Original Article

Microbiology Section

Serum Neopterin Estimation as an Indicator for Safe Blood Transfusion

SHAMEEM BANU A.S., LATHA.P., KAVERI K., JAYAKUMAR S.

ABSTRACT

Background and Objective: Neopterin is regarded as an early biomarker of the cellular immune response. Neopterin concentrations in body fluids are raised with high sensitivity infections. The determination of neopterin is an innovative tool for monitoring diseases which are associated with the activation of cell-mediated immunity. There is not much data of India available on serum neopterin estimation among voluntary blood donors attending the blood bank for assessing various transfusion transmitted diseases which are necessary for this study.

Materials and Methods: Blood samples were collected from Government General Hospital blood bank by venipuncture and serum was obtained by centrifugation. Serum antibodies against Human Immunodeficiency Virus (HIV-1&2), Hepatitis C Virus (HCV), *Treponema pallidum*, Cytomegalovirus (CMV-IgM) and Hepatitis B Virus surface antigen (HBsAg) were determined in all donor samples by routine ELISA method. Screening was done for malaria and filarial parasite by making smears. Serum neopterin was measured by a commercially neopterin enzyme immunoassay kit.

Results: A total of 304 donors were screened out of which 58 had elevated neopterin levels contributing to 19.07%. A total of 43 samples were positive for any one or more of the screening tests. All the 43 samples well correlated with neopterin elevation.

Conclusion: We conclude that the risk of transmitting new pathogens may be reduced using neopterin assay as a routine in blood banks.

Key Words: Transfusion Transmitted Infection, Human Immunodeficiency Virus (HIV-1&2) Hepatitis B Virus surface antigen (HBsAg) and Hepatitis C Virus (HCV)

KEY MESSAGE

- Neopterin concentrations in body fluids are raised with high sensitivity infections.
- Neopterin measurements provide an insight into the present state of cell-mediated immune response.
- Risk of transmitting new pathogens to recipient is reduced using neopterin assay as a routine in blood banks.

INTRODUCTION

The first case of transmission of a viral illness through blood transfusion was reported in 1943. Laboratory testing for viral transfusion-transmitted viruses began in 1969 with testing for hepatitis B surface antigen (HBsAg) [1].From the beginning till today it has been our aim to save life and also to ensure that the individual does not acquire any transfusion transmitted disease by following transfusion.

The diseases transmitted through blood can be classified based on the group of organisms such as bacterial, viral, protozoal and so on. Only in case of known pathogens,there are specific tests available for the detection of these microorganisms. At first to reduce the frequency of transfusion transmitted disease, screening of the blood donors for elevated levels of liver enzymes in the blood for hepatitis, followed by Hepatitis B and then Hepatitis C screening was done [2]. Later risk of contracting HIV through blood transfusion was noted and screening for it became mandatory. It seems reasonable for the blood banks to apply a limited primary set of specific tests to exclude the most dangerous infection. But a battery of screening tests is increasing for blood donors in order to ensure safe blood for the recipient. As the number of screening tests increases, one blood bag becomes costly and also difficult to face screening for the presence of too many pathogens.

Specific tests for known pathogens may have miss evolving ones in blood donors. A multi-specific gatekeeper test would reduce the residual risk of unknown pathogens [3]. The determination of neopterin levels in human body fluids offers a useful and innovative tool to monitor diseases associated with the activation of cell-mediated immunity. Neopterin is one such marker which is early, sensitive and non-specific marker of cellular immunity. Increasing neopterin levels in various infections precede the clinical manifestation and seroconversion. Normally samples are not tested for all possible infections. Its upper limit of normal in healthy adults is 9-10 nmol/l [4]. Serum levels above 10 nmol/l are regarded as elevated. Therefore, the measurement of neopterin in blood donor samples is a useful tool in order to reduce the risk of infections via blood transfusion [5].

