

Chronic neutrophilic leukemia with an associated V617F JAK2 tyrosine kinase mutation

Chronic neutrophilic leukemia (CNL) is a rare disease and can cause considerable diagnostic difficulty. Although the V617F JAK2 mutation has been described by several groups to be associated with classical myeloproliferative disorders (MPD), this same mutation has been detected with a low incidence in atypical MPD, such as CNL. Here we report the presence of the V617F mutation in a CNL patient, who is unusual for having survived for more than 96 months, with little disease progression. It remains to be established what role this mutation, which gives cells a proliferative advantage, might play in the pathogenesis and prognosis of rare atypical MPD.

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Chronic neutrophilic leukemia (CNL) is a rare disease categorized under the World Health Organization (WHO) classification¹ as a chronic myeloproliferative disease. In essence it is a diagnosis of exclusion, in a patient presenting with neutrophilia and splenomegaly with no evidence of chronic myeloid leukemia or a reactive neutrophilia. Diagnostic difficulties are not uncommon and it is likely that many previous cases considered to be CNL were misclassified. The stated figures for prognosis vary widely. Reilly² performed a survival analysis of 33 cases of patients felt to have 'true chronic neutrophilic leukemia' and suggested an overall median survival of 30 months and a 5-year survival of 28%. Treatment approaches to individuals with this disorder appear to be heterogeneous.

It is unusual for CNL individuals to survive for long, although it has been described,³ and we have one such individual attending our clinic. This patient presented at the age of 61 years with a 2-month history of fatigue and an influenza-like illness. Examination revealed only moderate hepatosplenomegaly. Hematologic findings were a hemoglobin of 14.8 g/dL, total white cell count of $54 \times 10^9/L$ (with neutrophils $48 \times 10^9/L$), and a platelet count of $316 \times 10^9/L$. Examination of blood films revealed a large population of segmented neutrophils with few immature granulocytes and no circulating myeloblasts. Bone marrow aspirate demonstrated a hypercellular marrow secondary to granulocytic proliferation with neither overt dysplastic features nor an increase in the blast percentage. The trephine biopsy did not indicate any fibrosis, increase in plasma cells or megakaryocytic proliferation. Conventional cytogenetic analysis demonstrated a normal male karyotype and the patient was negative for the BCR/ABL transcript by polymerase chain reaction analysis. Since no cause was established for a secondary neutrophilia he fulfilled the WHO⁶ diagnostic criteria for CNL and the management strategy at this stage was one of observation only. Recently published data⁴⁻⁸ have shown the acquired V617F Janus Kinase (JAK) 2 mutation is widespread amongst classical myeloproliferative (MPD) disorders, albeit in a heterogeneous manner. JAK2 is a member of the JAK non-receptor protein tyrosine kinase family and this mutation is located in the highly con-

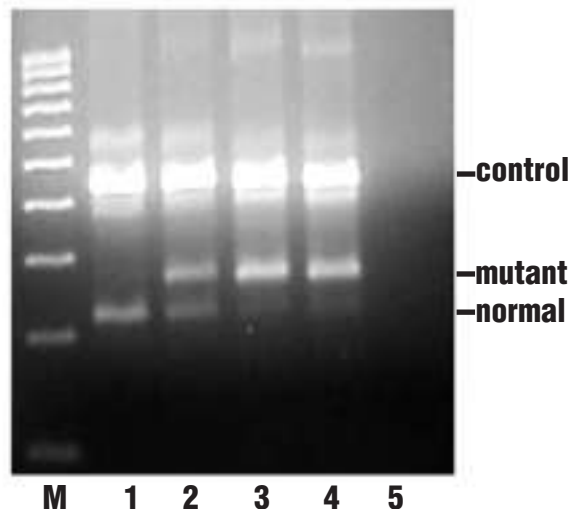


Figure 1. ARMS assay for the detection of the JAK2 2343 G→T base change. The wild type allele JAK2 allele and the mutant V617F allele are indicated by the presence of 229bp and 279bp bands. The PCR amplification control band is located at 463bp. The CNL patient, (Lane 4) strongly exhibited the 279bp band and was thus scored as homozygous. Lane 1: homozygous wild type control; Lane 2: heterozygous control; Lane 3: 2343 G→T homozygous control; Lane 5: PCR negative control; M: 1kb DNA ladder (ABgene, Epsom, Surrey, UK).

served JAK homology 2 (JH2) pseudokinase region. Loss of valine at position 617 in this region would result in constitutive tyrosine kinase phosphorylation activity by disruption of the intrinsic auto-inhibitory activity of the JH2 domain.⁹ Consequently, cells possessing the V617F JAK2 mutation would exhibit cytokine hypersensitivity and possess a proliferative advantage.

