

PDGF expression in IMF subtypes bone marrow trephines from patients with prefibrotic, cellular IMF (n=28) and advanced myelofibrosis (n=29) were retrieved from the archive and classified according to the degree of myelofibrosis essentially as described by Buhr *et al.*⁵ The control group (n=26) comprised cases displaying reactive hyperplasia of megakaryocytopoiesis without any evidence of a neoplastic proliferation.

RNA was extracted from total bone marrow cells and real-time reverse-transcription polymerase chain reaction assays were performed essentially as described elsewhere.⁷ Cases of advanced IMF displayed significant overexpression of the PDGF isoforms as well as of the PDGF-R α as compared to the expression in cases of cellular IMF and in the control group. Unexpectedly, expression in cases of cellular IMF did not differ substantially from that in non-neoplastic hematopoiesis (Figure 1). The overexpression in advanced IMF was prominent for PDGF-B and PDGF-R α , with an up to 15-fold ($p=0.001$) and 32-fold ($p<0.0005$) increase, respectively. Since fibroblasts in advanced IMF could be a considerable source for PDGF-R α we applied immunohistochemistry with a monoclonal antibody (MAB322, R&D systems, Minneapolis, USA) in order to delineate cellular origin.

While considerable numbers of megakaryocytes in both cellular IMF and advanced IMF displayed positive cytoplasmic labeling no other cell types, and in particular no stromal cells or endothelial cells, showed demonstrable PDGF receptor α labeling. Megakaryocytes in non-neoplastic hematopoiesis were constantly negative for PDGF-R α staining (*data not shown*). There was considerable heterogeneity of labeling intensities for PDGF-B in cellular IMF, advanced IMF, and non-neoplastic hematopoiesis. PDGF-B immunolabeling was not a very sensitive marker to indicate increased fibrosis because a considerable number of cases of advanced IMF with manifest fibrosis stained negative (*data not shown*). Since the immunohistochemical approach failed to reveal fibroblasts as a relevant source of PDGF-R α in advanced IMF, other PDGF-R subtypes (such as the β -type) might be involved in the activation of fibroblasts in IMF. Besides complex cellular interactions between the neoplastic clone (i.e. megakaryocytes) and the stroma in IMF, megakaryocytic PDGF-R α labeling in IMF strongly suggests that PDGF have a role apart from that in the purely fibrogenic process, e.g. involvement in autocrine activation.⁸ We conclude that increased expression of PDGFs in advanced IMF reflects disease progression and discriminates the cellular from the fibrotic stage of IMF.

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Malignant Lymphomas

Is bone marrow trephine biopsy always mandatory in staging Hodgkin's disease?

We reviewed data from 690 adult patients with Hodgkin's disease (HD) to determine whether bone marrow trephine biopsy (BMTB) is mandatory for all patients. The data suggest that it is not necessary in clinical stage I-IIA. However, bilateral BMTB is recommended in the presence of B symptoms also in patients with localized stage disease.

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The recommended staging procedures for patients with HD include BMTB. The value of this procedure is, however, controversial. The Ann Arbor conference recommendations for staging required BMTB to be carried out in the presence of peripheral blood cytopenia, when bone marrow involvement is doubtful and in clinical stages III and IV.¹ The Cotswolds conference recommended BMTB for patients with clinical stages II-IV.² Literature data report that about 4-15% of patients with HD have bone marrow involvement (BMI); however, the incidence of BMI in patients clinically staged as I-II A has been shown to be <1%.^{3,4} Many studies have shown that BMI is not, by itself, an adverse prognostic factor and does not define a specific high risk group requiring a different therapeutic approach.⁴

We retrospectively examined data from 690 adult patients (over 20 years) treated at our institution between 1993 and 2003, with the aim of evaluating whether BMTB is mandatory for all patients with HD. All patients were submitted to standard staging procedures, including bilateral BMTB. One hundred and fifty patients (22%) were defined as having initial stage disease, 373 (54%) as having intermediate stage disease and 167 (24%) as having advanced stage disease. Initial stage included patients staged I-IIA without risk factors (bulky mediastinal mass, extranodal involvement, massive splenic involvement,

Table 1. Clinico-biological features of patients with advanced stage Hodgkin's disease with and without bone marrow involvement.

