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### **Frequency and clinical correlates of JAK2 46/1 (GGCC) haplotype in primary myelofibrosis.**

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myeloablative regimens, especially total body irradiation, result in lasting growth disturbance, high risk of alloimmune disease, second cancers and early mortality, as described in the editorial and the reference cited above. For this reason, HSCT does not and by its very nature will not fulfill the second goal.

With regard to the third goal, there is no realistic possibility that HSCT can ever meet this because of its high consumption of costly human and material health resources in this nation and a world so deficient in them, as reflected in the maternal and child health statistics of this nation and the millions of deaths of mothers, infants and children globally that could be easily prevented or successfully treated by simple low cost measures.

Twenty-five years ago, it became apparent that treatment of childhood acute leukemias needed to be targeted to their genetic disorders.<sup>6</sup> The 'sledge hammer,' hazardous, resource-consuming non-specific measures such as combination chemotherapy, radiation and HSCT must be replaced by specific gene-targeted approaches. Two highly effective and minimally toxic gene-targeted treatments, tretinoin for acute promyelocytic leukemia and imatinib mesylate for Ph-positive chronic myelocytic leukemia and ALL, were developed in the late 1980s and early 1990s.<sup>7,8</sup> Now in general use, they demonstrate the value of this approach. However, basic and clinical research for other agents targeted to specific genetic aberrations in leukemia has been slow to develop.

The data of the Children's Cancer Group concerning survival of children with ALL published by Nguyen *et al.*<sup>9</sup> included nearly 10 000 patients treated over 20 years.<sup>9</sup> They show no significant improvement in 5-year event-free survival of children treated for ALL over this period. It is time for diversion of federal and private research funds from large expensive studies of relatively minute details of 'sledge hammer therapy' and flawed comparisons that have not yielded significant improvement in cure rates in recent years to basic laboratory and clinical research to identify specific gene-targeted agents that are not only effective but safer and more likely to be accessible to all children.

#### Conflict of interest

The author declares no conflict of interest.

## Frequency and clinical correlates of *JAK2* 46/1 (GGCC) haplotype in primary myelofibrosis

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About sixty-percent of patients with primary myelofibrosis (PMF) harbor the *JAK2*V617F mutation while about 8–10% present a mutation at codon 515 in *MPL* exon 10, W515L/K/A.<sup>1</sup> These mutations are shared by the other Philadelphia-chromosome negative myeloproliferative neoplasms (MPNs), which include polycythemia vera (PV), essential thrombocythemia (ET), and refractory anemia with ring sideroblasts and thrombocytosis (RARS-T), and represent a major criteria for MPN diagnosis according to the 2008 WHO classification.<sup>2</sup> Presence of a *JAK2*V617F mutated genotype and/or higher V617F allele

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burden have been variably associated with hematological and clinical characteristics in patients with PV or ET.<sup>3</sup> In case of PMF, a significant association of *JAK2*V617F mutated status with a more pronounced myeloproliferative phenotype favoring raised hemoglobin level and leukocyte count and larger splenomegaly has been reported, although not universally.<sup>3</sup> However, the most relevant finding in two independent studies in PMF patients, was that a low *JAK2* allele burden at diagnosis represented a strong surrogate marker associated with shortened survival.<sup>4,5</sup>

Overall prognosis in PMF is poorer compared to other classic MPNs. An international prognostic score system (IPSS), which allows to discriminate four categories of patients with significantly different median survival, has been developed recently by

the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT).<sup>6</sup> However, identification of additional factors associated with prognosis is of considerable importance for driving therapeutic decisions. For example, cytogenetic abnormalities have an IPSS-independent value.<sup>7</sup> Furthermore, Tefferi *et al.*<sup>8</sup> recently reported that nullizygoty for the *JAK2* 46/1 haplotype was associated with inferior survival regardless of IPSS score or V617F allele burden.

