Clinical Value of Soluble IgG Fc Receptor Type III in Plasma From Patients With Chronic Idiopathic Neutropenia

By Harry R. Koene, Masja de Haas, Marion Kleijer, Tom W.J. Huizinga, Dirk Roos, and Albert E.G.Kr. von dem Borne

Previous studies have shown that the plasma level of soluble IgG Fc receptor type III (sFcγRIII) is a measure of the total body neutrophil mass. The aim of this study was to determine whether the plasma level sFcγRIII is associated with the risk of contracting bacterial infections in patients with neutropenia. We collected blood from 66 patients suffering from acquired idiopathic neutropenia, whose blood was sent to our laboratory for diagnostic evaluation of neutropenia (neutrophil count <1,500 cells/μL). Soluble FcγRIII levels were measured in plasma. Genotype distibutions of FcγR polymorphisms were determined. Clinical data were obtained from the patient files. Patients were assessed as to whether or not they had suffered from a bacterial infection 3 months before to 3 months after a single sFcγRIII measurement. In addition, longitudinal data were obtained from 21 patients. Of the 66 neutropenic patients who were included, 15 had suffered from a bacterial infection in the period 3 months before to 3 months after sFcγRIII measurement. The age and sex distribution was equal among the groups with and without infections, as were the genotype frequencies of neutrophil FcγR polymorphisms. Both neutrophil count and plasma level sFcγRIII were significantly lower in the patient group with infections, compared with the noninfected group (*P* = .03 and *P* < .0001, respectively). No infections were reported for patients who had plasma sFcγRIII levels above 100 arbitrary units (AU; normal value, 30 to 200). After matching each infected patient with two noninfected patients having the same neutrophil count, sFcγRIII plasma levels remained significantly lower in the group with infections (*P* = .0001). For the patients who were followed in time, no infections were reported when sFcγRIII levels were above 100 AU. In conclusion, our population of patients with chronic idiopathic neutropenia with plasma sFcγRIII levels above 100 AU did not show an increased risk of contracting bacterial infections.

NEUTROPHILIC GRANULOCYTES play an important role in the host defence against bacteria. Qualitative, as well as quantitative, neutrophil disorders increase the risk of contracting bacterial infections. Normally, the majority of body neutrophils is in the bone marrow storage pool, whereas circulating neutrophils comprise less than 5% of the total body amount of neutrophils. Nevertheless, it is well established that a low number of circulating neutrophils is correlated with an increased infection risk. Prophylactic treatment with antibiotics or with granulocyte colony-stimulating factor (G-CSF) is common practice in neutropenic patients, especially in case of severe neutropenia (<500/μL). Because not all neutropenic patients will contract a bacterial infection, prophylactic therapy is not always necessary. Therefore, a parameter that would allow a more adequate treatment indication would be welcome. Human neutrophils express two different IgG Fc receptors (FcγR) on their cytoplasmic membrane, both of which bind IgG complexes (reviewed in De Haas et al[1]). These receptors play a crucial role in the process that leads to the degradation of IgG-opsonized particles. Each neutrophil carries 10,000 to 30,000 copies of FcγRIIa (CD32) and 100,000 to 300,000 copies of FcγRIIIb (CD16). Genetic polymorphisms on both FcγRIIIb and FcγRIIa influence the receptor-ligand interaction.[2-5]

Soluble (s) FcγRIII is present in plasma and other body fluids and is mainly derived from neutrophils.[6-8] Previous data suggest that the level of sFcγRIII might be an additional parameter in the evaluation of quantitative neutrophil disorders. The concentration of sFcγRIII in plasma correlates with the production of neutrophils in the bone marrow.[9,10] Depletion of the storage or marginating neutrophil pool does not affect plasma sFcγRIII levels, whereas changes in bone marrow neutrophil production are followed by changes in plasma sFcγRIII levels after a few days.[9,10] Neutrophils release FcγRIIIb during apoptosis in vitro.[11] In vivo, the lag time between changes in number of circulating neutrophils and levels of sFcγRIII suggests that plasma sFcγRIII originates from apoptotic tissue neutrophils and that neutrophils have a tissue phase of approximately 6 days. From these data, we hypothesized that the level of sFcγRIII in plasma of neutropenic patients could be used as an additional parameter to determine the total body neutrophil mass and thus the infection risk in these patients.

