technical brief

Increased Prevalence of IgM Antibodies to Epstein–Barr Virus and Parvovirus B19 in Blood Donations with Above-Normal Neopterin Concentration, *Harald Schennach, Peter Mayersbach, Diether Schönitzer* (Central Inst. for Blood Transfusion and Immunol., Univ. Hosp., Anichstrasse 35, A-6020 Innsbruck, Austria), *Dietmar Fuchs, Helmut Wachter, and Gilbert Reibnegger*[1] (Inst. of Med. Chem. and Biochem., Univ. of Innsbruck, Fritz Pregl Str. 3, A-6020 Innsbruck, Austria; [1] corresponding author: fax 43-512-507-2865, E-mail gilbert.reibnegger@uibk.ac.at)

In the Austrian Tyrol, voluntary blood donations have been routinely tested for neopterin concentration since 1986. The rationale for this additional testing of donated blood is the increase in safety of blood donations with regard to transmission of diseases, particularly infectious diseases. Neopterin, a product of interferon-γ-activated macrophages (*1*), is a sensitive indicator of cell-mediated immune activation and thus represents a general, early and highly sensitive marker for viral infections (*2, 3*). Since June 17, 1993, by order of the Austrian Federal Government, all Austrian blood banking institutions have had to include in their panel of laboratory tests for blood donations an assay to detect cellular immune activation; at present, neopterin measurement is considered the most sensitive test available for this. Its concentrations can be measured in serum by various methods, including HPLC (*4*), RIA (*5*), and ELISA (*6*).

We have previously presented data on >75 000 donations that were tested for neopterin. Donors were invited for a more thorough physical and laboratory evaluation if neopterin was >10 nmol/L (*7*). This cutoff value was chosen as the 98th percentile of the distribution of observed neopterin concentrations (*7*) and has also been used by others (*5*). In about one-quarter of those reexamined this way, a cause for the increase in neopterin could be detected retrospectively. In the remaining cases, the reason for the neopterin increase remained obscure even after reexamination. Bearing in mind the logistic difficulties of such a reexamination, however, this result was not unexpected.

To investigate additional suspected causes for increased neopterin even in these unexplained donors, we tested their blood with specific assays for other infections not routinely screened for in transfusion medicine. Testing blood for IgM antibodies against cytomegalovirus (CMV) has demonstrated a 20-fold higher prevalence of acute CMV infection in patients whose blood contains increased neopterin vs patients with normal neopterin concentrations: odds ratio = 20.0 (95% confidence interval, 6.9–59; $P < 0.0001$) (*8*). Here we report results of our testing donated blood for evidence of acute infections with Epstein–Barr virus (EBV) or parvovirus B19 (PV B19). Neopterin is known to be a sensitive marker in EBV-related diseases (*9, 10*).

We tested for EBV because of the high frequency of acute EBV infections in young adults, who represent a major group of voluntary blood donors, and because of the danger of posttransfusion complications such as infectious mononucleosis, chronic active EBV infection, polymyalgia, chronic fatigue syndrome, or even multilocalized lymphomas in immunosuppressed blood recipients. We therefor examined 987 consecutive blood donors who had passed th routine physical examination but had blood neopterin con centrations of >10 nmol/L. Neopterin was determined by commercial RIA (Immutest; Henning-Berlin, Berlin, Ger many), and EBV IgM was determined by enzyme immu noassay (Enzygnost Anti-EBV/IgM; Behring, Marburg Germany). For controls, we used the results from 502 ran domly selected donors with neopterin concentrations <1(nmol/L. All procedures followed were in accordance witl the ethical standards of the Ethical Committee of the Uni versity of Innsbruck.

Whereas only 10 of the 502 controls (2.0%) gave positiv EBV IgM results, 54 of 987 (5.5%) donors with greate neopterin concentrations were seropositive for EBV IgM Thus, the chance of acute EBV infection is almost threefol greater in donors with increased neopterin than in thos with normal neopterin: odds ratio = 2.85 (95% confidenc interval, 1.5–5.6; $P = 0.0022$).

