Selenium status in the body and proliferative activity of malignant cells

Sir,

Avanzini and co-workers recently reported the results of their study on serum selenium concentrations in patients with newly diagnosed lymphoid malignancies.[1] Interestingly, as compared to controls, selenium (Se) levels were significantly lower in patients with non-Hodgkin's lymphoma (in a representative sample) in IV stage and/or in those with high grade disease. However, literature reports of serum Se levels in patients with lymphoid malignancy or solid tumors are discordant.[2-4] These discrepancies may be due to case series that are not directly comparable among themselves or with healthy control cohorts.

It must be borne in mind that the elevated variability of serum Se may be due to factors other than cancer such as age, sex, body mass, dietary habits, life style (alcohol, smoking), intercurrent disease and medications.

When considering the various biological roles of Se (proliferation and expression of oncogenes by both normal and malignant cells, carcinogen metabolism, cellular immune response, prevention of oxidative stress, apoptosis) and when addressing the topic of whether Se is a risk factor or a protection against cancer,[5] one must evaluate selenium levels both in serum and in biological material that integrates selenium intake and reflects its status over the medium and long term (4 months for red blood cells, 12 months or longer nails and hair). These studies are however methodologically complex, and at present various types of investigations (prospective, environmental, epidemiological, ecological) have failed to provide conclusive results.

Several factors may influence Se exchanges between labile pools, deposits and cancer tissue, and we wish to point out one important factor which has so far received little attention but which might influence selenium profiles, namely the proliferative activity of cancer cells. Of particular interest along this line of thought is the finding by Avanzini and co-workers of an inverse relationship between serum selenium and β2-microglobulin, an important index of the turnover of neoplastic cells.

In an ongoing study on Se levels in the serum and hair of women with breast cancer (unpublished data), we observed that patients recruited at an early clinical stage had lower serum Se and higher Se hair content with respect to patients at a more advanced stage or to healthy controls:

Stage 0-I (n=42): serum Se mean value 76.2±21.7 μg/L, hair Se content geometric mean 416.5 μg/g

Stage II-IV (n=44): serum Se mean value 81.5±22.4 μg/L, hair Se content geometric mean 335.2 μg/g

Controls (n=86): serum Se mean value 88.6±26.4 μg/L, hair Se content geometric mean 370.5 μg/g

Though our data fell short of statistical significance, due in part to the wide spread of values in the series, the findings are suggestive in light of the kinetic properties of tumor cells.[6] Indeed the relationship between Se and cellular growth emerged from our *in vitro* studies that showed different Se accumulation and effect according to cell density in the lymphocyte cultures used in our experiments.[7] Breast cancer shows a Gompertz-type growth curve with an exponential increase in the early proliferative phases. These events, whose underlying mechanisms also require further investigation in terms of host-tumor interactions, might influence different aspects of Se distribution in various body districts.

In conclusion, further experimental and epidemiological studies are warranted to better elucidate the relationships between Se status and carcinogenesis; however, determination of Se levels in subjects with malignancies will only contribute data useful for the clinician when it is performed across several body districts (blood, depots, healthy and diseased tissue) and interpreted in the light of the proliferative characteristics of the tumor and of the intricate tumor host relationships.

*Lino Piccinini**
Paola Borella°
Annalisa Bargellini°

**Dipartimento di Scienze Mediche, Oncologiche e Radiologiche; °Dipartimento di Scienze Biomediche; Università degli Studi di Modena, Italy*

References

1. Avanzini P, Vinceti M, Ilariucci F, et al. Selenium status in the body and proliferative activity of malignant cells. Haematologica 1995; 80:505-11.
2. Stampfer MJ, Colditz GA, Willett WC. The epidemiology of selenium and cancer. Cancer Surv 1987; 6:623-33.
3. Deffuant C, Celerier P, Boiteau HL, Litoux P, Dreno B. Serum selenium in melanoma and epidermotropic cutaneous T-cell lymphoma. Acta Derm Venereol 1994; 74:90-2.
4. Piccinini L, Borella P, Bargellini A, Incerti Medici C, Zoboli A. A case-control study on selenium, zinc and copper in plasma and hair of subjects affected by breast and lung cancer. Biol Trace Elem Res 1996; 51:23-30.
5. Clark LC, Alberts DS. Selenium and cancer: risk or protection? J Natl Cancer Inst 1995; 87:4735.
6. Orfao A, Ruiz-Arguelles A, Lacombe F, Ault K, Basso G, Danova M. Flow cytometry: its applications in hematology. Haematologica 1995; 80:69-81.
7. Borella P, Bargellini A, Incerti Medici C. Chemical form of selenium greatly influences metal uptake and effects on cultured human lymphocytes. Biol Trace Elem Res 1996; 51:43-54.

Correspondence: Prof. Lino Piccinini, Divisione Oncologia Medica, Policlinico, via del Pozzo 71, 41100 Modena, Italy

Selenium and lymphoid malignancies (Reply)

Sir,

Piccinini *et al.*'s hypothesis that the proliferative activity of malignant lymphoid cells and of cancer cells in general might influence selenium status is interesting and is partially supported by prior data, including our own.[1] However, due to the limited evidence available on this topic, further data are needed to confirm that enhanced selenium uptake by neoplastic tissue may vary according to the mitotic activity of the cancer cells.