MATERIALS AND METHODS

Patients. EDTA-anticoagulated blood was sent to the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB) for diagnostic screening for the presence of neutrophil (auto)antibodies. Sixty-six consecutive adult patients (aged >18 years) with chronic (ie, ≥6 months) acquired idiopathic neutropenia were included from a total of approximately 300 diagnostic requests. Informed consent and clinical information was obtained via the treating physicians. Neutropenia was defined as an absolute neutrophil count (ANC) below 1,500/μL. No autoantibodies were detected in the direct immunofluorescence test. Patients were excluded when (1) the direct immunofluorescence test was positive, ie, the neutropenia was (auto)immune-mediated, (2) another autoimmune disease or an immunodeficiency

From the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, and Department of Hematology, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; and the Department of Rheumatology, University Hospital Leiden, Leiden, The Netherlands.

Submitted September 17, 1997; accepted December 23, 1997.

Supported by Grant No. 900-512-092 from the Netherlands Organization for Scientific Research (NWO).

Address reprint requests to Albert E.G.Kr. von dem Borne, MD, PhD, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Department of Experimental Immunohematology, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands.

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0006-4971/98/9110-0003$3.00/0

apart from neutropenia was present, or (3) the neutropenia was drug-induced. The patients were evaluated as to whether or not they had suffered from one or more bacterial infections in the period 3 months before until 3 months after analysis. Bacterial infections were scored on the basis of a positive bacterial culture result, in addition to clinical symptoms. None of the patients received prophylactic therapy. Twenty-one patients were followed in time.

Cell separation. Blood from healthy controls or patients was centrifuged over a Ficoll-Hypaque gradient (Pharmacia Fine Chemicals, Uppsala, Sweden) with a specific gravity of 1.076 g/mL. Peripheral blood mononuclear cells (PBMC) were harvested from the interphase for DNA isolation. Erythrocytes in the pellet were lysed with isotonic NH_4Cl at 4°C. The remaining cells were >95% neutrophils.

Soluble FcγRIII enzyme-linked immunosorbent assay (ELISA). Levels of sFcγRIII in (EDTA-) plasma or serum were measured essentially as previously described.[12] Briefly, ELISA plates (Nunc Immunoplate Maxisorp, Rockslide, Denmark) were coated with 5 μg/mL CD16 monoclonal antibody (MoAb) CLBFcRgran1 in 0.1 mol/L $NaHCO_3$, pH 9.6. Unbound sites were blocked with phosphate-buffered saline (PBS) containing 2% (vol/vol) milk (PBS/milk). Patient plasma samples were diluted in high performance ELISA (HPE) buffer (CLB, Amsterdam, The Netherlands). After washing the plates with PBS containing 0.02% Tween-20 (vol/vol), the wells were incubated with 25-fold and 50-fold dilutions of plasma or serum samples at room temperature in duplicate. The plates were then incubated for 1 hour with appropriate concentrations of a biotin-labeled polyclonal antipan-FcγRIII antibody, diluted in HPE buffer. After 30 minutes of incubation with horseradish peroxidase-labeled streptavidin, diluted in PBS/milk, 100 μL of substrate buffer was added. The colorimetric reaction was stopped by addition of 2 mol/L H_2SO_4, and the absorbance at 450 nm was measured in a Titertek multiscan ELISA reader (Flow Laboratory, Rockville, MD). Pooled plasma of 90 healthy individuals was used to obtain a calibration curve. The concentration of sFcγRIII in this pool was set at 100 arbitrary units (AU; approximately 5 nmol/L; normal range, 30 to 200 AU; approximately 1.5 to 10 nmol/L).[12]

