Eosinophilic leukaemia

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The last few years have seen much progress in our understanding of, and treatments for, eosinophilic leukaemia. In preparing this review, we used Pubmed and the archives of well-known Haematology journals to search for relevant research papers and reviews published in the last 5–10 years. In this article, we review the differential diagnosis and sub-classification of eosinophilic leukaemia, and go on to discuss clinical features, investigation and treatment of these disorders. We are increasingly able to classify clonal eosinophilias based on the underlying molecular genetic abnormalities, and prognosticate and treat patients according to this. The successful treatment of certain of these patients with imatinib, followed by a greater understanding of the mechanism of this treatment, has revolutionized the outlook for many patients with eosinophilic leukaemia. New similar tyrosine kinase inhibitors and other promising therapies are on the horizon.

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Introduction

The term ‘eosinophilic leukaemia’ can be used to describe any haematological neoplasm in which a raised eosinophil count is the dominant abnormality; eosinophils are increased in blood and bone marrow, and the eosinophils are a part of the neoplastic clone. An eosinophil count greater than $1.5 \times 10^9/l$ is often used as one of the criteria for making this diagnosis. Eosinophilic leukaemia may include a number of different disorders, defined according to clinicopathological characteristics as in the World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues\(^1\) or defined, at least in part, according to the underlying cytogenetic/molecular genetic abnormality.\(^2\)

In recent years, there has been a great deal of progress in our understanding of these conditions. As knowledge of the molecular genetic abnormalities that are implicated increases, so does our ability to more precisely define the disease entity in a given patient, and to prognosticate and decide upon the best treatment options. We are now able to tailor our therapies according to the underlying abnormality and

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also continue to make progress towards the design of further specific targeted therapies.

**Differential diagnosis of eosinophilia**

Any patient diagnosed with eosinophilia (eosinophils \( >0.5 \times 10^9/l \)), falls into one of the three categories of disease described as follows:

(i) Reactive eosinophilia
(ii) Clonal eosinophilia
(iii) Unexplained (idiopathic) eosinophilia

**Reactive eosinophilia**

Eosinophilia may be described as reactive when the eosinophils are non-clonal, and are produced from haemopoietic precursors in increased numbers as a response to an underlying disorder. Their increased production is brought about by a number of different mechanisms, resulting in the increased production of interleukin (IL)-5—the main eosinophilopoietic cytokine, and other cytokines including IL-3 and granulocyte/macrophage colony-stimulating factor (GM-CSF). These cytokines are mainly produced by T-helper cells. There are many underlying diseases which can cause an increase in eosinophil production in this manner—these can be broadly divided into infective, allergic and neoplastic conditions, although there are a variety of other causes, including vasculitis. A mild eosinophilia can occur during recovery from a bacterial infection, but significant infection-related eosinophilia is the result of parasitic infection. Worldwide, by far the most common cause of eosinophilia is helminth infection, particularly by nematodes and trematodes. Eosinophilia is usually greatest during acute infection or during tissue migration. Eosinophilia is less prominent with cestode infection, unless there is rupture of cysts, and protozoans usually cause only mild eosinophilia. In Europe and North America, the commonest cause of eosinophilia is allergy, including asthma. Eosinophils may also be increased as a reactive phenomenon in neoplastic conditions, including, rarely, haematological neoplasms. To avoid confusion, it is helpful to bear in mind that eosinophils in these conditions are not part of the neoplastic clone. Reactive eosinophilia can occur in lymphomas, particularly Hodgkin lymphoma and T-lineage non-Hodgkin lymphoma, and also in acute lymphoblastic leukaemia (ALL); in a minority of patients with lymphoid malignancy, the eosinophilia is not reactive, the eosinophils being shown to be a part of the neoplastic clone. Another situation in which...
the eosinophilia is reactive to a haematological disorder is when an abnormal lymphocyte population (often but not always demonstrably clonal) produces an excess of IL-5. These populations have an abnormal immunophenotype, and the condition sometimes later transforms into an overt T-cell lymphoma. The designation ‘lymphoproliferative variant of hypereosinophilic syndrome’ is sometimes used. The clinical features and treatment of this particular disorder are discussed later.

**Clonal eosinophilia**

Conditions in which eosinophils have been shown to be part of the neoplastic clone include: chronic eosinophilic leukaemia (CEL), systemic mastocytosis (SM), chronic myelomonocytic leukaemia (CMML), juvenile myelomonocytic leukaemia (JMML), chronic myeloid leukaemia (CML), atypical chronic myeloid leukaemia (aCML), acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS), and rarely ALL.

