

activity, may be explained, as in other forms of anemia,<sup>4,5</sup> by the chronic anemia that characterizes TM, which may lead to a persistent, appropriate sinus tachycardia and a sustained decrease in autonomic fluctuations. Additionally, the expansion of blood volume during transfusion could represent an uncontrolled stimulation of cardiac receptors with sympathetic afferents, leading to a further decrease in vagal modulation of heart rate.<sup>6</sup> Of interest, we recorded VLP in 31.5% of TM patients, which is well above the prevalence reported in normal subjects (0-7%).<sup>7</sup> This result may be explained by intracellular iron deposition and myocardial fibrosis, which may create heterogeneous ventricular depolarization,<sup>8</sup> and could lead to abnormal excitability of iron-loaded heart cells.<sup>9,10</sup> The higher incidence of ventricular arrhythmias that we observed in patients with VLP demonstrates the usefulness of signal-averaged ECG in identifying those patients who have an increased risk of potentially malignant arrhythmias.<sup>11,12</sup>

No relationship was found between signal-averaged ECG parameters and hematologic data. This could be explained by a small amount of storage proteins in the heart cells, or greater sensitivity to iron-induced oxygen free radicals. Thus, the presence of VLP appears to be a critical component of the arrhythmogenesis rather than a reflection of the severity of the disease. The comparison of TM patients with healthy control individuals may represent a limitation of this study. Further investigations in a large cohort of TM patients might confirm the prognostic value of these parameters.

In conclusion, analysis of HRV and VLP may be helpful in TM patients by detecting an underlying electrophysiologic substrate predisposing to arrhythmias. Naturally, this needs confirmation from both larger prospective and electrophysiologic studies.

Ferdinando Franzoni,\* Fabio Galetta,\* Carmine Di Muro,\*  
Giuliana Buti,<sup>o</sup> Ferdinando Pentimone\* Gino Santoro\*

\*Department of Internal Medicine; <sup>o</sup>Department of Pediatrics,  
University of Pisa, Italy

Key words: arrhythmias, ECG monitoring, iron overload, signal-averaged ECG, thalassemia major.

Correspondence: Fabio Galetta, MD, Department of Internal Medicine, University of Pisa School of Medicine, via Roma 67, 56126 Pisa, Italy. Phone: international +39.050.993373. Fax: international +39.050.553414. E-mail: fgaletta@med.unipi.it

## References

1. Modell B, Berdoukas V. The clinical approach to thalassemia. New York, NY: Grune Et Stratton. 1984.
2. Engle MA. Cardiac involvement in Cooley's anemia. Ann NY Acad Sci 1969;119:694-702.
3. Zurlo MG, De Stefano P, Borgna-Pignatti C, Di Palma A, Piga A, Melevisi C, et al. Survival and causes of death in thalassemia major. Lancet 1989;2:27-30.
4. Sezen AB, Demirel S, Akkaya V, Kudat H, Tukek T, Yeneral M, et al. Autonomic dysfunction in vitamin B12 deficiency: a heart rate variability study. J Auton Nerv Syst 1998;71:25-7.
5. Romero Mestre JC, Hernandez A, Agramonte O, Hernandez P. Cardiovascular autonomic dysfunction in sickle cell anemia: a possible risk factor for sudden death? Clin Auton Res 1997; 7:121-5.
6. Veglio F, Melchio R, Rabbia F, Molino P, Cat Genova G, Martini G, et al. Blood pressure and heart rate in young thalassemia major patients. Am J Hypertens 1998;11:539-47.
7. Danes P, Santarelli P, Hauser RG, Uretz EF. Quantitative analysis of the high-frequency components of the terminal portion of the body surface QRS in normal subjects and in patients with ventricular tachycardia. Circulation 1983;67:1129-38.
8. Olson LJ, Edwards WD, McCall JT, Ilstrup DM, Gersh BJ. Cardiac iron deposition in idiopathic hemochromatosis: histologic and analytic assessment of 14 hearts from autopsy. J Am Coll Cardiol 1987;10:1239-43.
9. Livrea MA, Tesoriere L, Pintaudi AM, Calabrese A, Maggio A, Freisleben HJ, et al. Oxidative stress and antioxidant status in  $\beta$ -thalassemia major: iron overload and depletion of lipid-soluble antioxidants. Blood 1996;88:3608-14.
10. Kuryshv YA, Brittenham GM, Fujioka H, Kannan P, Shieh C, Cohen SA, et al. Decreased sodium and increased transient outward potassium currents in iron-loaded cardiac myocytes. Implications for the arrhythmogenesis of human siderotic heart disease. Circulation 1999;100:675-83.
11. Denereaz D, Zimmermann M, Adamec R. Significance of ventricular late potentials in non-ischaemic dilated cardiomyopathy. Eur Heart J 1992;13:895-901.
12. Gomes JA, Winters SL, Stewart D, Horowitz S, Milner M, Barreca P. A new noninvasive index to predict sustained ventricular tachycardia and sudden death in the first year after myocardial infarction: based on signal-averaged electrocardiogram, radionuclide ejection fraction and Holter monitoring. J Am Coll Cardiol 1987;10:349-57.

