Hypereosinophilic syndrome: diagnosis and treatment

Tatjana Peroš-Golubičić[a] and Silvana Smojver-Ježek[b]

Purpose of review
Hypereosinophilic syndrome is increasingly recognized as a heterogeneous group of disorders, in some cases with precisely defined pathogenesis, which has led to changes in diagnostic approaches and therapeutic strategies. An update on causes and modern therapy is presented here.

Recent findings
Clonal eosinophilias belong to the group of myeloid malignancies. Karyotypically occult FIP1L1- platelet-derived growth factor receptor alpha and beta rearranged eosinophilic disorders respond to imatinib mesylate with almost 100% efficacy. If standard therapies fail, the FIP1L1-platelet-derived growth factor receptor-negative cases of hypereosinophilic syndrome should also be considered for treatment with imatinib. The recognition of acquired resistance to imatinib has aroused interest in developing new tyrosine kinase inhibitors. Other subgroups of clonal eosinophilias have been molecularly defined, but the curative verification of pathogenetic relevance has not been certified. Hypereosinophilic syndrome patients with abnormal T-cell populations have benefited from the treatment with anti IL-5 monoclonal antibodies.

Summary
The FIP1L1- platelet-derived growth factor receptor alpha and beta-positive patients, and those with abnormal T-cell populations are currently the only clearly defined treatable subgroups of hypereosinophilic syndrome. The FIP1L1-platelet-derived growth factor receptor alpha-negative responders to imatinib pose a question as to the existence of subentities with unrecognized tyrosine kinases-based mutation. The search for such cases and other treatable subgroups of hypereosinophilic syndrome has already begun.

Keywords
eosinophilia, hypereosinophilic syndrome, imatinib mesylate, tyrosine kinase inhibitors

[a]University of Zagreb Medical School and [b]Department of Cytology, University Hospital for Lung Diseases 'Jordanovac', Zagreb, Croatia

Correspondence to Tatjana Peroš-Golubičić, MD, PhD, Associate Professor, pulmonologist, University of Zagreb, Medical School, University Hospital for Lung Diseases 'Jordanovac', 10000 Zagreb, Jordanovac 104, Croatia
Tel: +385 1 23 85 142; fax: +385 1 23 48 345; e-mail: tperos-golubicic@net.hr

Introduction

The term hypereosinophilic syndrome (HES) was first introduced in 1968 by Hardy and Andersen [1]. Inclusion criteria for idiopathic HES were defined by Chusid *et al.* 1975 [2]. HES has since been characterized by persistently elevated eosinophil count ($\geq 1.5 \times 10^9$/L) in the peripheral blood for at least 6 months, with evidence of damage to the organs involved and a lack of known causes [3]. Our perception of HES has changed significantly over the past few years. Recent achievements in the comprehension of pathogenesis and the necessary diagnostic procedures are presented, with direct influence on therapy decisions.

Acquired hypereosinophilias are currently classified into primary and secondary, and primary further into clonal or idiopathic [4•].

Secondary eosinophilia

The highest prevalence of patients with HES have secondary eosinophilias, where eosinophilia is an epiphenomenon that occurs alongside a primary phenomenon, such as infection, atopy, drug reactions, connective tissue disorders, vasculitis, malignancies, and some other rare conditions. In all instances eosinophilia emerges as the response to eosinophilopoetic cytokines such as IL5, IL3 and granulocyte/macrophage colony-stimulating factor (GM-CSF), produced by T lymphocytes. Whereas IL3 and GM-CSF have activities on other hemopoetic lineages, IL5 is specific to the eosinophilic lineage, making it a prime target for therapeutic intervention [5].

Eosinophilia could also be a reaction to an abnormal T-cell population which causes excessive production of cytokines, especially IL5 [6]. Abnormal cytokine-secreting T-cells can occasionally be demonstrated to be clonal by T-cell rearrangement analysis. Patients with HES and aberrant T cells show quite similar clinical pattern: most are females, with a history of atopy (predominantly with skin, lung and gastrointestinal tract manifestations), hypergammaglobulinemia and high immunoglobulin E levels. These patients rarely develop endomyocardial

fibrosis. WHO definition of idiopathic HES requires the absence of an aberrant T cell population.

