Mild eosinophilia with values of less than 1000 eosinophils/µL is commonly seen in the clinical practice and can be secondary to parasitic, inflammatory or allergic diseases or to drug reactions. Additionally, eosinophilia may be due to connective tissue disorders, infections and occasionally to hematopoietic malignancies or solid tumors. The criteria established in the 1970s, for the definition of idiopathic hypereosinophilic syndrome is today unsatisfactory to characterize all conditions described as eosinophilia. Now these conditions are better understood due to the evolution of cellular and molecular biology. This knowledge has helped to characterize distinct disorders involving myeloid and lymphoid lineages. Hence, eosinophilia is categorized as reactive, clonal or idiopathic. With the introduction of anti-tyrosine kinase (imatinib mesylate) therapy, which is effective for the FIP1L1/PDGFRα rearrangement, there is a possibility to control or cure chronic eosinophilic leukemia. For this reason, precise and fast diagnosis is necessary for ideal therapeutic decisions before organic lesions that are irreversible, such as heart injury, become established. The aim of this manuscript is to review eosinophilia and offer an update on diagnostic and therapeutic investigations.

Keywords: Leukemia, eosinophilic, acute; Receptor, platelet-derived growth factor alpha; Receptor, platelet-derived growth factor beta; Receptors, fibroblast growth factor; Fusion proteins, bcr-abl; Hypereosinophilic syndrome

Introduction

Eosinophils are cells of 8 to 15 µm in diameter, with bilobed nuclei usually characterized by the presence of intracytoplasmic granules that have a high affinity for eosin. These granules contain eosinophil peroxidase, cationic proteins and eosinophil major basic protein (MBP). MBP constitutes the highest proportion of protein granules; it harms many parasites as well as respiratory epithelial cells. (1,2) Eosinophils make up about 3% of the bone marrow cells of normal individuals with concentrations that vary from 50 to 500 cells/µL in the peripheral blood. (3) Peripheral cell counts vary diurnally in humans with lower concentrations in the morning and higher in the afternoon as the level of estrogen decreases throughout the day. (4)

Eosinophils originate from CD34-positive hematopoietic precursor cells of the bone marrow after stimulation by cytokines such as interleukin-3 (IL3), IL5 and granulocytic-macrophage colony-stimulating factor (GM-CSF). These cytokines are soluble immunoregulatory factors released by T lymphocytes in the bone marrow after an appropriate stimulus, but may also be released by CD4+ and CD8+ cells in the peripheral blood as well as by inflamed tissues. (3) Eosinophils are considered predominantly tissue cells as they tend to accumulate in end-organs such as in the gastrointestinal tract, lungs and skin. After entering these tissues, eosinophils no longer return to circulation. The number of eosinophils in the tissue can remain high even when the concentration in the peripheral blood is low. (4)
The half-life of circulating eosinophils is about 18 hours however this period may be extended in abnormal conditions due to the action of eosinophil-activating cytokines. Once activated, eosinophils acquire distinct morphological, phenotypic and functional characteristics. These changes include: reduction in the density and increases in antibody-dependent cytotoxic function, increases in the synthesis of mediators such as the leukotrienes (LCT4), increases in the production of cytokines (IL3, IL4, IL5, IL10, IL13, GM-CSF and IFNγ) that have autocrine and paracrine effects and that have a regulatory action on T-helper lymphocytes (Th2) at the site of inflammation, and increases in adhesion to the vascular endothelium and in the capacity to migrate to the tissues. More than two nuclear lobes inside eosinophils suggest that they have been activated. Thus, activation of eosinophils is a dynamic and continuous phenomenon in the course of which cells undergo simultaneously or sequentially a series of modifications in order to stop being circulating cells and infiltrate tissues. Upon activation, eosinophils become complex multifunctional cells as they operate 1) on inflammation with cytotoxic functions linked to their ability to release protein inflammatory mediators and lipids, and 2) with a regulatory action on tissue inflammatory response through cytokine secretion and direct interaction between membrane molecules and other cell types, particularly cells related to immunity.

