

Guidelines for the diagnosis and treatment of eosinophilia.

Final version, April 2009.

The Nordic study group on myeloproliferative disorders (NMPD) decided in 2007 to write a proposal for guidelines on hypereosinophilic states, based on already existing national and international recommendations. The aim has been to write a document that can be used in all Nordic countries for clinical as well as educational purposes. Therefore, numerous illustrations are given with references, including on-line linking from the document to relevant websites, which may all be used, some with permissions as stated at the end of the document in a separate section.

Hypereosinophilia in haematology is one of the very rare conditions, and solid evidence based on large protocols or randomized trials are lacking. This proposal for guidelines tend to give current best evidence and interpretation in making decisions, based upon the development reported in diagnostic work-up and therapy.

The guidelines are written for health professionals with a speciality or interest in haematology. They incorporate the new diagnostic criteria established by the World Health Organization 2008. We plan further updates on a bi-annual basis, and it is therefore recommended that colleagues use the on-line version, rather than to print and copy paper versions of the document, and to send comments for improvements.

Writing committee:

- Ole Weis Bjerrum, Copenhagen e-mail: ole.weis.bjerrum @ rh.regionh.dk
- Tarja-Terttu Pelliniemi, Turku e-mail: tarja-terttu.pelliniemi @ utu.fi
- Hans Wadenvik, Gothenburg e-mail: hans.wadenvik @ medic.gu.se

for the Nordic MPD Study Group, April 2009.

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Introduction

The eosinophilic granulocyte – the eosinophil – was originally described as the acidophilic leukocyte by Paul Ehrlich in 1879 based on work with synthetic and selective aniline stains. The name was given due to the coarse orange / red granulae, clearly visible by light microscopy in the cytoplasm, when stained with eosin. Eos is the Greek goddess of the dawn (Fig. 1). The physiology and function of eosinophils, as well as its pathophysiological role has been of almost ever growing interest during the last century. The eosinophil is still a very popular and intriguing subject. A bibliographic search on “pubmed.org” for “eosinophil” results in more than 22.000 articles and more than 3000 articles for hypereosinophilic syndrome.

This guideline gives an update on the fascinating cell and intends to bring the eosinophil in focus in a clinical spectrum of very variable disorders, where the cell is either reactive or the cause of disease itself. The pathophysiological clarification has improved during recent years, and even though much still remains to be discovered, the present algorithms for diagnosis and treatment may be updated and revised. In addition, the guideline serves as a review and material may be used for educational purposes and spread of information.

Figure 1.



Paul Ehrlich, 1854 - 1915
Nobel Prize Winner 1908



Eos, daughter of Hyperion and Thelia,
Sister of Helios (god of Sun) and Selene (Moon)

The eosinophil – development, structure and function.

Eosinophilopoiesis only takes place in the bone marrow. As mobile granulocytes equipped with a vast armamentarium the eosinophils circulates in blood stream, being distributed to almost all organs where they spend the major part of their lifetime, acting as metabolically and functionally highly active and interactive cells with specialized functions. Eosinophil granulocytes are normally involved in host defence against parasites, modulators of innate and adaptive immunity, inflammatory responses and tissue repair (1,2,3).

Three classes of transcription factors: PU.1, C/EBP and GATA-1 are involved in the lineage commitment and differentiation of eosinophils. Pluripotent CD34+ stem cells in the red bone marrow gives rise to a hybrid precursor cell, which later differentiates to either the basophil or the eosinophil granulocyte (4). GATA-1, which is a zinc family member (5), is considered to be the most important transcription factor for eosinophilic lineage specification, because of the observation of an absolute eosinopenia in a viable, mouse model without a high-affinity palindromic GATA-binding site, normally mediating positive autoregulation in the GATA-1 promotor (6).

