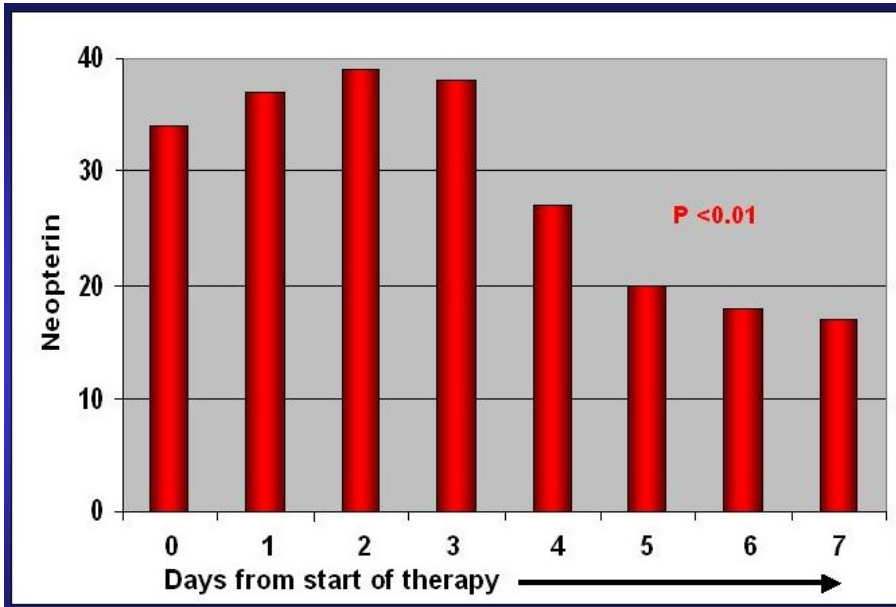
Figure 7: Schematic course of neopterin levels in HIV infected individuals during therapy with zidovudine (azidothymidine): in case of successful therapy neopterin levels decrease within a few days [49].

In patients with HIV infection a tight connection between neopterin concentrations in cerebrospinal fluid (CSF) and the development of neuropsychiatric symptoms was found. HIV-associated dementia is associated with strong intrathecal formation of neopterin, in this case neopterin levels in CSF reach significantly higher values than in serum [55,56]. By measurements of neopterin levels in CSF it is possible to monitor the efficacy of antiretroviral therapy to penetrate through the blood-brain barrier [49].

4.1.2. Infections with parasites and intracellular bacteria

Increased neopterin levels are detected in infections with intracellularly living bacteria and parasites such as tuberculosis [57,58], leprosy [59], melioidosis [60], malaria [61,62] and schistosomiasis [63]. In nearly every patient with acute malaria increased neopterin levels are found, moreover even asymptomatic children with low-grade parasitaemia present with increased neopterin levels. Besides, a temporal connection between the development of malaria symptoms and neopterin levels in serum was demonstrated by experimental infection of healthy volunteers with Plasmodium falciparum [64]. In that study it was shown that the increase of neopterin levels may even precede fever reactions.

In patients with pulmonary tuberculosis neopterin levels [57] correspond to the extent and the activity of the disease. During therapy neopterin values show a prompter response than usually applied methods such as blood sedimentation rate or X-ray. Worsening of the disease is indicated early by rising neopterin concentrations.