The 46/1, or 'GGCC' haplotype, has been characterized in three independent studies as a germline haplotype block that is strongly associated (3–4 odds ratio) with the development of a *JAK2*V617F-mutated MPN.<sup>9–11</sup> Subsequent studies also showed significant association of this haplotype, with *JAK2* exon 12-mutated,<sup>12</sup> *JAK2*-wild type ET,<sup>13</sup> and *MPL*W515-mutated PMF or ET,<sup>14</sup> suggesting that this haplotype increases the risk of developing a MPN, regardless of the acquisition of the V617F mutation. However, in another recent study, no such association with *MPL* mutations was described.<sup>15</sup>

The aim of this study was to evaluate the association of *JAK2* 46/1 haplotype with *JAK2*V617F mutational status, clinical characteristics and prognosis in a series of patients with PMF. We obtained permission from the local institutional review board of the two institutions where the patients were referred to. We included 202 patients with a diagnosis of PMF according to the 2008 World Health Organization (WHO) criteria; in the case diagnosed earlier, a careful review of the clinical records and of the bone marrow biopsy was performed to assure compliance with the WHO criteria. All were typical fibrotic forms of myelofibrosis; pre-fibrotic myelofibrosis were not included. Measurement of *JAK2*V617F allele burden was performed in genomic DNA purified from granulocytes, obtained within one year from diagnosis in the absence of cytotoxic therapy, using a quantitative real time (QRT-PCR) assay, as previously described.<sup>5</sup> Genotyping for *MPL* W515L/K

mutation was performed as described.<sup>16</sup> Screening for 46/1 haplotype was performed in granulocytic DNA using a tag SNP, rs12343867, that is in complete linkage disequilibrium with the 46/1 haplotype. A commercially available SNP RT-PCR assay (No. C\_31941689\_10, Applied Biosystem, Foster City, CA, USA; <http://www2.appliedbiosystems.com>) was employed in an allelic discrimination assay using the Abi Prism 7300 Detection System. This SNP consists in a T to C shift, with the C allele being associated with the 46/1 haplotype. We used published criteria<sup>9</sup> for scoring SNPs as homozygous or heterozygous, to cope for skewed allelic ratio caused by uniparental disomy (UPD) at *JAK2* locus in cases with large homozygous *JAK2* V617F mutated clone. In 11 patients with >90% *JAK2*V617F allele who were homozygous for either C ( $n=6$ ) or T ( $n=5$ ) allele, only one allele was included in the final analysis, considering that no SNP information could be obtained for the residual *JAK2* wild-type allele.<sup>9</sup>

The *JAK2* mutated allele burden and the rs12343867 CC, TT, or CT variants were considered as ordered categorical variables. The chi-square or Fisher's exact test (two-tailed) or chi-square test for trend (larger contingency table) with Bonferroni correction, were used to compare the variables among the different groups of patients which had been categorized according to mutational status, haplotype status, or mutated allele burden. The analysis of continuous variables among the groups was performed using the Mann-Whitney *U* test or Kruskal-Wallis test for multiple comparison. Kaplan-Meier analysis and the log-rank test were used to estimate overall survival. A *P* value of <0.05 was considered to indicate statistical significance; all tests were two-tailed. Data were processed using the SPSS (Statsoft Inc, Tulsa, OK, USA) software.

The main clinical and laboratory characteristics of the 202 PMF patients included in the study are reported in Table 1. Median follow-up was 65.4 months, ranging from 1 to 312

**Table 1** Characteristics of the 202 patients with PMF included in the study, stratified according to the rs12347867 genotype