Piccinini and colleagues also addressed a fundamental issue in epidemiologic and clinical research on the health effects of selenium: the methodology for exposure assessment and, in particular, the use of biomarkers as surrogate measures of selenium exposure (represented in most individuals by dietary intake). Selenium content of serum, plasma, erythrocytes, whole blood, hair, toenails, and urine are among the biomarkers most frequently used in epidemiologic and clinical studies. Serum, plasma and urine selenium are short-term markers of exposure, whereas the remaining indicators tend to reflect long-term selenium intake. The limitations of these indicators as surrogate measures of intake have been reviewed.[2] Selenium-dependent glutathione peroxidase activity has also been evaluated as a possible biomarker of exposure, but it does not appear to be a reliable indicator of selenium intake since the correlation between the two parameters is not linear[2] and, what is more, glutathione peroxidase activity may be induced by oxidizing agents[3] (including selenium itself).[4,5]

In our clinical studies[1,6] we evaluated selenium exposure through determination of serum selenium content, a sensitive short-term selenium marker,[2] because we were interested in a possible relationship between the clinical characteristics of lymphoid malignancies and recent changes in selenium status. Obviously the characteristic that makes serum selenium content of interest in clinical research, i.e. its ability to reflect short-term selenium intake, also represents a limitation in an epidemiologic setting, particularly in retrospective studies where selenium status is likely to be affected by the disease, at least in some body tissues. This is why we did not consider our results to be contradictory to the prior hypothesis of a direct association between selenium exposure and the risk of lymphoid malignancies,[7] though they did not add any evidence to support this hypothesis.

Biomarkers, however, may not adequately reflect selenium intake due to factors such as gender, body mass, medical

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treatment.[8-10] Moreover, concurrent exposure to other factors such as cadmium or methionine may modify the distribution (and therefore the levels) of selenium in peripheral tissues.[11] An influence of cadmium on selenium metabolism might explain at least in part why lower selenium levels have generally been observed in smokers as opposed to non-smokers,[8,9,12] in spite of the fact that tobacco smoke represents a source of selenium exposure.[13] The limitations of biomarkers in exposure assessment have suggested the usefulness of evaluating average selenium exposure through estimation of usual dietary intake, but unfortunately this methodology is also subject to limitations due to the high variability in the selenium content of foodstuffs in several geographical areas.[12,14] The degree of correlation between selenium intake, as estimated through food diaries or other techniques, and biomarkers as surrogate measures of exposure has been found to be satisfactory in some studies but not in all.[8,10]

An important advantage to estimating dietary intake would be the possibility of determining the specific chemical forms of selenium to which the human body has been exposed before any in vivo metabolic conversion occurs. Whichever approach is adopted in the assessment of selenium exposure – dietary intake or biomarkers – other factors such as exposure to heavy metals, which can markedly influence the biological activity of selenium, should also be considered.[5]

Despite the complexity of epidemiologic studies on the relationship between selenium and cancer, we agree with Piccinini *et al*. on the need to investigate this issue further, especially in the light of the results of two recent studies that have analyzed the effect of selective long-term selenium exposure (self-administered or accidental) on cancer risk.[7,12]

*Paolo Avanzini**
Marco Vinceti°
*Fiorella Ilariucci**

**Day-Hospital Ematologico, Arcispedale S. Maria Nuova, Reggio Emilia, and °Sezione di Igiene e Microbiologia, Dipartimento di Scienze Biomediche, Università di Modena, Italy*

References

1. Avanzini P, Vinceti M, Ilariucci F, Masini L, D'Incà M, Vivoli G. Serum selenium concentrations in patients with newly diagnosed lymphoid malignancies. Haematologica 1995; 80:505-11.
2. Levander OA. Considerations on the assessment of selenium status. Fed Proc 1985; 44:2579-83.
3. Chow CK, Dillard CJ, Tappel AL. Glutathione peroxidase system and lysozyme in rats exposed to ozone and nitrogen dioxide. Environ Res 1974; 7:311-9.
4. Csallany AS, Su L, Menken BZ. Effect of selenite, vitamin E and N,N'-diphenyl-p-phenylenediamine on liver organic solvent-soluble lipofuscin pigments in mice. J Nutr 1984; 114:1582-7.
5. Davis RL, Spallholz JE. Inhibition of selenite-catalyzed superoxide generation and formation of elemental selenium (Se°) by copper, zinc, and aurintricarboxylic acid (ATA). Biochem Pharmacol 1996; 51:1015-20.
6. Avanzini P, Vinceti M, Ilariucci F, Merli F, Masini L, D'Incà M. Il dosaggio del selenio sierico è utile nel monitoraggio clinico dei linfomi non Hodgkin (LNH)? Proceedings of 35th Congress of the Italian Society of Hematology, 1995:233.
7. Vinceti M, Rovesti S, Gabrielli C, et al. Cancer mortality in a residential cohort exposed to environmental selenium through drinking water. J Clin Epidemiol 1995; 48:1091-7.
8. van den Brandt PA, Goldbohm RA, van't Veer P, Bode P, Hermus RJJ, Sturmans F. Predictors of toenail selenium levels in men and women. Cancer Epidemiol Biomarkers Prev 1993; 2:107-12.
9. Robberecht H, Deelstra H. Factors influencing blood selenium concentration values: a literature review. J Tace Elem Electrolytes Health Dis 1994; 8:129-43.
10. Longnecker MP, Stram DO, Taylor PR, et al. Use of selenium concentration in whole blood, serum, toenails, or urine as a surrogate measure of selenium intake. Epidemiology 1996; 7:384-90.
11. Salbe AD, Levander OA. Effect of various dietary factors on the deposition of selenium in the hair and nails of rats. J Nutr 1990; 120:200-6.
12. Garland M, Morris JS, Stampfer MJ, et al. Prospective study of toenail selenium levels and cancer among women. J Natl Cancer Inst 1995; 87:497-505.
13. Bogden JD, Kemp FW, Buse M, et al. Composition of tobaccos from countries with high and low incidence of lung cancer. I. Selenium, polonium-210, Alternaria, Tar, and nicotine. J Natl Cancer Inst 1981; 66:27-31.
14. Mariottini E, Capei R, Bigazzi Grasso C. Selenium content in foods. J Prev Med Hygiene 1995; 36:55-60.