Neutrophil FcγRIIIb-neutrophil antigen (NA) phenotyping. Neutrophils were phenotyped with NA1- and NA2-specific MoAbs by an indirect immunofluorescence test as described previously.[13] Briefly, neutrophils were incubated with MoAb for 30 minutes at room temperature and subsequently stained with fluorescein isothiocyanate (FITC)-labeled goat antimouse Ig. Binding of MoAbs was assessed by fluorescence microscopy. When the amount of isolated neutrophils was too small to perform phenotyping, genomic DNA was isolated from PBMC of the patient, and the genotype was determined by polymerase chain reaction (PCR). Previous work has shown that NA-genotyping and NA-phenotyping results are in complete concordance.[14]

Neutrophil FcγRIIIB and FcγRIIA genotyping. Genotyping for the NA system on FcγRIIIb was performed as previously described, based on two PCR-based allele-specific primer annealing (ASPA) assays.[14] A new PCR-based allele-specific restriction analysis (ASRA) was developed to type for the *FcγRIIA-131H/R* polymorphism. Briefly, two *FcγRIIA*-specific primers were used to amplify a 77-bp fragment (sense: 5′-CAA GCC TCT GGT CAA GGT C-3′; antisense: 5′-TGG AGA AGG TGG GAT CCA TA-3′). The antisense primer introduced an *Nde*I restriction site only in *FcγRIIA-131H*. The amplified *FcγRIIA*-specific fragment was digested with restriction endonuclease *Nde*I after which fragments were electrophoresed in polyacrylamide gels and visualized with ethidium bromide and UV light. The *FcγRIIA-131R/H* gene frequencies in a healthy Caucasian population (n = 93) were equal to the published ones.[2]

Statistical analysis. GraphPad Instat (GraphPad Software, San Diego, CA) was used for statistical calculations. After initial analysis of all the included patients, each patient with an infection was matched with two patients without infections who had an equal ANC to correct for any effect of the neutrophil count on the infection incidence. Data were analyzed with Student's *t* test. When differences in standard deviations were significant, Welch's approximate *t*-test was used. FcγR genotype and phenotype distributions were compared with the χ^2 test. A two-sided *P* value below .05 was considered significant. When comparing the test characteristics of the ANC and the plasma sFcγRIII level, receiver operating characteristics (ROC, ie, sensitivity *v* 1-specificity) curves were used to determine the optimal cut-off point of the ANC.

RESULTS

Sixty-six patients with neutropenia of unknown origin were divided in two groups who either did or did not suffer from a bacterial infection in the period 3 months before to 3 months after sFcγRIII measurement. Table 1 shows the clinical and phenotypical data of all included patients. Fifteen patients suffered from a bacterial infection in the study period, as determined on the basis of the clinical picture and a positive culture. Among the diagnosed infections were recurrent upper respiratory tract infections (n = 4), pneumonia (n = 3), urinary tract infections (n = 3, one urosepsis), recurrent skin infections (n = 2), aphtous mouth ulcers (n = 2), and infections of the gastrointestinal tract (n = 1). All infections were treated successfully with antibiotics. As shown in Table 1, no significant differences were observed regarding sex and age distribution among the two groups. Fifty-three patients were typed for the FcγRIIa-131R/H and the FcγRIIIb-NA(1,2) polymorphism. No genomic DNA was available from 13 patients. The *FcγRIIA* and *FcγRIIIB* genotype frequencies in the infected and noninfected groups were not significantly different and did not differ from frequencies in the normal (Dutch) population.[15] No differences were observed in sex and age between neutropenic patients with and without a bacterial infection 3 months before

Table 1. Clinical and Laboratory Data of Neutropenic Patients With and Without Infections

