Role of Serum Neopterin in Diagnosis of Early Onset Neonatal Sepsis

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Abstract

Sepsis is the commonest cause of neonatal mortality and is probably responsible for 30-50% of the total neonatal deaths each year in developing countries. Diagnosis of neonatal sepsis remains a major challenge, as early signs of sepsis are often non-specific and the laboratory criteria are also not fully reliable. This leads to unnecessary exposure to antibiotics before the presence of sepsis has been proven with potentially poor outcomes. Several attempts have been made to use physiologic parameters, hematologic indices, and cytokine profiles, at the time of onset of the suspected sepsis episode to identify accurately neonates with sepsis. Elevated serum level of neopterin has been shown to be an early specific and sensitive marker responsible for activation of the cellular immune system and has also been proposed to aid in the diagnosis of bacterial infection.

KeyWords:

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Introduction:

Neopterin was first isolated from larvae of bees, in worker bees and in royal jelly in 1963, and subsequently from human urine by Sakurai and Goto in 1967 (1).

Neopterin or 2-amino-4-hydroxy-6-(D-erythro-1′,2′,3′-trihydroxypropyl)-pteridine is produced from guanosine triphosphate via guanosine triphosphate cyclohydrolase I (GTPCH I) by activated monocytes, macrophages, dendritic cells, and endothelial cells and to a lesser extent in renal epithelial cells, fibroblasts, and vascular smooth muscle cells upon stimulation mainly by interferon gamma and to a lesser extent by interferon alpha and beta with its release being enhanced by tumor necrosis factor (2, 3).

GTPCH I mRNA expression is synergistically and independently induced by interferon gamma through the Jak2/Stat pathway of nuclear transcription regulation and through TNF by the NF-kappaB pathway (4).

Release in response to cytokines released by T-lymphocytes and natural killer cells make neopterin an indicator of activation of cell mediated immunity including release by infections associated with activation of T-lymphocytes and natural killer cells, malignancies, autoimmune diseases, rejection of transplanted organs, and atherosclerosis (3).

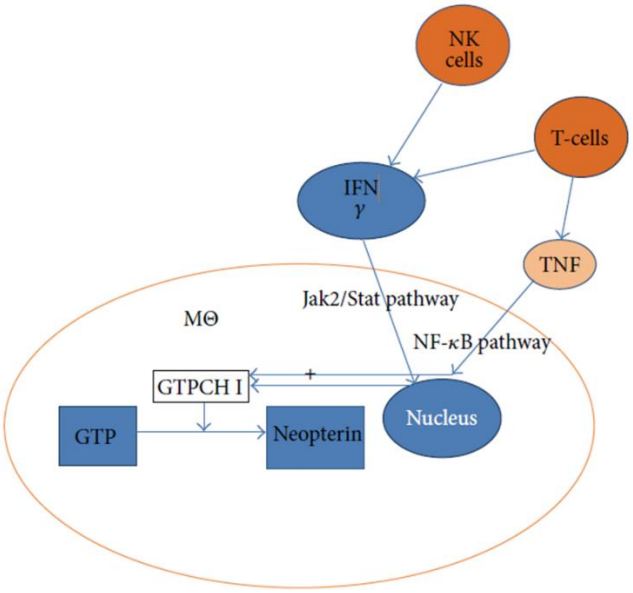

Figure (1): Pathways for induction of neopterin production (MΘ: macrophage, Jak: Janus kinase, Stat: Signal Transducer and Activator of Transcription, and GTPCH: guanosine triphosphate cyclohydrolase). (3).

At its first isolation in the 1960s neopterin was detected in the pupae of bees by anion exchange chromatography followed by paper chromatography (1).

In the seventies gas chromatographic-massfragmentographic methods were described allowing measurement in urine. Subsequently detection and quantification of neopterin

succeeded in serum, urine, and other body fluids using standard high pressure and by reverse-phase high-performance liquid chromatography with fluorescence detection. Later simpler radioimmunoassays and more recently enzyme-linked immunosorbent assays have been developed which are suitable for large numbers of samples. Semiquantitative measurement with a dipstick system using polyclonal antineopterin antibodies has been validated and may be suitable for bedside testing and in the setting of developing countries (5).

Neopterin Levels in Bacterial Infections:

Patients with bacterial infections with species other than mycobacteria showed significantly lower urinary neopterin levels compared to patients with viral infections in one study (6) but no statistically significant difference in a more recent study (7).