It is well known that eosinophils have a beneficial function by destroying parasites and mediating inflammation in asthma and allergies, but also a harmful function as they release cationic enzymes or pro-inflammatory mediators on activation. Indeed, the precise function of these cells in allergic inflammation and asthma remains controversial. There is no single cell type responsible for the immunopathology aspects of inflammation in asthma; other than eosinophils, T lymphocytes, mast cells and neutrophils, among others are implicated.

Eosinophilia

Mild eosinophilia is when the eosinophil count is between 500 and 1500 cells/µL, moderate from 1500 to 5000 cells/µL and severe when > 5000 cells/µL cells in peripheral blood. The term hypereosinophilic syndrome was coined by Hardy & Anderson in 1968 to define a pathological situation of severe prolonged eosinophilia of unknown etiology. A significant and sustained increase (> 5%) of circulating eosinophils is usually due to parasitic (severe eosinophilia) and allergic diseases (mild to moderate eosinophilia) and inflammation or to rarer clonal or idiopathic situations, which evolve with severe tissue damage as a result of eosinophilic infiltration.

The criteria established in the 1970s by Chusid et al. for the definition of idiopathic hypereosinophilic syndrome, which included eosinophilia ≥ 1500 cells/µL for more than six consecutive months or death before six months associated with signs and symptoms of hypereosinophilic disease, lack of evidence of parasitic infection, allergy or other causes of eosinophilia and signs and symptoms of organic disease related to hypereosinophilia became insufficient to describe all entities classified under the term eosinophilia that today are better understood thanks to advances in cell and molecular biology which provide the characterization of distinct diseases and that involve cells of myeloid and lymphoid lineages.

Reactive eosinophilia

Reactive eosinophilia is the most common type and is primarily due to an inflammatory reaction against parasitic infestation, allergic phenomena, skin lesions or even non-hematologic malignancies. Table 1 lists some of the different diseases which may cause reactive eosinophilia. In these situations, the eosinophilia is not clonal but is caused by an increase in cytokines, as previously described.

Eosinophilia secondary to parasitic infections is usually due to the biological intratissular cycle of helminths. The eosinophilic reaction is a result of contact between the parasite and the cells of the organism. The severity of the eosinophilia reflects the complexity of the parasitic cycle inside the organism: the more complex - passing through the liver (Fasciola hepatica), lung (Ascaris) and muscles (Trichina) - the worse the eosinophilia. When parasites are confined to the gut (whipworms, tapeworms), eosinophilia is transitory. Ectoparasites, such as myiasis, can also cause eosinophilia. Eosinophilia varies with the developmental stages of the parasite. In ascariasis, eosinophilia increases in the first three weeks reaching levels of from 1000 to 5000 eosinophils/µL and then slowly decreases in subsequent weeks. In infestations by filarial and visceral larva migrans, eosinophilia persists at high levels for prolonged periods. In cases of strongyloidiasis, the eosinophilia worsens at every cycle of re-infestation (autoinfestation).

The association between eosinophils and allergic diseases is very important. There is a correlation between bronchial hyperresponsiveness and peripheral eosinophilia in relation to asthma. Various drugs can trigger eosinophilia or induce different manifestations including DRESS syndrome, Stevens-Johnson syndrome and toxic epidermal necrolysis. DRESS syndrome (drug rash with eosinophilia and systemic symptoms), a reaction against antibiotics, antipsychotic agents, anti-hypertensive drugs and some other medications, is characterized by high fever, exanthem and facial swelling. Peripheral lymphadenopathy may occur and the patient may be at risk of death if this disease is associated with fulminant hepatitis or immunological interstitial nephropathy.