## Cytokines

### Circulating levels and promoter polymorphisms of interleukins-6 and 8 in pediatric cancer patients with fever and neutropenia

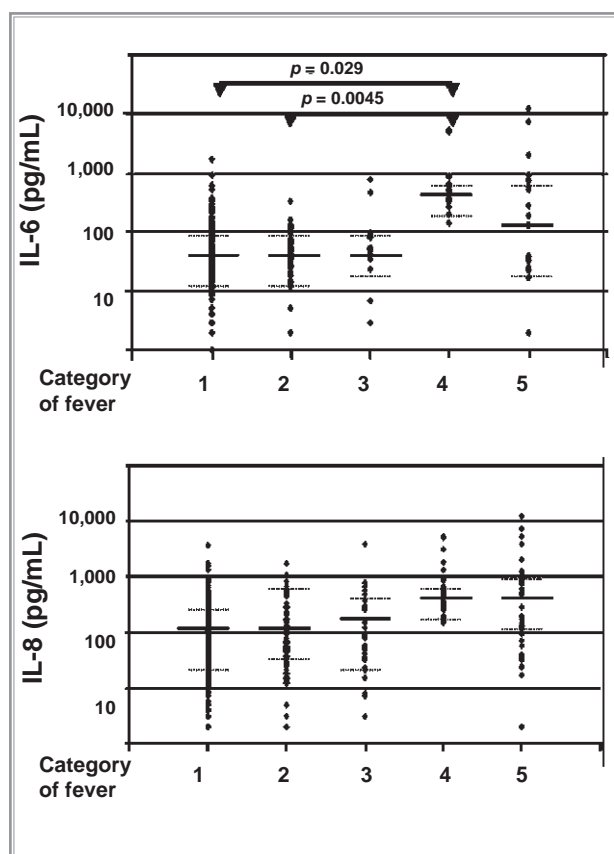
We evaluated interleukin (IL)-6 and IL-8 as early markers of serious infection in febrile neutropenic children and found that both molecules had limited diagnostic value. Although the promoter polymorphisms IL-6 G-174C and IL-8 A-251T influence serum concentrations of the respective cytokines, genotyping for these polymorphisms does not improve the diagnostic value of IL-6 and IL-8 measurements.

haematologica 2004; 89:234-236  
(http://www.haematologica.org/2004/2/234)

Several small studies<sup>1-5</sup> suggest that the assessment of interleukin (IL)-6 and IL-8 concentrations at the time of admission is a valuable tool for predicting serious infection in febrile neutropenic cancer patients. Since serum levels of both cytokines are influenced by known promoter polymorphisms,<sup>6,7</sup> we evaluated whether genotyping of IL-6 and IL-8 promoter polymorphisms improves the diagnostic value of these cytokines.