Correspondence: Paolo Avanzini, MD, Day-Hospital Ematologico, Arcispedale S. Maria Nuova, viale Umberto I 50, 42100 Reggio Emilia, Italy

When to perform peripheral blood progenitor cell collection in hematological patients?

Sir,

we read with interest the paper by Torretta *et al*.[1] on the experience of Pavia University regarding circulating progenitor cell collection in cancer patients. The authors stated that, using daily flow cytometric monitorization of $CD34^+$ cells in the peripheral blood (PB), collections were started when these cells reached a value of 20 µL. However, the possibility of harvesting even though circulating $CD34^+$ cells were below 20 µL (between 10 and 20 µL) was always considered in relation to the particular clinical history, state of disease and therapeutic strategy adopted for each patient.

In agreement with this latter statement, we would like to describe our experience on this topic. As Torretta *et al*. reported, we usually start leukapheresis when white cells in the PB, evaluated in the morning just before collection, are greater than 1000 µL and $CD34^+$ cells greater than 20 µL. However, when making clinical decisions, we have to consider that some patients, due to their clinical situation such as secondary myelodysplasia, exhausted marrow, heavy previous chemo- or radiotherapy may have serious problems in mobilizing $CD34^+$ progenitor cells in the blood.[2,3] This leads to the need for a larger number of procedures to obtain the optimum yield of progenitor cells for a safe engraftment. In this setting, some authors suggest a threshold dose of 2×10^6/kg $CD34^+$ cells, while others refer that less than 5×10^6/kg, although able to restore hemopoiesis in most cases, can be responsible for delayed engraftment or defective platelet reconstitution and recommend collecting not less than 8×10^6/kg $CD34^+$ cells to ensure a rapid, complete and sustained hematopoietic recovery.[4,5] We retrospectively analyzed our data on 54 patients suffering from hematological malignancies (21 multiple myelomas, 14 acute myeloid leukemias, 10 non-Hodgkin's lymphomas, 9 Hodgkin's disease) who received high-dose mobilizing chemotherapy plus growth factor administration (G-CSF and in some cases GM-CSF) at our Institution between April 1993 and July 1996. The total number of leukapheresis performed was 159 (mean number per patient 2.9, range 1-4). About 9 liters of blood were processed for each patient.

The amount of $CD34^+$ cells collected at each leukapheresis was analyzed in relation to the number of $CD34^+$ cells in the PB, in order to evaluate the predictive yield capacity of the latter. A

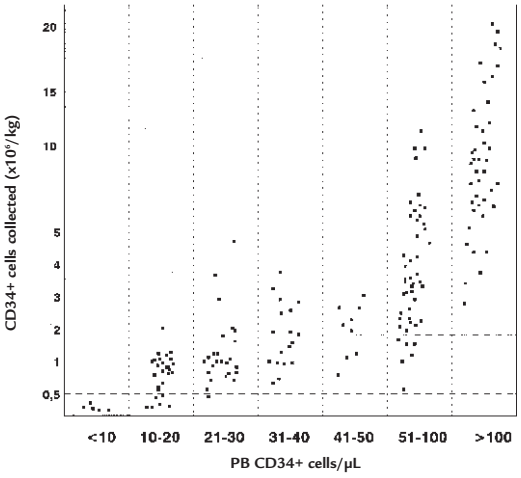

Figure 1. $CD34^+$ progenitor cell content (x 10^6/kg) in leukapheresis in relation to the PB $CD34^+$ cell concentration (/mL).

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strong correlation was found between the two variables ($r = 0.86$; $p<0.0001$), as suggested by linear regression analysis. The best results were obtained when the number of $CD34^+$ cells in PB was greater than 50 µL, while the worst results were related to a level of less than 10 µL (Figure 1). Only in 5 cases, because of the particular clinical history of the patients, did we perform leukapheresis when circulating $CD34^+$ cells were < 10 µL. None of these reached 0.5×10^6/kg $CD34^+$ cells (mean value collected was 0.23, range $0.05\text{-}0.4\times10^6$/kg). With more than 10/mL $CD34^+$ cells in PB we collected no less than 0.5×10^6/kg, with rare exceptions. When $CD34^+$ cells exceeded 20/mL we usually reached more than 1×10^6/kg. No statistical difference was found in patients with $CD34^+$ cell values ranging from 21 to 50 µL. Finally, in our hands, more than 50 µL circulating $CD34^+$ cells ensured a collection greater than 2×10^6/kg in most patients.