Pulmonary eosinophilia, characterized by lesions histologically identified as infiltrations of eosinophils, may be due to bronchopulmonary aspergillosis. Dermatological
diseases such as very extensive contact eczema or atopic dermatitis and bullous pemphigus may present with eosinophilia. Connective tissue diseases, such as lupus erythematosus, polyarteritis nodosa and scleroderma, can cause moderate eosinophilia as can chronic inflammatory conditions, such as ulcerative colitis and Crohn's disease. Paraneoplastic eosinophilia is also a well known phenomenon concomitant to the metastatic process. Radiotherapy can also trigger transitory eosinophilia.

**Clonal eosinophilia**

Clonal eosinophilia involves clonal aberrations. Identification of the clonality of eosinophils, as such, is difficult and thus evidence that the disease is part of a clonal disease of the bone marrow is accepted.

According to the classification of the World Health Organization, clonal eosinophilia may be defined according to the pathophysiological characteristics or associated with genetic-molecular changes such as: lymphoid and myeloid malignancies associated with eosinophilia and abnormalities of the platelet-derived growth factor receptor alpha (PDGFRα), platelet-derived growth factor receptor beta (PDGFRβ) or fibroblast growth factor receptor (FGFR1) which includes chronic eosinophilic leukemia (CEL) with FIP1L1/PDGFRα. Characterized by myelo- or lymphoproliferation, i.e., there may be a manifestation of CEL, acute myeloid leukemia or even lymphoma with persistent eosinophilia usually > 1500 cells/µL; organic infiltration by eosinophils or mast cells; rearrangements involving PDGFRα, PDGFRβ or FGFR1, absence of the Philadelphia chromosome or rearrangement of the fusion proteins BCR/ABL1 and < 20% blasts in bone marrow. CEL is more common in men, with peak incidence at around 40 years of age. The serum tryptase and vitamin B12 levels are elevated.

**PDGFRα rearrangements:** The most common is a deletion of the CHIC2 gene, located on chromosome 4q12[del(4)(q12q12)], resulting in the juxtaposition of FIP1L1/PDGFRα. This mutation occurs in multipotent progenitor cells and patients with this condition meet the criteria for CEL or systemic mastocytosis with eosinophilia. The interstitial microdeletion is best detected by fluorescence in situ hybridization (FISH), but can also be observed by reverse transcriptase polymerase chain reaction (RT-PCR). This fusion encodes the FIP1L1/PDGFRα protein with constitutive tyrosine kinase activity and is highly susceptible to inhibitors such as imatinib mesylate.

In CEL, there is autonomous and clonal proliferation of eosinophilic precursors, resulting in persistent myelo- or lymphoproliferation in the bone marrow, peripheral blood and tissues. Organ damage is a result of leukemic infiltration and release of cytokines.

About 10% of patients are diagnosed by chance
because they are asymptomatic. In others, symptoms such as fever, fatigue, cough, angioedema, muscle pain, rash and diarrhea are common.(11) Anemia, thrombocytopenia, mucosal ulceration, endomyocardial fibrosis and splenomegaly are also not rare.

The most important clinical finding is related to endomyocardial fibrosis with restrictive heart failure, which is irreversible. Heart disease triggered by the infiltration of eosinophils in the endocardium has a necrotic initial stage which lasts for an average of five weeks. In this stage the disease is not clinically recognized and generally goes unnoticed at echocardiography and angiography as ventricular wall thickening has not occurred. Sometimes, just endomyocardial biopsy of the right ventricular allows diagnosis in this stage. The second thrombotic stage, with an average duration of 10 months, involves the formation of mural thrombi with potential embolization to the brain. Finally, the third late fibrotic stage after two years is characterized by endomyocardial fibrosis resulting in mitral and/or tricuspid regurgitation which may require valve replacement. The clinical presentation of dyspnea, chest pain, congestive heart failure and cardiomegaly is evident as is T-wave inversion at electrocardiography.

Peripheral neuropathy, central nervous system dysfunction and pulmonary symptoms may also be present.