| | Patients without BMI | Patients with BMI | p |
|--------------------------------|----------------------|-------------------|-------|
| Total | 139 | 28 | |
| Male/Female (%) | 79/60 (57/43) | 16/12 (57/43) | |
| Clinical stage before BMTB | | | |
| I B | 0 | 2 (7%) | |
| IIE | 3 (2.2%) | 0 | |
| III | 73 (52.5%) | 22 (79%) | |
| IV | 63 (45.3%) | 4 (14%) | |
| Histology | | | |
| Nodular sclerosis | 94 (67.6%) | 14 (50%) | 0.11 |
| Mixed cellularity | 27 (19.5%) | 9 (32%) | 0.21 |
| Lymphocyte predominance | 4 (2.9%) | 1 (3.6%) | 0.68 |
| Lymphocyte depletion | 2 (1.4%) | 1 (3.6%) | 0.99 |
| Classical HD, unspecified | 12 (8.6%) | 3 (10.7%) | 0.99 |
| B symptoms | 118 (85%) | 23 (82%) | 0.93 |
| Bulky | 28 (20%) | 4 (14%) | 0.64 |
| Splenic involvement | 30 (21.6%) | 5 (17.8%) | 0.85 |
| Extranodal sites other than BM | 63 (45.3%) | 4 (14%) | 0.004 |

BMI: bone marrow involvement; BMTB: bone marrow trephine biopsy; Bulky: a mediastinal mass > 1/3 of the transverse diameter of the thorax or a > 5 cm mass identified by CT scan; BM: bone marrow.

Table 2. Response to first line treatment and last follow-up of patients with advanced stage HD with and without bone marrow involvement.

| Response | Advanced stage without BMI | Advanced stage with BMI | p |
|---|----------------------------|-------------------------|-------|
| Complete remission | 131 (94%) | 25 (89%) | 0.58 |
| Progression | 6 (4%) | 3 (11%) | 0.36 |
| Relapse | 13 (10%) | 4 (16%) | 0.58 |
| Lost to follow-up during first line therapy | 1 (1%) | 0 | |
| Death during first line therapy | 1 (1%) | 0 | |
| Median follow-up (range) | 47 months (2-115) | 36 months (3-116) | |
| Alive in complete remission | 124 (89%) | 24 (86%) | 0.83 |
| Alive with disease | 4 (3%) | 0 | 0.82 |
| Death | 8 (6%) | 1 (3%) | 0.99 |
| Lost to follow-up | 3 (2%) | 3 (11%) | 0.096 |

erythrocyte sedimentation rate (ESR) >50 mm/h and 3 or more lymph node areas involved). Intermediate stage included patients staged I-IIA with risk factors, I-IIB and IIIA without risk factors. Twenty-eight patients (4%) had BMI; of these, 14 (50%) had monolateral involvement.

Twenty-six (93%) of these 28 patients had already been judged, on clinical ground, as having advanced stage disease (22 stage III, 4 stage IV) before BMTB so their treatment plan did not change on the basis of the bone marrow result. Only 2 patients (7%) of the 28 patients had been clinically considered as having intermediate stage disease; thus, BMTB was determinant in changing stage and treatment plan only in these 2 patients. Both these patients were over 40 years old (45 and 60 years), had B symptoms (fever) and a high ESR; one of these had bulky disease. No patient clinically staged as I-IIA had a positive bone marrow biopsy.

The characteristics of the patients with advanced stage HD are reported in Table 1. At diagnosis, no significant differences were observed between patients with BMI and those without BMI with regard to male/female ratio (1.33 versus 1.31), median age (33 years, range 21-62 versus 33 years, range 20-68), B symptoms (82% versus 85%), ESR (median 70 mm/h in both groups), rate of anemia, defined as Hb <10.5 g/dL, (53% versus 54%), leukocytosis or lymphocytopenia (11% versus 27%). According to literature data, although patients without BMI showed a higher complete response rate and lower progression of disease and relapse rate, in our series we did not find significant differences in terms of response rate (complete response=89% in the presence of BMI versus 94% in the absence of BMI, $p=0.58$) and overall prognostic likelihood (Table 2). The mixed cellularity histological subtype was more frequent in patients with BMI (32% versus 19.5%), though the difference did not reach statistical significance.

In conclusion, the probability of having a positive bone marrow biopsy in HD is higher in the presence of advanced disease, B symptoms, mixed cellularity histologic subtype, male sex, high ESR, anemia and lymphopenia, but the difference is not statistically significant. These observations are concordant with literature data.⁴⁻⁶ Furthermore, considering the low incidence of BMI in patients with clinically defined initial stage disease without risk factors, we conclude that BMTB should not be performed as a routine staging procedure in HD patients with clinical stages I-IIA in the absence of risk factors. However BMTB should always be performed in patients with clinically staged I-II HD with risk factors and in those with advanced stage disease. Finally, when required, BMTB should be done bilaterally because of the frequent focal BM involvement in HD.⁵

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Thrombosis

Relationships between white blood cell count and levels of serum homocysteine and cysteine in healthy subjects

Concentrations of serum thiols and white blood cell (WBC) count were examined in a group of 124 healthy volunteers. Univariate Pearson's analysis showed a close correlation between WBC count and total homocysteine and WBC and cysteine.

