CASE REPORT

CHRONIC EOSINOPHILIC LEUKAEMIA ASSOCIATED WITH *JAK2* EXON 13 INSERTION/DELETION MUTATIONS

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Short title: Chronic eosinophilic leukaemia associated with JAK2 Exon 13 insertion/deletion mutations

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Abstract

Chronic eosinophilic leukaemia, not otherwise specified (CEL, NOS) is a diagnosis of exclusion, made in cases in which there is clonal eosinophilia but an absence of genetic aberrations that define other disease subtypes. There is a need for further characterisation of these cases in order to inform risk stratification and management. The importance of *JAK2* mutations in myeloproliferative neoplasms (MPN) as a whole is well-established, although their role specifically in eosinophilic disorders is less clear, with only a minority of cases demonstrating *JAK2* abnormalities. Here we report two cases with an exon 13 insertion-deletion (indel) mutation in *JAK2*; one with CEL-NOS, and the second with an unspecified eosinophilic disorder. *JAK2* indels were not detected in a screen of suspected MPN cases (n=592) without eosinophilia that tested negative for common MPN driver mutations. Our findings thus provide further evidence for a specific association between this rare mutation and clonal eosinophilic disorders.

Introduction

Eosinophilia is defined as an increase in the number of eosinophils in the peripheral blood above the upper limit of normal; generally regarded as $0.5x10^9$/L; whilst the term 'hypereosinophilia' is used to describe a persistent eosinophilia of $>1.5x10^9$/L [1]. Meanwhile, the 'hypereosinophilic syndrome' (HES) describes cases in which hypereosinophilia is accompanied by eosinophil-mediated end-organ damage [2].

The eosinophilias may be broadly categorised as primary (clonal), secondary to other aetiologies (reactive), or idiopathic [3]. Within primary eosinophilias, the World Health Organization (WHO) now consider those associated with rearrangements of *PDGFRA*, *PDGFRB* or *FGFR1*; or the *PCM1-JAK2* rearrangement; as specific disease entities [4]. Alternatively, some cases will meet the criteria for other haematological neoplasms and can be categorised accordingly. Chronic Eosinophil Leukaemia, not otherwise specified (CEL, NOS) is diagnosed in cases of hypereosinophilia with an increase of blast cells in blood or marrow and/or evidence of clonality by a molecular or cytogenetic abnormality other than the specific disease-defining rearrangements defined above, which do not meet the diagnostic criteria for any other neoplasm [5].

In contrast to the molecularly defined group of eosinophilias, in which the recognition of the role of fusion tyrosine kinase genes has facilitated more precise prognostication and management strategies, there remains a paucity of evidence regarding CEL, NOS. Whilst patients with this rare diagnosis have been found to have poor outcomes overall [6], further investigation of this group of patients may reveal subsets with differing prognoses and responses to treatment.

This report describes two cases with eosinophilic neoplasms with a specific insertion-deletion (indel) mutation within exon 13 of *JAK2*, previously reported in only six other cases [7,8].

Case 1

An 82-year old female was referred to Haematology clinic in 2018 for investigation of eosinophilia. She had a background of polymyalgia rheumatica, for which she was on long-term prednisolone (11mg daily at the time of referral). At the time of assessment, her main symptom was generalised fatigue. She was mobilising with a stick due to hip pain, but examination otherwise was relatively unremarkable, with no organomegaly or lymphadenopathy palpable. WHO performance score was 1.

The full blood count (shown in table 1) revealed a raised eosinophil count of $3.0x10^9$/L, as well as a mildly elevated haemoglobin, haematocrit, and neutrophil count. Tests of renal and liver function, lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), and mast cell tryptase, were all within normal ranges. Anti-neutrophil cytoplasmic antibody (ANCA) and anti-nuclear antibody screens were negative.

Computerised tomography scan of chest, abdomen and pelvis revealed pancolonic diverticulosis but no other significant abnormalities of the abdominal viscera, no lymphadenopathy, and no thoracic disease. Spirometry and transthoracic echocardiography demonstrated normal physiology with no evidence of end-organ damage.

Bone marrow examination (BME) (shown in Fig. 1) demonstrated increased eosinophils and a slight expansion of the erythroid lineage. Trilineage haematopoiesis was preserved, with no significant dysplasia of any lineage. The overall cellularity and architecture were normal, with no fibrosis seen. There was no evidence of lymphoma or other malignant infiltration, and no excess of blasts (<1%).