Three cytokines: IL-3, IL-5 and GM-CSF are involved in eosinophil development and maturation. The three haemopoietic growth signalling polypeptides are encoded by genes linked on chromosome 5, produced by T-cells and bind to receptors, which share a common beta chain (7). IL-5 is considered to be the most important eosinophilopoietin (8), because of the observation of relative eosinopenia in mice with an IL-5 gene deletion following allergen challenge (9) and absolute eosinophilia in homozygote transgenic mice with aberrant expression of the IL5-gene and elevated IL-5 levels in blood (10).

Three different agents: IL-5, eotaxins and antigens / allergens are involved in eosinophilic trafficking from bone marrow to various tissues following selective chemoattractant gradients. The agents may act independently of each other, and others may act locally. The major population of eosinophils, also compared to the bone marrow, is normally located in the lamina propria in all segments of the gastrointestinal tract. The localization occurs early in development, and is independent on viable intestinal microbiological flora. Normally, eosinophils are also found in some tissues associated with the external surface, including lower respiratory tract and the genitourinary tract, spleen and lymph nodes, but very scarce or not at all in most other tissues including skin, brain and various glands in the adult (1,11,12, 13).

Eosinophils pass through the same maturation stages as neutrophils during development. They comprise some 3 % of the total bone marrow population, and are present in equal amounts as eosinophilic promyelocytes, myelocytes and mature eosinophilic granulocytes, respectively, and are decreasing in size during development, which terminate in the band and segmented forms. The mature eosinophil is morphologically distinct with a typically bilobated, Pelger-like nucleus. In the bone marrow mature eosinophils may be recognized at the late myeloblast or early promyelocyte stage. In the blood eosinophils are 12 – 17 μm , with characteristic ovoid granules, which seems to occupy most of the cytoplasm. Eosinophils are therefore normally almost double the size of erythrocytes and a little larger on average than neutrophils. The nucleus may be more segmented upon activation of the cell – or due to vitamin B₁₂ deficiency (3,14). Eosinophils are rather fragile and susceptible to damage by preparation of blood smear, and their morphology with respect to density and cytoplasmic content may often differ in patients with eosinophilia, i.e. showing vacuoles or altered granula size (12).

Normally, eosinophils qualitatively represent 1 – 3 % of the circulating leukocytes and quantitatively below $0.5 \times 10^9 / \text{l}$. The half-life in blood is estimated to be 18-25 hours, but may differ among species and be prolonged in patients with eosinophilia. Number and circulation time is a function of bone marrow production, tissue egress after cell tethering and transmigration of endothelium following rolling along the border and emigration through postcapillary venules. The lifetime in tissues is unknown, but cells may be cultured for as long as three weeks in the presence of T-cell conditioned medium (3,15,16).

The ultrastructure of mature eosinophils in light and in electron microscopy is normally dominated by the bilobed nucleus and the coarse or large (eosinophilic) granula. The nucleus is mostly exentric localized and may have prominent nucleoli. The Golgi complex and rough endoplasmic reticulum diminish considerably after granula formation has ceased after the myelocyte stage. Mitochondria are present also in the mature cell, which contains primary, secondary and small granules, glycogen particles, lipid bodies, and vesicotubular structures (fig. 2), (3, 12, 14).

The primary granula are scarce, making up some 5 % of the granula and store an enzyme, which form the Charcot-Leyden crystals in tissues or fluids, considered as a hallmark of eosinophil activity in inflammatory reactions, such as asthma or parasitic infections. The crystals are bipyramidal, may be up to 50 μm , consisting of a pair of hexagonal pyramids joined at their bases, and are typically seen as signs of eosinophil activation. It appears slender and pointed at both ends in the light microscope, and was first observed in 1851 by von Zenker, and later further described by Charcot and Robin (1853) and von Leyden (3,12). The enzyme was for many years considered to be a weak lysophospholipase, but nowell detailed analysis has clarified that the Charcot-Leyden crystal protein has a near structural familiarity with the galectin super-family and has therefore been designated galectin-10. It comprises up to 10 % of total eosinophil protein, and has no lysophospholipase activity. Instead, it binds and interacts with eosinophil lysophospholipase in vitro and known inhibitors of this lipolytic activity (17). The precise role or function has not really been settled later, but some data indicate that changes in the expressions of galectin-10 is important for myeloid cell differentiation into specific lineages, being upregulated in both eosinophil and neutrophil differentiations of HL-60 cells (18).

