Factors Influencing Serum Neopterin Concentrations in a Population of Blood Donors, Harald Schennach,1 Christian Murr,2 Elmar Gichter,1 Peter Mayersbach,1 Diether Schöntzer,1 and Dietmar Fuchs2 (1 Central Institute for Blood Transfusion, University Clinic Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria; 2 Institute of Medical Chemistry and Biochemistry, Leopold-Franzens University, and Ludwig Boltzmann Institute for AIDS Research, Fritz Pregl Strasse 3, A-6020 Innsbruck, Austria; * author for correspondence)

Neopterin, a product of interferon-γ-activated monocytes/macrophages is a sensitive indicator of cell-mediated immune activation (1). In humans, increased concentrations of neopterin in serum and urine have been found during allograft rejection episodes and in various malignant disorders, autoimmune diseases, and viral infections, including HIV type 1 (HIV-1) (2–8). 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 (9).

In the Austrian Tyrol, ~900,000 voluntary blood donations have been routinely screened for increased neopterin concentrations since 1986. According to the results of previous studies, the cutoff for neopterin concentrations was set to the 98th percentile, thus tolerating a donation loss of 2% (9).

Although the behavior of neopterin concentrations during the course of acute viral infections, for example, is well known, there are fewer data regarding the association of neopterin concentrations and physiologic findings in a healthy population. In this study, we looked for associations between neopterin concentrations and other laboratory tests that are routinely performed on blood donations at our institute. Associations between neopterin concentrations and physiologic conditions of blood donors obtained during the blood-donation procedure were also investigated.

Blood samples from 1156 consecutive blood donors from the Austrian Tyrol (693 males and 463 females) were collected in January 2001. A questionnaire was administered, which asked about current smoking habits, medical conditions, and use of medications. Of the blood donors, 262 (22.7%) reported being smokers (range of cigarettes smoked daily, 1–50), 56 (4.8%) were taking antihypertensive drugs, and 52 (4.5%) reported using antiinflammatory drugs, such as acetylsalicylic acid. The body mass of the donors was classified by body mass index (BMI) as described earlier (10). Arterial blood pressure was measured indirectly at the upper arm, and body temperature was determined from the external auditory canal by an infrared thermometer (LighTouch® LTX; Exergen). Hemoglobin values were measured on a Hemocue® hemoglobin data management analyzer immediately before blood collection. Hemoglobin values <128 g/L (0.78% of the blood donors) led to exclusion from blood donation, but the data of these donors were included in this study.

Blood samples were drawn by venipuncture. The blood was allowed to clot at room temperature, and serum was obtained by centrifugation at 3220 g rpm for 15 min. All analyses were performed within 1 day after blood collection. To exclude infections hazardous to blood recipients, serum antibodies against HIV-1 and -2, HCV, T. pallidum, and HBV surface antigen were determined in all donor samples; all samples were negative. The absence of viral RNA or DNA (HCV, HBV, HIV-1) was determined by PCR pool testing. Serum neopterin was measured by a commercially available ELISA method (ELItest® Neopterin; BRAHMS) with a detection limit of 2 nmol/L neopterin and interassay CVs of 3.9–8.2%. Upper limits of the reference interval (95th percentiles) for neopterin concentrations depend on age, ranging from 8.7 nmol/L (19–75 years) to 13.5 nmol/L (<19 years) and 19.0 nmol/L (>75 years), as described earlier (11). Serum cholesterol was measured enzymatically according to the principle of Stadtman with a Dade Dimension® AR automated analyzer and commercial reagent sets (Dade Behring), and the laboratory reference value used for cholesterol was <5.2 mmol/L (200 mg/dL). ALT activity was measured at 25°C on a Dade Dimension AR (Dade Behring) by a method optimized according to the criteria of the German Society of Clinical Chemistry. Individuals with ALT >40 U/L were regarded as suspicious for hepatic infections, and their donations were therefore excluded from transfusion.