Immunophenotyping showed no clinically significant clonal lymphocyte population. The karyotype was 46,XX. She was negative for *FIP1L1-PDGFRA* and *BCR-ABL1* rearrangements by reverse transcriptase polymerase chain reaction (PCR), and also *KIT* D816V mutation by digital PCR on peripheral blood. The only abnormality identified by targeted next generation sequencing (NGS) analysis was an indel mutation within exon 13 of *JAK2* (NM_004972:c.1747_1756delinsT [p.Leu583_Ala586delisnSer]), at a variant allele frequency of 15%.

Over the subsequent 26 months, her eosinophil count gradually increased, eventually reaching $10.1x10^9$/L, despite continuing on a fluctuating maintenance dose (5-10mg daily) of prednisolone. Other counts are shown in table 1. LDH had increased to 373 IU/L. Serum erythropoietin was low (1.3mU/ml). Clinical assessment revealed a gradual worsening of fatigue and decline in functional status but no specific evidence of end-organ damage. Echocardiogram demonstrated normal cardiac function and abdominal ultrasound showed no organomegaly.

A decision was made to start treatment. An application for compassionate use of ruxolitinib, made to NHS England, was unsuccessful. She was instead started on PEGylated interferon alfa (PEG-IFN), and subsequently imatinib (up to a dose of 400mg daily), but failed to demonstrate a clinical or haematological response to either agent. At the time of analysis, she was alive and without overt end-organ damage at 36 months from presentation.

Case 2

An 87-year old female with a background of chronic obstructive pulmonary disease was referred to Haematology clinic in 2020 with a gradually rising eosinophil count over at least seven years (shown in table 2).

At the time of assessment her main symptoms were related to bilateral leg swelling and recent episodes of cellulitis (deep vein thrombosis had been excluded by ultrasound). Her eosinophil count was $7.9x10^9$/L and she had a mild polycythaemia (haemoglobin 156g/l). Blood film confirmed eosinophilia but showed no other morphological abnormalities of the erythroid or myeloid lineages. LDH was raised at 674 IU/L. ANCA screen was negative.

Peripheral blood tests for *BCR-ABL1*, *FIP1L1-PDGFRA*, and *KIT* D816V mutation were negative, but a *JAK2* exon 13 indel mutation (NM_004972:c.1748_1757delinsA [p.Leu583_Ala586delinsGln]) was detected by Sanger sequencing. No previous samples were available to determine whether this mutation was detectable earlier in the disease course.

Around 2 months after her initial assessment, she died unexpectedly due to a cerebral haemorrhage following a fall at home, precluding further investigations. Consequently, a definitive haematological diagnosis was not established.

Discussion

The *JAK2* gene encodes a tyrosine kinase involved in intracellular signalling through the JAK-STAT pathway [9]. Mutations of *JAK2* are well-established as driver mutations in myeloproliferative neoplasms (MPN) [10]. By far the most common is the V617F substitution (*JAK2*^{V617F}) within exon 14, seen in almost all of cases of polycythaemia vera (PV) and 50-60% of cases of essential thrombocythaemia and primary myelofibrosis [11], but also in up to 4% of cases with hypereosinophilia [12]. The V617F mutation results in constitutive activation of *JAK2* by inducing a conformational change in its pseudokinase domain, thus abrogating the autoinhibitory function of this domain [13]. A number of other nearby mutations within exons 12-15 have been identified in patients with MPN, and have been speculated to confer a similar effect [14].

Although *JAK2*^{V617F} is uncommon in primary eosinophilic disorders [15], the role of JAK2 dysregulation through other mechanisms in these conditions has gained recognition in recent years. Genetic rearrangements giving rise to the *JAK2* fusion genes *PCM1-JAK2, ETV6-JAK2* and *BCR-JAK2* have been implicated in myeloid neoplasms with eosinophilia [4]; whilst it has also been shown that JAK2 is a key downstream mediator of proliferation in cells with the *FIP1L1-PDGFRA* rearrangement; the most common genetic aberration seen in cases of chronic eosinophilic leukaemia (CEL) [16].