The above disorders are divided according to their clinicopathological features, including the involvement of lineages other than that of the eosinophil, and the presence or absence of an increased number of highly immature cells (blast cells). The conditions in which eosinophilia may be dominant are essentially CEL, CMML with eosinophilia and aCML with eosinophilia. In CEL, hypereosinophilia is the predominant feature of the disease, whereas when there is eosinophilia associated with CMML, monocytes are also increased and similarly, in aCML with eosinophilia, circulating granulocyte precursors are also increased. Criteria that should be fulfilled in order to diagnose CEL are:

(i) An eosinophil count \( \geq 1.5 \times 10^9/l \) and increased bone marrow eosinophils.

(ii) Exclusion of reactive eosinophilia, including that secondary to haematological disorders such as lymphoma.

(iii) Exclusion of other haematological neoplasms in which eosinophils are part of the clone, including CML, AML, other myeloproliferative disorders and myelodysplastic syndromes.

(iv) Exclusion of an abnormal T-cell population with abnormal cytokine production.

(v) Evidence of clonality of the eosinophils (i.e. demonstration of a clonal abnormality in myeloid cells by cytogenetic or molecular genetic techniques) or the presence of increased blasts in the peripheral blood (\( > 2\% \)) or bone marrow (5–19%).
Another way of classifying the clonal eosinophilic disorders is according to the underlying molecular genetic abnormality, if known.\textsuperscript{2,6} This has the advantage that the genetic abnormalities of the different syndromes may be more fundamental to the nature of the condition that the exact number of eosinophils, monocytes or granulocyte precursors. Very importantly, they may also be predictive of response to specific drugs. The main genetic abnormalities recognized are rearrangement of particular genes encoding receptor tyrosine kinases involved in eosinophil proliferation, particularly \textit{PDGFRA}, \textit{PDGFRB} and \textit{FGFR1}. A common feature of these molecular genetic abnormalities is that a translocation or interstitial deletion leads to the formation of a fusion gene that encodes an aberrant constitutively activated tyrosine kinase, active in one of the signalling pathways between the cell surface and the nucleus.

These ‘syndromes’ will subsequently be described in further detail.

\textit{Idiopathic eosinophilia}

Idiopathic hypereosinophilia is diagnosed when, after extensive investigation, causes of reactive hypereosinophilia (including abnormal T-cell populations) have been excluded and no evidence of clonality of the eosinophils has been found.

A subgroup within this classification is that of the idiopathic hypereosinophilic syndrome which may only be diagnosed if the following additional criteria are fulfilled:

(i) Eosinophil count is $\geq 1.5 \times 10^9/l$;
(ii) Eosinophilia has been present for more than 6 months;
(iii) There is evidence of end organ damage.

It should be emphasized that the terms ‘idiopathic eosinophilia’ and ‘idiopathic hypereosinophilic syndrome’ are used when no specific diagnosis is possible; such patients must be kept under close observation and may even require treatment prior to the discovery of the true nature of the condition. A proportion of patients who, until quite recently, would have been diagnosed as having the idiopathic hypereosinophilic syndrome, are now known to have eosinophilic leukaemia as a result of a \textit{PDGFRA} rearrangement;\textsuperscript{7} use of the term of ‘idiopathic’ to refer to such patients is now inappropriate.

The diagnostic process that should be followed before a case is classified as idiopathic is shown in Figure 1 and Table 1.
Clinical features of the eosinophilic leukaemias

These may be considered in two separate groups:

First, irrespective of the cause of an eosinophilia, the presence of increased eosinophils in the blood can lead to organ damage and thus to clinical symptoms and signs—this damage is mediated by release of the contents of eosinophilic granules. Tissue damage is more likely if degranulated eosinophils are numerous, e.g. greater than $1 \times 10^9/l$. Damage particularly affects the heart, leading to congestive cardiac failure and arrhythmias; thromboembolism and skin, pulmonary and central nervous system involvement can also occur.

Secondly, clinical and laboratory features can relate to the leukaemic process itself: splenomegaly, lymphadenopathy (in some subtypes), cytopenias, raised lactate dehydrogenase (LDH) and hyperuricaemia due to high cell turnover, raised serum vitamin B₁₂ consequent on the increased production of B₁₂-binding proteins by granulocytes.