Table 1 reports characteristics of the study participants. Notably, 8.1% of the studied donors had neopterin concentrations above the 95th percentile, and 2.16% of them had neopterin concentrations >11.0 nmol/L, which excluded their donation from transfusion. Because the distributions of observed values were nonnormal for some indices, correlations between the investigated variables were assessed by Spearman’s rank correlation coefficients. There was a weak but highly significant correlation of neopterin concentrations with age \( r = 0.259; \text{confidence interval (CI), 0.202–0.313; } P < 0.0001 \) and diastolic blood pressure \( r = 0.132; \text{CI, 0.073–0.190; } P < 0.0001 \), and an inverse correlation was observed between neopterin concentrations and the number of cigarettes smoked daily \( r = -0.130; \text{CI, -0.188 to 0.07; } P < 0.0001 \). Similarly, a weaker positive correlation was found between neopterin concentrations and BMI \( r = 0.084; \text{CI, 0.025–0.143; } P < 0.01 \). There were no significant correlations between neopterin concentrations and serum cholesterol, serum ALT, blood hemoglobin, body temperature, or systolic blood pressure. Blood donors >41.5 years, the median of the distribution, had higher neopterin concentrations (median, 6.1 nmol/L) than those <41.5 years (median 5.5 nmol/L; Mann–Whitney test, \( U = 128500; P < 0.0001 \)). Similarly, patients with diastolic blood pressure >10.9 kPa, the median of the distribution, had higher neopterin concentrations (median, 6.0 nmol/L) than those with diastolic blood pressure ≤10.9 kPa (median, 5.6 nmol/L; Mann–Whitney test, \( U = 146400; P = 0.0003 \)), and...
nonsmokers showed higher neopterin concentrations (median, 5.8 nmol/L) than smokers (median, 5.45 nmol/L; Mann–Whitney test, \( U = H100599, P = 0.0001 \)).

To test the relationship of serum neopterin concentrations with age, smoking status, diastolic blood pressure, and BMI, we performed four-way ANOVA using the program BMDP2V (BMDP Statistical Software, 1990 Ed.; University of California Press). In this analysis (Fig. 1), the factors age, diastolic blood pressure, and BMI were dichotomized by the median of the observed distribution, and the variable smoking status was dichotomized in smokers and nonsmokers. Because variances in the 15 subgroups formed on the basis of age, smoking status, diastolic blood pressure, and BMI were different, a reciprocal transformation of neopterin concentrations was performed before analysis. Three factors, age (\( F = H100526.86, P = 0.0001 \)), smoking status (\( F = H100515.08, P = 0.0001 \)), and diastolic blood pressure (\( F = H10054.87, P = 0.0273 \)), showed an effect on neopterin concentrations, whereas BMI did not (\( F = H10050.83 \); not significant). All the interaction terms (e.g., age vs smoking status, age vs diastolic blood pressure, smoking status vs diastolic blood pressure, diastolic blood pressure vs age vs smoking status, and so forth) were not statistically significant, suggesting that interaction effects were negligible.

The significant increase of neopterin concentrations with age is in good agreement with previous studies in this field (11–15) and is also evident from the age dependency of the reference values (see Table 1). The reason for this phenomenon is still a matter of discussion. Possibly the higher incidence of still unrecognized disease processes associated with immune activation and increased neopterin production in the elderly, e.g., arteriosclerosis (16, 17) or neurodegenerative disorders (18), might contribute to higher reference values in the elderly. This possibility assumes that some of the individuals in the reference population already have a pathologic process that is not yet detectable clinically, which would be in line with the observation made in nonagenarians that higher neopterin concentrations were associated with reduced residual life span (14).

In agreement with an earlier study (12), smokers showed lower neopterin concentrations than nonsmokers.

### Table 1. Baseline characteristics of blood donors in the study (n = 1156; 693 males and 463 females).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>First quartile</th>
<th>Median value</th>
<th>Third quartile</th>
<th>Range</th>
<th>Below</th>
<th>Above</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>32.8</td>
<td>41.5</td>
<td>51.0</td>
<td>18.1–64.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neopterin, nmol/L</td>
<td>5</td>
<td>5.7</td>
<td>6.9</td>
<td>3.4–19.6</td>
<td>94</td>
<td>156</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.7 (1800)</td>
<td>5.3 (2060)</td>
<td>6.0 (2320)</td>
<td>2.9–11.3 (1120–4380)</td>
<td>647</td>
<td>56.0</td>
</tr>
<tr>
<td>Alanine amino transferase, U/L</td>
<td>12</td>
<td>15</td>
<td>20</td>
<td>4–97</td>
<td>15</td>
<td>1.3</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>150</td>
<td>160</td>
<td>169</td>
<td>124–201</td>
<td>1</td>
<td>158</td>
</tr>
<tr>
<td>Diastolic blood pressure, kPa (mmHg)</td>
<td>10.1 (76)</td>
<td>10.9 (82)</td>
<td>12.0 (90)</td>
<td>6.9–16.0 (52–120)</td>
<td>145</td>
<td>12.5</td>
</tr>
<tr>
<td>Systolic blood pressure, kPa (mmHg)</td>
<td>16.9 (127)</td>
<td>18.3 (137)</td>
<td>19.9 (149)</td>
<td>13.3–28.8 (100–216)</td>
<td>133</td>
<td>11.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.5</td>
<td>24.5</td>
<td>26.8</td>
<td>17.3–43.9</td>
<td>13</td>
<td>509</td>
</tr>
<tr>
<td>Body temperature, ºC</td>
<td>36.2</td>
<td>36.4</td>
<td>36.7</td>
<td>35.0–38.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers (n = 262), cigarettes per day</td>
<td>10</td>
<td>15</td>
<td>20</td>
<td>1–50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( a \) n, number of observations.