Since 1986, neopterin screening of blood donations has been done in the Austrian Tirol to detect potentially hazardous donations [3]. It is a bioactive substance released by the activated monocyte/macrophage, and an early and sensitive marker used for reflection of cellular immune activation status induced by the lymphocyte macrophage system. It has been suggested that it is an excellent marker for the activation of the monocyte/macrophage axis in some clinical situations [6,7].

Neopterin (6-D-erythro-trihydroxypropylpterin) is a low molecular-weight compound derived from guanosine triphosphate GTP [8], a pteridine derivative released into circulation from activated macrophages. The activated monocyte-macrophage is an important source of neopterin. Serum neopterin (s-neo) level is an indicator of both macrophage activation and interferon gamma (IFN-γ) activity, a major macrophage activating factor. Neopterin can be measured more easily and accurately than IFN-γ levels in serum [9].

Increased amounts of neopterin in body fluids are associated with a variety of diseases in which activation of the cellular immune mechanism is involved, such as certain malignancies, infections, allograft rejection, autoimmune disease of cardiac and renal failure, coronary artery disease and myocardial infarctions [10]. Elevated neopterin levels were observed in silicotic individuals, rheumatoid arthritis, neuropsychiatric abnormalities, Kaposi.s sarcoma, intrahepatic cholestasis of pregnancy, pulmonary tuberculosis and follow-up of antituberculosis treatment, activation of cell-mediated immunity (CMI) during pregnancy and severe burn sepsis [11]. 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.

Determination of neopterin levels reflects the stage of activation of the cellular immune system which is Important in the pathogenesis and progression of various diseases. Therefore, so-called "non-specific test" like measurements of neopterin concentrations would be useful to detect various disorders which represent a certain risk for blood transfusion recipients.

MATERIALS AND METHODS

Blood samples were collected after obtaining consent from voluntary donors who are attending Government General Hospital blood bank by venipuncture and serum was obtained by centrifugation. All analyses were performed within 1 day after blood collection. To exclude infections hazardous to blood recipients, serum antibodies against HIV-1&2, HCV, *T. pallidum*, and HBV surface antigen were determined in all donor samples. HBsAg – Micro screen ELISA test, HIV-Lab systems, Rapid Plasma Regain test –Span diagnostics, HCV antibody detection (ELISA) –LG HCD3.0, CMV IgM ELISA Novum Diagnostica were performed. Screening was done for malaria and filarial parasite by making smears. In addition p24 antigen assay-Innotest HIV antigen mAb was done. Neopterin ELISA-IBL neopterin enzyme immunoassay kit was used. Serum neopterin was measured by a commercially available ELISA method (Neopterin GenWay Biotech Inc) with a detection limit of 0.7 nmol/l and the specificity is about 99.95%.

RESULT

Out of 304 voluntary blood donors screened 253 (83.22%) were males and 51 (16.77%) were females. Age wise distribution of male and female donors and number of donors with elevated neopterin level is shown in [Table/Fig-1]. A total of 304 donors were screened, out of which 58 had elevated neopterin levels contributing to 19.07%. Neopterin concentrations were ranging from 10.1 nmol/l to 35 nmol/l. The distribution of elevated levels (range wise) of neopterin among donors is shown in [Table/Fig-2]. A total of 43 samples were positive for any one or more of the screening tests and all samples correlated with neopterin elevation. Prevalence of transfusion transmitted diseases among donors and its correlation

Age Group (In years)	Male Donors	Female Donors	Elevated Neopterin Level
15-20	51	22	7
21-25	110	12	24
26-30	51	3	14
31-35	12	3	3
36-40	16	9	6
41-45	9	1	4
46-50	4	1	0
Total	253	51	58

[Table/Fig-1]: Age wise Distribution of Male and Female donors and number of donors with elevated neopterin level.