Most patients with PDGFRα rearrangements respond to imatinib mesylate at a dose of 100 mg/day. However, about one third of cases of hyper-eosinophilia who respond to imatinib do not present with the FIP1L1/PDGFRα rearrangement, indicating the existence of other as yet unknown clonal alterations.(12) Indeed, as well as FIP1L1, three other gene partners fuse to the PDGFRα to encode proteins with constitutive tyrosine kinase activity that drive clonal eosinophilic proliferation: BCR/PDGFRα, KIF5B/PDGFRα and DK5AP2/PDGFRα.(10)

Conventional G-band karyotyping of a bone marrow sample should be systematically performed when CEL is suspected as it allows the detection of the clonal abnormalities observed in this disease or other abnormalities that lead to the diagnosis, for example, the presence of the Philadelphia chromosome, which indicates that this is chronic myeloid leukemia (CML) with a component of eosinophilia.

Thanks to cytogenetic studies, the BCR/PDGFRα rearrangement with the translocation t(14;22)(q11;q11) was described in two patients with CEL(13) while the KIF5B/PDGFRα has been observed in a single case with a complex karyotype involving chromosomes 3, 4, 10 and perhaps 13(14) and the CDK5AP2/PDGFRα rearrangement in patients with CEL and ins(9;4)(q33;q12q25). Another similar insertion, the ins(9;4)(q43;4q12q31), with slightly different break points, was also described by Bacher et al.(16) as was the t(8;9)(p21;p24) or PCM1/JAK2 rearrangement.(16)

Translocations between 4q12 and other chromosomes were also found, including t(3;4)(p13;q12), t(4;7)(q11;q32) and t(4;7)(q11;p13), but the genes involved have not yet been identified.(17)

Trisomy 8 or i(17q) can confirm the diagnosis of CEL. However these anomalies occur in several other hematopoietic diseases and therefore the differential diagnosis should be carefully investigated.

Myeloid neoplasms with rearrangements involving the PDGFRβ are also observed, but less commonly than with PDGFRα. PDGFRβ, located on 5q33, and rearrangements that include this region are observed more frequently in chronic myelomonocytic leukemia (CMML) or chronic myeloproliferative diseases that evolve with eosinophilia, such as t(5;12)(q33;p13) or the ETV6/PDGFRβ rearrangement.

Rearrangements involving the short arm of chromosome 8, 8p11, FGFR1, the so-called 8p11 syndrome including t(8;13)(p11;q12), t(8;9)(p11;q32-q34) and t(6;8)(q27;p11), are characterized by the involvement of lymphoid/myeloid multipotent stem cells and although eosinophilia is one, usually short-term manifestation in the chronic phase, the disease progresses to acute myeloid leukemia, or lymphoblastic T-cell or occasionally B-cell leukemia/lymphoma within 12 to 24 months of diagnosis.(2,10) Translocations involving t(1;4)(q44;q12), t(5;11)(p15;q13), t(8;9)(p22;p23) or t(5;9)(q32;q33) have also been described.(18)

Some patients may present with KIT D816V-associated systemic mastocytosis in which case the disease differs from that of FIP1L1/PDGFRα as it is not always responsive to imatinib mesylate. Systemic mastocytosis may have an eosinophilic component. A mutation involving the JAK2 gene has been described in patients with chronic myeloproliferative diseases and, in this case, it may evolve with eosinophilia. The t(9;11)(p24;p13) with rearrangement of the JAK2/ETV6 genes also characterizes a hematologic disease with eosinophilia.(10)

Eosinophilia that evolves with hematologic malignancies

CEL can be distinguished from other clonal hematopoietic diseases in which eosinophilia is part of the neoplastic clone, such as chronic myeloid leukemia, chronic myeloproliferative diseases (polycythemia vera, myelofibrosis and essential thrombocythemia), myelodysplastic syndromes, acute myeloid leukemia, and in particular, myelomonocytic leukemia with inv(16) or the CBFβ/MYH11 rearrangement and acute myeloid leukemia with maturation t(8;21) or the ETO/AML1 (RUNX1/RUNX1T1) rearrangement.(9)