|   | rs 12347867 genotype |                  |                  |                  | P    |
|---|----------------------|------------------|------------------|------------------|------|
|   | All patients         | CC               | CT               | TT               |      |
| No.   | 202                  | 39               | 84               | 79               |      |
| Age <sup>a</sup>  | 58 (16–90)           | 60 (16–83)       | 57 (26–90)       | 57 (19–89)       | 0.38 |
| Follow-up, months <sup>b</sup>                            | 65.6 (1–312)         | 58.5 (2.4–237.3) | 68.5 (3.0–312.0) | 66.2 (1.0–308.9) | 0.51 |
| Females, no. (%)  | 75 (37.1)            | 21 (53.8)        | 30 (35.7)        | 24 (30.3)        | 0.05 |
| <i>JAK2</i> V617F, no (%)                                 | 133 (65.8)           | 31 (79.5)        | 56 (64.3)        | 48 (60.7)        | 0.13 |
| <i>JAK2</i> V617F burden <sup>a</sup>                     | 50 (5–100)           | 63 (11–100)      | 53 (12–100)      | 46 (5–100)       | 0.06 |
| WBC × 10 <sup>9</sup> /l <sup>a</sup>                     | 9.3 (0.6–106.1)      | 10.6 (1.4–37.7)  | 8.8 (1.9–90.8)   | 9.3 (0.6–106.1)  | 0.37 |
| Hb g/l <sup>a</sup>                                       | 123 (46–180)         | 122 (77–170)     | 125 (56–175)     | 119 (46–180)     | 0.45 |
| PLT × 10 <sup>9</sup> /l <sup>a</sup>                     | 355 (19–2522)        | 346 (19–1300)    | 347 (38–1568)    | 367 (41–2522)    | 0.44 |
| LDH U/l <sup>a</sup>                                      | 614 (162–3426)       | 757 (198–2645)   | 602 (162–3426)   | 548 (202–2981)   | 0.65 |
| Blast ≥ 1%, no (%) <sup>c</sup>                           | 51 (30.2)            | 14 (41.2)        | 19 (27.1)        | 18 (27.7)        | 0.49 |
| CD34 <sup>+</sup> cells % <sup>a</sup>                    | 0.24 (0–9.5)         | 0.22 (0–5.58)    | 0.25 (0.01–9.5)  | 0.25 (0–7.55)    | 0.98 |
| CD34 <sup>+</sup> cells × 10 <sup>9</sup> /l <sup>a</sup> | 26.2 (0–5454)        | 47.0 (0–789)     | 22.5 (0.73–5020) | 27.97 (0–5454)   | 0.70 |
| Spleen > 15 cm from LCM, no (%)                           | 60 (29.7)            | 17 (43.6)        | 18 (21.4)        | 25 (31.6)        | 0.15 |
| Constitutional symptoms, no (%) <sup>c</sup>              | 72 (43.7)            | 19 (55.9)        | 28 (40.0)        | 25 (38.4)        | 0.26 |
| IWG-MRT score, no (%) <sup>c</sup>                        |                      |                  |                  |                  |      |
| Low   | 61 (36.1)            | 7 (20.6)         | 28 (40.0)        | 26 (40.0)        |      |
| Int-1   | 38 (22.5)            | 8 (23.5)         | 16 (22.8)        | 14 (21.5)        | 0.40 |
| Int-2   | 40 (23.7)            | 9 (26.5)         | 18 (25.7)        | 13 (20.0)        |      |
| High  | 30 (17.7)            | 10 (29.4)        | 8 (11.4)         | 12 (18.5)        |      |
| Leukemia transformation, no. (%)                          | 25 (12.4)            | 4 (10.2)         | 11 (13.9)        | 10 (12.6)        | 0.95 |
| Deaths, no (%)  | 42 (20.8)            | 11 (28.2)        | 14 (16.7)        | 16 (20.2)        | 0.33 |

Abbreviations: PMF, primary myelofibrosis; IWG-MRT, International Working Group for Myelofibrosis Research and Treatment.

<sup>a</sup>Values are expressed as the median (range).

<sup>b</sup>Values expressed as the mean (range)

<sup>c</sup>Information were available in 169 patients.

months; 42 patients died during the observation period (20.8%). Leukemia transformation occurred in 25 patients (12.4%). According to the IPSS score, 36.1, 22.5, 23.7 and 17.7% of the evaluable patients ( $n=169$ ) were distributed among the four risk categories, comparable to other series.<sup>6,8</sup> One-hundred thirty-three patients were *JAK2V617F* mutated (65.8%), with a median allele burden of 50% (range 5–100%); their distribution in *V617F* allele burden quartiles was 11.3, 39.1, 22.5, and 27.1%.