	No Infections (n = 51)	Infections (n = 15)	Two-Sided *P* Value
Male:female	20:31	3:12	NS*
Age	53 (range: 18-88)	61 (31-82)	NS
ANC (cells/μL)†	1,170 ± 320‡	860 ± 477	.03
Range (cells/μL)	600-1,500	50-1,500	
Plasma sFcγRIII (AU)	109 ± 66	49 ± 26	<.0001
Range (AU)	23-315	11-95	
Bone marrow data			
Not available	19 (37%)	4 (25%)	
Normal marrow	25 (49%)	5 (31%)	
Decreased myelopoiesis	2 (4%)	1 (6%)	
Increased myelopoiesis	2 (4%)	2 (13%)	
Possible dysplasia	2 (4%)	2 (13%)	
Lymphocytosis	1 (2%)	1 (6%)	
FcγR genotype§			
FcγRIIA-131R	0.59	0.61	
FcγRIIA-131H	0.41	0.39	NS
FcγRIIIB-NA1	0.29	0.32	
FcγRIIIB-NA2	0.71	0.68	NS

*NS, not significant.

†ANC, absolute neutrophil count.

‡Mean ± SD.

§Genotype frequencies in a control (Dutch) population are 0.47 and 0.53 for *FcγRIIA-131R* and *-131H,* respectively and 0.37 and 0.63 for *FcγRIIIB-NA1* and *-NA2,* respectively.[2]

or after the sFcγRIII measurement. The sFcγRIII levels were significantly lower in the patients with infections compared with the patients without infections (49 ± 26 AU *v* 109 ± 66 AU, respectively; Welch's approximate *t*-test; $P < .0001$). As shown in Table 1, the results of diagnostic bone marrow examinations were essentially the same in the two groups. The ratios between mitotic and postmitotic cells of the neutrophilic lineage were not available. Like the sFcγRIII levels, the mean number of circulating neutrophils was significantly lower in the patients with infections, compared with the patients without infections (860 ± 477 cells/μL and 1,170 ± 320 cells/μL, respectively; Welch's approximate *t*-test; $P = .03$). To identify the level of sFcγRIII as an independent predictor of infection risk, 14 neutropenic patients with infections were each matched with two neutropenic patients without infections, but with equal ANC. The remaining patient (sFcγRIII: 24 AU; ANC: 50 cells/μL) in the group with infections could not be matched and was excluded. Figure 1 shows the distribution of sFcγRIII levels in plasma of neutropenic patients with and without bacterial infections, matched for ANC. The plasma levels of sFcγRIII were significantly lower in the neutropenic patients with infections, compared with the uninfected patients (54 ± 26 AU *v* 113 ± 62 AU, respectively; Welch's approximate *t*-test; $P = .0001$). The mean difference between sFcγRIII levels in patients with and without infections was 59 AU (95% confidence interval [CI]: 31 to 87 AU). Again, no infections were reported for patients who had sFcγRIII levels above 100 AU. All patients who suffered from an infection in the defined period had plasma sFcγRIII levels below 100 AU. The level of sFcγRIII was not statistically correlated to age or ANC. The two groups had similar phenotype distributions of the FcγRIIIb-NA and the FcγRIIa-131R/H polymorphisms.

We compared the prognostic values of a plasma level of sFcγRIII below 100 AU and an ANC below 1000 cells/μL