IL-6 and IL-8 concentrations were measured in duplicate by ELISA (R&D) in children with febrile neutropenia (>38.5°C, absolute neutrophil count (ANC)  $\leq$ 500/ $\mu$ L) at the time of admission and 24 hours later. Children with fever for longer than 24 hours prior to admission were excluded. Febrile episodes were classified as bacteremia with Gram-negative or Gram-positive organisms, microbiologically or clinically documented localized infection, pneumonia or fever without an identifiable source (FUO).

Genomic DNA isolated from peripheral blood was used for genotyping the promoter polymorphisms IL-6 G-174C



**Figure 1.** Individual levels, calculated median (solid bar), and 25<sup>th</sup> and 75<sup>th</sup> percentiles (dotted bars) for IL-6 (above) and IL-8 (below) measured in neutropenic children with febrile episodes at the time of admission without an identifiable source of infection. Category of fever: FUO (1), localized infection (2), pneumonia (3), bacteremia due to Gram-negative organisms (4) and bacteremia due to Gram-positive organisms (5).

and IL-8 A-251T by means of polymerase chain reaction (PCR) and allele-specific restriction digestion.<sup>8,9</sup>

The Wilcoxon's rank sum test was used for comparisons of the individual groups categorized by the type of infection. The *p* values are two-tailed and are considered statistically significant if  $<0.05$ . All statistical calculations were done with the statistical software package BiAS (Version 7.07 for Windows 95/NT). The study protocol was approved by the local ethics committee.

One hundred and forty-six patients treated in three pediatric cancer centers were included in this study [63 boys, 83 girls, mean age 9 years (0.5–28 years)]; the diagnoses were acute lymphoblastic and myeloid leukemia ( $n=48$  and  $n=15$ ), lymphoma ( $n=16$ ), solid tumor ( $n=66$ ), and histiocytosis ( $n=1$ ). Overall, there were 311 febrile episodes which were categorized as FUO ( $n=209$ ), localized infection ( $n=48$ ), pneumonia ( $n=15$ ), and bacteremia with Gram-negative ( $n=18$ ) and Gram-positive organisms ( $n=21$ ).

IL-6 levels on admission were significantly higher in patients with bacteremia with Gram-negative organisms than in patients with FUO or with localized infections, but did not differ from the IL-6 levels in patients with pneumonia or bacteremia with Gram-positive organisms (Figure 1). IL-8 levels did not differ significantly across the different types of infection (Figure 1).

**Table 1.** Diagnostic value of plasma levels of IL-6 and IL-8 as indicators of serious infection in febrile neutropenic children.

	IL-6		IL-8	
Limit* (pg/mL)	235	1,000	320	500
Sensitivity	89	11	56	44
Specificity	91	99	79	89
PPV <sup>o</sup>	38	33	14	20
NPV <sup>f</sup>	99	95	97	96

\*Thresholds were chosen according to previous results.<sup>3</sup> <sup>o</sup>PPV: positive predictive value; <sup>f</sup>NPV: negative predictive value. Negative predictive value: percentage of children below threshold with febrile episodes without an identifiable source, localized infection, pneumonia, or bacteremia due to Gram-positive organisms; positive predictive value: percentage of children above threshold with bacteremia due to Gram-negative organisms; sensitivity: percentage of children with bacteremia due to Gram-negative organisms and/or clinical sepsis above threshold; specificity: percentage of children with febrile episodes without an identifiable source, localized infection, pneumonia, or bacteremia due to Gram-positive organisms below threshold.

Despite the fact that the sensitivity, specificity, and positive and negative predictive values of IL-6 as an early marker for serious infection in febrile neutropenic children are better than those of the IL-8, assay of this cytokine is insufficient as a single test for clinical application (Table 1). The assessment of IL-6 or IL-8 on day 2 did not increase the diagnostic value (*data not shown*).

In order to analyze whether knowledge of the genotype increases the diagnostic value of IL-6 and IL-8 concentrations, we genotyped an additional 49 children. In these patients, IL-6 and IL-8 levels had been measured in 111 episodes of fever and chemotherapy-induced neutropenia as part of a former study.<sup>3</sup> These patients were comparable to the patients of the present study (*data not shown*).

