

# Serum Neopterin: A Potential Marker for Screening Blood Donors

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## ABSTRACT

**Objective:** To determine serum neopterin levels in blood donors of local population and its association with transfusion transmitted infections.

**Study Design:** A cross-sectional observational study.

**Place and Duration of Study:** Department of Physiology, Liaquat National Hospital and Medical College (LNHMC) in collaboration with Basic Medical Sciences Institute (BMSI) and Jinnah Postgraduate Medical Centre (JPMC), Blood Bank, Karachi, Pakistan, from January to June 2015.

**Methodology:** During this period, a total of 174 blood donors were selected through random sampling technique. All participants fulfilling the inclusion criteria involving apparently healthy blood donors of either gender within the age bracket of 18 - 60 years and consenting to participate were selected. The participants were screened for transfusion transmitted infections as per WHO recommendations through the standard procedures used for screening at the JPMC blood bank. The demographic profile, anthropometric measurements and vitals were recorded for every participant. Serum neopterin was measured using ELISA kits. Data was analysed on SPSS version 21. ANOVA and chi-square tests were applied as tests of significance at a p-value of <0.05.

**Results:** The neopterin content in the sera of disease negative blood donors was  $6.23 \pm 2.19$  nmol/l as compared to disease positive blood donors, in whom the neopterin level was increased to  $15.10 \pm 4.93$  nmol/l ( $p = 0.001$ ).

**Conclusion:** The neopterin assay has the potential to detect a number of transfusion transmissible viral diseases; which may, or may not be revealed by the usually employed battery of routine tests. We conclude that the risk of transfusion transmitted pathogens in our population can be reduced significantly, using neopterin assay as a routine in blood banks.

**Key Words:** Blood donors. Screening. Neopterin. Blood transfusion. Transmissible infections.

## INTRODUCTION

Blood transfusion is an important component of health-care which saves millions of lives; however, this life-saving procedure may sometimes be associated with risks including the transmission of disease from an infected donor to the recipient. Every blood transfusion, therefore, carries a potential risk for transmissible diseases.<sup>1</sup>

The transfusion of blood-borne infections is the most important transfusion associated risk, especially in the developing countries. Knowledge of the infectious agents, particularly those which are endemic in a region, is crucial for minimising the risk of transmission of these infections.<sup>2</sup>

Screening for transfusion transmissible infections (TTIs) ensures that blood transfusion is safe. Unsafe blood transfusion is damaging from both a human and an

economic point of view. Each unit of blood transfused potentially carries 1% risk of transfusion related problems, including TTIs such as human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), syphilis and malaria. In Pakistan, with an only nascent culture of voluntary donations, a strong dependence on replacement and paid donors and the lack of systematic screening strategy. The infection risks are formidable since the commercially remunerated blood donors and family replacement donors are more likely to transmit TTIs as compared to the voluntary donors. The morbidity and mortality, resulting from transfusion of infected blood, have far reaching consequences for the recipients and their families.<sup>3</sup>

As far as the local blood donors are concerned, the studies conducted show that the prevalence of blood transmitted infection is very high in commercial blood donors. Around 20% of them are positive for hepatitis C and 10% for hepatitis B infections. Amongst the family replacements/donors, prevalence of 5% for hepatitis B and 2.5% for hepatitis C infections was found; whereas, in voluntary blood donors the incidence was 2% for hepatitis B and 0.5% for hepatitis C infections.<sup>4</sup>

For screening of the donated blood, the World Health Organisation (WHO) has recommended core tests for hepatitis B surface antigen (HBsAs), antibody to hepatitis C and HIV subtypes 1 and 2, and serological test for syphilis.<sup>5</sup> However, specific testing cannot control newly emerging and/or unrecognised infections.