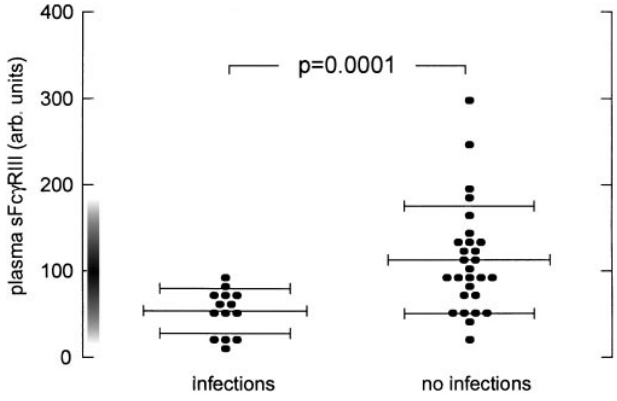

Fig 1. Plasma sFcγRIII levels (AU) in neutropenic patients with (n = 14) or without infections (n = 28) in the period 3 months before to 3 months after a single sFcγRIII measurement. Each patient with an infection was matched with two patients without infections who had approximately the same neutrophil count. The mean sFcγRIII levels (± standard deviation [SD]) in patients with and without infections were 54 ± 26 AU and 113 ± 63 AU, respectively (Welch's approximate *t* test; two-tailed *P* value = .0002). No statistically significant differences were observed between the two groups regarding sex and age distribution. FcγRIIa-131R/H and FcγRIIIb-NA(1,2) phenotype distributions were similar in the two groups (3 × 2 contingency table; *P* = .91 and *P* = .53, respectively).

Table 2. Prognostic Value of the Plasma Level of sFcγRIII in Patients With Neutropenia of Unknown Origin (n = 66) Regarding the Risk of Infection

	Cut-off	
	sFcγRIII < 100 AU	ANC < 1,000/μL*
Sensitivity†	1.00 (15/15)	0.60 (9/15)
Specificity	0.41 (21/51)	0.67 (34/51)
Positive predictive value	0.33 (15/45)	0.35 (9/25)
Negative predictive value	1.00 (21/21)	0.85 (34/40)

*The optimal cut-off point for ANC was determined with ROC curve.
†A positive test was defined as an sFcRIII level below 100 AU. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated with 2 × 2 tables (infection *v* sFcγIII < 100: n = 15, infection *v* sFcγRIII > 100: n = 0, no infection *v* sFcγRIII < 100: n = 30, no infection *v* sFcγRIII > 100: n = 21; infection *v* ANC < 1,000: n = 9, infection *v* ANC > 1,000: n = 6, no infection *v* ANC < 1,000: n = 17, no infection *v* ANC > 1,000: n = 34).

regarding the infection risk in the unmatched neutropenic patient group (n = 66). The ANC cut-off point of 1,000 cells/μL was determined by ROC curves. This cut-off value resulted in optimal specificity and sensitivity in our patient group (not shown). As shown in Table 2, the sensitivity and negative predictive value of the level of sFcγRIII are both 1.0, using a cut-off point of 100 AU. The sensitivity and negative predictive value of the ANC, with a cut-off value of 1,000/μL, were 0.6 and 0.7, respectively.

Longitudinal data were obtained from 21 of the 66 patients (32%). These patients were followed for a mean period of 9.4 months (range, 1 to 17) in which at least two sFcγRIII levels were measured (mean, 2.95; range, 2 to 5). Laboratory findings were correlated to the clinical situation of the patient at the time of blood sampling. To investigate whether sFcγRIII levels were constant in the healthy population, five healthy individuals served as controls. Blood from these donors was collected every 3 months for 1 year, and neutrophil counts and plasma sFcγRIII levels were measured. In these controls, both parameters remained constant over time, and no bacterial infections were reported in the study period (data not shown). The neutropenic patients were divided into three groups: a group with sFcγRIII levels above 100 AU without infections, a group with sFcγRIII levels below 100 AU without infections, and a group with sFcγRIII below 100 AU with infections. As shown in Fig 2, sFcγRIII levels above 100 AU were only observed in patients without infections. In 2 particular patients (Table 3, data not plotted in Fig 2), an increase in sFcγRIII level to values above 100 AU was coincident with a resolution of infection periods (not shown).