The frequency of C allele, that stands for 46/1 haplotype, was significantly higher in the whole series of PMF patients, compared to the local control population comprising of 235 subjects (0.386 vs 0.253;  $P=6.37 \times 10^{-6}$ ) (Table 2). Such difference was largely due to the category of *JAK2V617F* mutated patients (C allele frequency, 0.413;  $P=8.29 \times 10^{-7}$  vs local controls). In fact, *JAK2* wild-type patients displayed an increased representation of the C allele compared to controls (frequency, 0.333), which did not reach the level of statistical significance if compared with local controls ( $P=0.063$ ). However, if we considered the WTCCC data that included 1492 subjects, as a reference,<sup>9</sup> also the difference with *JAK2* wild-type patients reached the significance level ( $P=0.036$ ). We interpret these data as supportive of the association of 46/1 haplotype with both *JAK2V617F* mutated and wild-type PMF patients, although the power of association with the latter group of patients is significantly lower and could be missed when the number of alleles considered is relatively small. On the other hand, we observed a significant correlation between the burden of *V617F* allele and the frequency of rs12343867 allele. In *JAK2V617F* mutated patients, with an allele burden lower than 25% the frequency of C allele was 0.333, similar to the *JAK2* wild-type population ( $P=0.331$ ), while a C allele frequency of 0.416 was found in those with greater than 25% *V617F* allele burden ( $P=7.83 \times 10^{-7}$  vs local controls). Furthermore, by dividing *JAK2V617F* mutated patients in quartiles of allele burden, the frequency of C allele increased progressively from 0.333 to 0.500 ( $P<0.0001$ ,  $\chi^2$  test for trend).

In our series, we found 7 *JAK2V617F* wild-type/*MPLW515* mutated patients among 190, in whom the information was available; two were *W515K* and five *W515L*. The frequency of C allele was 0.50, and the difference with local controls was statistically significant ( $P=0.038$ , OR 2.95 (95% CI, 1.01–8.58)). However, the low number of subjects considered precludes any conclusion.

We found no difference regarding hematological parameters, large splenomegaly, presence of constitutional symptoms, categorization according to the IPSS, rate of transformation to leukemia or death according to the rs12343867 genotypes. Also, we found no difference in overall survival in the three rs12343867 genotypes even after stratification of the patients according to their *JAK2V617F* mutational status (Figures 1a–c).

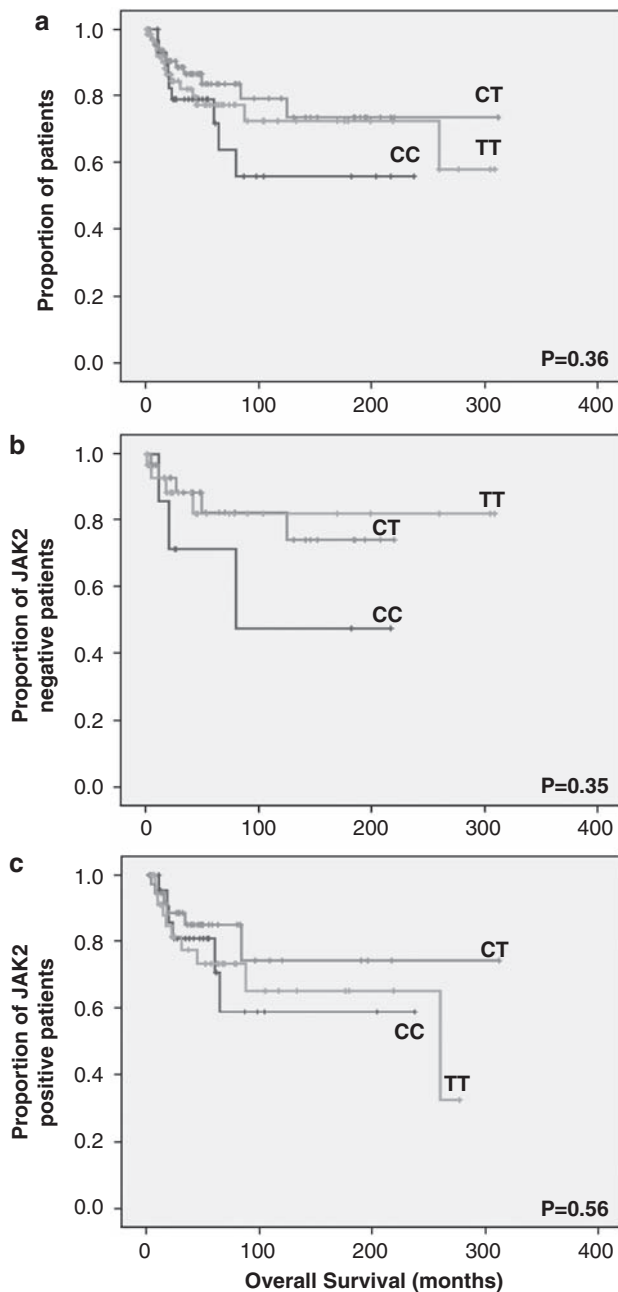
In the original reports, the 46/1 haplotype was found to bear strong association with *JAK2V617F*-positive MPN whereas the association with *V617F*-negative cases was more variable.<sup>9–11</sup> However, in subsequent analysis from the Mayo Clinic in PMF<sup>8</sup> and ET,<sup>13</sup> the frequency of the rs12343867 C-allele (i.e., the 46/1 haplotype) was found significantly increased in both *V617F*-mutated and wild-type patients; similar results have been found in a large series of ET patients without *V617F* mutation, either *MPL* exon 10 mutated or *JAK2/MPL* wild-type.<sup>14</sup> Present data in the largest PMF population analyzed till date confirm the significant association of 46/1 haplotype with PMF, and point to a major contribution of *JAK2V617F*-mutated patients. In line with Tefferi *et al.*,<sup>8</sup> we observed that the C allele was over-represented in patients showing the highest *V617F* allele burden; this underlines the contribution of UPD in the progressively increasing rate of 46/1 haplotype according to *V617F* allele burden.