Clonal eosinophilia

Demonstration of eosinophil clonality is not required, and is seldom feasible. Clonal eosinophilia could be substantiated either by a cytogenetic or molecular marker of clonality in myeloid cells, or bone marrow histological features that are congruent with an otherwise classified myeloid malignancy. They can be grouped according to clinicopathological findings (as with the WHO classification) [3], or semimolecular classification [7,8•]. The proposal of a new, alternative semimolecular classification of chronic myeloid disorders is the logical consequence of a rapidly increasing repertoire of primary clonogenic mutations in some of the disease subentities.

Eosinopilia could be a feature of acute myeloid leukemia, chronic myeloid leukemias, myelodysplastic syndrome (MDS), and classic and atypical myeloproliferative disorders (MPD). Atypical MPDs are subdivided into clinicopathologically assigned atypical MPDs and molecularly defined atypical MPDs.

Clinicopathologically assigned atypical MPDs include several entities which are not molecularly defined, where eosinophilia is a prominent feature, like in chronic eosinophilic leukemia (CEL), systemic mastocytosis, chronic myelomonocytic leukemias, and unclassified MPDs.

Most cases of molecularly defined atypical MPDs are accompanied by prominent blood eosinophilia. Molecularly defined atypical MPDs are a subgroup of clonal eosinophilias associated with causative genetic abnormalities, although the same genetic abnormality may be accompanied by different disease phenotypes [9••].

PDGFRA-rearranged eosinophilic disorders

Most frequently the platelet-derived growth factor receptor alpha (PDGFRA) -rearranged eosinophilic disorders have been documented, most often the microdeletion on chromosome 4q12, which results in FIP1L1- PDGFRA fusion [10]. Genes that fuse to PDGFRA encode constitutively active tyrosine kinases, which drive the clonal cell proliferation. Fusion tyrosine kinases (FTK), the product of hybrid genes, initiate malignant transformations, including proliferation and protection from apoptosis, but also induce resistance to genotoxic treatment. These effects of BCR/ABL kinases have been much better characterized than those induced by other FTKs, so it is impossible to exclude the possibility of FTK-specific phenomena in drug resistance and genome instability [11•]. The chromosomal abnormalities are most probably initiated by oxidative stress, radiation, genotoxic chemicals or DNA replication stress. FIP1L1-PDGFRA fusion has also been documented in patients

who received chemotherapy for non-Hodgkin lymphoma, and 8 years later developed CEL. It raises the possibility of therapy-induced genetic damage, which would add chemotherapy to the list of causes of eosinophilia [12•].

PDGFRA-rearranged eosinophilic disorders occur most often in karyotypically normal patients. It is important to recognize these patients, since therapy with imatinib is very effective but response to corticosteroids is very poor. Features of this group include male predominance, anemia, thrombocytopenia, increased serum vitamin B12 and mast cell tryptase levels, endomyocardial and bone marrow fibrosis, splenomegaly, and occasionally lung fibrosis [13,14]. Summarizing this information and other currently reported data [15•], we suggest that FIP1L1- PDGFRA-positive HES, with myeloproliferative features, should be considered a distinct clinical entity.

The prevalence of FIP1L1- PDGFRA mutations in unselected patients is quite low: among 741 patients with eosinophilia, Pardanani *et al.* found mutations only in 3% of cases [16••]. The prevalence is considerably higher in a subgroup of patients who satisfy the WHO classification criteria, ranging from 12–88% [17]. Reported frequencies vary markedly, most probably due to incoherence of the groups studied, but also due to the difficulties in verifying the molecular diagnosis.