Neopterin Range (nmol/l)	Number of Donors screened			Percentage
	Males (253)	Females (51)	Total (304)	
10-15	35	4	39	67.24
15.1-20	9	2	11	18.97
20.1-25	5	0	5	8.62
25.1-30	0	0	0	0
30.1-35	3	0	3	5.17
Total	52	6	58	100

[Table/Fig-2]: Distribution of elevated levels (range wise) of neopterin among donors

S. No.	Screening Test	Number of Positives (Out of 304)	Number of Positives with elevated neopterin level (>10nmol/l)
1	HBsAg	8 (2.63%)	8 (100%)
2	HIV 1,2 Ab	5 (1.64%)	5 (100%)
3	MP/MF (Leishman's smear)	0	0
4	Rapid Plasma Reagin Card test	0	0
5	HCV Ab	2 (0.657%)	2 (100%)
6	CMV(IgM-ELISA)	22 (7.24%)	22 (100%)
7	p24 Ag	3 (0.986%)	3 (100%)

[Table/Fig-3]: Prevalence of transfusion transmitted diseases among donors and its correlation with neopterin

Total number of samples positive for screening tests- 40
Total number of positive samples with neopterin elevation-40

Neopterin Range (nmol/l)	HBsAg	HIV 1,2 Ab	HCV Ab	CMV (IgM-ELISA)	p24 Ag
10-15	3	1	2	16	1
15.1-20	5	1	0	3	0
20.1-25	0	3	0	1	1
25.1-30	0	0	0	0	0
30.1-35	0	0	0	2	1
Total	8	5	2	22	3

[Table/Fig-4]: Distribution of elevated neopterin levels in various transfusion transmitted diseases

with neopterin is shown in [Table/Fig-3]. Distribution of elevated neopterin levels in various transfusion transmitted diseases is shown in [Table/Fig-4].

DISCUSSION

Study was conducted on voluntary blood donors attending the blood bank for a period of 3 months at Government General Hospital. Three hundred and four voluntary blood donors were selected and the age group in our study was in the distribution of 18-46 years, whereas study on the voluntary donors in Tirol region in Austria the age distribution was 17-64 years [3]. Among 304 donors, 253 were males and 51 were females. Majority of male donors (110) were in the 21 to 25 age distribution while in the females majority (22) was in the 15 to 20 age group.

In our study out of 304 donors, 58(19.07%) of them showed elevated neopterin levels whereas study by Honlinger et al showed only 1.6% [12]. Out of 58 neopterin elevated donors 67.74% were having values in the range of 10-15nmol/l. The Tirol study showed 0.09% donors with values above 25 nmol/l [12] whereas in our study we observed this value in 5.17% donors.

All the donors screened for routine basic screening test (HIV 1 &2 Ab, HBsAg, HCV and RPR). After screening, out of 58, only 15 donors were positive for infections screened, which includes HBsAg(8), HIV(5), HCV (2) respectively. All these 15 donors who were positive for infection, correlated with elevated level neopterin. The remaining 43 donors with elevated neopterin level was screened for CMV IgM, MP/MF and p24 Ag. Out of 43 donors, CMV IgM was positive in 22 and p24 Ag was positive in 3.

Among blood donors CMV IgM were positive in 22 donors (7.24%) when compared to study done by Honlinger et al it was almost double which showed 3.7% positivity [12]. 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. Similarly donors with increased neopterin levels the occurrence 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 [13].

In our study out of 304 screened, 8 (2.6%) were positive for HbsAg. The neopterin content in the sera of viral B hepatitis was 19.9%+ –5.7nmol/l. Among the eight HbsAg positive donors, 5 of them showed neopterin levels in the range of 15-20nmol/l. The data was obtained for an evident increase in neopterin levels associated with virus B hepatitis this was well supported by another study done by Somsonov et al [14].