Association of the rs12343867 genotype with clinical characteristics and disease prognosis was also evaluated. We failed to identify statistically significant correlations between any of the possible genotypes (CC vs CT vs TT) and a number of hematological or clinical variables. In particular, we found no association with the rate of leukemia transformation, categories of risk, and overall survival. These results are at variance with a recent study, where the nullizygosity for the 46/1 haplotype, that is, harboring the rs12343867 TT-allele, was significantly associated with shortened survival, particularly in the group of *JAK2V617F* wild-type patients.<sup>8</sup> There is no obvious explanation for these discrepancies. One possibly meaningful difference relates to the fact that the number of patients who reached the survival end-point in the Mayo Clinic study was greater than that observed in this series (59 vs 21%). Therefore, we surmise that additional studies are required in order to confirm whether the germline configuration of 46/1 haplotype represents a surrogate marker of worse prognosis in PMF patients, independent of IPSS score.

**Table 2** Results of genotyping for the 46/1 tag SNP rs12343867 in relation to the *JAK2V617F* mutational status

|                            | No. of cases | No of C alleles | No. of T alleles | Frequency of C allele | vs local controls     |                  | vs WTCCC controls      |                  |
|----------------------------|--------------|-----------------|------------------|-----------------------|-----------------------|------------------|------------------------|------------------|
|                            |              |                 |                  |                       | P value               | OR (95% CI)      | P value                | OR (95% CI)      |
| Local controls             | 235          | 119             | 351              | 0.253                 | —                     | —                | 0.9817                 | 0.99 (0.80–1.25) |
| WTCCC                      | 1492         | 757             | 2227             | 0.252                 | 0.9817                | 0.99 (0.80–1.25) | —                      | —                |
| All patients               | 202          | 156             | 237              | 0.386                 | $6.37 \times 10^{-6}$ | 1.94 (1.45–2.59) | $1.84 \times 10^{-9}$  | 1.93 (1.55–2.41) |
| <i>JAK2</i> wt             | 69           | 46              | 92               | 0.333                 | 0.063                 | 1.47 (0.98–2.22) | 0.036                  | 1.47 (1.02–2.11) |
| <i>JAK2 V617F</i> -pos     | 133          | 110             | 145              | 0.413                 | $8.29 \times 10^{-7}$ | 2.24 (1.62–3.09) | $7.69 \times 10^{-10}$ | 2.23 (1.72–2.90) |
| <i>V617F</i> allele burden |              |                 |                  |                       |                       |                  |                        |                  |
| <25%                       | 15           | 10              | 20               | 0.333                 | 0.331                 | 1.47 (0.67–3.24) | 0.319                  | 1.47 (0.68–3.15) |
| ≥25%                       | 118          | 98              | 125              | 0.416                 | $7.83 \times 10^{-7}$ | 2.31 (1.65–3.24) | $1.43 \times 10^{-9}$  | 2.30 (1.75–3.04) |
| 1–25%                      | 15           | 10              | 20               | 0.333                 | 0.434                 | 1.47 (0.67–3.24) | 0.319                  | 1.47 (0.68–3.15) |
| 26–50%                     | 52           | 39              | 65               | 0.375                 | 0.012                 | 1.77 (1.13–2.77) | 0.0051                 | 1.76 (1.18–2.65) |
| 51–75%                     | 30           | 25              | 35               | 0.416                 | 0.007                 | 2.11 (1.21–3.66) | 0.004                  | 2.10 (1.25–3.53) |
| 76–100%                    | 36           | 36              | 25               | 0.500                 | $5.14 \times 10^{-8}$ | 4.25 (2.45–7.37) | $3.07 \times 10^{-9}$  | 4.23 (2.52–7.10) |