4.1.3. Differential diagnosis between viral and bacterial infections
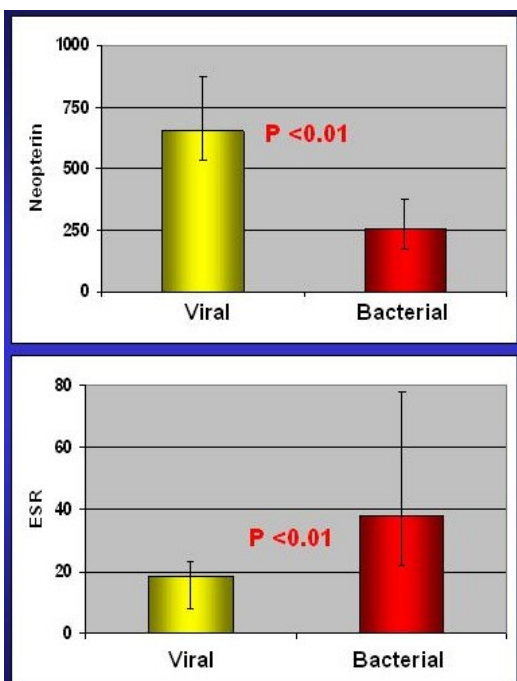

Figure 8: Neopterin levels (upper) and erythrocyte sedimentation rate (ESR, lower) in patients with viral and bacterial pneumonias. In contrast to lower ESR, neopterin levels are significantly higher in patients with viral infections than in bacterial infections [65].

Neopterin measurements are able to support differential diagnosis between viral and bacterial infections [65]. In contrast to highly increased neopterin levels in patients with virus infections, in patients with acute bacterial infections usually normal or only slightly increased values can be detected (Fig.8). It could be shown that neopterin measurements are even better able to distinguish between acute viral or bacterial infection than blood sedimentation rate or leucocyte counts [65], although in this study both the latter methods were used for differential diagnosis of infections. It is important to note that protracted bacterial infections often are associated with increased neopterin levels.

4.1.4. Multiple trauma and sepsis

In traumatized or post-operative patients in intensive care medicine serum neopterin levels were shown to predict the development of septic complications. In patients with multiple trauma significantly higher levels are found in patients who later develop sepsis [66] than in aseptic patients. In septic patients neopterin concentrations predict survival, non-survivers presenting with higher neopterin levels than survivors. Similar results were shown in patients with acute pancreatitis [67]. Already at the time of admission at the hospital neopterin concentrations allowed a significant prediction of patients' survival. The predictive value of serum neopterin levels was superior to that of, e.g., C-reactive protein.

4.1.5. Cerebral infections

Neopterin penetrates through the blood-brain-barrier, so that there usually exists a linear relationship between neopterin levels in blood and in cerebrospinal fluid (CSF) [55]. In contrast during the course of cerebral infections also intrathecal neopterin formation occurs, and in this case neopterin levels in CSF reflect the activity of intrathecal disease very closely [34]. In patients with acute Lyme neuroborreliosis extremly high neopterin levels in CSF (>100 nmol/l) were detected [68], which closely reflected disease activity and subsequently also the impact of, e.g., antibacterial treatment on the infectious process was indicated. In serum of these patients one usually can find only minor changes which take place within the normal range. Analogously high neopterin levels in CSF are common in encephalitis of viral and bacterial origin [34]. Because neopterin levels in serum are normal in absence of a systemic infection, neopterin determinations in CSF and serum are helpful for the differential diagnosis of, e.g., febrile convulsions and meningitis in children [69,70].

4.2. Autoimmune diseases and other inflammatory diseases

Neopterin levels in autoimmune diseases correlate with the fluctuating course and reflect the severity of the disease in a sensitive and convenient way.