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Potential hazardous pathogens may remain undetected either because they are known but not screened, or they are undiscovered; thus current screening technologies are ineffective against them. It is also possible that blood is donated during the window period, when the donor is already diseased but the specific antibody production has not yet started. Non-specific screening with immune response markers like neopterin, could decrease this threat.<sup>5</sup>

Neopterin is a sensitive indicator of activated cell mediated (T-helper cells type-1) immune response. Activation of the immune system and subsequent rise in neopterin concentration is a key feature of various pathologies, specifically viral infections. Since neopterin is stable in biological fluids, it can be used to evaluate the intensity of cell-mediated immune responses. Various studies worldwide have established the role of neopterin as a biomarker for the onset, progression, and outcome of different diseases.<sup>6</sup>

Raised levels of serum neopterin are found in chronic HBV positive subjects. In hepatitis B carriers, raised neopterin levels may be an indicator of replication.<sup>7</sup> In HCV positive patients, elevated neopterin levels are more often associated with a positive PCR.<sup>8</sup>

Serum neopterin levels are found to be raised in HIV infected patients not only during the acute phase but also in the window period and correlate with the extent and severity of the disease.<sup>9</sup> Studies have demonstrated higher serum neopterin concentrations in Dengue fever, which has the potential of transfusion transmission.<sup>10</sup> Neopterin levels are also found to be elevated in malaria.<sup>11</sup>

After many years of trials, the nationwide screening for elevated neopterin was introduced in Austria in 1994, when its significant role in blood screening was recognised.<sup>12</sup> Since then, a number of studies done in Austria and various other countries, have proved the capability of neopterin screening to improve safety of blood donations regarding the transmission of virus infections.<sup>13,14</sup>

The objective of this study was to estimate serum neopterin levels in blood donors of local population and to determine its association with TTIs. If found effective, we might be able to use this marker for additional safety of blood transfusions in our population.

## METHODOLOGY

This cross-sectional study was carried out in the Department of Physiology, Liaquat National Hospital and Medical College (LNHMC) in collaboration with Basic Medical Sciences Institute (BMSI) and Jinnah Postgraduate Medical Centre (JPMC), Blood Bank, Karachi. Data collection was for a period of 6 months from January to June 2015. During this period, a total of

174 blood donors were selected through random sampling technique. All participants fulfilling the inclusion criteria, involving apparently healthy blood donors of either gender within the age bracket of 18 - 60 years and consenting to participate, were selected on odd dates of the month excluding the official holidays.

The selected participants' blood was screened for WHO recommended diseases through the standard procedures used for screening at the JPMC blood bank. Serum neopterin was measured with commercial enzyme linked immunoassay (ELISA) kits. Blood samples were obtained in 4-5 ml evacuated sterile gel tubes. After centrifugation of plasma, the serum obtained was stored at -20°C until neopterin assay was performed. Since neopterin is photosensitive, all samples were kept in dark during all procedures. The cut-off for serum neopterin concentration for healthy donors was taken as < 10 nmol/l.<sup>15</sup>

The data analysis was done on computer package SPSS (Statistical Packages of Social Sciences) version 21.0. Clinical characteristics were summarised in terms of frequencies and percentages for qualitative/categorical variables (age groups, blood groups, screening results of blood TTIs, etc.) and mean  $\pm$  standard deviation (SD) for quantitative variables (neopterin level in ng/ml). Chi-square test was used to find the association of qualitative/categorical variables and student t-test/ANOVA was used for quantitative data. In all statistical analyses, p-value <0.05 was considered significant. The study was approved by the Ethical Review Committee of respective institutions.

## RESULTS

A total of 174 blood donors were recruited in the study. All the donors were screened for routine basic screening tests (HIV 1 and 2, HBsAg, HCV, malaria and syphilis). After screening, out of 21 donors with elevated neopterin, 14 were positive for infections screened routinely which included HBsAg in 7 (4%), HCV in 6 (3.4%) and HCV + HIV co-infection in 1 (0.6%). The remaining 7 (4%) donors with elevated neopterin were further screened with an advanced panel of tests including cytomegalo virus (CMV) IgM and Dengue IgM antibodies. Three (1.72%) of these asymptomatic donors showed presence of CMV IgM antibodies.