Abbreviation: WTCCC, Wellcome Trust Case Control Consortium.<sup>9</sup>



**Figure 1** Kaplan-Meier analysis of overall survival in PMF patients. The plots show the overall survival according to the rs12343867 genotype (CC corresponds to homozygosity for the 46/1 haplotype) in all 202 PMF patients (a), in the 69 *JAK2* wild-type patients (b) or in the 133 *JAK2V617F* mutated patients (c).

#### Conflict of interest

The authors declare no conflict of interest.

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## Bone marrow fibrosis and vascular density lack prognostic significance in childhood acute lymphoblastic leukaemia

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Three recent papers by Norén-Nyström *et al.*<sup>1–3</sup> have collectively shown that higher levels of bone marrow fibrosis (measured as reticulin fibrin density (RFD)) and increased vascularity (microvessel density (MVD)) are associated with both relapse and higher minimal residual disease (MRD) in precursor B acute lymphoblastic leukaemia. Other studies have reported marrow fibrosis<sup>4</sup> or increased vascularity<sup>5</sup> in some acute lymphoblastic leukaemia (ALL) patients, but have not found a prognostic significance associated with higher levels.

To resolve this controversy and to determine whether RFD and/or MVD has a clinically useful prognostic role in our patients, we measured fibrosis, RFD and MVD in the 84 patients diagnosed with ALL between 2001–2007 at our institute who had good-quality biopsies and compared them with known sensitive prognostic markers, such as chromosome ploidy and MRD. The patients had been treated according to study 7 (before 2003, 24 patients, 5 relapses) or study 8 (55 patients, 8 relapses) of the Australian and New Zealand Children's Haematology and Oncology Group, or by some other therapy (5 patients). There is currently no difference in leukaemia-free survival between the two Berlin-Frankfurt-Munster (BFM)-based protocols at our centre; therefore, it seemed reasonable to analyse the patients as a group. The 84 patients included 44 males and 40 females who ranged in age from 11 months to 17 years with a median age of 5 years.

New trephine sections were cut and stained for reticulin fibrosis, collagen fibrosis and von Willebrand factor (VWF) immunohistochemistry.<sup>6</sup> A hematoxylin and eosin (H&E) stain was performed to facilitate the determination of cellularity. The bone marrow fibre content was determined by using a grading system (modified Bauermeister scale) that included five grades.<sup>7</sup> The percentages of patients with different grades of fibrosis were grade 0: 2.5%, grade 1: 33.3%, grade 2: 37%, grade 3: 19.7% and grade 4 (collagen +): 7.5%. RFD was quantified as previously described by Norén-Nyström *et al.*<sup>1</sup> Briefly, RFD was calculated using a 121-point eye piece graticule at  $\times 400$  magnification. The intersections of reticulin fibres with the crossing points of the graticule were counted in 10 different areas in the section. RFD was defined as volume of reticulin fibres per volume reference tissue. Fibrosis grade showed a strong positive correlation with RFD ( $P < 0.001$ ).