Within the group of bacterial infections it was shown that patients with symptoms for at least 5 days had significantly higher neopterin concentrations than patients with acute illness. This applied particularly to bacterial pneumonia. Patients with urinary tract infections were found to have similar levels to patients with viral infections with data on urinary neopterin concentrations but not serum concentrations. Thus it remains unclear whether local production of neopterin takes place in urinary tract infections and serum neopterin would stay low. There was no significant difference in neopterin levels between patients with febrile neutropenia and underlying haematological and oncological conditions and gram-negative versus gram-positive infections (8).

In patients on an intensive care unit with sepsis and septic shock urinary neopterin/creatinine ratios were found to be significantly higher compared to patients with other forms of systemic inflammatory responses syndromes (9) and serum neopterin levels were higher in nonsurvivors compared to survivors of sepsis and multiorgan failure scores correlated with neopterin levels (10). In this context it was however noted that neopterin levels correlated negatively with reduced renal function reflecting renal failure causing a reduced excretion of neopterin. Future studies could correct for reduced excretion due to reduced renal function by calculation of the serum neopterin/creatinine ratio.

Investigations on critically ill patients on intensive care units evaluated neopterin levels as tool to discriminate patients with systemic inflammatory response syndrome with and without infectious etiology. Neopterin levels were found to have a specificity of 78% for discriminating infectious and noninfectious etiology of critical illness (11).

Bacterial meningitis was associated with both elevated serum and CSF neopterin levels compared to controls. In Lyme neuroborreliosis—a late complication of infection by the tick-born spirochete Borrelia burgdorferi—high neopterin concentrations were found in CSF of patients, whereas serum neopterin levels were not markedly increased, confirming intrathecal neopterin production. Infection with Treponema pallidum subsp. pallidum (syphilis) was not associated with elevated neopterin levels. In melioidosis by Pseudomonas pseudomallei neopterin concentrations were found to be significantly higher than controls (3).

In brucellosis neopterin levels were with a mean 52.5 mmol/mL significantly higher than healthy controls and patients with tuberculosis (12).

In leprosy caused by Mycobacterium leprae 75% of patients with tuberculoid and lepromatous leprosy presented with elevated urinary neopterin excretion (13).

Serum Neopterin Level in Early Onset Neonatal Sepsis:

Elevated levels of neopterin have been shown to be early specific and sensitive marker responsible for activation of the cellular immune system in several clinical settings including allograft regection, acute bacterial infections, inflammatory and malignant diseases (14).

Boseila et al., (15) revealed that serum neopterin was significantly increased in the infected and suspected groups than the control group.

Mitaka, (16) observed that neopterin level have been increased in patients progressing from gram negative sepsis to septic shock and it was reported that neopterin level are higher in patient with septic shock than in patients with non infectious SIRS and explained that neopterin biosynthesis in inflammatory state might be caused by increased levels of endogenous interferon gamma which was directly related to the extent of systemic T-lymphocyte activation. This finding can be easily explained because neopterin closely reflects the activation of both monocytes macrophages and endothelial cells, which have a central role in the

pathogenesis of septic shock.

TasdelenFisgin et al., (17) observed that neopterin is a coparameter in the diagnosis of bacterial infection as elevated concentration of neopterin are found to be relevant to the endothelial damage and septic complication, so neopterin is found to be a prognostic factor in patients with sepsis.

Baydar et al., (9) observed that serum neopterin level were significantly higher in all patients than in the control and explained that by neopterin is a biomarker of cellular immunity and therefore increased level of neopterin may reflect septic complications.

TasdelenFisgin et al., (17) found a significant correlation between serum neopterin level and the mortality rate in patients with sepsis who explained that neopterin may contribute to tissue damage caused by increased cellular apoptosis.

Ruokonen et al., (11) observed that increased neopterin level in non survived are not soley related to the activity of the inflammatory response but also to the severity of the illness.

Neopterin Release in Viral Infections:

Relationship of Neopterin Levels to Time Course of Viral Infections

Serum neopterin levels were also found to be significantly elevated in symptomatic dengue virus infections with levels higher than in measles and influenza virus disease (18).

Levels correlated with duration of fever and severity of disease (18).