PDGFRA may also fuse to other partner genes; the PDGFRA breakpoint is faithful, and is restricted to exon 12, however the BCR-PDGFRA, and recently the KIF5B-PDGFRA and CDK5RAP2-PDGFRA fusions have been described [18•,19].

PDGFRB-rearranged eosinophilic disorders

Platelet-derived growth factor receptor beta (PDGFRB) -rearranged disorder is a rare cause of chronic eosinophilic leukemia, with cytogenetically apparent 5q31-33 translocation. Molecular analysis is mandatory, since imatinib therapy induces complete remission in most cases of confirmed PDGFRB-rearrangement [20].

Previous data affirm the remarkable association between activating mutations of certain receptor tyrosine kinases and eosinophil-associated MPD. A recent study on mice suggests that FIP1L1- PDGFRA gene is not sufficient to induce a hypereosinophilic, myeloproliferative disease, but requires a second event associated with IL-5 overexpression, thus reflecting the intricacy of events leading to eosinophil-associated MPD [21••].

FGFR1-rearranged eosinophilic disorders

The rearrangement and activation of fibroblast grow factor receptor 1 (FGFR1) is associated with a syndrome

known as the 8p11 myeloproliferative syndrome, or stem cell leukemia lymphoma syndrome, that frequently presents with eosinophilia. This syndrome has a grave prognosis, and rapidly transforms into acute leukemia [22].

Other genetic abnormalities
Clonal hypereosinophilia is occasionally detected in some patients with BCR/ABL positive chronic myeloid leukemia (CML) and KITD816 or FIP1L1- PDGFRA positive systemic mastocytosis. Rearrangement of transcription factor ETV6 at 12p13 is associated with eosinophilia in chronic hematologic malignancies, ETV6-SYK rearrangements in MDS with eosinophilia, and many other genetic abnormalities have been described in patients with HES [23•,9••].

Other examples of mutations with reputed pathogenetic relevance in MPD cases, usually without eosinophilia, include BCR/ABL mutation in CML, JAK2V617F in polycythemia vera, essential thrombocythemia and primary myelofibrosis, JAK2 exon 12 mutations in polycythemia vera, and MPLW515L/K in primary myelofibrosis and essential thrombocythemia [24].

Idiopathic eosinophilia
If the causes of secondary eosinophilia, clonality or the presence of aberrant T-cells have not been documented, the diagnosis of idiopathic HES is proposed. WHO criteria for the diagnosis of idiopathic HES also have to be applied; if these criteria are not fulfilled then the diagnosis of benign hypereosinophilia is postulated.

Clinical features of idiopathic HES are rather diverse: some patients are almost asymptomatic, while others show grave symptoms with multiple organ involvement. Although almost any organ is prone to eosinophil-associated organ damage, the heart, the nervous system, the skin and respiratory tract are most frequently targeted. The pulmonary manifestations (infiltrates and nodules, pleural effusion) arise in about 40% of patients [25,26], and rarely as the sole organ presentation. Thromboembolic disease of the heart chambers and peripheral vessels is also occasionally observed.

Diagnostic procedures
The initial step is to exclude all the known causes of HES [4•]. If secondary eosinophilia is ruled out, laboratory tests should aim to either exclude or confirm clonality of eosinophils/myeloid cells or aberrant T cell populations.

The minimum of investigation is blood count with leukocyte differential, microscopic examination of peripheral blood, serum IgE, IgG, IgA, IgM, vitamin B12, leukocyte alkaline phosphatase score, lymphocyte phenotyping, TCR gene rearrangement analysis, karyotyping, abdominal ultrasound and echocardiogram [27].

A striking feature of peripheral blood morphology is eosinophilia, composed mainly of mature eosinophils, eosinophilic myelocytes and promyelocytes in small numbers. Eosinophilic abnormalities are present and include sparse granulation with morphologically visible clear cytoplasm, enlarged size, and hypersegmentation or hyposegmentation of nuclei. Findings are not specific as secondary, idiopathic, and clonal hypereosinophilias all share the same eosinophilic morphology [3,23•]. Neutrophilia is often present, in some cases monocytosis and basophilia could be found. Finding more than 2% of blasts in peripheral blood would correspond with clonal hypereosinophila.