4.2.1. Rheumatoid arthritis

In patients with rheumatoid arthritis neopterin levels correspond with the extent of the disease [71]. Average values of neopterin in patients with rheumatoid arthritis even in stage 1 are already higher than in patients with osteoarthritis, so neopterin detection also may support differential diagnosis in these cases. With the
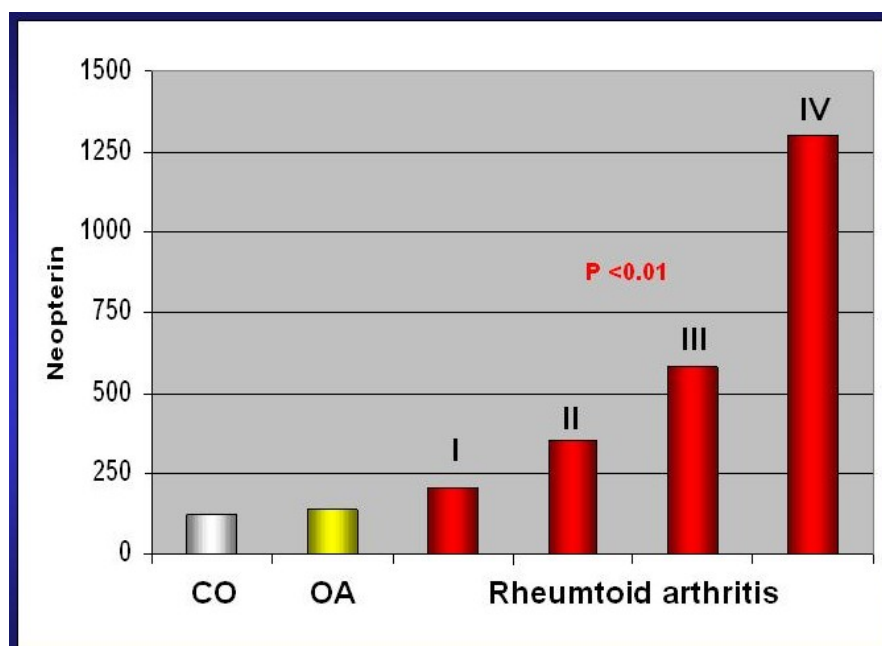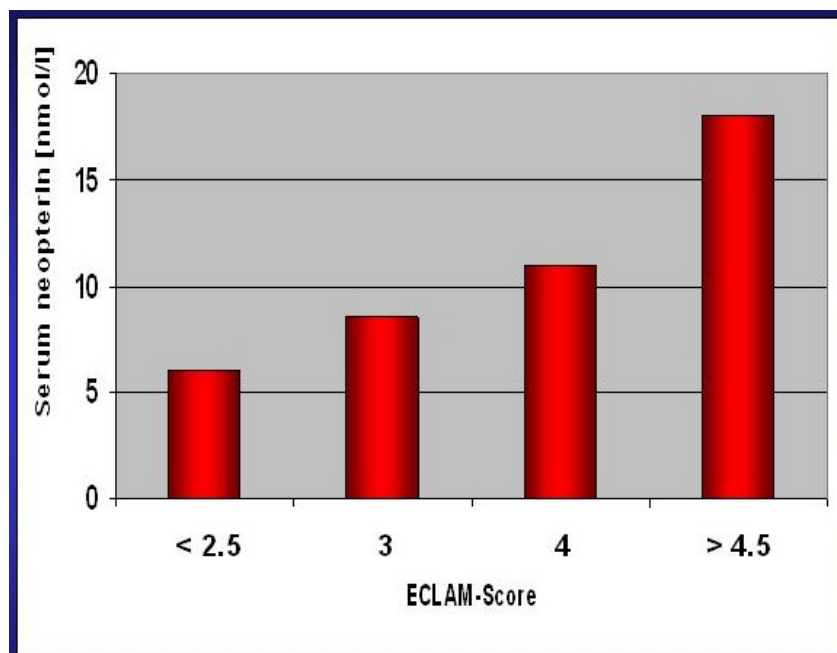

Figure 9: Neopterin levels in patients suffering from rheumatoid arthritis: patients with rheumatoid arthritis show increased levels in comparison to controls (CO) and patients with osteoarthritis (OA). In patients with rheumatoid arthritis a close association between higher neopterin levels and the activity of the disease was demonstrated (activity scores I - IV) [71].

progression of the disease higher neopterin levels are found. The influence of the clinical activity of the disease on neopterin levels is even stronger than that of the stage of the disease (Fig.9). Neopterin levels in follow-up show changes of the activity very sensitively which is especially important to indicate periods in which therapy has to be applied. It is to point out that neopterin concentrations in synovial fluid of patients suffering from rheumatoid arthritis are higher than in serum, and the concentrations of neopterin in synovial fluid reflect the activity of disease even better than serum or urine concentrations do [72].