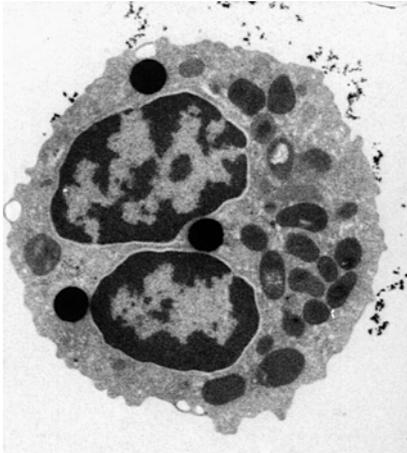
The secondary (or specific) granules are elliptical in shape posses an electron-dense non-crystalloid matrix that provides storage for highly charged basic proteins like eosinophilic cationic protein (ECP), eosinophil-derived neurotoxin (END) and eosinophil-derived peroxidase (EPO), which accounts for \sim 40 % of granula protein by mass. The crystalloid core provides storage for the major basic protein (MBP), and this protein accounts for \sim 30 % of granula proteins stored by eosinophils (fig. 2) (3,12).

The small granules increase in number with maturity and are less electron dense in the tissue eosinophils. In particular two enzymes: arylsulphatase B and acid phosphatase are related to this granule population.

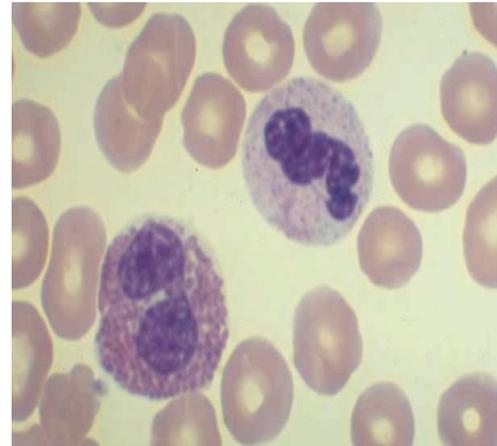
Lipid bodies are round cytoplasmic storage compartments, not surrounded by a membrane and serve as sites of arachidonic acid storage and metabolism and are considered to be the principal site of eicosanoid production in eosinophils (3,19). Their numbers increase upon activation of eosinophils (fig. 2).

Vesicle extrusion and *vesicotubular structures* possible represents a specific degranulation activity in eosinophils, besides the classical exocytosis, and regulated by soluble attachment protein receptors, controlling a protein assembly-disassembly pathways, involved in differential release of eosinophil granule content (3,12, 20) (see section – “degranulation” page 14).

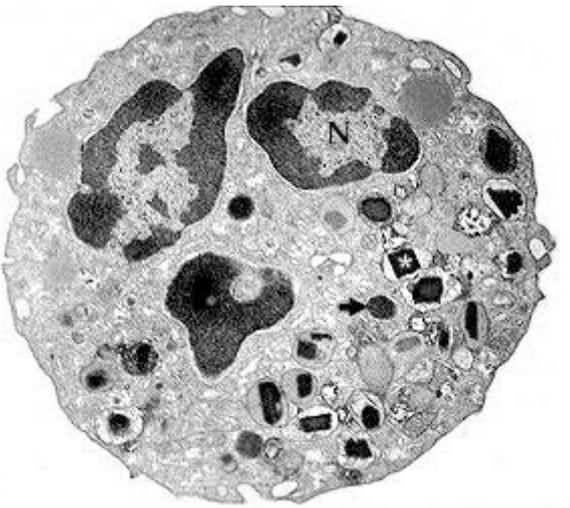
Figure 2.