Investigations into the physiological functions of neopterin in viral infections revealed that it is able to delay the development of the cytopathic effect of coxsackie B5 virus in Hep-2 cells. A proposed mechanism is the stimulation of inducible nitric oxide synthase expression leading to an increase in nitric oxide production. Other mechanisms include the induction of the translocation of the nuclear factor-kappa B to the nucleus. (19).

Neopterin as Diagnostic and Prognostic Marker in HIV Infection

Testing of 328 samples of 29 HIV infected individuals found that 44/68 (64.7%) of samples, which were HIV-1 RNA and p24 antigen positive had elevated neopterin levels (>10 nmol/L). 6/216 (2.8%) samples, which were both HIV-1 and p24 antigen negative had elevated neopterin levels (20).

Studies investigated markers of immune activation for their usefulness as prognostic markers in HIV infection and showed an increase of neopterin levels in people with HIV infection compared to patients without HIV infection. Neopterin levels were found to predict HIV related mortality (21).

Neopterin as Surrogate Marker for Viral Load to Monitor Response to Antiretroviral Treatment

In a land mark study the effects of dual reverse transcriptase inhibitor (RT) therapy and highly active antiretroviral therapy (HAART) on neopterin levels in patients with HIV infection were compared to HIV uninfected controls, HIV infected patients not on treatment, and patients who had stopped treatment (22).

Neopterin levels in patients who discontinued HAART became similar to untreated HIV patients. (19).

Neopterin may be a particularly useful surrogate marker for monitoring of control of HIV replication in settings in developing countries where HIV RNA viral load measurement is not available and may be a cheaper alternative particularly if semiquantitative dip stick tests are used for urine samples (5).

Longitudinal serial measurements in the same individual could overcome difficulties with interpretation in settings where chronic parasitic (malaria) or bacterial (tuberculosis) infections may elevate the baseline neopterin level and could allow monitoring of response to antiretroviral treatment in the absence of resistance testing and provide means to monitor compliance in the outpatient setting (3).

Table (1): Applications of neopterin measurements for monitoring of treatment response in infectious diseases.

Infection	Treatment	Type of sample	Change in neopterin levels observed
Human immunodeficiency virus	Reverse transcriptase inhibitors and highly active antiretroviral treatment	Blood, cerebrospinal fluid	Mean of 15.6 ng/mL for treated versus a mean of 22.3 for untreated HIV patients
Hepatitis C virus	Pegylated interferon combined with ribavirin	Blood	The rate of response was twofold higher among patients with pretreatment neopterin levels <16 nmol/L than in patients with levels ≥16 nmol/L
Mycobacterium tuberculosis	Antituberculotic treatment	Blood, urine	Levels declined on twice weekly measurements in all monitored patients with pulmonary tuberculosis on treatment and fell to below tolerance limits within 10 weeks of treatment in 6/10 patients

Viral Hepatitis:

Alanine aminotransferase levels in HCV infected persons correlated significantly with neopterin levels. Serum neopterin levels were found to be a useful predictor of response to treatment of chronic HCV infection with pegylated interferon combined with ribavirin. Neopterin concentrations were evaluated in 260 HCV patients treated by pegylated

interferon combined with ribavirin. Mean and median pretreatment neopterin concentrations were lower in patients with sustained virological response than in nonresponders. The rate of response was twofold higher among patients with pretreatment neopterin levels <16 nmol/L than in patients with neopterin levels ≥16 nmol/L, even after controlling for HCV genotype status (24).

This may have been due to large standard deviations and small numbers in groups. A more recent investigation found that in chronic hepatitis the mean ± SD serum neopterin levels were 14.2 ± 5.6 nmol/L, 20.3 ± 7.9 nmol/L in patients with liver cirrhosis and 5.2 ± 1.4 nmol/L in the control group. Serum neopterin levels were significantly higher in patients with chronic hepatitis (P = 0.005) and cirrhosis patients (P = 0.008) than in control subjects. Cirrhotic patients had significantly higher serum neopterin levels than patients with chronic hepatitis (26).

Viral Respiratory Tract Infections

Neopterin has been investigated as a marker to distinguish viral from bacterial lower respiratory tract infections. The investigators found that serum neopterin levels were elevated (>10 nmol/L) in 96% of patients with viral LRTI. The median serum neopterin concentration was almost 2-fold higher in the viral LRTI group than bacterial LRTI patients (30.5 versus 18.7 nmol/L) and 5-fold higher than those in healthy controls. The specificity for correct identification of viral LRTI was 69.5% for a cut-off of >15 nmol/L (27).