The cases presented in our report were found to have exon 13 insertion/deletion mutations within *JAK2* (*JAK2*^{ex13InDel}). To our knowledge, two other groups have identified patients with eosinophilic disorders associated with these mutations, in only six patients. Pohlkamp et al. detected *JAK2*^{ex13InDel} in two of 52 patients with 'hypereosinophilia of undetermined significance' [7], whilst Patel et al. presented four separate cases with *JAK2*^{ex13InDel} [8]. The c.1747_1756delinsT mutation involving insertion of serine, seen in our first case, was also reported in two patients by Patel et al. and in one patient by Pohlkamp et al, whilst the c.1748_1757delinsA mutation, involving insertion of glutamine, was reported in one case from each study.

Patel et al. went on to investigate the in vitro effects of *JAK2*^{ex13InDel}, using cell lines transfected with retroviral constructs for its expression [8]. They demonstrated that cells expressing *JAK2*^{ex13InDel} exhibited phenotypic differences from those expressing *JAK2*^{V617F}, with their results suggesting that *JAK2*^{ex13InDel} may promote differentiation towards the eosinophil lineage through cytokine-independent IL-5/STAT5 signalling.

Despite this functional evidence supporting a direct role for *JAK2*^{ex13InDel} in promoting eosinophil expansion, it remains unclear if the association between this abnormality and eosinophilia is due to ascertainment bias. To address this, we screened 592 cases of suspected MPN for *JAK2*^{ex13InDel} using our targeted NGS assay with a limit of detection of approximately 1-2%. All cases were referred for routine investigation and tested negative for *JAK2* (V617F and exon 12), *CALR* and *MPL* driver mutations. None tested positive for a *JAK2*^{ex13InDel}, supporting the hypothesis that this abnormality is a specific driver of eosinophilia.

A number of studies have sought to identify the range of genetic abnormalities seen in CEL, NOS; as well as in patients previously categorised as idiopathic hypereosinophilia (IHE). One recent analysis of 17 patients with CEL, NOS identified seven different categories of karyotypic abnormality (most commonly trisomy 8) and mutations in eight different genes (*ASXL1*, *IDH1*, *TP53*, *SRSF2*, *SH2B3*, *STAT5B*, *KDM6A*, and *NF1*), but not *JAK2* [17]. Meanwhile, three studies investigating IHE populations found mutations within genes involving DNA methylation and chromatin modification, such as *DNMT3A, TET2,* and *ASXL1*, to be amongst the most prevalent [7,18,19], whilst another found *NOTCH1* to be the most frequently mutated gene [20]. Taking just these four studies into account, *JAK2* mutations were seen in just 6 of 57 (11%) cases of mutation-positive IHE, including the two patients with $JAK2^{ex13InDel}$ reported by Pohlkamp at al., plus a patient with $JAK2^{V617F}$ reported in the same study [7], as well as other patients with unspecified *JAK2* mutations. Whilst the cases described in our report provide further evidence for the association of a specific *JAK2* exon13 mutation, $JAK2^{ex13InDel}$, with an eosinophilic phenotype, clearly $JAK2^{ex13InDel}$ remains an uncommon finding amongst the wide range of abnormalities seen in patients with CEL, NOS or IHE.

Patel et al. reported that two of their four patients with $JAK2^{ex13InDel}$-associated CEL also met the WHO diagnostic criteria for PV [8]. However, we note that these patients were judged to meet the 'major criteria' for PV on the grounds that they had i) a raised haematocrit, and ii) a *JAK2* mutation (which assumes that $JAK2^{ex13InDel}$ can be considered equivalent to the $JAK2^{V617F}$ or exon 12 mutations specified by the WHO); rather than based on the BME findings. Our first case, considered in the same way, would also meet the diagnostic criteria for PV; although her polycythaemia was mild and intermittent, and her BME findings were not typical for PV. We would argue that this patient, according to the current WHO criteria, would therefore be most appropriately classified as CEL, NOS. Our second case could not be fully investigated; but is likely to have been classified in the same way, given that her polycythaemia was at a similar level, and her peripheral blood investigations were not suggestive of any other diagnosis. However, our findings support the suggestion by Patel et al. that $JAK2^{ex13InDel}$ appears to cause a unique mixed phenotype featuring both hypereosinophilia and polycythaemia, albeit possibly with a milder degree of polycythaemia than is typically seen with $JAK2^{V617F}$ or exon 12 mutation.