4.2.3. Systemic lupus erythematosus

In acute systemic lupus erythematosus (SLE) neopterin levels are highly increased and correspond tightly with the activity of the disease (Fig.10) [73,74]. In a mutlivariate analysis of several standard laboratory parameters and different markers of immunological activity, e.g. soluble tumor necrosis factor receptor, soluble interleukin-2 receptor and soluble CD8 neopterin in a combination with blood sedimentation rate was found to be the best indicator for the activity of SLE [68].

Figure 10: Neopterin levels compared to the clinical activity (European Consens Lupus Activity Score, ECLAM) in patients suffering from systemic lupus erythematosus [73].

4.2.4. Wegener's granulomatosis, dermatomyositis

About two thirds of patients with Wegener's granulomatosis show increased neopterin levels in serum. Neopterin values correlate significantly with the activity of the disease as expressed by the Birmingham vasculitis activity score [75]. Similar results are found in patints suffering from dermatomyositis [76], where again a correlation with the activity of the disease was demonstrated. Particularly in patients with polymyositis extremly high neopterin levels are detectable.

4.2.5. Inflammatory diseases of the gastrointestinal tract

As in rheumatoid arthritis and SLE also in inflammatory diseases of the gastrointestinal tract like Crohn's disease [77], ulcerative colitis [78] or celiac disease [79] neopterin levels are tightly connected with the clinical activity, e.g., in patients suffering from Crohn's disease with the help of neopterin values an activity score was developed, which is based on only two further parameters, namely haematocrit and frequency of liquid stools. This simple activity index achieves similar significant results as the well established Crohn's Disease Activity Index (CDAI) [77]. Moreover quantification of neopterin in inflammatory diseases of the gastrointestinal tract is helpful to monitor treatment. In children suffering from celiac disease a rapid decline of neopterin concentrations is seen during gluten-free diet [79].

4.2.6. Lung sarcoidosis

In the majority of patients with active sarcoidosis neopterin levels are increased correlating to disease activity. Neopterin concentrations in serum and urine and also in the bronchoalveolar lage fluid rise in a

direct relationship to the roentgenologic stages [80]. In the follow up of patients clinical improvement is indicated by decreasing, worsening by increasing neopterin levels.

4.2.7. Multiple sclerosis

Neopterin levels in cerebrospinal fluid (CSF) of patients with multiple sclerosis are significantly higher in the acute phase of disease than in stable periods [81]. Moreover detection in CSF may also support differential diagnosis, e.g., it is important to note that patients with amyotrophic lateral sclerosis have normal neopterin levels in serum and CSF [82].

4.2.8. Cardiovascular diseases

Patients with dilated cardiomyopathy show increased neopterin levels, the values corresponding with the stage of the disease [83]. Furthermore correlations with the extent of cardiac impairment were found. In children with Kawasaki disease also increased neopterin levels are detected [84], during follow up neopterin concentrations reflect the efficacy of, e.g. therapy with immunoglobulins. Also patients with acute rheumatic fever show increased neopterin levels with high frequency [85]. In this case higher neopterin levels indicate an increased risk for the development of valve lesions. Healthy individuals with an increased risk for arteriosclerosis present with statistically higher neopterin levels than persons with lower risk, but the analized changes of the neopterin levels usually remain within the normal ranges for healthy individuals [86]. In contrast in patients after myocardial infarction temporarily increased neopterin levels were reported [87].