In keeping with earlier reports,<sup>3</sup> RFD was significantly higher in precursor B acute lymphoblastic leukaemia cases than in T-ALL (Table 1) and there was a negative correlation between RFD and white blood cell count at diagnosis, with high RFD being associated with lower initial white blood cell count: mean  $10.7$  vs  $35.3 \times 10^9/l$  for lower RFD values. Similarly, there was a negative correlation between RFD and the percentage of

blast cells in the peripheral blood (Spearman's correlation  $r = -0.278$ ,  $P = 0.018$ ). A higher RFD was also correlated with a higher cellularity in the bone marrow ( $r = 0.292$ ,  $P = 0.007$ ). This could imply that some types of leukaemia create a higher degree of fibrosis than others and that these blasts are then 'trapped' in the marrow.

We found no difference between the RFD values of NCI (National Cancer Institute risk score) high- or low-risk patients, or between patients who relapsed vs those who continue in complete remission (Table 1). The most important prognostic factor in our patients was MRD. PCR-based MRD analysis was performed on day 33 and 79 in study 8 patients, that is, at the

**Table 1** Reticulin fibre density, microvascular density and prognostic factors

| Clinical characteristics                  | RFD |      |       | MVD |      |    |
|---|-----|------|-------|-----|------|----|
|   | n   | Mean | P     | n   | Mean | P  |
| <i>Immunophenotype</i>                    |     |      |       |     |      |    |
| B-precursor                               | 77  | 10.1 | 0.041 | 75  | 4.7  | NS |
| T-cell                                    | 7   | 5.2  |       | 4   | 3.2  |    |
| <i>WBC count at diagnosis</i>             |     |      |       |     |      |    |
| $< 26.2 \times 10^9/l$ (Mean value)       | 63  | 10.7 | 0.045 | 61  | 4.7  | NS |
| $> 26.2 \times 10^9/l$                    | 21  | 6.5  |       | 18  | 4.4  |    |
| <i>NCI risk group<sup>a</sup></i>         |     |      |       |     |      |    |
| Standard risk                             | 57  | 9.4  | NS    | 55  | 4.6  | NS |
| High risk                                 | 20  | 11.9 |       | 20  | 4.8  |    |
| <i>Disease status<sup>a</sup></i>         |     |      |       |     |      |    |
| Remission                                 | 64  | 10.5 | NS    | 63  | 4.6  | NS |
| Relapsed                                  | 13  | 7.7  |       | 12  | 4.9  |    |
| <i>Cytogenetic groups<sup>a</sup></i>     |     |      |       |     |      |    |
| Normal                                    | 9   | 8.0  | 0.002 | 9   | 4.7  | NS |
| t(12;21)                                  | 19  | 5.9  |       | 18  | 4.2  |    |
| Trisomies 4,10,17                         | 14  | 17.0 |       | 13  | 5.0  |    |
| t(9;22)                                   | 2   | 7.3  |       | 2   | 4.9  |    |
| Other abnormalities                       | 23  | 13.6 |       | 23  | 4.7  |    |
| <i>Ploidy<sup>a</sup></i>                 |     |      |       |     |      |    |
| Hypodiploid                               | 6   | 3.9  | 0.006 | 6   | 4.7  | NS |
| Diploid                                   | 35  | 8.0  |       | 34  | 4.3  |    |
| Hyperdiploid (47–50)                      | 13  | 10.3 |       | 13  | 5.1  |    |
| High-hyperdiploid (51–61)                 | 22  | 15.3 |       | 21  | 4.7  |    |
| <i>MRD groups (day 33–35)<sup>a</sup></i> |     |      |       |     |      |    |
| $< 10^{-3}$                               | 46  | 11.0 | NS    | 45  | 4.7  | NS |
| $> 10^{-3}$                               | 5   | 11.6 |       | 5   | 4.0  |    |

Abbreviations: MRD, minimal residual disease; MVD, microvessel density; NS, not significant ( $> 0.05$ ); RFD, reticulin fibrin density; WBC, white blood cell.

<sup>a</sup>Prognostic grouping for precursor-B ALL patients only ( $n = 77$ ).