Unspecified chronic eosinophilic leukemia is characterized by persistent eosinophilia > 1500 cells/µL, organ lesions such as endomyocardial fibrosis, presence of blasts in peripheral blood or bone marrow (< 20%), absence of evidence of other chronic myeloproliferative neoplasms or myelodysplasia, and absence of the Philadelphia chromosome, (BCR/ABL1), PDGFRα, PDGFRβ, FGFR1, inv(16), t(16;16) or t(5;12).
Idiopathic hypereosinophilic syndrome, which is similar to the above but without the presence of blasts and without clonality, lasts for more than six months.\(^{(11)}\)

**The investigation of eosinophilia**

The investigation of a patient with hypereosinophilia should be systematic, since the accurate detection of CEL can hasten diagnosis and anticipate the initiation of therapy before cardiac injury becomes established. A careful clinical history is the first step to guide the investigation.

**Investigation of reactive eosinophilia**

Investigation of parasitic eosinophilia begins with epidemiological questionnaire about travel; did the patient visit endemic areas or not, swimming in rivers or lakes, contact with sand, eating habits, hobbies, contact with animals, insect bites, etc..

Depending on the suspected parasite, the investigation focuses on where evidence may be found, such as feces, urine, blood or skin. Stool testing is the easiest to be performed; three samples should be requested to increase the chances of detecting eggs or larvae. Sometimes serum antibodies need to be investigated.\(^{(15)}\) Other laboratory investigations may include serology for schistosomiasis, filariasis, strongyloidiasis and toxocariasis. Additionally, focal findings may require specific tests such as urine, cerebrospinal fluid or tissue biopsy.\(^{(2)}\)

A detailed history of drug use, even the so-called alternatives, may identify a possible medication etiology.

**Investigation of clonal eosinophilia**

Clinical symptoms or signs suggestive of tissue damage mediated by eosinophils require careful physical examination in particular in respect to skin lesions, lymphadenopathy, hepatosplenomegaly, and changes in cardiovascular and pulmonary systems.

In the event of cardiopulmonary disease, a chest x-ray, echocardiography, pulmonary function test and troponin T test are needed.

A peripheral blood smear may only help to some extent, as it does not differentiate between clonal and reactive eosinophilic. However, the presence of blasts can lead to investigations of CEL, AML or ALL; the observation of an increased number of lymphocytes or anomalous lymphocytes suggests possible lymphoma or an aberrant T cell population; monocytesis requires an investigation of PDGFR\(\alpha\) rearrangements; thrombocytopenia and/or anemia may indicate the presence of a hematopoietic disease, while dysplasia may suggest myelodysplastic syndrome.\(^{(2,10,19)}\) A myeloproliferative disorder is suspected if vitamin B12 and serum tryptase levels are elevated, although tryptase levels are also high in systemic mastocytosis.\(^{(15,19)}\) Knowledge of the IgE concentration is needed and, when elevated, the patient may benefit from corticosteroids.

Immunophenotyping of lymphocytes may reveal the presence of an abnormal T cell population and may demonstrate clonality.

An evaluation of the bone marrow should be made as soon as a reactive cause has been rejected or when a hematopoietic disease is suspected.

Analyses of the FIP1L1/PDGFR\(\alpha\), PDGFR\(\beta\) and BCR/ABL gene rearrangements in peripheral blood are important as are the investigation of cytogenetic abnormalities in bone marrow. Clonal aberrations, which are observed in some CEL cases,\(^{(16)}\) can be detected by karyotyping. This technique can also direct other investigations. Table 2 summarizes the necessary examinations.

