

Table 1. Maternal demographic data and body iron status in the study and control groups.

	Study group (n=29)	Control group (n=58)	P value
Age (years)	29.48±6.20	29.29±5.35	NS
Weight (kg)			
Booking	55.79±7.87	57.72±8.00	NS
Pre-delivery	70.55±10.71	70.46±11.06	NS
Height (cm)	158.76±7.17	161.27±6.12	NS
BMI (kg/m ²)			
Booking	22.10±2.62	22.20±2.61	NS
Third trimester	27.96±3.73	27.04±3.93	NS
Hb (g/dL)			
Booking	10.01±1.70	11.78±1.05	0.000
Third trimester	10.21±1.22	11.74±0.92	0.000
Parity	2.31±2.05	2.10±1.41	NS
Ferritin (ng/mL)	53 [10 - 261]	10 [4 - 121]	0.0000
Iron (μmol/L)	19.37±10.62	10.37±5.65	0.0060
Transferrin (μmol/L)	35.04±8.81	45.17±8.71	0.0011
Transferrin saturation (%)	30.54±23.95	12.14±7.39	0.0006

and GDM.⁷ A higher incidence of GDM was found in patients suffering from α thalassemia trait than in a control group.⁷ Lao *et al.* suggest that iron excess might not be the explanation of the high prevalence of GDM in women with α thalassemia trait, because pancreatic alpha cell overactivity with increased glucagon response has also been shown in thalassaemic patients with impaired glucose tolerance.⁷ However, two recent new prospective case-control studies confirmed the association between stores of iron and the incidence of diabetes.^{8,9} These studies showed that higher iron stores are associated with an increased risk of type 2 diabetes in healthy populations, independently of known diabetes risk factors. Our results confirmed this hypothesis. In our study the median ferritin concentration and the mean transferrin saturation in the study group were significantly higher than in the control group. In the study group, the median ferritin values were statistically different in patients with GDM ($p=0.004$). Despite the higher incidence of GDM in the study group, there were no differences in the incidence of obstetrical complications or perinatal outcome.

Our results support the hypothesized association between heterozygous forms of hemoglobinopathies with higher iron stores and the impairment of carbohydrate regulation.¹⁰ The increased risk of GDM could have a substantial impact on preconception counseling and the antenatal management of patients with heterozygous forms of hemoglobinopathies. Further studies are needed to clarify the origin of the impaired glucose regulation in patients heterozygous for hemoglobinopathies. It would be useful to apply higher iron stores as a diabetes risk factor and to compare the incidence of diabetes mellitus between two groups with heterozygous forms of hemoglobinopathies with high and low ferritin concentrations and transferrin saturation. If this identifies an increased incidence of GDM in patients with elevated ferritin con-

centrations and transferrin saturation, one may suspect that the higher body iron store is the main factor in impaired glucose regulation.

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Chronic Myeloid Leukemia

e6a2 BCR/ABL1 fusion with cryptic der(9)t(9;22) deletions in a patient with chronic myeloid leukemia

This is the first report of e6a2 and e1a2 BCR/ABL1 positive chronic myeloid leukemia (CML) with cryptic deletions of the 5'ABL1 and 3'BCR in separate clones which differ in genomic regions of the deleted der(9). Both deletions were detected throughout monitoring. Imatinib mesylate stabilized this CML with rare genetic aberrations for a relatively long time.

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A 37-year old man, was admitted to the Haematology Unit, Perugia General Hospital because of persistent fever, scotoma, and sternal pain. A peripheral blood count showed anemia (hemoglobin 10.9 g/dL), and leukocytosis (WBC $38 \times 10^9/L$). The blood film showed

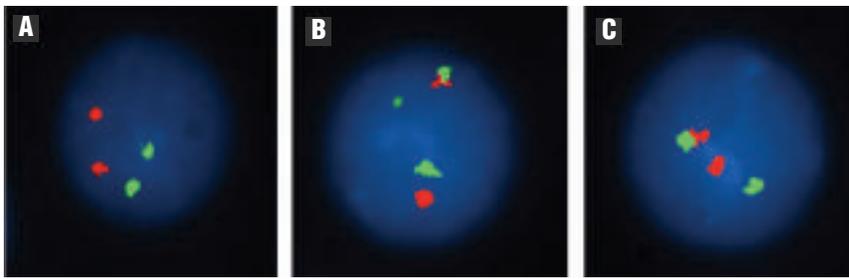


Figure 1. FISH experiments with the LSI BCR/ABL1 dual color dual fusion probe (Vysis). **A)** a normal nucleus showing two green and two red signals; **B)** one red, two green and one fusion signal in a BCR/ABL1 positive nuclei carrying a deletion of the 5'ABL1; **C)** one red, one green and one fusion signal in a BCR/ABL1 positive nuclei with a deletion of both the 5'ABL1 and the 3'BCR.