Five HIV positive donors correlated with neopterin assay whereas three (0.986%.) donors with elevated neopterin level had p24 Ag which the routine test missed. A study by Fuchs et al showed increased neopterin levels in all 100% HIV positive donors. Three quarters of asymptomatic persons with HIV infection have increased neopterin levels [3], and these levels are even higher during acute HIV infection [15]. In addition, in human immunodeficiency virus (HIV) infection, neopterin levels increase in parallel with progressive disease, are inversely correlated with $CD4^+/CD8^+$ T-cell subset ratios and are of predictive significance [16].

Hepatitis C virus infection also correlated with elevated neopterin levels. The two(0.6%) HCV positive samples had neopterin values in the range of 10.1–15nmol/l. Similar result was obained by Harald Schennach et al [17].

In almost all the patients with acute viral infections neopterin levels were increased, irrespective of the specific nature of the virus, this was demonstrated in patients with acute hepatitis A or B [18], patients with Epstein-Barr-virus infection (infectious mononucleosis) and cytomegalovirus (CMV) infection but also in patients with measles [19]. Usually neopterin concentrations 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 [20].

Eighteen samples which had elevated neopterin values but were not positive for any one of the screening tests. Follow up of these donors and repeat neopterin after 4 weeks would throw more light on it. In our opinion keeping the safety of the patients in mind, these eighteen samples should not be used for transfusion. To improve the safety of blood donations, additional neopterin testing of blood donations became mandatory for all Austrian blood-transfusion services in addition to testing for HIV-1 and -2 antibodies, hepatitis C virus (HCV) antibodies, hepatitis B virus (HBV) surface antigen, alanine aminotransferase (ALT), and *Treponema pallidum* antibodies [21]. The neopterin assay thus detects a variety of potentially harmful diseases or conditions which would not be revealed by the usually employed battery of routine tests. Hence we conclude that the risk of transmitting new pathogens may be reduced using neopterin assay as a routine in blood banks.

REFERENCES

[1] Dwyre DM, Fernando LP, Holland PV. Hepatitis B, hepatitis C and HIV transfusion-transmitted infections in the 21st century. *Vox Sanguinis* 2011; 100(1): 92–98.

[2] NIH consensus development panel on infectious disease testing for blood transfusions: Infectious disease testing for blood transfusions. *JAMA* 1995; 274:1374–79

[3] Honlinger M, Fuchs D, Hausen A, et al. Serum-Neopterin best immung zur zusatzlichen Sicherung der Bluttransfusion. *Dtsch Med Wochenschr*. 1989;114:172-76.

[4] Shaw AC. Serum C-reactive protein and neopterin concentrations in patients with viral or bacterial infection. *J Clin Pathol* 1991;44:596-99

[5] Inci Fisenk B, Durdal US, Osman I. Ozcebe & Gulsen Hascelik. The value of increased Neopterin levels in reducing transfusion-transmitted virus infections: Detection of a donation from a HbsAg positive chronic carrier by screening of neopterin in Turkish blood donors. *Scandinavian Journal of Infectious disease* 2005; 37:599-604.

[6] Werner-Felmayer G, Werner ER, Fuchs D, Hausen A, Reibnegger G, Wachter H. Neopterin formation and tryptophan degradation by a human myelomonocytic cell line (THP-1) upon cytokine treatment. *Cancer Res* 1990;50:2863-67.

[7] Fuchs D, Weiss G, Wachter H. The role of neopterin as a monitor of cellular immune activation in transplantation, inflammatory, infectious and malignant diseases. *Crit Rev Clin Lab Sci* 1992;29:307-41.

[8] Reddy MM, GRIECO MH. Neopterin and Alpha and Beta Interleukin-1 Levels in Sera of Patients with Human Immunodeficiency Virus Infection. *J Clin Microbiol* 1989; 27(9): 1919-23.

[9] Chandra Immanuel, Lalitha Victor, K Silambu Chelvi, C Padmapriyadarsini, Fathima Rehman et al. Serum neopterin levels in HIV infected patients with & without tuberculosis. *Indian J Med Res* 2005;121:220-25.