4.3. Malignant diseases

Certain malignant diseases are associated with elevated neopterin concentrations. However, since neopterin is released by cells of the immune system but not by tumor cells neopterin is not a tumor marker in the common sense: T-cell activation, probably induced by malignant cells, leads to formation of cytokines and to activation of monocytes/macrophages for neopterin production. The sensitivity of neopterin changes depends very much on the location and the type of the malignant process [88]. Most patients with hematological neoplasias show increased neopterin levels whereas in patients suffering from breast cancer hardly increased levels can be found. But in general neopterin concentrations in body fluids of patients with malignant diseases correlate with the extent of the disease, respectively the tumor mass, and indicate a higher risk for progression of the disease. Consequently the utility of neopterin measurments in malignant diseases does not lie in the so-called tumor screening but in judging prognosis and in the monitoring of therapy [89-91]. A significant predictive value for prognosis of the neopterin levels before therapy was found, e.g., in patients with gynecological and hematological neoplasias, in patients with bronchus carcinoma, cancer of the prostate, hepatocellular carcinoma and with gastrointestinal cancer. During follow-up of
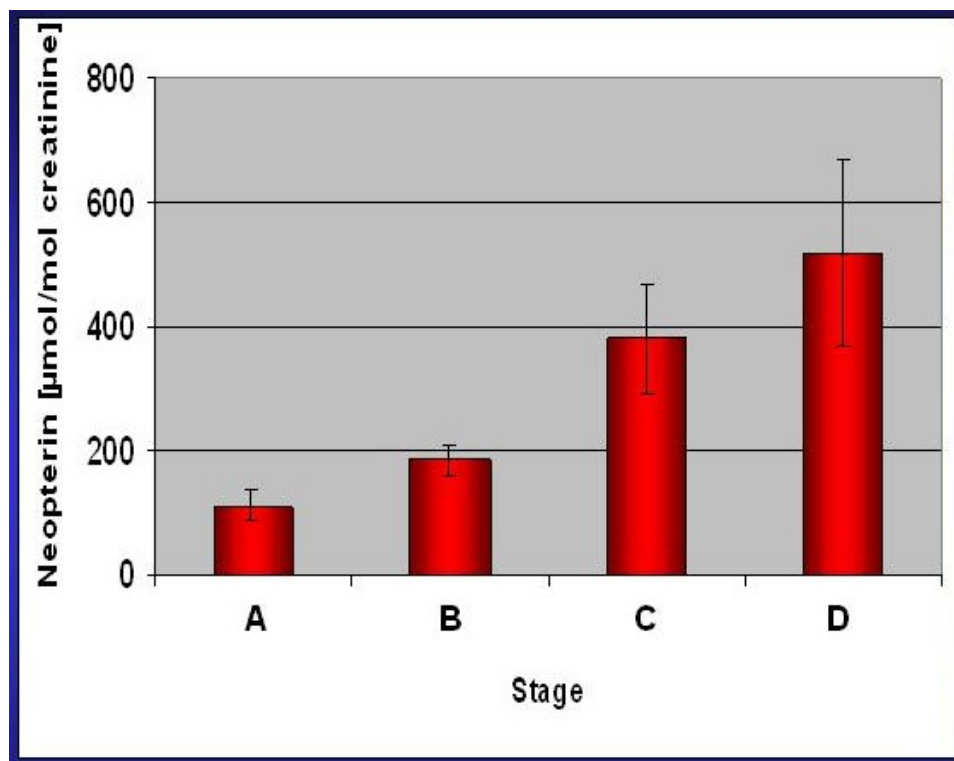

Figure 11: In patients with malignant tumors(example shown: carcinoma of the prostate) neopterin levels (mean + S.D.) increase with higher stage (stages A – D). Higher neopterin concentrations are associated with shortened survival).

patients a tight connection between an uncomplicated course of the disease and normal neopterin levels was demonstrable. In contrast, incomplete removal of the tumor or recurrence of the malignant event correlates with remainingly high or increasing neopterin levels [90]. Therefore, the measurement of neopterin represents a possibility for additional monitoring especially during the follow-up of patients. In this case increasing neopterin levels indicate the need of further diagnostical measures because neopterin changes are due to immune deterioration which not necessarily must be a result of the tumor disease. Besides neopterin detection may be used also for diagnosis as an additional indicator to differentiate between benign or malignant tumor diseases.