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**Treatment**

Reactive eosinophilia disappears with treatment of the underlying disease. Imatinib mesylate must be introduced immediately in cases with the FIP1L1/PDGFR\(\alpha\) rearrangement. Start with a dose of 100 mg/day. The dose should be increased to 400 mg/day if there is no response or if residual disease is identified. In general, patients achieve hematologic remission with complete disappearance of symptoms and signs within about thirty days. Although the drug is easily tolerated, some patients develop cardiogenic shock during the first week of treatment with reduced left ventricle ejection fraction. Prednisone (1 mg/kg/day) prescribed before initiating with imatinib is recommended for patients who present with
alterations in troponin T or in echocardiogram tests. Currently, it is believed that a low maintenance dose of imatinib should be used indefinitely if molecular remission is achieved.\(^{10,19}\) Oligospermia may be a side effect of treatment.\(^2\) Cases with PDGFRα rearrangements may also benefit from imatinib mesylate at a dose of 400 mg/day.

Asymptomatic patients with HES should remain under observation, but should be regularly evaluated by echocardiography and for troponin T alterations. Those with organ damage should receive corticosteroids (1mg/kg/day). If no response, chemotherapy with hydroxycarbamide (hydroxyurea) should be initiated. Vincristine, chlorambucil, cyclophosphamide, etoposide, cyclosporine, and 2-chlorodeoxyadenosine (2CDA) can be used as second line drugs. Alpha-interferon can induce long-term hematologic and cytogenetic responses. Remission has been considered with symptom improvement, reduction in splenomegaly and the disappearance of skin lesions, and heart and thromboembolic complications.\(^{17,19}\)

As a final option, the use of higher doses of imatinib mesylate for cases without evidence of PDGFRα and PDGFRβ rearrangements may eventually yield results, even partial.\(^{10}\)

The role of allogeneic bone marrow transplantation is not well established because, despite the success in selected cases, acute and late complications are frequent.\(^{10,19}\)

Advances in heart surgery prolong survival of patients with end-stage heart disease involving endomyocardial fibrosis, mural thrombus and valvular insufficiency. Replacement of the mitral and tricuspid valves or endomyocardial surgery for the disease in the third stage may improve heart function.\(^{17}\)

Leukapheresis may provide a temporary reduction of the high eosinophil counts but with no long term effect.

The use of anticoagulation and antiplatelet agents has shown variable success in preventing recurrent thromboembolism.\(^{17}\)

Resumo

A eosinofilia é frequente na prática clínica, principalmente quando os valores estão entre 500 e 1000 eosinófilos/μL, e indica a presença de doença parasitária, alérgica ou reação a medicamentos. Afora essas situações, a eosinofilia pode ser devida a doenças do tecido conjuntivo, infecções e, mais raramente, a doença hematológica maligna ou a tumores sólidos. Os critérios estabelecidos na década de 70 para a definição para a definição da síndrome hipereosinofílica idiopática se tornaram insuficientes para caracterizar todas as entidades albergadas sob o termo eosinofilia e, hoje, melhor compreendidas graças aos avanços na biologia celular e molecular, que proporcionaram a caracterização de doenças distintas e que envolvem células das linhagens mieloide e linfóide. Nesse contexto, as eosinofilia sanguíneas são categorizadas como reacionais, clonais e idiopáticas (SHE). O advento de terapia antitirosinoinibina (a exemplo do mesilato de imatinibe), eficaz para os casos com o rearranjo gênico FIP1L1/PDGFRA, também abriu novas perspectivas para o controle ideal da leucemia eosinofílica crônica.

Daí a importância do diagnóstico preciso e rápido para a indicação terapêutica ideal, antes que os se instalem as complicações orgânicas, em especial cardiácas, que são irreversíveis. O presente manuscrito objetiva rever as situações de eosinofilia sanguínea e oferecer uma atualização da investigação diagnóstica e terapêutica.

Descritores: Leucemia eosinofílica crônica; Receptor tipo alfa para fator de crescimento derivado de plaquetas; Receptor tipo beta para fator de crescimento derivado de plaquetas; Receptores de fator de crescimento de fibroblastos; Proteínas de fásao bcr-abl; Síndrome hipereosinofílica

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