Investigation of suspected eosinophilic leukaemias

An eosinophilia will generally be initially diagnosed from a full blood count. Other features of the full blood count, supplemented by careful
examination of a blood film, may be useful in the differential diagnosis: raised monocytes in CMML with eosinophilia, circulating granulocyte precursors in aCML with eosinophilia, raised lymphocytes or cytologically abnormal lymphocytes if the underlying disorder is lymphoproliferative, cytopenias in AML, circulating blast cells in AML and to a lesser extent in the chronic eosinophilic leukaemias, dysplasia in MDS, CMML and aCML. The morphology of the eosinophils themselves is, however, unhelpful in narrowing down the diagnosis. Many morphological abnormalities are being described including abnormalities of size, granulation and nuclear lobulation, but eosinophils can look relatively normal in eosinophilic leukaemia and may show gross abnormalities in reactive conditions.

Especially if the eosinophil count is very high at diagnosis and/or there is evidence of end organ damage, it is important to exclude a treatable eosinophilic leukaemia at an early stage in the investigation.

Returning to the requirements for diagnosis of eosinophilic leukaemia; initially, reactive causes for the hypereosinophilia should be excluded. The choice and extent of investigation will depend on history, symptoms, clinical signs and geographic origin of the patient.

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<tr>
<th>Table 1 Investigations that are indicated in a patient with unexplained persistent hypereosinophilia</th>
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<td>Investigation</td>
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<td>Blood film</td>
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<td>Investigation for parasitic infection</td>
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<td>Immunoglobulin E and tests for allergy</td>
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<td>Bone marrow aspiration and trephine biopsy</td>
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<td>Cytogenetic analysis on bone marrow aspirate</td>
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<td>Molecular analysis on peripheral blood cells for FIP1L1–PDGFRA fusion gene</td>
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<td>Serum tryptase</td>
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<td>Immunophenotyping of peripheral blood T cells</td>
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<td>Computerised tomography (CT) scan of chest and abdomen</td>
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It is generally useful at a minimum to send stool for microscopy for parasites, and often to undertake serological investigations to rule out certain parasitic infections. Occasionally, such an infection may coexist with a haematological disorder, in which case it is important to treat the infection, as this may result in resolution/amelioration of the eosinophilia and its consequences despite persistence of the haematological disease. Other investigations may focus on diagnosing an allergic condition or malignancy, depending on the clinical picture.

Other initial investigations may include serum vitamin B₁₂ and mast cell tryptase assays, both being raised in myeloproliferative disorders; mast cell tryptase is raised to a greater extent in SM than in CEL and other myeloproliferative disorders.

If initial investigations do not disclose a cause for the eosinophilia, a bone marrow aspirate and trephine biopsy, supplemented by cytogenetic analysis, are indicated. Morphological assessment of the aspirate may allow diagnosis of AML/MDS/lymphoma. Trephine biopsy may show a lymphomatous infiltrate, or increased mast cells, indicating that a diagnosis of SM should be considered. An increase of mast cells is also seen in the PDGFRA-rearranged disorders. Mast cell immunostaining (e.g. immunohistochemistry for mast cell tryptase, possibly supplemented by CD117) should be performed in all cases of eosinophilia where the diagnosis is not immediately apparent. Immunophenotyping of eosinophils is not helpful, since no specific abnormalities have been described. Cytogenetic analysis of the marrow may allow diagnosis of eosinophilic leukaemia, for example with a translocation involving PDGFRB; all of the translocations described to date are cytogenetically apparent i.e. a translocation is visible on standard cytogenetic analysis. Other cytogenetic abnormalities that may be shown include the Philadelphia chromosome in CML, but this condition presents with dominant eosinophilia only in the accelerated phase.

Molecular genetic analysis for the presence of FIP1L1–PDGFRA fusion need not await bone marrow examination since it can be performed on peripheral blood cells. Either fluorescence in situ hybridisation (FISH) or the polymerase chain reaction (PCR) can be used, the more sensitive nested PCR being needed for the detection of some cases.

The importance of early diagnosis of patients with rearrangement of either PDGFRα or PDGFRB is that both conditions can be treated successfully with imatinib, and the sooner the treatment is commenced, the more likely it is that tissue damage by eosinophils can be halted or even reversed.