The eosinophilic peroxidase content in hypereosinophilias is usually normal, regardless of its etiology. Finding napthol ASD chloroacetate esterase suggests the clonal origin of hypereosinophilia, although more studies in reactive cases are needed. There are no reports of specific abnormality of the eosinophil immunophenotype [3,23•].

Serum tryptase and vitamin B-12 should be measured, since increased concentrations suggest a myeloproliferative disorder. The immunophenotyping of the lymphocytes in peripheral blood, measurements of serum IL5 levels and TCR gene rearrangement analysis should suffice to either exclude or confirm the presence of aberrant T cells.

Bone marrow is usually hypercellular, with eosinophils ranging between 10 and 30%. Charcot-Leyden crystals are often present, as well as increase in mast cells. Signs of dysplastic feature in all lineages with increased number of blasts between 5 and 19% suggest clonal origin, but not prove it [3].

Cytomorphology combined with cytogenetic analysis, interphase fluorescent in situ hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR) are necessary to demonstrate clonality. Combination of nested RT-PCR, interphase FISH and quantitative RT-PCR can be used for detecting PDGFRA rearrangements. PCR is effective for establishing TCR rearrangement and FISH analysis for BCR-ABL abnormalities [28]. It is possible to monitor molecular response to treatment with peripheral blood screening for PDGFRA fusion performing either FISH or RT-PCR [4•]. The value of real-time quantitative PCR (RQ-PCR) in eliciting the molecular response to treatment and molecular remission is under investigation.

Eosinophil-mediated organ damage must also be demonstrated. The echocardiogram, chest x-ray, pulmonary function tests and serum troponin levels should be performed. Complementary roles for transthoracic, trans-

esophageal and contrast echocardiography in the evaluation of cardiac involvement in idiopathic HES have recently been reported [29•].

Treatment of clonal eosinophilia

The most exciting change in the management of HES is the discovery of a subgroup that can be treated with tyrosine kinase inhibitors [10]. There is general consensus that the PDGFRA and PDGFRB-rearranged disorders are efficiently treated with imatinib [9••,16••]. Imatinib mesylate is one of the first selective protein kinase inhibitors, and is orally administered; treatment should commence with the dose as low as 100 mg/day. If a molecular or clinical relapse is detected, the dose should be increased up to 400 mg/day [30]. Of approximately 1700 patients in various clinical trials, the most common untoward side effects of imatinib were fluid retention, diarrhea, nausea, vomiting and muscle cramps [31].

Kerkela *et al.* [32••] recently described 10 patients who developed severe congestive heart failure while on imatinib, and also ventricular contractile dysfunction in imatinib-treated mice, with histological findings suggestive of toxic myopathy. It was also shown that imatinib causes stress in the endoplasmatic reticulum, which in turn induces cadiomyocyte cell death. These findings suggest that endoplasmic reticulum stress response is pivotal to the cardiotoxicity associated with imatinib. Further investigations are needed to define the frequency of imatinib-associated cardiotoxicity, as well as closer monitoring for cardiac problems in patients on imatinib [33•]. It has already been recommended that patients on imatinib with high troponin T levels should be synchronously treated with corticosteroids to prevent the cardiotoxicity [34].

Imatinib should be administered indefinitely, with an empirically derived schedule of low doses of 50–100 mg/daily, becoming intermittent (from once daily to once weekly), in order to prevent relapses [16••]. The follow-up armamentarium of tests should include the tools to determine the molecular response to therapy.