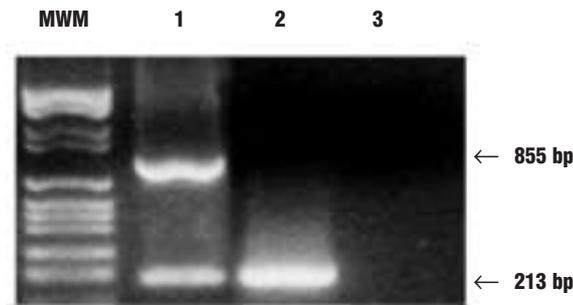


Figure 2. Ethidium bromide-stained agarose gel. MWM, molecular weight marker VI (Roche, Applied Science). Lane 1: RNA from the patient with e1a2 and e6a2 BCR-ABL transcripts. Lane 2: e1a2 positive control. Lane 3: negative control.

mature and immature myeloid cells with marked increases in myelocytes and mature neutrophils. Neutrophil alkaline phosphatase was normal. Chronic phase chronic myeloid leukemia (CML) was diagnosed on bone marrow biopsy and aspirate. Imatinib mesylate at 400 mg/daily was well tolerated.

The karyotype on G-banded metaphases at diagnosis was: 46,XY,t(9;22)(q34;q11)[10/16]/46,XY[6/16]. At diagnosis fluorescence *in situ* hybridization (FISH) performed with the LSI BCR/ABL dual color dual fusion translocation probe (Vysis, Abbott Laboratories, Olympus, Milan, Italy) gave two red and two green signals (2R2G) in 20% of nuclei as expected in normal cells; one red, two green, and one fusion signal (1R2G1F) in 50% of nuclei as expected in cells bearing the BCR/ABL1 rearrangement and a deletion of the 5'ABL1; one red, one green, and one fusion signal (1R1G1F) in 30% of nuclei, as expected in cells bearing the BCR/ABL1 fusion and deletion of the 5'ABL1 and the 3'BCR¹ (Figure 1).

FISH detected BCR/ABL1 in 25% of nuclei (1R2G1F in 15%; 1R1G1F in 10%) after 1 month's therapy, in <1% after 8 months, in 45% (1R2G1F in 27%; 1R1G1F in 18%) after 13 months and in 14% (1R2G1F in 9%; 1R1G1F in 5%) after 20 months. This pattern shows that neither clone had a selective advantage.

At diagnosis, experiments with RP11 clones mapping at 9q34, centromeric to the 5' end of ABL1 (centromere-409K20-138E2-202H3-88G17-618A20-17L7-telomere) gave one hybridization signal in 50-60% of nuclei, confirming deletion of DNA sequences at the 5'ABL1 which extends for at least 1 megabase. DNA clones for the 22q11 band at the 3'BCR, RP11-143F12 and RP11-71G19, gave two hybridization signals in 95% and 96% of nuclei and the third clone, RP11-248J22, gave one signal in 30% of nuclei indicating a deletion in a BCR/ABL1 positive subclone, which extends for 145kb.

Reverse transcription polymerase chain reaction (PCR) was performed according to BIOMED¹ to investigate the BCR/ABL1 rearrangements.² Nested PCR was done with forward primer BCR e1-C (5'-CAGAACTCGCAA-CAGTCCTTC-3') and reverse primer ABL a2-D (5'-CAGACCCTGAGGCTCAAAGTC 3') which detected two different bands (Figure 2). Sequence analysis of the PCR product showed two fusion transcripts, e1a2 BCR/ABL1 and e6a2 BCR/ABL1. It was beyond the scope of this paper to assess whether the two transcripts were due to alternative splicing mechanisms or to different breakpoints on the Philadelphia chromosome as well as on the derivative chromosome 9.