Another diagnosis to consider and attempt to rule out, especially if initial investigations have revealed no cause for the eosinophilia, is that of the lymphoproliferative variant of the hypereosinophilic syndrome. Immunophenotyping of peripheral blood or bone marrow lymphocytes
may be helpful to rule out an abnormal population or lymphoproliferative disorder. If this diagnosis is thought to be likely, IL5 levels and T-cell receptor rearrangement analysis (to demonstrate clonality of lymphocytes) are useful.

Other important investigations in patients with eosinophilia focus on the detection and assessment of end-organ damage. Depending on the height of the eosinophil count and the presence of symptoms/signs, suitable investigations may include troponin T measurement, echocardiography, electrocardiography, chest imaging and lung function tests.

If all the above investigations have all been carried out, and still no cause for the eosinophilia is apparent, then a diagnosis of idiopathic eosinophilia may be made. As described above, ‘idiopathic hypereosinophilic syndrome’ is diagnosed if there is tissue damage also.

The individual ‘syndromes’

PDGFRA rearrangements

Since the discovery of the cytogenetically silent FIP1L1–PDGFRA fusion in a group of patients previously categorized as having ‘idiopathic hypereosinophilic syndrome,’ it is now thought that 30–60% of such patients actually have eosinophilic leukaemia. The fusion gene encodes a constitutively active tyrosine kinase, which, via downstream signalling pathways, is thought to confer a proliferative and survival advantage on myeloid cells. Although the causative mutation arises in a pluripotent stem cell with the potential to give rise to eosinophils, neutrophils, monocytes, B lymphocytes, T lymphocytes and mast cells, markedly abnormal proliferation is largely confined to eosinophils and, to a lesser extent, neutrophils and mast cells.

Patients with this fusion gene are predominantly male, and tend to have splenomegaly and raised serum B12 and mast cell tryptase levels. The marrow is generally hypercellular, and an increase of mast cells is also usually demonstrable, sometimes leading to a diagnosis of systemic mastocytosis; we consider this diagnosis is inappropriate and likely to lead to confusion since SM with mutation of the KIT gene is a quite different disease. Even if patients fulfil the WHO criteria for SM it is now generally recommended that the FIP1L1–PDGFRA fusion syndrome be regarded as a separate entity.

Three other fusion genes involving PDGFRA have been described more recently. The fusion partner genes are BCR, KIF5B and CDK5RAP2. Unlike FIP1L1–PDGFRA fusion, these genetic abnormalities are all apparent on standard cytogenetic analysis. Experimental data showing that 4% of patients with ‘idiopathic
hypereosinophilia’ overexpress the PDGFRA kinase domain, suggests that further fusion partners have yet to be discovered in such cases.\textsuperscript{11}

**PDGFRB rearrangements**

In a small number of patients with CEL or CMML or other myeloproliferative/myelodysplastic conditions, usually with eosinophilia, a translocation involving the PDGFRB gene at 5q33 is found.\textsuperscript{13} The most frequently observed abnormality is t(5;12)(q33;p13) with resultant ETV6–PDGFRB fusion. Patients are very largely male with a wide age range (median 42 years), and usually splenomegaly. A minority of cases transform to AML.

Other similar patients have had fusion genes that involved HIP1, H4, CEV14, RAB5 (all summarized by Steer et al.),\textsuperscript{13} PDE4DIP,\textsuperscript{14} NIN,\textsuperscript{15} TP53BP1,\textsuperscript{16} HCMO1T1,\textsuperscript{17} WDR48,\textsuperscript{18} GOLGA4,\textsuperscript{18} KIAA1509,\textsuperscript{19} and TMP3.\textsuperscript{20} All reported abnormalities have been apparent on cytogenetic analysis but FISH analysis can be useful to confirm PDGFRB rearrangement, since not all translocations with breakpoints in this region involve this gene.

**FGFR1 rearrangements**

In a very small proportion of patients with hypereosinophilia, a translocation involving the FGFR1 gene at 8p11 can be demonstrated.\textsuperscript{21} These translocations all give rise to a fusion gene encoding a constitutively active tyrosine kinase. This group of patients are often said to have the ‘8p11 myeloproliferative syndrome’ or ‘stem cell leukaemia/lymphoma syndrome’. The responsible mutation occurs in a pluripotent stem cell able to give rise to myeloid cells, T cells and B cells. The most frequently observed cytogenetic abnormality is t(8;13)(p11;q12) leading to the formation of a ZNF198–FGFR1 fusion gene. Other translocations involving 8p11 and FGFR1 lead to fusion genes involving FGFR10P1 (FOP), CEP110, BCR (all reviewed by Macdonald et al.\textsuperscript{21}), HERVK,\textsuperscript{22} TIF1,\textsuperscript{23} MYO18A\textsuperscript{24} and BCR.\textsuperscript{25}