DISCUSSION

Less than 5% of the total body amount of neutrophils circulate in the peripheral blood. Nevertheless, extremely low numbers of circulating neutrophils (<500/μL) correlate with the incidence of bacterial infections.[16] However, the infection risk in patients with moderate to mild neutropenia (500 to 1,500/μL) is difficult to estimate, and an additional parameter to evaluate the extent of neutrophil defense is desirable. Previous work has shown that the concentration of sFcγRIII in plasma is a reflection of the total body neutrophil mass and is correlated

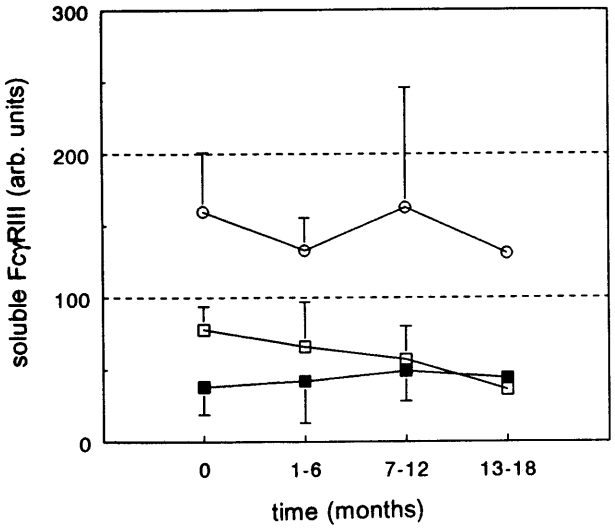

Fig 2. Soluble FcγRIII levels in neutropenic patients who were followed longitudinally. Mean follow-up was 9.4 months (range, 1 to 17) in which at least two blood samples were tested (mean, 2.95; range, 2 to 5). Patients without infections were divided in two groups: one with sFcγRIII levels above 100 AU (○; n = 9) and one with sFcγRIII levels below 100 AU (□; n = 6). All patients with reported infections had sFcγRIII below 100 AU (■; n = 6).

with the production of neutrophils in the bone marrow.[9,10] In the present study, we identified the concentration of sFcγRIII in plasma as a parameter that can be of additional value in the prediction of the infection risk in patients with chronic idiopathic neutropenia. We tested the hypothesis that a normal sFcγRIII level in plasma from neutropenic patients reflects a normal total body amount of neutrophils and therefore indicates a normal neutrophil defense and thus no increased infection risk. Patients with an established decrease in neutrophil production (due to malignancies in the bone marrow or drugs) or increased neutrophil destruction (due to circulating neutrophil antibodies) were excluded. The latter group of patients was excluded because part of the autoantibodies in immune-mediated neutropenia has anti-FcγRIII specificity,[17] which might interfere with sFcγRIII measurement. Moreover, the fate of FcγRIIIb derived from destroyed neutrophils is unknown, rendering interpretation of sFcγRIII levels in plasma from patients with immune-mediated neutropenia difficult.

In 66 patients with neutropenia of unknown origin, no infections were reported when plasma sFcγRIII levels were above 100 AU. Overall, the mean plasma sFcγRIII level, as well as the mean neutrophil count, were significantly lower in neutropenic patients with infections compared with patients without infections. The lower plasma sFcγRIII level was probably not caused by the infection because recent work has shown that bacterial infections do not affect neutrophil FcγRIIIb expression.[18] In our group of patients, both neutropenia and low levels of plasma sFcγRIII reflected a compromised neutrophil defense, predisposing to bacterial infections, but the plasma sFcγRIII level proved to be a more sensitive indicator. When we eliminated the effect of the ANC on the infection incidence by matching the patients with and without infections for the ANC, the differences in sFcγRIII levels between the two groups persisted. As in the unmatched group, none of the neutropenic patients with a plasma sFcγRIII level above 100 AU suffered from an infection in the study period. A similar conclusion can be drawn from the longitudinal data: infections were not reported for patients whose sFcγRIII levels were above 100 AU during the follow-up period. Apparently, the number of circulating neutrophils alone underestimates the neutrophil defense in these patients. Because the range of sFcγRIII levels in healthy individuals with normal neutrophil counts is large (30 to 200 AU), with 60% having levels below 100 AU, the interpretation of sFcγRIII levels below 100 AU in neutropenic patients is complicated. Most of these neutropenic patients may have a level of sFcγRIII in the lower part of the normal range together with an unusual distribution of neutrophils, reflected by a low number of circulating neutrophils. Low levels of sFcγRIII can also be expected in the case of low neutrophil FcγRIIIb expression. The latter was found in healthy individuals with heterozygous *FcγRIIIB* gene deficiency, a trait that exists in about 10% of the population.[12,19] Neutrophils from these individuals show half the membrane FcγRIIIb expression and half the amount of plasma sFcγRIII of individuals with two *FcγRIIIB* genes. The lower level of sFcγRIII in neutropenic patients with infections seems not to be caused by a higher prevalence of heterozygous *FcγRIIIB* gene deficiency because there was no increased prevalence of FcγRIIIb-NA(1+2-) or -NA(1-2+) patients. Furthermore, the finding that *FcγRIIIB* gene deficiency is not associated with an increased susceptibility to infections[14] indicates that the gene deletion is probably equally distributed among the infected and noninfected neutropenic groups. Quantitative Southern blotting to determine the number of *FcγRIIIB* genes could definitively settle this question.