The primary resistance to imatinib in PDGFRA and PDGFRB rearranged disorders has not been registered. The acquired resistance in two cases has been shown to be due to a specific point mutation in the fusion gene (T674I), the mechanism being identical of resistance in CML. Researchers have accordingly developed novel, more potent tyrosine kinase inhibitors that can overcome not only BCR/ABL-dependent mechanisms of resistance seen in CML, but also the indepenent [35••]. PKC412 has been efficient in imatinib resistance *in vitro* [36], as well as sorafenib [37•]. It has been recently shown that AMN107 (nilotinib), a novel aminopyrimidine tyrosine

kinase inhibitor is active, and as equipotent as imatinib against cells expressing the FIP1L1- PDGFRalpha fusion genes, with projections for its efficacy *in vivo* [38•]. Dasatinib, which is 100 times more potent as a tyrosin kinase inhibitor than imatinib, is under investigation [39].

All-trans retinoic acid (ATRA) is well-established in the treatment of promyelocytic leukemia. ATRA is currently used in molecular targeted therapy directed at the chimeric protein generated by the specific chromosomal translocation [40]. Its efficacy has been tested on primary HES-derived cells. ATRA has been shown to inhibit eosinophil colony formation of HES-derived bone marrow cells, and act as a powerful inducer of apoptosis of the EOL-1 cell line; this makes ATRA a promising new candidate drug for treatment of clonal HES [41•].

Treatment of idiopathic HES

Patients with HES have traditionally been treated with prednisone, and this is still the first-line drug of choice. The initial dose of 1mg/kg daily rapidly achieves excellent clinical and laboratory results in most instances: the symptoms wane and eosinophil count declines to normal values. Although 70% of patients are responders, a considerable number experience relapse while on corticosteroids. Second-line drugs, interferon-α (IFN-α) or hydroxyurea induce remission in the majority of patients. There have been additive effects reported from the combination of IFN-α and prednisone, while combining IFN-α and hydroxyurea allows dosage reduction of IFN-α and better control of hypereosinophilia than with either agent alone [42].

Patients not responding to above mentioned therapy regimes received various cytotoxic drugs (vincristin, cyclophosphamide, 6-thioguanine, methotrexate, cytarabine) sporadically, with variable results [43••].

The institution of imatinib therapy in a subset of FIP1L1- PDGFRA-negative patients has been considered, but this is quite controversial. Some patients have responded, but responses were slower and required higher imatinib doses than FIP1L1- PDGFRA-positive cases [10]. According to some experts, imatinib should be considered the third-choice drug in these circumstances [4•].

The management of asymptomatic patients with HES, who do not have signs of organ damage, is still under debate. If therapy is not instituted, as is the preferential choice of most experts, close monitoring is mandatory. Measurement of serum troponin level every 3–6 months and an echocardiogram every 6–12 months is also advised.

The efficacy of two monoclonal antibodies has been recently tested. Mepolizumab targets IL-5, and alemtuzumab the CD52 antigen expressed by eosinophils. They showed promising effects, acting with equal efficacy, reducing the number of eosionophils, abolishing the symptoms and improving quality of life [44,45], although there have been concerns as the rebound eosinophilia has been noticed after treatment cessation [46].

Human intravenous immunoglobulin (IVIg) preparations may be useful in the management of HES; it has recently been shown that they exert cytotoxic effects on purified human blood eosinophils as well as eosinophils obtained from patients with HES. These effects are attributed to anti-Siglec-8 autoantibodies present in IVIg preparations [47•].

If all above-mentioned therapies fail, bone marrow transplantation is considered. This approach has been successful, in some cases effective in the long-term and with eradication of causative genetic abnormalities [48].

Conclusion

A small but important subgroup of patients with HES has been recognized and defined by molecular techniques. The consequence was a significant change in treatment strategy, which included advocating the inhibitors of tyrosine kinases and monoclonal antibodies into the therapy, which produced excellent results. These achievements aroused lively interest from researchers in diverse scientific fields, as well as bedside physicians. We expect the definition of new molecular genetic abnormalities, which are manageable with imatinib and other tyrosin kinase inhibitors, or other treatable causes, and consequently the prospect of improving the prognosis for yet more HES patients.

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