Increased neopterin levels in different tumor diseases reflect the importance of immune activation for the clinical course of the disease. As in HIV infected persons associations between neopterin levels and the presence of anemia or cachexia in these patients can be found [93,94], which points to the role of inhibitory cytokines in the pathogenesis of these symptoms. The same cytokines that are mainly responsible for neopterin formation seem to have an essential importance for the development of anemia and weight loss [95]. Recent results demonstrate that neopterin metabolism may even be involved directly in the malignant event, namely by interfering with intracellular signal-transduction pathways which are sensitive to oxidative stress, because neopterin and dihydroneopterin are able to interfere with redox equilibria in cells and were shown to promote the expression of protooncogene c-fos [28].

4.4. Monitoring of graft recipients

In recipients of solid allografts (kidney, liver, pancreas and heart) daily neopterin monitoring during the hospital stay represents a sensitive way to detect immunological complications like rejection or infections early [96]. On average already two days, sometimes even 7 days, before clinical complications may occur neopterin levels begin to increase significantly (Fig.12). But increased neopterin values can only be interpreted as a warning signal for developing immunological complications and have to become further clarified by additional differential diagnostic measures. In follow-up it was also shown that neopterin levels during the post-operative phase are associated with the long-term graft survival [97], higher neopterin levels are associated with a significantly shorter graft survival.
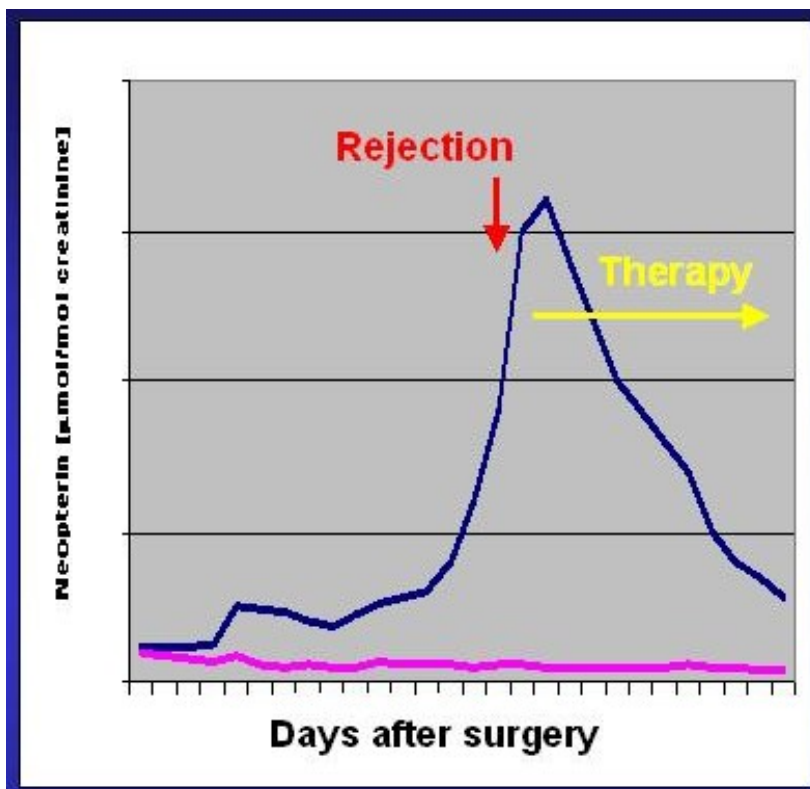
Figure 12: Schematic course of neopterin levels in a patient after kidney-transplantation (blue) as compared with an uncomplicated course (purple). Rejection episodes are indicated by increasing neopterin levels on average approximately 2 days before clincial diagnosis is established by convential means [96]; successful immunosuppressive therapy of the rejection leads to a decrease of neopterin levels.