Clinical presentation is heterogeneous.\textsuperscript{21} Lymphadenopathy, splenomegaly and systemic symptoms are common. Patients may present in chronic phase with eosinophilia, neutrophilia and, less often, monocytosis. Two patients had a previous history of polycythaemia vera. Others present with, or transform to, T-lymphoblastic lymphoma/leukaemia (at least 16 cases)\textsuperscript{21} and a few present with or transform to B-lineage lymphoblastic leukaemia/lymphoma (at least four cases).\textsuperscript{21,26,27} Transformation to myeloid sarcoma or AML is also quite common.
The disease has a rapidly progressive clinical course and an extremely poor prognosis with conventional therapy.

**Other genetic abnormalities**

Hypereosinophilia may occur in KIT-mutated SM and with miscellaneous translocations involving ETV6, JAK2, SYK and ABL, all genes encoding tyrosine kinases. The JAK2\(^{V617F}\) mutation, seen most commonly in polycythaemia vera, has also been described in some patients with hypereosinophilia.\(^{28}\)

**Treatment of eosinophilic leukaemia**

Depending on the specific molecular genetic abnormality, many patients will respond to imatinib. This is a small molecule tyrosine kinase inhibitor, which is active against many abnormal constitutively active receptor tyrosine kinases implicated in the pathogenesis of haematological malignancies. It was initially used to treat Philadelphia-positive CML, where it inhibits the BCR–ABL fusion product and thus removes the proliferative drive to CML cells, resulting in remission in the vast majority of cases. It is even more active against the FIP1L1–PDGFRA fusion product and efficacy is demonstrated or predicted for other PDGFRA fusion products. Most of the fusions involving PDGFRB are also responsive to imatinib.\(^{29,30}\)

Just as the BCR–ABL fusion gene may undergo further mutation, resulting in a change in structure of the fusion product, and resistance to imatinib binding/action, a similar mutation had been described in a small number of cases of FIP1L1–PDGFRA positive CEL. In at least three cases, the T674I mutation developed during imatinib therapy.\(^{7}\)

New receptor tyrosine kinase inhibitors may be of benefit in this circumstance. Since these are not yet widely available, treatment options for those conditions, which are unresponsive to imatinib are as for idiopathic hypereosinophilia.

FGFR1-rearranged disorders are a special case as they have such a dismal prognosis—in younger patients allogeneic stem cell transplantation should be considered in chronic phase or after induction of remission, at least until such time as an effective targeted treatment becomes available. In this regard, PKC412 appears promising.\(^{31}\)

Imatinib is not useful.
Treatment of idiopathic hypereosinophilic syndrome

This disorder has historically been fairly difficult to treat, and often fatal. As more patients are found to have the FIP1L1–PDGFRA fusion or abnormal lymphocyte populations, the proportion of patients in whom a diagnosis of idiopathic hypereosinophilic syndrome remains appropriate has considerably decreased. Treatment options for these patients include steroids and hydroxycarbamide (previously known as hydroxyurea), but many other agents have been successful in small numbers of patients. The role of a trial of imatinib therapy for these patients remains controversial. There have been a number of reports of response to imatinib in patients with hypereosinophilia but without the FIP1L1–PDGFRA fusion, and this may be sufficient evidence to justify a trial, especially in those patients who are unresponsive to steroids.

New treatments under development, apart from the new tyrosine kinase inhibitors, include anti-IL5 monoclonal antibodies, which may be especially useful in those patients with abnormal T-cell populations (i.e. a different group from those with idiopathic eosinophilia).

Conclusion

Our understanding of and treatment options for patients with eosinophilic leukaemia and the idiopathic hypereosinophilic syndrome have improved greatly in recent years, the major advance being the discovery of the cytogenetically silent FIP1L1–PDGFRA fusion and the response of the associated syndrome to imatinib therapy. Thus, the prognosis of a subset of patients with these disorders has greatly improved. It is likely that our ability to precisely diagnose these disorders will continue to improve, and that new treatment options will soon become available, improving outcomes in those syndromes, which are currently difficult to treat, for example the FGFR1-rearranged disorders, and the mutated FIP1L1–PDGFRA fusion product that is unresponsive to imatinib.

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