In this study, out of 174 subjects, 154 (88.5%) were negative for the screening of TTIs. The neopterin content in the sera of these blood donors was  $6.23 \pm 2.19$  nmol/l; whereas in the blood donors who tested positive for TTIs either by routine screening or through advanced panel of screening markers, the neopterin level was elevated to  $15.10 \pm 4.93$  nmol/l. When compared statistically, the difference between these values was highly significant ( $p=0.001$ , Table I).

When neopterin levels were assessed in blood donors suffering from various TTIs, significant increase in its levels was observed as compared to healthy donors (p=0.001, Table II).

Table III shows the variation in neopterin levels in various TTIs. Hepatitis B seropositive donors showed increased neopterin level between 10.1 - 22.0 nmol/l. Hepatitis C seropositive donors showed elevated neopterin level between 10.1 - 22.0 nmol/l. A single donor who was positive for HIV+HCV co-infection showed raised neopterin level of 25.01 nmol/l. The asymptomatic CMV positive blood donors also showed elevated neopterin level between 14.1-22.0 nmol/l. However, syphilis positive donors showed normal range of neopterin level, i.e. 3.1-10.0 nmol/l in their serum.

Sensitivity =  $17/21 \times 100 = 80.9\%$ , specificity =  $150/153 \times 100 = 98.0\%$ , and accuracy =  $167/174 \times 100 = 96.0\%$ .

Using chi-square test for association of elevated (>10 nmol/l) and serum neopterin level with screening test

**Table I:** Serum neopterin levels in donors with positive and negative screening tests (n=174).

Screening test	No. (%)	Number of TTI positive with elevated neopterin level (>10 nmol/l)*	Neopterin level (nmol/l)	
			Mean ± S.D	p-value
Negative	154 (88.51)	4 (2.6%)	6.23 ±2.19	0.001
Positive	20 (11.49)	17 (85.0%)	15.10 ±4.93	
Total	174	21	-	-

Sensitivity =  $17/21 \times 100 = 80.9\%$ ; Specificity =  $150/153 \times 100 = 98.0\%$ ; and Accuracy =  $167/174 \times 100 = 96.0\%$ .

\* Using Chi-square test for association of elevated (>10 nmol/l) serum neopterin level with screening test (negative / positive) and t-test for actual value of neopterin level (mean ± S.D) with negative and positive screening test.

**Table II:** Serum neopterin levels in specific transfusion transmitted infections (n = 174).

Transmitted disease	No. (%)	Number of TTI positive with elevated neopterin level (>10 nmol/l)	Neopterin levels nmol/l	p-value
Hepatitis B	7 (4.02)	7 (100%)	16.39 ±3.21	0.001
Hepatitis C	6 (3.45)	6 (100%)	15.59 ±1.63	
HIV + Hepatitis C	1 (0.57)	1 (100%)	25.01 ±0.00	
CMV	3 (1.72)	3 (100%)	17.03 ±1.83	
Syphilis	3 (1.72)	0	5.86 ±0.78*	

\*Using ANOVA for comparison of neopterin level (nmol/l) with transmitted infection.

**Table III:** Variation of neopterin levels in transfusion transmitted infections (n=174).

Neopterin range (nmol/L)	Subject (%)	Transfusion transmitted disease				
		Hep "B"	Hep "C"	Hep "C" & HIV	CMV	VDRL
3.1 - 6.0	70 (40.2%)	0	0	0	0	1
6.1 - 10.0	83 (47.7%)	0	0	0	0	2
10.1 - 14.0	4 (2.3%)	2	1	0	0	0
14.1 - 18.0	11 (6.3%)	3	4	0	2	0
18.1 - 22.0	5 (2.9%)	2	1	0	1	0
22.1 - 26.0	1 (0.6%)	0	0	1	0	0
Total	174	7	6	1	3	3

(negative / positive) and t-test for actual value of neopterin level (mean ± S.D) with negative and positive screening test.