[10] Li Yanchun, Hu Zhidong. Significance of humoral neopterin in clinical diagnostics and prognosis. *J Med Colleges of PLA* 2011;26(1): 45-51.

[11] Pingle KS, Tumane RG, Jawade AA. Neopterin: Biomarker of cell-mediated immunity and potent usage as biomarker in silicosis and other occupational diseases. *Indian Journal of Occupational and Environmental Medicine* 2008;12(3):107-111.

[12] Hönlinger M, Fuchs D, Hausen A, Reibnegger G, Schönitzer D, Werner ER et al. Serum neopterin determination for the additional safeguarding of blood transfusions. Our experiences with 76,587 blood donors. *Dtsch Med Wochenschr* 1989;114(5): 172-76.

[13] Schennach H, Mayersbach P, Schönitzer D, Fuchs D, Wachter H, Reibnegger G. Increased prevalence of IgM antibodies to Epstein-Barr virus and parvovirus B19 in blood donations with above-normal neopterin concentration. *Clin Chem* 1994;40: 2104-5.
[14] Somsonov Mlu, Golban TD, Nasonov EL, Masenko VP. Serum neopterin in hepatitis B. *Klin Med* (Mosk) 1992; 70(3-4): 40-42.
[15] Zangerle R, Schonitzer D, Fuchs D, Most J, Dierich MP, Wachter H. Reducing HIV transmission by seronegative blood. *Lancet.* 1992;340: 130-31.
[16] Fuchs D, Hausen A, Reibnegger G, Werner ER, Dierich MP, Watchter H. Neopterin as a marker for activated cell-mediated immunity: application in HIV infection. *Immunol Today*. 1988; 9(5):150-55.
[17] Harald Schennach Diether Schoenitzer Dietmar Fuchs. Association between Chronic Hepatitis C Virus Infection and Increased Neopterin Concentrations in Blood Donations. *Clinical Chemistry* 1998;44(10): 2225-6.
[18] Reibnegger G, Auhuber I, Fuchs D, et al. Urinary neopterin levels in acute viral hepatitis. *Hepatology* 1988;8:771-74.
[19] Johnson RT, Vaisberg A. Immune activation during measles: interferon gamma and neopterin in plasma and cerebrospinal fluid in complicated and uncomplicated disease. *J Infect Dis* 1990;161:449-53.
[20] Prior C, Fuchs D, Hausen A, et al. Potential of urinary neopterin excretion differentiating chronic non-A, non-B hepatitis from fatty liver. *Lancet* 1987; ii:1235-7.
[21] Schennach H, Murr C, Ga¨chter E, Mayersbach P, Scho¨nitzer D, Fuchs D. Factors Influencing Serum Neopterin Concentrations in a Population of Blood Donors. *Clinical Chemistry* 2002; 48(4): 643-45

AUTHOR(S):

1. Dr. Shameem Banu A.S.
2. Dr. Latha P.
3. Dr. Kaveri K.
4. Dr. Jayakumar S.

PARTICULARS OF CONTRIBUTORS

1. MBBS, MD (Microbiology), Department of Microbiology, Saveetha Medical College & Hospital, Saveetha University, Thandalam, Kancheepuram - 602 105 Tamilnadu, India.
2. MBBS, MD (Microbiology), Assistant Professor, Department of Microbiology, Madras Medical College, Chennai.
3. MBBS, MD (Microbiology), Deputy Director, Virology Section, Department of Preventive Medicine, King Institute, Guindy, Tamil Nadu, India.
4. MBBS, MD (Microbiology), Associate Professor, Department of Microbiology, Saveetha Medical College & Hospital, Saveetha University, Thandalam, Kancheepuram - 602 105.Tamilnadu, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr.Shameem Banu A.S.
Professor & Head, Department of Microbiology,
Saveetha Medical College & Hospital, Saveetha University,
Thandalam, Kancheepuram District – 602 105
Tamilnadu, India.
E mail: shameembanu10@rediffmail.com
Mobile Number: 9940127670

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