The survival of our patients (considered from the first timepoint at which the eosinophil count was $\geq 1.5 \times 10^9$/L) appears superior to that of the CEL, NOS populations previously studied, in whom median survival has been reported to be in the region of 14-22 months [6,17,18]. We observed gradual progression of eosinophilia over several years in both cases, with our first case also remaining free of overt end-organ damage at the time of analysis; although we note that this is in contrast to the patient presented by Patel et al. [8].

Whilst there is currently no standard of care for patients with CEL, NOS, responses have been reported with treatments including interferon-alfa, hydroxycarbamide, and imatinib [21]. Alternatively, some authors recommend intensive chemotherapy followed by allogeneic stem cell transplantation in selected cases [3]. Amongst the documented cases with $JAK2^{ex13InDel}$, one patient demonstrated a response to ruxolitinib, whilst another failed to achieve a reduction in eosinophil count with PEG-IFN [8]. Our first case also failed to respond to PEG-IFN, as well as imatinib. It may be that her ongoing prednisolone treatment (originally started for polymyalgia rheumatica) provided some degree of disease

control. Further investigation is required to clarify the optimal management in patients with JAK2^{ex13InDel}, as well as CEL, NOS as a whole.

STATEMENTS

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Statement of Ethics

Subjects, or their next of kin, have given their written informed consent for the publication of their case.

Conflict of Interest Statement

No conflicts of interest are declared.

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Author Contributions

Nicholas Lafferty: Main author of the manuscript.

Matthew Salmon: Laboratory analysis of samples from two cases; screen of suspected MPN cases using targeted NGS assay.

Nicholas C.P. Cross: Laboratory analysis of samples from two cases; screen of suspected MPN cases using targeted NGS assay; supervision and editing of the manuscript.

Iain Singer: Provision of case details for patient 2; supervision and editing of the manuscript.

Aaron Cooney: Co-author of the manuscript.

Ram Jayaprakash: Supervision and editing of the manuscript.

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FIGURE LEGENDS

Fig. 1. A. Bone marrow aspirate showing increased numbers of eosinophils (7% of nucleated cells) [May-Grunwald-Giemsa, 50x magnification]. B. Bone marrow trephine biopsy showing normal cellularity [Haematoxylin and Eosin, 2.5x magnification]. C. Bone marrow trephine biopsy showing increased numbers of eosinophils (eosinophils highlighted with arrows) [Haematoxylin and Eosin, 40x magnification]. D. Bone marrow biopsy with immunohistochemistry for CD235a, showing a modest increase in erythroid cells (approximately 40% of total cellularity) [20x magnification].

Table 1. Full blood count results for patient 1.