If neopterin detection in serum or plasma is utilized for the follow-up of patients after kidney transplantation it is necessary to calculate a quotient of neopterin versus creatinine concentrations (similar to the proceedure for neopterin measurements in spot urine specimens). By this way changes of neopterin concentrations due to renal impairment but not caused by an underlying immune response can be at least partly taken into account (see also chapter 3.3.).

Monitoring of neopterin concentrations in other body fluids such as bile fluid or pancreatic juice of patients after allograft transplantations provides further support for differential diagnosis, e.g., neopterin measurements in pancreatic juice [98] after combined kidney/pancreas transplantation allows to identify the

organ which is experiencing immunologal complications. In a similar way contemporary monitoring of neopterin concentrations in bile fluid and serum in liver graft recipients further helps to find out whether a patient is concerned either with rejection or systemic versus hepatic infections [32].

During the course before and after bone marrow transplantation the aplastic phase caused by chemotherapy and/or whole body irradiation before transplantation is associated with low or even decreased neopterin levels [99]. Hematological reconstitution is associated with increasing neopterin levels and the so-called "hematological take" is indicated approximately 7 days in advance. During and before viral infections or graft-versus-host (GvH) disease neopterin values usually rise dramaticaly. Thus, neopterin measurements are useful for the follow-up after bone marrow transplanation to distinguish between uncomplicated and complicated courses with viral infections or GvH reactions.

4.5. Immunomodulating therapy and follow-up of therapy

Because of its formation during the course of cell-mediated immune response neopterin monitoring allows to determine the effects of therapeutical interventions which are assigned to interfere with the degree of immune activation. Particularly in therapies with cytokines such as interferons, interleukins or tumor necrosis factor-α a dose-dependent increase of neopterin occurs, so that monitoring of neopterin concentrations allows to define an optimal dosage of immune-modulating therapy [100]. Beside measurement of neopterin concentrations is also performed for monitoring patients with malignant diseases during treatment with immunotherapy applying antitumoral peptides.

Except the monitoring of therapeutical measures that operate directly on the immunologic network neopterin determinations allow to examine therapeutic effects during clinical situations in which neopterin concentrations reflect the activity of the disease. Usually successful surgical intervention to remove a malignant tumor leads to a decrease of neopterin concentrations [89]. Similarly successful antibacterial treatment of, e.g. pulmonary tuberculosis, is associated with rapidly decreasing neopterin levels [57,58]. Therefore efficacy of treatment or even the compliance of patients can be monitored by neopterin measurements. Similar results were found in patients with acute rheumatic fever [85], Lyme neuroborreliosis [68] or autoimmune diseases.

4.6. Blood transfusion and bone banking

Transfusion of blood or blood-products bears a certain risk for transmitting infectious agents or probably also malignant processes. Untill now only very few specific tests for antibodies against the most dangerous agents, namely HIV and hepatitis viruses, are performed on a regular basis. Because of logistic reasons it is only possible to introduce a limited number of such specific tests for the routine screening of blood donations. Since there are various different pathogens which cannot be screened for by this strategy, anamnestic examinations of donors should at least partly fill this gap. Therefore, a so-called "nonspecific test" like measurements of neopterin concentrations would be useful to detect various disorders which represent a certain risk for blood transfusion recipients. Infections with viruses including retroviruses, intracellularly living bacteria but also autoimmune processes and certain malignant diseases lead to an activation of the cellular immune system and consequently to increased neopterin concentrations in individuals even when they still may feel free of any symptom. The sensitivity of neopterin measurements is high to detect such disorders, and a reasonable number of investigations demonstrates that the measurement of neopterin is well practicable as a screening test in the field of blood transfusion [101]. Thereby it has to be pointed out that neopterin levels are already increased in an early stage of an infection, even before specific antibodies are formed (compare chapter 4.1.) to be able to further close the so-called "window period" which still exists when antibody screening is performed.