Duration of pyrexia in SARS patients correlated positively with neopterin levels. Patients on steroid therapy had significantly lower neopterin levels. Measurement of neopterin in isolation and in relationship to other inflammatory markers like procalcitonin and C-reactive protein were investigated for discriminatory power between viral and bacterial lower respiratory tract infections. Investigators used the CRP/neopterin ratio (C/N ratio) to discriminate viral and bacterial etiology of respiratory tract infections. In a study conducted in Hong Kong sera obtained on the day of hospitalization for LRTI from 139 patients with confirmed bacterial etiology and 128 patients with viral etiology were examined. A further 146 sera from healthy Chinese subjects with no infection were included as controls. The area under the receiver operating characteristic (ROC) curve (area under curve [AUC]) for distinguishing bacterial from viral infections was 0.838 for CRP and 0.770 for PCT. The AUC for distinguishing viral from bacterial infections was 0.832 for neopterin. When the markers were used in combination, AUC of ROC of the C/N ratio was 0.857, whereas (CRP × PCT)/neopterin was 0.856 (27).

In a subsequently reported study the median of the C/N ratio was 10 times higher in patients with bacterial aetiology than with viral aetiology (12.5 versus 1.2 mg/nmol; P < 0.0001) and 42 times higher than those in healthy subjects (12.5 versus 0.3 mg/nmol; P < 0.0001). The area under the receiver operator characteristic curve for the C/N ratio was 0.840. A cut-off value of "C/N ratio >3" for ruling in/out bacterial/viral infection yielded optimal sensitivity and specificity of 79.5% and 81.5%, respectively (28).

Viral Central Nervous System Infections

Early studies showed elevated neopterin levels in cerebrospinal fluid (CSF) of patients with aseptic meningitis and herpes simplex and measles virus encephalomyelitis (29).

CSF levels of neopterin seem to reflect intrathecal production by microglia as neopterins have a low permeability across the blood brain barrier with a serum-to-CSF distribution at a quotient of 1/40.It has recently been established that normal CSF neopterin is brain-derived. The interindividual variation of CSF neopterin in healthy adults was found not to depend on serum neopterin concentration variation (coefficient of variation, CV-CSF = 9.7% < CV-serum = 24.5%). Additionally individual normal CSF neopterin concentrations were found to be invariant to the variation of the albumin quotient, QAlb; that is, CSF neopterin does not derive from leptomeninges (30).

Patients with viral meningitis had elevated CSF neopterin levels compared to healthy controls but normal serum levels (29).

CSF neopterin levels correlated hereby with CSF monocytic cell count. Patients with various forms of encephalitis including those caused by herpes simplex virus, varicella zoster virus, and tick borne encephalitis virus had significantly elevated CSF neopterin levels compared to controls and higher levels than in patients with viral meningitis without overlap of levels in the two conditions. In HIV infection there was a clear relationship between the severity of AIDS-related dementia and CSF neopterin levels (23).

Higher CSF HIV viral loads were associated with

higher CSF neopterin levels (31).

After commencement of combination antiretroviral therapy (ART), CSF neopterin decreased markedly but remained slightly above normal levels in a substantial number of patients despite several years of receiving ART (23).

Even patients with systemic virological failure exhibit a substantial reduction of CSF neopterin concentrations, though above that of virologically suppressed patients (32).

In patients on combination ART, the lowest CSF neopterin levels have been found in patients with the lowest CSF viral loads (<2.5 copies/mL) (23).

No significant difference in CSF neopterin concentrations was found between those treated with protease inhibitor- and nonnucleoside reverse transcriptase based regimens in combination with 2 nucleoside analogues (33).

This would support the idea that viral replication within or close to the CSF, at least to some extent, is partly driving the inflammatory response. It has also been suggested that an inflammatory response, once triggered, may lead to a self-sustaining state of cellular activation as has been seen in patients with herpes simplex virus type-1 encephalitis (3).

HIV RNA levels measured in CSF or plasma were not significantly associated with CSF neopterin trajectories. In addition, all study participants had experienced virologic control to the limit of standard detection as a result of their treatment and CSF neopterin levels were the only factor strongly associated with subsequent decay rates and the ultimate set-point levels (23).

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