Though the quality of a leukapheresis does not depend only upon the absolute number of $CD34^+$ progenitors present,[6,7] our data confirm that daily estimation of the circulating $CD34^+$ cell number by flow cytometry may guide our clinical decisions and may offer a useful tool for predicting the number of procedures to perform.

Giovanni D'Arena
Pellegrino Musto
Mario Carotenuto

Division of Hematology, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy

References

1. Torretta L, Perotti C, Dornini G, et al. Circulating progenitor cell collection: experience from 275 leukapheresis in various malignancies and in healthy donors. Haematologica 1996; 81:208-15.
2. Haas R, Mohle R, Fruhauf S, et al. Patient characteristics associated with successful mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. Blood 1994; 83:3787-94.
3. Bensinger W, Appelbaum F, Rowley S, et al. Factors that influence collection and engraftment of autologous peripheral-blood stem cells. J Clin Oncol 1995; 13:2547-55.
4. Gordon MY, Blackett NM. Some factors determining the minimum number of cells required for successful clinical engraftment. Bone Marrow Transplant 1995; 15:659-62.
5. Siena S, Bregni M, Di Nicola M, et al. Durability of hematopoiesis following autografting with peripheral blood hematopoietic progenitors. Ann Oncol 1994; 5: 935-41.
6. D'Arena G, Cascavilla N, Musto P, et al. Flow cytometric characterization of CD34+ hematopoietic progenitor cells in mobilized peripheral blood and bone marrow of cancer patients. Haematologica 1996; 81:216-23.
7. Caligaris Cappio F, Cavo M, De Vincentiis A, et al. Peripheral blood stem cell transplantation for the treatment of multiple myeloma: biological and clinical implications. Haematologica 1996; 80:356-75.

Correspondence: Giovanni D'Arena, MD, Division of Hematology, IRCCS Casa Sollievo della Sofferenza Hospital, 71013 San Giovanni Rotondo, Italy. Tel. international +39.882.410539. Fax: international +39.882.411705.

All-trans retinoic acid might also induce apoptosis in freshly isolated chronic myeloid leukemia cells

Sir,

we read with interest the recent letters by Martinelli and coworkers[1] and Zinzani and coworkers[2] on the induction of apoptosis by the nucleoside analogs fludarabine (FAMP), 2-chlorodeoxyadenosine (2-CdA) and 2-deoxycoformicin (DCF), whether used alone or in combination with α-interferon (α-IFN), in freshly isolated leukemic cells from chronic myeloid leukemia (CML). Apoptotic cell death, as demonstrated by electrophoretic gel DNA fragmentation pattern, was induced by both FAMP[1] and 2-CdA,[2] either alone or in combination with α-IFN, whereas DCF,[2] with or without α-IFN, failed to do so. The authors focused on the opportuneness of promoting further *in vitro* and *in vivo* studies with these two promising adenosine analogs, possibly employing assays able to measure programmed cell death. We agree with the authors about the timeliness of exploring new effective drugs capable of driving the CML clone into apoptosis, and on this point we would like to provide further evidence in support of their *in vitro* findings.

We recently tested the *in vitro* capability of FAMP and all-trans retinoic acid (ATRA) to drive peripheral myeloid cells from untreated Ph+ CML patients in chronic phase into apoptosis. Apoptosis was measured by using simple and reliable flow cytometric methods based on decreased forward light, increased right angle scatter and reduced propidium iodide fluorescence stainability.[3] These methods, as compared to electrophoretic gel DNA fragmentation assays, which allow only bulk apoptosis measurement, are able to detect programmed cell death on a single cell basis. In our model, CML cells cultured alone in standard complete medium (RPMI 1640 plus 10% FCS) showed a low apoptotic cell rate (6.8% at 96 hours of culture) at all the different time points tested (24, 48, 72, 96 hours). By contrast, when cultures were performed in the presence of FAMP (5 µM) apoptosis reached 26.3% and 31.7% at 72 and 96 hours, respectively. Similar results were obtained when FAMP was substituted with ATRA (3 µM). This agent, which was previously shown to induce apoptosis in both acute promyelocytic leukemia[4] and chronic lymphoproliferative disorders,[5] also drove CML cells into apoptotic cell death (28.6% at 72 and 40.5% at 96 hours of culture). Apoptosis occurred mainly via terminal myeloid differentiation of the leukemic clone, as demonstrated by cytospin morphological and cytochemical examinations.

Taken together, these findings further support the importance of focusing on inducers of programmed cell death as promising new agents in the management of chronic phase CML, and provide a rationale for the employment of either purine analogs or ATRA in pilot clinical trials.