\( b \) Reference values: \( \leq 13.5 \) nmol/L (<19 years), \( \leq 8.7 \) nmol/L (19–75 years), \( \leq 19 \) nmol/L (>75 years).

\( c \) Reference values: \( \leq 5.2 \) nmol/L (2000 mg/L).

\( d \) Reference values: \( \leq 40 \) U/L.

\( e \) Reference values: males, 130–180 g/L; females, 120–160 g/L.

\( f \) Reference values: \( \leq 12.7 \) kPa (95 mmHg).

\( g \) Reference values: \( \leq 21.3 \) kPa (160 mmHg).

\( h \) Reference values: 19–25 kg/m².

Fig. 1. Untransformed mean values and SDs of the eight formed subgroups.

Values were established on the basis of a three-factor ANOVA with the following significant factors: age (<41.5 years and \( \geq 41.5 \) years); smoking status; and diastolic blood pressure. LP denotes \( \leq 10.9 \) kPa; HP denotes \( >10.9 \) kPa. The box plots extend from the mean ± 1 SD, with a horizontal line at the mean.
The authors of several studies have observed increased intraalveolar cytokine production (19) or increased CD4+ T lymphocytes in smokers (20). A suppressive effect of tobacco smoke on the human immune system has also been reported (21), which could explain the lower neopterin production of smokers in our study.

We noted a correlation between neopterin concentrations and diastolic blood pressure values. Although an explanation for this correlation is not immediately evident, the possibility of a connection between immunoactivation and increased diastolic pressure cannot be excluded definitively. Recently, a correlation was reported between ischemic attack and both neopterin and the potent endothelial-derived, vasoconstrictive peptide endothelin-1 (22). Conditions such as atherosclerosis (16) or infections by Helicobacter pylori (23), which are associated with immune activation and higher neopterin production, might possibly lead to vasoconstriction and thereby increased diastolic blood pressure because of increased endothelin-1 production.

The positive correlation that was found between neopterin concentrations and BMI, a correlation that was not an independent one, supports results of an earlier study in individuals with non-insulin-dependent diabetes mellitus (13), and it would be in line with earlier suggestions that long-lasting immune activation, even if only moderate, may contribute to the development of obesity (24).

Nevertheless, our study of blood donors indicates that neopterin concentrations are associated with age, smoking status, diastolic blood pressure, and BMI, although the reasons for these associations are still unclear. The interindividual variation of neopterin concentrations among healthy individuals may, in part, be attributable to variations caused by these variables rather than analytical variation of the assay used.

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References


Serologic Diagnosis of Hantaan Virus Infection Based on a Peptide Antigen, Zheng Li, Xuefai Bai and Huijie Bian (1 School of Chemistry and Material Science, Shaanxi Normal University, 710062, Xi’an, China; 2 Department of Infectious Disease, Tangdu Hospital, 3 College Engineering Research Centre, Fourth Military Medical University, 710032, Xi’an, China; * author for correspondence: fax 86-29-3293906, e-mail bjh@pub.xaonline.com)

Hantavirus (HV) is the causative agent of a severe type of hemorrhagic fever with renal syndrome (HFRS), with an annual incidence in China of 50 000–100 000 cases. Hantaan virus (HTNV) 76–118 is the prototype strain of the HV genus and the hantaan serotype (1). HV has a single-stranded, negative-sense tripartite RNA genome, the segments of which are designated large, medium, and small. The RNA genome encodes the viral RNA polymerase, envelope glycoproteins (G1, G2), and nucleocapsid protein (NP) (2). A serologic investigation (3) showed that HTNV NP has strong antigenicity and immunogenic-