The rare breakpoint on the BCR gene (e6a2), encoding for a protein of 185kD, has been described in one patient during progression of chronic myelomonocytic leukemia (CMML)³ and in four patients with CML,^{4,5} as well as in a T-cell acute lymphoblastic leukemia cell line (MHH-TALL1).⁶ Male predominance was observed (4/5), but no other specific clinical feature has emerged. The fusion was associated with an aggressive course with early death in three out of the four patients for whom follow-up information is available.⁴ The e6a2 BCR breakpoint occurs in the middle of the (GEF)/dbl-like, which is partially retained in BCR/ABL1 fusion protein. The full domain mediates interactions with several G proteins that are involved in signal transduction, and the missing part seems to enhance the oncogenic/leukemogenic activity of the chimeric protein derived from the e6a2 BCR/ABL1 fusion.⁷ Despite this stimulating hypothesis, the prognostic impact of e6a2 BCR/ABL1 cannot really be assessed because the patients received different treatments. Two patients were treated with imatinib mesylate therapy. One man who obtained only a reduction in the WBC died of infectious complications after 42 days.⁴ In the second patient, real-time PCR showed a reduction in the BCR/ABL1 transcripts after three months of therapy.³

Loss of a tumor suppressor gene or genes could explain the poor prognosis associated with der(9)t(9;22) deletions after treatment with interferon- α and/or chemotherapy. The prognostic impact in patients treated with imatinib mesylate remains to be defined. Huntly *et al.*⁸ suggested that patients with der(9) deletions had a lower response rate than those without, whereas in a multivariate analysis by Quintas-Cardama *et al.* der(9) deletion had no significant impact on response, survival, or response duration.⁹ To date, this is the first observation of e6a2 BCR/ABL1 positive CML with separate clones which differ in the genomic regions of the deleted der(9)t(9;22). In response to imatinib mesylate disease stabilized in our patient over a 21 month follow-up, suggesting the prognosis of e6a2 positive CML with accompanying deletion may no longer be so poor.

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Multiple Myeloma

Baseline Tc^{99m}-MIBI scanning predicts survival in multiple myeloma and helps to differentiate this disease from monoclonal gammopathy of unknown significance

We performed baseline Tc^{99m}-MIBI scanning in 43 patients with multiple myeloma (MM) and in 31 with monoclonal gammopathy of unknown significance (MGUS) patients. We identified two groups of MM patients whose actuarial survival correlated with low or high MIBI scores. MGUS patients had normal or very low scores.

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The ability of Tc^{99m}-MIBI scanning to detect bone marrow involvement in MM has been known since 1996¹ but is scarcely used. The aim of this prospective study was to determine whether Tc^{99m}-MIBI uptake can be used as a prognostic factor in MM and whether it can differentiate between MM and MGUS.

We studied 43 MM and 31 MGUS patients (33 men and 41 women), aged between 43 and 83 years (mean 69±7.9). Tc^{99m}-MIBI scanning and plain X-rays were performed before any therapy. The median follow-up was 57 and 44 months in the MM and MGUS groups, respectively. The Tc^{99m}-MIBI scans were scored for intensity and pattern according to Pace;² there were four possible intensity levels (normal, +, ++ and +++) and four possible patterns (normal (N), focal (F), diffuse (D) and focal + diffuse (F+D)). Two specialists in nuclear medicine, blind to the patients' diagnosis, evaluated the patterns and scores. We also received the percentage of bone marrow cells and biochemical data related to prognosis: β₂-microglobulin (0.7-1.8 µg/mL), C-reactive protein (0.01-0.5 mg/dL), albumin (3.4-4.8 g/dL), creatinine (0.5-1.3 mg/dL) and lactate dehydrogenase (80-480 U/L). The units and normal ranges in our laboratory are given in brackets. All symptomatic patients with active disease but two received VBCMP/ VBAD as first line therapy. Radiotherapy and/or autologous stem cell transplantation were applied when appropriate.

The patients were categorized into groups according to Tc^{99m}-MIBI uptake and MIBI pattern and the differences between groups were analyzed with the Kruskal-Wallis test and stepwise multiple regression. We used the Kaplan-Meier method for survival analysis, and log rank (Mantel-Cox), Breslow-Gehan-Wilcoxon, Tarone-Ware, Peto-Peto-Wilcoxon and Harrington-Fleming tests to investigate differences between groups. In cases of a *p* value <0.05 in any test we assumed a difference in survival and the significance was checked with the post-hoc Bonferroni-Dunn test. MM and MGUS groups were analyzed separately and together. Table 1 shows the variables correlated with MIBI uptake and pattern with the Kruskal-Wallis test (A), and with multiple stepwise regression (B). Only β₂-microglobulin, C-reactive-protein and lactate dehydrogenase were selected by multiple regression in MM patients. The percentage of bone marrow plasma cells was also selected if all the patients were considered together. Twenty-eight of the 31 MGUS patients had negative MIBI scores. Of the three cases with positive scores, two had a very low intensity (+) diffuse pattern and one a very low intensity (+) focal pattern