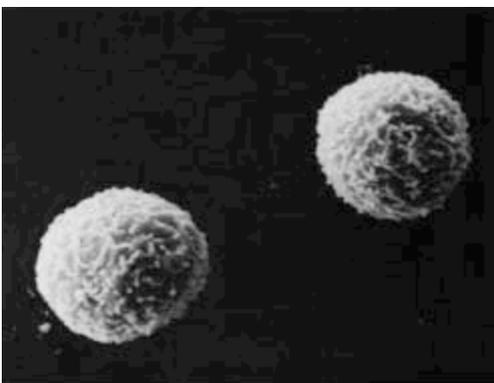
EM of human eosinophil
 Ref.: dr. Per Venge
[Uppsala University](http://www.uppsala.se)



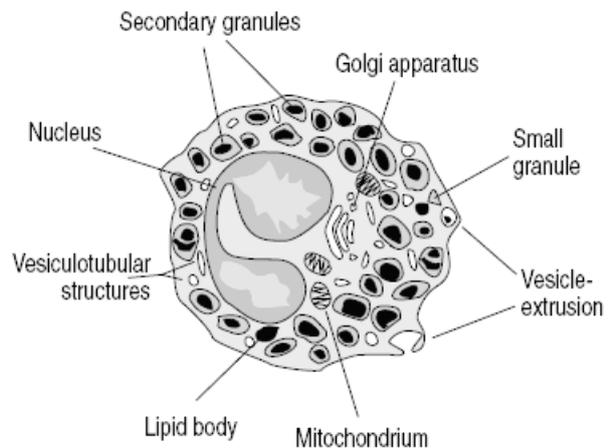
A band eosinophil and mature neutrophil in human blood, x 100
 Ref.: [Bloodline](http://www.bloodline.com)



Ultrastructure of eosinophil in electron micrograph shows 3 portions of the bilobed nucleus (N), a primary granule (dark arrow), and multiple specific granules (* marks one) that stain red with the H&E stain (see above). Primary granules contain Charcot-Leyden crystal protein. The specific granules have a dark crystalloid core and a paler, non-crystallized matrix. The dark core contains major basic protein, and the matrix contains eosinophil peroxidase, neurotoxin, and cationic protein. Ref.: Martha L. Warnock & Marcia J. McCovin
[University of California](http://www.universityofcalifornia.edu)



Scanning electron micrograph of human resting eosinophils (12)



Schematic drawing of eosinophil granulocyte showing characteristic organelles (12)

The eosinophil – constituents and function.

Eosinophils are equipped with many preformed, enzymatic or non-enzymatic protein-constituents, some of which are highly charged and the eosinophils are able to produce many substances in order to fulfil its function. Some of the factors are specific for eosinophils, like major basic protein and eosinophil-derived neurotoxin. Other factors are also engaged in the function of other cells, like the production of reactive oxygen species. This armamentarium is used in a controlled process, although unleashing many of the constituents in normal tissue will cause deleterious (side-)effects on local cells (toxicity or cell-death) or functions (bronchoconstriction or diarrhoea). Table 1 gives an overview of the most essential factors related to eosinophil activity, but many more factors (i.e. gelatinase) are localized in the intracellular compartments (1,3,12,21).

Being an interactive, mobile granulocyte the eosinophils are highly responsive and communicating by membrane-bound surface antigens and receptors. Even though more may be known than illustrated (fig. 3), the schematic representation illustrates the ability of eosinophils – like other leukocytes – to communicate at the exterior surface.

Figure 3.