Fabio Stagno
Ugo Consoli
Emma Cacciola jr

Institute of Hematology, University of Catania, Italy

References

1. Martinelli G, Zinzani PL, Farabegoli P. Purine analogs (fludarabine and 2-chlorodeoxyadenosine) as apoptosis-inducing drugs in CML therapy. Haematologica 1996; 81:286-7.
2. Zinzani PL, Martinelli G, Buzzi M, Farabegoli P, Bendandi M, Tura S. Apoptosis induction with purine analogs on freshly isolated chronic myeloid leukemia cells. Haematologica 1995; 80:390-1.
3. Stagno F, Impera S, Italia P, Cacciola E jr, Corsaro F, Guglielmo P. In vitro drug-induced apoptosis in freshly isolated leukemic cells: a flow cytometric analysis. J Biol Res 1995; 71:99-103.
4. Chomienne C, Ballerini P, Balitrand N, et al. All-trans retinoic acid in acute promyelocytic leukemias. II. In vitro studies: structure-function relationship. Blood 1990; 76:1710-7.
5. Stagno F, Cacciola RR, Impera S, Guglielmo P. Apoptotic effect of all-trans retinoic acid (ATRA) on B cells from chronic lymphoproliferative disorders [abstract]. Exp Hematol 1995; 23:889.

Correspondence Fabio Stagno, MD, Istituto di Ematologia, Ospedale Ferrarotto, via S. Citelli 6, 95124 Catania, Italy. Tel. international +39.95.7435968. Fax. international +39.95.365174.

Cryosupernatant in thrombotic thrombocytopenic purpura (TTP): is it really useful?

Sir,

Perotti *et al*. in their paper entitled *Cryoprecipitate-poor plasma fraction (cryosupernatant-CPP) in the treatment of thrombotic thrombocytopenic purpura at onset. A report of four cases*[1] demonstrated that CPP can induce a more rapid improvement in the clinical manifestations of TTP than fresh frozen plasma, while laboratory parameters show slow normalization. We treated six patients with plasma exchange (PE) and CPP (two relapses at four and six years, respectively; four cases at first diagnosis).

The clinical manifestations of these patients at onset are reported in Table 1.

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Table 1. Laboratory parameters and clinical manifestations at onset in six patients with TTP.

	Hb g/dL	Plt x10⁹/L	LDH U/dL	Neurological findings	Kidney findings
RR	6.5	9.0	2750	no	no
MD	6.8	5.0	1498	no	no
DA	11.0	2.0	5786	yes	yes
CM	7.5	13.0	2450	no	no
PLM	6.7	14.0	1743	yes	yes
BS	7.2	11	1570	yes	no

All patients received the first plasma exchange employing fresh frozen plasma, and CPP was used in the following procedures. PE was performed daily until normalization of the platelet count and serum LDH levels.

Two patients (B.S. and P.M.L.) died 8 and 5 days, respectively, after beginning plasma exchange, and the addition of high-dose iv immunoglobulins in one case did not produce any effect. Clinical remission was reached in four patients. In one case, neurological disorders at onset such as amnesia and paresthesia improved after three PE procedures.

Normalization of platelet count was obtained after 7, 11, 13 and 17 plasma exchange procedures, respectively. Our experience confirms the data reported by Perotti *et al*. regarding hematological recovery, while the neurological disorders that appeared during the treatment in two patients did not seem to be influenced by the addition of CPP.

These observations seem to limit the efficacy of CPP as compared to fresh frozen plasma in the treatment of TPP.

Riccardo Centurioni
*Cristina Refe**
Alessandro Cecapolli

*Istituto di Clinica Medica Generale, Ematologia ed Immunologia Clinica, Università di Ancona and *Servizio di Immunoematologia e Trasfusionale, Ancona, Italy*

References

1. Perotti C, Torretta L, Molinari E, Salvaneschi L. Cryoprecipitate-poor plasma fraction (cryosupernatant) in the treatment of thrombotic thrombocytopenic purpura at onset. A report of four cases. Haematologica 1996; 81:175-7.

Correspondence: Riccardo Centurioni, MD, Istituto di Clinica Medica, Ospedale di Torrette, 60020 Ancona, Italy.

Lymph node myeloid metaplasia associated with chronic neutrophilic leukemia

Sir,

chronic neutrophilic leukemia (CNL) is a rare disorder characterized by neutrophilia due to mature elements and organ infiltration, including both hepatosplenomegaly and lymph node infiltration.[1,2] Around 78 cases have been reported up to now and no one else has described the presence of myeloid metaplasia in lymph nodes as we have in this study.

A 68-year-old man presented weight loss, enlarged lymph nodes, hepatosplenomegaly and ascites. WBC count was 156×10^9/L (84% neutrophils, 1% monocytes, 2% basophils, 3% bands, 2% atypical lymphocytes, 3% metamyelocytes, 5% myelocytes). Hemoglobin was 8.7 g/dL, platelets 68×10^9/L, uric acid was 15.8 mg/dL, alkaline phosphatase 2457 U/L, LDH 915 U/L, granulocytic alkaline phosphatase 300 U. Renal and liver function was normal. Paracentesis revealed ascitic liquid with 3.6×10^6/L cells (85% mature neutrophils). Bone marrow biopsy displayed increased cellularity and granulocytic hyperplasia compatible with CNL. Cytogenetic study was normal (46,XY). Molecular biology did not demonstrate a bcr-abl translocation. A lymph node biopsy showed massive substitution of the normal lymph node architecture by hematopoietic cells, including elements of all three series; within the granulocytic line numerous polynuclear cells were conspicuously intercalated among immature cells. Megakaryocytes were also frequent, and some of them presented phagocytic phenomena. The patient was treated with hydroxyurea, which provided good WBC count control; however, two months later he developed hepatorenal failure and died on day +71 after diagnosis.