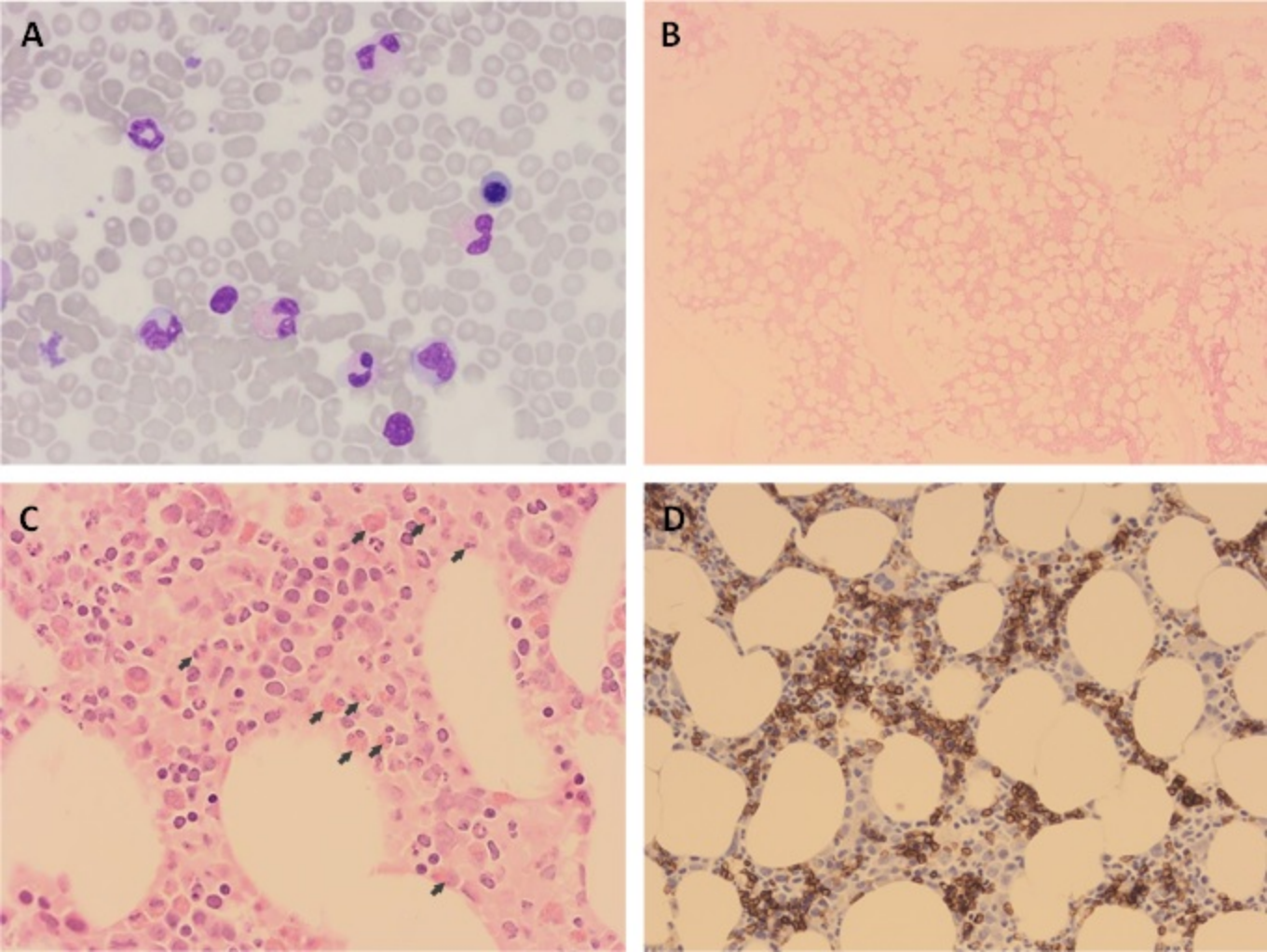
Table 2. Full blood count results for patient 2.

Table 1

Parameter	**June 2014**	**June 2017**	**May 2018 (diagnosis)**	**March 2020**	**July 2020 (pre-treatment)**
Haemoglobin (g/l)	139	139	155	159	151
Mean corpuscular volume (fL)	97.1	97.1	95	90.8	90
Haematocrit	0.410	0.433	0.478	0.496	0.470
White cell count ($x10^9$/L)	6.9	12.5	12.6	20.2	23.7
Neutrophil count ($x10^9$/L)	2.9	6.7	7.8	7.8	9.6
Eosinophil count ($x10^9$/L)	**1.0**	**1.9**	**3.0**	**9.2**	**10.1**
Monocyte count ($x10^9$/L)	0.4	1.0	0.3	0.7	1.1
Basophil count ($x10^9$/L)	0.0	0	0.0	0.1	0.1
Platelet count ($x10^9$/L)	260	303	301	349	355
Lymphocyte count ($x10^9$/L)	2.7	2.9	1.5	2.4	2.9

Table 2

Parameter	**January 2013**	**July 2016**	**September 2017**	**August 2018**	**June 2020 (diagnosis)**
Haemoglobin (g/l)	141	142	142	142	156
Mean corpuscular volume (fL)	93.7	100.7	90.6	89.1	94.7
Haematocrit	0.446	0.422	0.416	0.425	0.486
White cell count ($x10^9$/L)	8.9	11.3	10.7	12.3	18.7
Neutrophil count ($x10^9$/L)	5.1	5.7	4.5	4.8	7.8
Eosinophil count ($x10^9$/L)	**0.5**	**2.4**	**2.5**	**4.7**	**7.9**
Monocyte count ($x10^9$/L)	0.8	0.7	0.8	0.8	0.9
Basophil count ($x10^9$/L)	0.0	0	0.1	0.1	0.1
Platelet count ($x10^9$/L)	304	315	300	313	357
Lymphocyte count ($x10^9$/L)	2.5	2.4	2.2	2.0	1.9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