Table 3. Soluble FcγRIII Levels (AU), Neutrophil Counts (mm^{-3}), and Clinical Data of Two Neutropenic Patients in Whom Resolution of Repeated Infection Periods Occurred

	Patient A*			Patient B		
Time	PMN	sFcγRIII	Infection	PMN	sFcγRIII	Infection
Initial	770	76	+	200	22	+
3 mo				1000	110	−
6 mo	380	64	+	500	255	−
9 mo				330	141	−
12 mo	1300	151	−			

*Bone marrow of patient A showed no abnormalities, bone marrow examination of patient B was not performed.

In childhood populations, correlations with susceptibility to infections with several microorganisms have been described for both neutrophil FcγR polymorphisms.[2-4] However, we found no differences in *FcγRIIA-131R/H* and *FcγRIIIB-NA(1,2)* gene distributions between the neutropenic patients with and without bacterial infections, suggesting that, at least in adult neutropenic patients, these polymorphisms do not play a major role in the defense against bacteria.

We determined the test characteristics of sFcγRIII measurement based on our data. In our group of neutropenic patients, the sensitivity and negative predictive value of an sFcγRIII level above 100 AU regarding the occurrence of a bacterial infection was 100%. Comparison of this test with the prognostic value of an ANC below 1,000 cells/μL showed that measure-

ment of sFcγRIII is of additional value in the estimation of the infection risk in neutropenia. An ANC below 500/μL was a specific indicator for infection, although sensitivity was weak (specificity and sensitivity of 1.0 and 0.2, respectively). These data suggest that measurement of plasma sFcγRIII is especially useful in moderate to mild neutropenia (500 to 1,500 cells/μL). The main clinical implication of our data is that prophylactic antibiotics or treatment with G-CSF might be safely omitted in neutropenic patients with sFcγRIII levels above 100 AU.

Correlations between several disease states and sFcγRIII levels have been described.[20-22] However, most of these correlations can be attributed to a decreased bone marrow neutrophil production and consequently low levels of plasma sFcγRIII. In our patients, the results of bone marrow examinations were similar in the groups with and without infections. This might suggest that the plasma level sFcγRIII provides a more precise reflection of neutrophil production than does microscopic examination of the bone marrow itself. We only included patients with chronic idiopathic neutropenia in whom production and lifetime of neutrophils were thought to be normal. Our data show that in these patients an sFcγRIII level above 100 AU can support the clinician in his or her decision to adopt an expectative attitude. Moreover, the hypothesis that plasma sFcγRIII reflects bone marrow neutrophil production and the results of the present study suggest a possible applicability of sFcγRIII measurement in more common neutropenias, for example in assessing the severity of bone marrow infiltration in leukemic patients. Prospective evaluation of different patient groups may confirm this assumption.

ACKNOWLEDGMENT

The authors thank the Department of Leukocyte and Thrombocyte Serology of the CLB for collecting all of the patient samples and performing the neutrophil NA(1,2)-phenotyping.

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