Intriguingly, the V617F JAK2 mutation has also been described in both atypical MPD and myelodysplastic syndromes,^{5,10} albeit at a low the incidence. Steensma *et al.*¹⁰ analyzed six CNL patients and only one was found to possess a homozygous V617F JAK2 mutation. Of particular interest, this patient also had a history of B-cell lymphoma. This was not the case in our patient. Another study⁵ indicated that 20% of atypical or unclassified MPD were also positive for the V617F JAK2 mutation.

We wanted to establish whether our patient possessed the V617F JAK2 mutation we, therefore collected a blood sample, after gaining informed consent according to the Declaration of Helsinki. The sample was fractionated and genomic DNA isolated using a Nucleon BACC 1 DNA extraction kit (Nucleon Biosciences, Manchester, UK). The 2343 'G to T' JAK2 mutation (V617F) was by detected by an amplification refractory mutation system (ARMS) as described elsewhere⁴ except Thermo-Start DNA Polymerase (ABene, Epsom, Surrey) was used. ARMS PCR allows the detection of both the wild type JAK2 allele and the mutant V617F allele, as indicated by the presence of 229bp and 279bp bands on a 3% agarose gel (Figure 1). Our patient proved homozygous for the mutation in the granulocytes and macrophages. Sequencing data is shown in Figure 2.

At present, our patient remains symptomatically well and is maintained on hydroxycarbamide (also known as hydroxyurea) 500 mg daily which was commenced because his absolute neutrophil count continued to rise

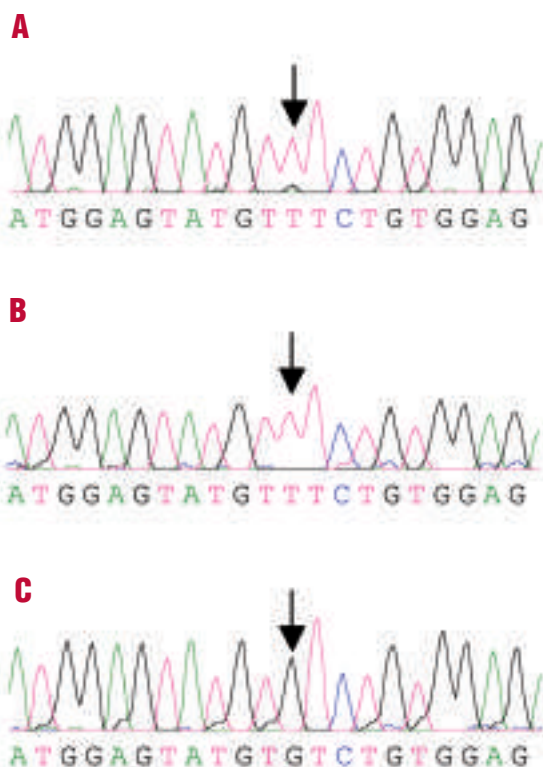


Figure 2. Detection of the G1849T mutation in the JAK2 gene. Sequencing of exon 12 of the JAK2 gene detected a base change of G to T at nucleotide 1849 in unfractionated blood (B), macrophages/granulocytes (C) as compared to wild type sequence (A). Bases are as follows: G: black; A: green; T: red; C: blue.

above $100 \times 10^9/L$. His neutrophil count now averages around $30\text{--}45 \times 10^9/L$. A trial of imatinib mesylate, 400 mg daily, produced no hematologic response.

As mentioned previously, this patient with CNL has had an unusually long survival (now 96+ months). Interestingly, it appears that in other MPD the *V617F* *JAK2* mutation is associated with a longer median disease duration.⁷

An analysis of all cohorts of patients with CNL will enable an evaluation of the prevalence and prognostic relevance of the *V617F* *JAK2* mutation in this disease.

However, it remains to be established how the presence of a *JAK* mutation, which provides a proliferative advantage, affects the pathogenesis of an atypical MPD.

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