Myeloid metaplasia has not been previously described in CNL. Chronic neutrophilic leukemia was recently characterized as a distinct myeloproliferative disease with a specific molecular marker (bcr/abl with C3/A2 junction).[3,4] Our finding of myeloid metaplasia is consistent with the myeloproliferative nature of CNL.

*José A. Pérez-Simón**
*Jesús M. Hernández-Rivas**
Teresa Flores°

*Servicio de Hematología, *Servicio de Anatomía Patológica, °Hospital Universitario de Salamanca, Paseo de San Vicente 58-182, Salamanca, Spain*

References

1. Gastearena J, Torúe MT, Uriz MJ, Pérez Equiza C, Calavia J, Gorosquieta A. Leucemia neutrofílica crónica. Sangre 1995; 40:499-503.
2. Zitton R, Rèa D, Hoang L, Ramond S. Chronic neutrophilic leukemia. A study of four cases. Ann Hematol 1994; 68:55-60.
3. Santucci MA, Saglio G, Tura S. Pathogenesis and progression of chronic myeloid leukemia. Haematologica 1996; 81:63-70.
4. Pane F, Frigeri F, Sindona M, et al. Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). Blood 1996; 88:2410-4.

Correspondence: José A. Pérez-Simón, MD, Servicio de Hematología, Hospital Universitario de Salamanca, Paseo de San Vicente 58-182, 37007 Salamanca, Spain.
Tel. international +33.23.291100-291200.
Fax. international +33.23.291131.

Prolonged low doses of oral etoposide may be effective in individual patients with advanced lymphoproliferative disorders refractory to aggressive chemotherapy

Sir,

etoposide, a semisynthetic podophyllin derivate, is currently employed in the treatment of several malignancies. The antitumor efficacy of etoposide is highly schedule dependent and it has been demonstrated that five-day administration is superior to single day administration.[1] Oral etoposide has greatly facilitated the use of multiple-day schedules and the drug displays good activity in many extrahematological malignancies as well as in lymphomas.[2,3] From December '93 to May '94, we treated with prolonged low doses of oral etoposide 24 patients with advanced hematological malignancies not eligible for further intensive approaches due to age >60 years and/or severe previous infective complications. Of these, ten patients had ALL, 8 AML, 4 NHL and 2 had CML.

Etoposide (50 mg/m^2 per day) was administrated orally for 21 consecutive days; patients who showed a response or stable disease on day 28 received a second and a third cycle of the same treatment. Responder patients received maintenance etoposide at the same dosage 10 days/month until relapse. Three patients (12.5%) died during the first cycle of intracerebral hemorrhage (1 patient) or infective complications (2 patients); no other toxic deaths were observed. All other patients received at least 2 cycles. Two ALL and 1 NHL patient (12.5%) obtained a complete remission of short duration (2, 3, 7 months); 1 ALL and 2 NHL patients (12.5%) achieved a partial response. No patient with myeloproliferative disease attained a response. Data from responder patients are shown in Table 1.

As for toxicity, 15/24 patients (62.5%) suffered febrile episodes during treatment (4 sepsis, 6 bronchopneumonia, 1 fungal sinusitis and 4 fever of unknown origin). One patient developed an intracerebral hemorrhage, while 8/24 patients (33.3%) displayed cutaneous hemorrhagic manifestations. Ten patients (41.6%) had mild nausea (WHO < 2) and vomiting, and 6/24 (25%) mucositis (WHO 2).

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Table 1. Data from responder patients.

Sex	Age	Disease	Disease status	Previous treatments	Response	N.cycles to response	Response duration (months)
F	22	ALL	2nd resistant relapse	VCR+DNR+CTX IDAC+IDA ARA-C+MTX+PDN	CR	2	2
M	34	ALL	resistant	AIL 7603 IDAC+IDA	CR	2	3
F	64	NHL	1st relapse	F-MACHOP	CR	3	7
F	41	ALL	1st relapse	MAGRATH	PR	3	4
F	77	NHL	1st resistant relapse	CHOP PROVECIP	PR	2	4
M	22	NHL	resistant	MACOP-B +ABMT	PR	2	3

Despite the heterogeneity and low number of patients treated, some preliminary observations can be made. The 25% overall response rate achieved is encouraging and compares favorably with other single-agent approaches, as well as with intravenous etoposide.[4]

Previous studies have also reported encouraging results using oral etoposide in untreated elderly AML patients.[5] By contrast, the results reported in ALL patients are disappointing.[4] No AML patient in our study responded. However, ALL patients showed a 30% response rate (2/10 CR and 1/10 PR). Overall, oral etoposide achieved better results in advanced lymphoproliferative disease (6/14 responder patients) than in advanced myeloproliferative disease (0/10 responder patients). The reason for this behavior is unclear.

The toxicity of the schedule was acceptable and no patient discontinued the treatment because of nausea. As expected from the advanced disease status and heavy pretreatment of these patients, median response and survival duration were short. The use of oral etoposide in less advanced disease and/or its association with other drugs are possible ways of improving these results.