Similarly, 1060 donors with above-normal neopterin an 462 with normal neopterin concentrations were tested fo the presence of IgM antibodies to PV B19 by enzyme im munoassay (Parvovirus-B19-IgM-ELISA; Progen Biotech nik, Heidelberg, Germany). This pathogen is very resistan to the usual plasma inactivation procedures and may caus acute hemolytic crises of chronic hemolytic anemia (e.g. sickle cell anemia), hydrops fetalis after maternal infec tion, severe and persisting adult polyarthropathy, an erythema infectiosum. Recently, Santagostino et al. (*11*) demonstrated that a solvent-detergent and terminally dry heated factor VIII concentrate could transmit PV B19 in fection in previously untreated hemophilia patients. A with EBV, the infection rate is comparatively high ir young adults.

Of the 1060 donors' sera with above-normal neopterin, 7 (6.9%) were found to be seropositive for PV B19 IgM; 1(seropositive samples were found among 462 donations witl normal neopterin content (2.2%). The difference is statisti cally significant: odds ratio 3.34 (95% confidence interval 1.7–6.4; $P = 0.0003$).

We conclude that the chances of finding acute infection with EBV and PV B19 in blood donors with increase neopterin concentrations are significantly higher than ir donors with normal neopterin concentrations. Clearly, onl a fraction of donors with increased neopterin will presen with acute infection with just these two pathogens; othe infections, e.g., CMV (*8*), may account for at least part o the remaining number of donors with increased neopterir concentrations. One of the dominant motivations in Aus tria for screening donated blood by quantifying neopterin i the desire to exclude unknown and (or) newly emerging viruses from being spread through blood transfusion. A our institution, donations with increased neopterin concen tration are withdrawn from transfusion; they may, how ever, be used for, e.g., production of albumin or other blood products for which efficient sterilization procedures are available.

We suggest the results are even more impressive when one considers the inherent logistical problems of investiga tions of this type. In particular, neopterin is known to

ncrease significantly more rapidly than specific antibodies n response to infection with DNA viruses (*12*), RNA viuses (*13*), or retroviruses (*14*). Thus, the interval during vhich increased neopterin and specific antibodies are deectable simultaneously is comparatively short (Fig. 1). Consequently, the figures we obtained for the odds ratios nost probably represent lower limits; the power of tests for ieopterin to detect early infections in which antibody reponse has not yet developed may be even greater. Answering this question unequivocally will require a prospective tudy in which donors with normal and above-normal conentrations of neopterin are tested for the presence of spefic IgM antibodies at certain intervals after they have lonated blood. The results of the present pilot study should ncourage such a project.

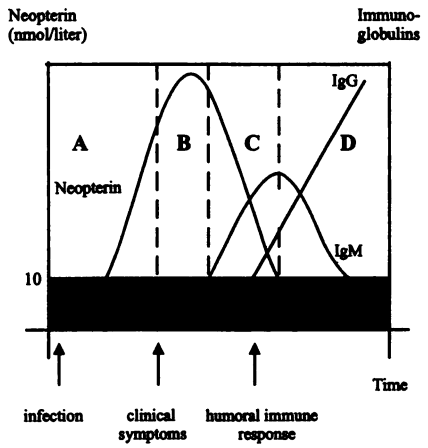

Fig. 1. Schematic time course of neopterin concentration in blood ind detectability of specific antibodies in viral infections.

After infection, neopterin concentrations start to increase, even before the end f the incubation period (*phase A*). Production of specific antibodies occurs hereafter, and neopterin concentrations usually decline and finally normalize vhen the pathogen is eliminated from the organism. Only during *phase C* are oth increased neopterin and specific IgM antibodies simultaneously detectble. The scheme also shows that a fraction of acutely infected subjects will robably have high neopterin and no IgM antibodies, and that another fraction vill have normal neopterin despite the presence of IgM antibodies. An exception of this typical temporal profile of neopterin concentrations after viral nfection is seen in infection by the human immunodeficiency virus type 1 (*14*), vhere neopterin after a strong rise in the early acute phase also declines but sually does not return to normal values.

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