For all IL-6 promoter polymorphisms (CC, CG and GG), IL-6 levels were significantly different between patients with bacteremia with Gram-negative organisms and patients with FUO ( $p = 0.0028$ ,  $0.025$ , and  $0.028$ , respectively) and between patients with bacteremia with Gram-negative organisms and patients with localized infections ( $p < 0.00001$ ,  $0.0045$ , and  $< 0.00001$ , respectively). In contrast, among patients with a certain genotype of the IL-8 A-251T promoter polymorphism (AA, AT and TT), IL-8 levels in patients with the different infections did not differ significantly. These results were similar to the overall results without taking the genotype into account. Thus, the sensitivity, specificity, and positive and negative predictive values of measured levels of IL-6 or IL-8 for predicting serious infection in children with febrile neutropenia was not improved by subdividing the patients according to their IL-6 or IL-8 promoter polymorphism genotype.

Our analysis shows that the assessment of IL-6 and IL-8 levels at the time of admission has limited diagnostic value for the reliable identification of serious infection in children with febrile neutropenia, a finding which contrasts with the results of a number of preliminary studies.<sup>1–5</sup> These studies were, however, single-center trials and included only small numbers of patients.

The lack of an association between the IL-6 G-174C and the IL-8 A-251T promoter polymorphisms and interleukin levels in our study population might be explained by the fact that the increases of IL-6 and IL-8 concentrations after endotoxin challenge, which can be several thousand-fold, probably override any genetically controlled, fine-tuning effect. Furthermore, recently described polymorphisms in

the 5'-flanking region of the IL-6 and IL-8 genes do not act independently of one another but as part of an extended promoter haplotype.<sup>10</sup> In summary, our data demonstrate that the assessment of IL-6 and IL-8 levels in febrile neutropenic children is of limited diagnostic value and is not improved by genotyping for promoter polymorphisms.

Thomas Lehrnbecher,\* Gudrun Fleischhack,° Mitra Hanisch,\* Frank Deinlein,\* Arne Simon,° Toralf Bernig,® Stephen J. Chanock,® Thomas Klingebiel\*

Departments of Pediatric Hematology and Oncology, University of Frankfurt,\* Bonn,° Würzburg,®  
 ®Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Funding: this study was supported by the Wilhelm Sander-Stiftung.

Acknowledgments: we thank the medical and nursing staff of the Departments of Pediatric Hematology and Oncology, Universities of Bonn, Frankfurt and Würzburg, Germany.

Key words: children, febrile neutropenia, chemotherapy, interleukin, polymorphisms.

Correspondence: Thomas Lehrnbecher, MD, Department for Pediatric Hematology and Oncology, Children's Hospital III, Johann Wolfgang Goethe-University, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany. Phone: international +49.69.63083481. Fax: international +49.69.63016700. E-mail: thomas\_lehrnbecher@yahoo.com

## References

- Engvall P, Granstrom M, Andersson B, Bjorkholm M. Moni-

- toring of endotoxin, interleukin-6 and C-reactive protein serum concentrations in neutropenic patients with fever. *Eur J Haematol* 1995;54:226-34.
- de Bont ES, Vellenga E, Swaanenburg JC, Fidler V, Visser-van Brummen PJ, Kamps WA. Plasma IL-8 and IL-6 levels can be used to define a group with low risk of septicemia among cancer patients with fever and neutropenia. *Br J Haematol* 1999;107:375-80.
- Lehrnbecher T, Venzon D, de Haas M, Chanock SJ, Kuhl J. Assessment of measuring circulating levels of interleukin-6, interleukin-8, C-reactive protein, soluble Fc gamma receptor type III, and mannose-binding protein in febrile children with cancer and neutropenia. *Clin Infect Dis* 1999;29:414-9.
- Bayer L, Schontube M, Dorffel W. [C-reactive protein, IL-6 and procalcitonin as infection parameters in children with oncologic diseases]. *Klin Padiatr* 2000;212:326-31.
- Kern WV, Heiss M, Steinbach G. Prediction of gram-negative bacteremia in patients with cancer and febrile neutropenia by means of interleukin-8 levels in serum: targeting empirical monotherapy versus combination therapy. *Clin Infect Dis* 2001;32:832-5. 1998; 102:1369-76.
- Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax* 2000; 55:1023-7.
- Foster CB, Lehrnbecher T, Samuels S, et al. An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. *Blood* 2000; 96:2562-7.
- Lehrnbecher T, Chanock SJ. Detection of common cytokine and colony stimulating factor gene polymorphisms. *Methods Mol Biol* 2003; 215:71-93.
- Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. *J Biol Chem* 2000; 275:18138-44.