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Several clinical studies have demonstrated that moderately increased levels of plasma total homocysteine (tHcy) are associated with atherosclerosis, cardiovascular disease, stroke and peripheral vascular disease.¹ The mechanism by which homocysteine exerts its effects has not been clearly defined, although it is generally accepted that the accumulation of homocysteine in plasma can damage the endothelium. It has been suggested that homocysteine may induce vascular injury (including endothelial dysfunction, smooth muscle cells proliferation and thiolation of lipoprotein) and affect platelet aggregation and coagulation.² Other studies have reported that elevated levels of total cysteine (tCys) are related to an increased risk of cardiovascular diseases and atherosclerosis.³ Atherosclerosis is a multifactorial disease associated with a variety of risk factors and among them infection and inflammation may contribute to vascular injury and atherogenesis. Inflammation may also promote atherosclerotic plaque rupture and thrombosis.⁴ White blood cells (WBC) may serve as an important biomarker for these disease processes. Elevated WBC may also be considered a risk factor for acute myocardial infarction, coronary artery disease and stroke.⁵ Although an *in vitro* association between homocysteine and inflammation has been previously observed⁶ there is little information about the relationship between plasma thiols and WBC count *in vivo*. We, therefore, measured plasma thiol levels and WBC counts in a sample of patients without evidence of cardiovascular disease in order to verify whether there are any associations between these parameters involved in severe pathological manifestations. We analyzed 124 healthy volunteers, 44 women and 80 men, aged 50±10 years. WBC were meas-

Table 1 Serum thiol levels, white blood cells count and other parameters of the healthy subjects studied (n = 124).

| | Mean (SD) | Range |
|--------------------------------------|--------------|---------------|
| Age (years) | 50 (10) | 34-65 |
| Sex, M/F (%) | 64.5/35.5 | |
| Body mass index (kg/m ²) | 25 (3) | 18-35 |
| tHcy (µmol/L) | 11.21 (3.84) | 5.63 - 27.55 |
| tCys (µmol/L) | 284 (43.9) | 186.3 - 406.9 |
| WBC (K/µL) | 7.31 (1.72) | 4.10 - 13.10 |
| Triglycerides (mg/dL) | 82.1 (38.3) | 15-190 |
| Total cholesterol (mg/dL) | 175.1 (34.6) | 128-198 |
| LDL cholesterol (mg/dL) | 109.2 (31.1) | 78-139 |
| HDL cholesterol (mmol/L) | 48.9 (14.8) | 25-69 |
| Glucose (mg/dL) | 90 (15) | 66-108 |
| Creatinine (mg/dL) | 1.0 (0.3) | 0.5-1.4 |
| Systolic BP (mmHg) | 119 (7) | 99-138 |
| Dyastolic BP (mmHg) | 75 (6) | 58-88 |

ured by a Cell-Dyn 1700 (Abbott) system within 30 minutes from taking the blood sample. Serum thiols were determined by capillary electrophoresis laser induced detection as described by Zinellu *et al.*⁷ A comprehensive description of the investigated parameters are reported in Table 1. Mean levels of homocysteine and cysteine were about 11 µmol/L (range 5.6–27.5 µmol/L) and 284 µmol/L (range 186.3–406.9 µmol/L), respectively. As might be expected for a healthy population-based sample, 90% of this group had homocysteine levels < 15 µmol/L. Since the initial analysis demonstrated that the frequency distribution of tHcy was skewed toward higher values we chose to improve the normality of the distribution by log transformation of all data. Univariate Pearson's correlation between WBC and serum thiols levels are illustrated in Figure 1. WBC counts showed a significant positive correlation both with tHcy ($r=0.25$, $p<0.005$) and tCys ($r=0.30$, $p<0.0005$). The association between these thiols and the immune system was also confirmed by our previous data⁸ that showed a positive correlation between cysteine and homocysteine levels and neopterin in serum. This molecule was primarily synthesized by human monocytes and macrophages after stimulation by interferon-gamma (IFN-γ) produced by activated helper T-cells. Nevertheless, it is commonly accepted that both cysteine and homocysteine and an activated immune system may contribute to vascular injury and atherogenesis.⁹ The biological mechanisms by which homocysteine exerts its noxious effects are still unclear, but it has been found that it stimulates the expression of biologically active monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8), two major chemokines for leukocyte trafficking, in humans monocytes. Zeng *et al.* focused on the characterization of the signal transduction pathway(s) mediating the stimulatory effect of homocysteine on both MCP-1 and IL-8 expression in human monocytes.⁶ These investigations indicated that oxidative stress had a determinant role in the stimulatory effect of homocysteine most likely via activation of the redox-sensitive transcription factor NF-κB. Homocysteine-induced B lymphocyte proliferation is mediated by oxygen radicals such as O₂, OH, and H₂O₂, generated