ROBERTA CARUSO*
FRANCESCO ALESIANI°
ROBERTO LATAGLIATA*

*Ematologia, Dipartimento di Biopatologia Umana, Università "La Sapienza" di Roma;
°Divisione Medica, Ospedale di S. Severino Marche (MC); Italy

References

1. O'Dwyer PJ, Leyland-Jones B, Alonso MT, et al. Etoposide (VP-16-213): current status of an active anticancer drug. N Engl J Med 1985; 312:692-700.
2. Johnson DH, Greco FA, Strupp J, Hande KR, Hainsworth JD. Prolonged administration of oral etoposide in patients with relapsed or refractory small-cell lung cancer: a phase II trial. J Clin Oncol 1990; 8:1613-7.
3. Hainsworth JD, Johnson DH, Frazier SR, Greco FA. Chronic daily administration of oral etoposide in refractory lymphoma. Eur J Cancer 1990; 26:818-21.
4. Bishop JF, Lowenthal R, Joshua D, Matthewes JP, Wolf MM, Cooper IA. Etoposide in leukemia. Cancer 1991; 67:285-91.
5. Evans JPM, Prentice HG. Remission induction in acute myeloid leukemia with oral etoposide. Lancet 1982; 2:1345-6.

Correspondence: Roberto Latagliata, MD, Ematologia, Dipartimento di Biopatologia Umana, Università "La Sapienza", via Benevento 6, 00161 Roma, Italy. Fax. international +39.6.44241984.

Circulating antiplatelet antibody specificity in children with immune thrombocytopenic purpura at onset

Sir,

immune thrombocytopenic purpura (ITP) is caused by the interaction of platelet reactive antibodies with platelet surface antigens, which determines accelerated platelet destruction of antibody-coated platelets. Two forms of childhood ITP may occur: a syndrome similar to adult chronic ITP, and an acute self-limiting form of the disease. Only few reports have been published about antiplatelet antibody specificity in paediatric ITP; some of them[1,2] studied the specificity of circulating antiplatelet antibodies by testing patient sera by immunoblotting. However, certain conformational antigens on platelet membrane are destroyed by this technique. Moreover, these reports concerned small pediatric ITP populations. It was suggested that the presence of circulating anti-GPIIb/IIIa antibodies may be useful in differentiating acute from chronic ITP in children;[3] however, in a recent pediatric survey[4] no difference between the two ITP forms was found. We investigated the specificity of circulating antiplatelet antibodies of ITP children at onset, in order to assess whether it may represent a marker of evolution of the disease. Sera were collected from 74 ITP children (4 months to 13 years, mean age of 5.5 years) at onset before beginning therapy. Forty-nine patients recovered within 6 months from the initial diagnosis (acute ITP), whereas 25 patients developed chronic disease (mean duration 2.2 years, range 1 to 5 years). Antibody specificity was assessed by indirect MAIPA assay refined according to Kiefel *et al*.;[5] we looked for anti-GPIIb/IIIa and anti-GPIb/IX IgG antibodies.

Anti-GPIIb/IIIa antibodies were found in 19/49 (38.8%) and in 8/25 (32.0%) acute and chronic ITP, respectively. Antibodies to GPIb/IX were detected in 15/49 (30.6%) acute ITP and in 7/25 (28.0%) chronic ITP. Thus, in our experience we did not find any significant difference between acute and chronic ITP, evaluating both anti-GPIIb/IIIa and anti-GPIb/IX antibodies. This study reports on the investigation of circulating antiplatelet antibodies specificity in the largest sample of acute and chronic ITP children at onset ever analyzed at our knowledge. We conclude that circulating antiplatelet IgG specificity in childhood ITP at onset does not represent a marker to the early recognition of those patients devoted to chronicize. We cannot rule out that autoantibodies against platelet antigens other than GPIIb/IIIa and GPIb/IX were responsible for thrombocytopenia in some of our patients; moreover, antiplatelet IgM should be investigated especially in acute ITP patients. Finally, more useful information could be obtained performing directly MAIPA on patient's platelets.

PAOLA BIGLINO
PAOLO PERUTELLI
PIER GIORGIO MORI

Hematology Laboratory, Hematology and Oncology Department, G. Gaslini Children's Hospital, Genoa, Italy

References

1. Winiarski J. IgG and IgM antibodies to platelet membrane glycoprotein antigens in acute childhood idiopathic thrombocytopenic purpura. Br J Haematol 1989; 73:88-92.
2. Fabris F, Cordiano I, Steffan A, Randi ML, Girolami A. Identification of anti-platelet autoantibodies by Western blot in 45 patients with idiopathic thrombocytopenic purpura (ITP). Haematologica 1992; 77:122-6.
3. Berchtold P, McMillan R, Tani P, Sommerville-Nielsen S, Blanchette VS. Autoantibodies against platelet membrane glycoproteins in children with acute and chronic immune thrombocytopenic purpura. Blood 1989; 74:1600.
4. Taub JW, Warrier I, Holtkamp C, Beardsley DS, Lusher JM. Characterization of autoantibodies against the platelet glycoprotein antigens IIb/IIIa in childhood idiopathic thrombocytopenic purpura. Am J Hematol 1995; 48:104-7.
5. Kiefel V. The MAIPA assay and its applications in immunohaematology. Transf Med 1992; 2:181-8.

Correspondence: Paola Biglino, MD, Hematology, Laboratory Hematology and Oncology Department, G. Gaslini Children's Hospital, largo G. Gaslini 5, 16147 Genova Italy. Fax. international +39.10.3776590.