## Chronic Myeloproliferative Disorders

### Imatinib mesylate can induce complete molecular remission in FIP1L1-PDGFR-α positive idiopathic hypereosinophilic syndrome

Recently, a fusion gene, FIP1-like 1 (FIP1L1)-PDGFR-α, has been found to be involved in some patients with idiopathic hypereosinophilic syndrome (HES) responsive to imatinib therapy. We report a new case of a patient with FIP1L1-PDGFR-α positive HES, treated with imatinib mesylate for more than 17 months, who obtained a complete molecular response.

*haematologica* 2004; 89:236-237  
 (<http://www.haematologica.org/2004/2/236>)

Idiopathic hypereosinophilic syndrome (HES) is a currently incurable chronic myeloproliferative disorder characterized by persistent hypereosinophilia. Imatinib mesylate (Gleevec)<sup>®</sup> - a tyrosine kinase inhibitor specifically directed against abl, bcr-abl, c-kit and platelet-derived growth factor receptors (PDGFR) - has recently shown therapeutic effects<sup>1-2</sup> in patients carrying the FIP1-like 1 (FIP1L1)-PDGFR-α fusion gene.<sup>2-3</sup> We report the first case of FIP1L1-PDGFR-α<sup>+</sup> HES with a documented complete molecular response to imatinib.

In December 2001, a 65-year old male with no significant past medical history presented with persistent leukocytosis (76×10<sup>9</sup>/L) with eosinophilia (42%). Molecular analysis did not detect BCR-ABL, FGFR1-BCR, or the PDGFα-TEL rearrangement. After 21 days of imatinib (600 mg/day), the white cell and eosinophil counts fell dramatically and have

since remained normal over 17 months of continuing treatment (Figure 1). No significant hematologic toxicity has been observed.

In March 2003, following informed consent, retrospective reverse transcriptase polymerase chain reaction (RT-PCR) analysis of FIP1L1-PDGFR-α was performed as reported elsewhere.<sup>2</sup> A new type of the FIP1L1-PDGFR-α fusion transcript was detected (in peripheral blood and bone marrow) both at diagnosis and after 9 months of treatment, but not at 17 months (Figures 1A and B). Mixing serially diluted total FIP1L1-PDGFR-α<sup>+</sup> RNA (diagnostic sample) with the HL60 cell line, we were able to amplify the transcript up to a 1:10<sup>4</sup> dilution. Sequence analysis confirmed that the breakpoints in PDGFR-α occurred in exons 12 and 8 of FIP1L1. The different bands represent splice variants (Figure 1B). It should be noted that while the hematologic response occurred rapidly within the first 3 weeks of imatinib therapy, most likely as a result of FIP1L1-PDGFR-α inhibition, as reported also by other authors,<sup>1,4</sup> complete molecular response seems to have occurred much later, at some time between 9 and 17 months.

Although we do not know the exact clinical significance (or prognostic value) of the complete molecular response recorded in our patient, it seems reasonable to assume that eradication of minimal residual disease is superior to complete morphologic response. It is also unclear how long imatinib therapy should be administered to a patient affected by FIP1L1-PDGFR-α<sup>+</sup> hematologic diseases (HES and systemic mastocytosis)<sup>5</sup> following a complete clinical or molecular response: for the present we have preferred to continue treatment. Nevertheless, we believe that imatinib treatment might be curative.

Giovanni Martinelli, Michele Malagola, Emanuela Ottaviani, Gianantonio Rosti, Elena Trabacchi, Michele Bacarani