## DISCUSSION

Blood transfusion is a life-saving procedure and helps innumerable people globally. It is however, an important mode of transfer of infections from blood donors to the recipients. Presently, in developing countries, the prevalence of TTIs is much higher and we are considerably away from attaining a zero-risk level.

In this study, out of 174 donors, 21 (12.06%) showed elevated neopterin level which is in accordance with the study done by Banu *et al.*, who found 58 (19.09%) donors with elevated neopterin level.<sup>13</sup> In another study conducted by Honlinger *et al.*, only 12 (1.6%) blood donors showed increased neopterin level; whereas in the study of Fisenk *et al.*, 57 (5%) donors showed elevated neopterin levels.<sup>16,17</sup>

Among blood donors, CMV IgM was positive in 3 donors (1.724%). When compared to study conducted by Honlinger *et al.*, it was almost half, which showed positivity in 12 (3.7%) donors.<sup>16</sup> The neopterin level in the sera of these CMV positive donors was  $17.03 \pm 1.83$  nmol/l which is in agreement with the studies of Schennach *et al.* and Parrak *et al.*, who found similarly elevated neopterin levels in CMV positive asymptomatic blood donors.<sup>17,18</sup> Schennach *et al.* reported a 20-fold increased incidence of acute CMV infection in blood donors with elevated neopterin level, where increased neopterin level was found even before CMV IgM seroconversion.<sup>17</sup>

In this study, out of 174 donors, 7 (4.02%) were positive for HbsAg. The neopterin content in the sera of hepatitis B positive donors was  $16.39 \pm 3.21$  nmol/l. Kalkan *et al.* in their study also found elevated neopterin levels of  $15.6 \pm 5.1$  nmol/l in chronic HBV positive subjects, which is in agreement with our study.<sup>19</sup> The study done by Banu *et al.* also found similar results of neopterin elevation in hepatitis B positive blood donors.<sup>13</sup>

In this study, one HIV positive donors showed elevated neopterin level of 25.01 nmol/l. The studies conducted by Fusch *et al.* also showed increased neopterin level in 100% HIV positive subjects.<sup>20</sup> They found that in HIV infection, neopterin levels increase with progressive disease and are inversely correlated with CD4+ t-cell count. The values are also of predictive significance. Recently, in their study, Nubling *et al.* concluded that the diagnostic sensitivity of neopterin screening during the HIV window phase is similar to p24 antigen test. Therefore, neopterin screening of blood donors may also identify the window phase of HIV infection.<sup>21</sup>

HCV infection also correlated with elevated neopterin levels. The 6 (3.4%) HCV positive samples had neopterin values of  $15.59 \pm 1.63$  nmol/l. Similar results of

neopterin elevation were obtained by Schennach *et al.* and Banu *et al.* in their studies.<sup>13,17</sup>

In this study, three of the donors tested positive for syphilis. The neopterin concentration in their serum was  $5.86 \pm 0.78$  nmol/l, which was below the cut-off level of 10 nmol/l. Similar findings were observed by N'gom *et al.* who did not find any neopterin elevation with syphilis.<sup>22</sup> This is probably due to the fact that in contrast to the viral infections which trigger the cytotoxic immune response involving TH1 cells, the systemic bacterial infections invoke humoral immunity, thus involving TH2 instead of TH1, and therefore, no change in neopterin levels was observed.<sup>22</sup>

A limitation of this study was the sample size which was restricted because of constraint in funds. The strengths of the study from the novelty of topic to the importance of neopterin levels as a screening tool cannot be overlooked. The random sampling technique supported the small sample size by decreasing the bias in the study.

## CONCLUSION

Neopterin screening has the potential to detect a number of blood transfusion transmissible viral diseases which may or may not be detected by conventional and routine tests. On the basis of our findings, we suggest and recommend that further extensive research be undertaken on a larger scale to explore the beneficial role of neopterin screening in blood donation in reducing TTIs in our population.

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