In Austria measurements of neopterin concentrations became obligatory for all blood-donations in 1994, in the Austrian province of the Tyrol screening of blood donations by neopterin measurements has already been performed since 1986 according to an order of the local government. In the meantime it was found that an additional screening with neopterin measurements of blood donation is able to reduce the risk of transmitting acute virus infections, e.g., neopterin screening was shown to reduce the risk of missing acute CMV infections in blood donations by a factor of 17; in donations with increased neopterin levels (>10 nmol/l) approximately a 17-fold higher frequency of acute CMV infections was found compared to donors with low neopterin levels [102]. Importantly in that study anamnestic examination was done as usual before the donation was allowed, but the acute infections were not recognized by anamnesis or by the donor himself. Moreover it was demonstrated that in an asymptomatic course of CMV infection the increase of neopterin even starts before CMV-IgM seroconversion, and CMV-IgM seropositivity correlated with the highest neopterin values (Fig.12). Similarly donors with increased neopterin levels the occurence of an acute Epstein-Barr-virus infection or parvovirus infection was 4 to 6 times more likely than in the donors with neopterin levels within the normal range [103].

Besides neopterin measurement are also nominated as an additional method for the screening of bone donors [104]. Particularly the diagnosis of malignant diseases and infections in donors becomes supported by additional neopterin measurements.
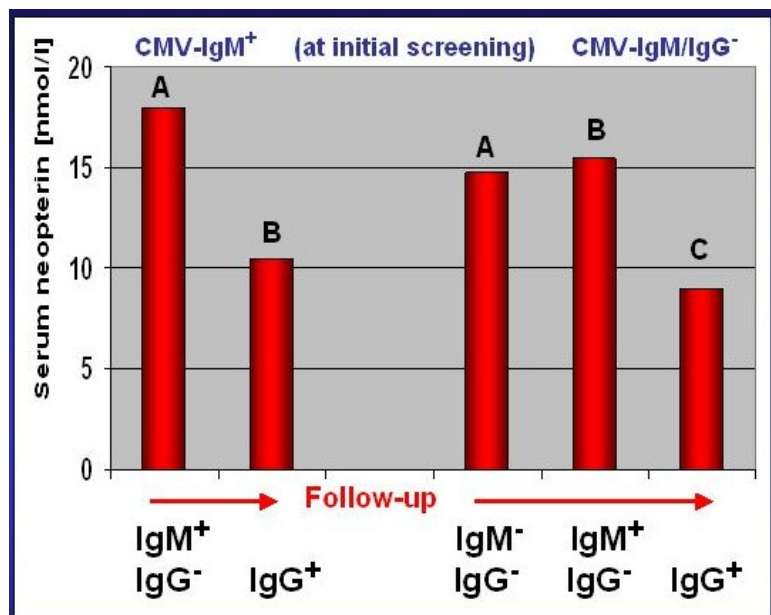
Figure 13: Neopterin levels in blood donors during asymptomatic acute CMV infection: in approximately 5% of blood-donors with neopterin levels above 10nmol/l acute CMV-infections can be confirmed by CMV-IgM seropositivity (CMV-IgM+, A) [102]; after complete seroconversion the neopterin levels of most of these donors decrease to the normal range (IgG+, B). Among donors with increased neopterin levels individuals were found who were still completely seronegative for CMV (CMV/IgM and IgG-, A), but in a subsequent donation became CMV/IgM+ and afterwards also developed CMV-IgG antibodies (C). Thus, CMV infection was detecable by increased neopterin concentrations not only before IgG-seroconversion but also before detectable IgM.

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