Hodgkin's disease in Brazil: a clinicopathologic study

Sir,

the behavior of HD in Brazil, namely its epidemiology, histological distribution, clinical and pathological stages, and the

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potent for cure with conventional treatment is not well- known but is of both practical and scientific importance. Spector *et al*.[1] described good results in treating 59 HD patients from rather low socioeconomic background using C-MOPP/ABV chemotherapy.[2] The authors carried out a study in 134 cases of adult patients (age above 15 years; 76 males and 58 females) from 1985 to 1994. HD appeared predominantly in males (R=1.31); the incidence peaked between 21 and 30 years and presented a descending curve after 30 years of age.

The most frequent histologic type was nodular sclerosis (NS)(50.4%) follwed by mixed cellularity (MC) (34.6%), lymphocyte depletion (LD) (9.0%) and lymphocyte predominance LP) (5.2%) (Table 1). NS-I type prevailed over NS-II (30.8% and 19.6%, respectively). The cervical region, followed by the spleen and retroperitoneum, was the most frequent primary sites of disease. A bulky mass was found in 20% of the cases, mostly in the mediastinum. Bone marrow (BM) was involved in 16.5% of the patients: none LP type, 3.0% in NS, 6.8% in MC and over 40% in LD type. Anemia was directly related to BM infiltration (p=0.003). B symptoms as well as higher levels of mucoproteins were more frequent in males (p=0.02 and 0.03, respectively). In contrast, early clinical stage occurred mainly in females (p=0.004). Probability of survival after 5 years was 83%. Disease free survival was 52%. No statistically significant differences were found between sexes, NS and MC types or NS-I and NS-II subtypes. Complementary factors that negatively influenced the survival were BM infiltration (p=0.02), lymphopenia (p=0.01) and serum mucoproteins above 7 mg/dL (p=0.01).

Table 1. Distribution of histologic types according to sex.

	Males		*Females*		*Total*	
histologic type	*n.*	*%**	*n.*	*%**	*n*	*%*
LP	4	5.2	3	5.2	7	5.2
MC	33	43.4	13	22.4	46	34.6
NSI°	17	22.3	25	43.1	41	30.8
NSII°	13	17.1	13	22.4	26	19.6
LD	8	13.1	4	6.9	12	9.0
unclassified	1	1.3	0	0	1	0.8
total	76	100	58	100	134	100

These findings demonstrate that the NS type is possibly the most frequent histologic type, at least in Southeast Brazil, a pattern similar to that found in developed countries.[3]

One interesting clinical feature concerns the period between the first sign of clinical syrnptoms and medical diagnosis. We observed that this period was significantly shorter in women and in NS-II type, when compared to other types, particularly NS-I. Among men, a history of disease up to 24 months could be detected. On the other hand, the shorter disease history interval among NS-II patients may be due to more rapid progress of lymph node enlargement. This fact points to different biological behavior for NS-I and NS-II, although this difference did not influence response to therapy or prognosis.

One of the most important aspects in our study is related to the distribution of patients in clinical stages. The validity of the Ann Arbor staging system has been established.[4] In our study, a large number of cases were in advanced stages (68.8%) and presented B symptoms (67.2%). This is different from developed countries, where such cases rarely exceed 50%. The larger extent of disease at diagnosis in our patients could also be documented by the number of sites of involvement, the prognostic significance of which has already been stressed.[5] All these features point to a delay in diagnosis of HD in our population. This is also confirmed by the high incidence of BM involvement, anemia, high ESR, and mucoprotein levels, as well as a high frequency of lymphopenia. Nevertheless, response to treatment was adequate according to the results described for the same stages and with the same therapy schedules.

*Cármino Antonio de Souza**
José Vassallo°
*Irene Lorand-Metze**

**Department of Internal Medicine; and °Department of Pathology, Faculty of Medicine, State University of Campinas, Brazil*

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References

1. Spector N, Costa MA, Pucheri W, et al. C MOPP/ABV yields good results in a public hospital population with Hodgkin's disease in Brazil. Cancer 1993; 71:2823-7.
2. Klimo P, Connors JM. MOPP/ABV hybrid program combination chemotherapy based on early introdution of seven drugs of advanced Hodgkin's disease. J Clin Oncol 1985; 3:1174-82.
3. Georgii A, Fischer R, Hubner K, Schwarze EW, Bernhards J. Classification of Hodgkin's disease biopsies by a panel of four histopathologists. Report of 1140 patients from German National trial. Leuk Lymphoma 1993; 9:365-70.
4. Carbone PP, Kaplan HS, Mushoff K Smithers DW, Tubiana M. Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res 1971; 31:1860-1.
5. Mendenhall NP, Cantor AB, Barr DM, Lynch JW, Million RR. The role of prognostic factors in treatment selection for early-stage Hodgkin's disease. Am J Clin Oncol 1994; 17:189-95.

Correspondence: Cármino Antonio de Souza, MD, Hematology-Hemotherapy Center, State University of Campinas, Cidade Universitaria "Zeferino Vaz", P.O. box 6198. 13081-970, Brazil. Tel. & Fax. international 55.19.2393181.

Meeting Announcements

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Any further information available through the Scientific Secretariat, Studio E.R. Congressi, via Riva Reno 47, 40122 Bologna, Italy. Tel. +39.51.235293. Fax +39.51.235296.

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