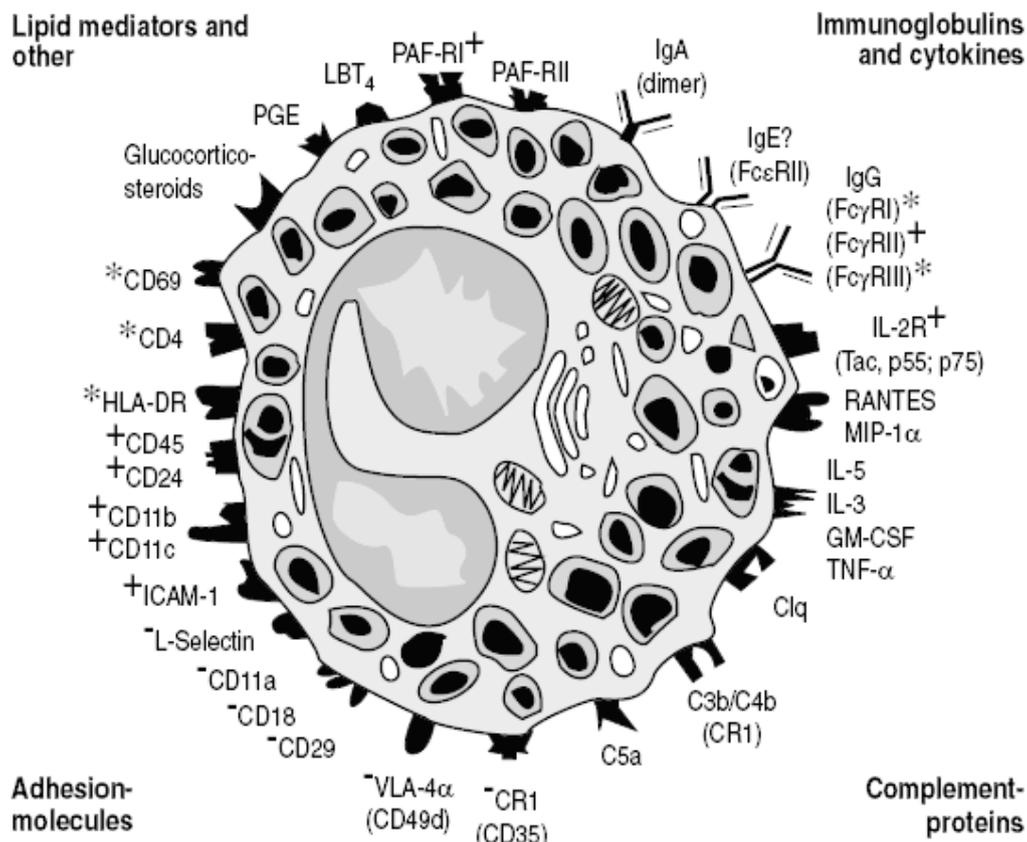


Fig. 3. Schematic representation of surface antigens identified on eosinophils. Some of the antigens are upregulated (+), downregulated (-) or induced () following recruitment from the circulation into tissue. The existence of an IgE receptor on eosinophils has been a matter of (?), but both so-called high- and low-FcεR (I & II) are present (3). CD: cluster differentiation; CR: complement receptor; FcγR: IgE-receptor; IgG-receptor; GM-CSF: granulocyte/macrophage colony-stimulating factor; HLA-DR: human leucocyte antigen-DR; ICAM: intercellular adhesion molecule-1; Ig: immunoglobulin; IL: interleukin; LTB₄: leukotriene β₄; MIP: macrophage inflammatory protein; PAF: platelet-activating factor; PAF-R1: high-affinity PAF receptor; PAF-RII: low-affinity PAF receptor; PGE: prostaglandin E; RANTES: regulated upon activation in normal T-cells expressed and secreted; TNF: tumour necrosis factor; VLA: very late activation antigen (12).

Table 1. Eosinophil constituents

COMPONENT	CHEMICAL CHARACTER	MW kDa	GENE on #	LOCATION	BIOLOGICAL FUNCTION	REF.
Major basic protein	Non-enzyme protein, lectin	14	11	Core specific granule	Cytotoxicity against helminths, tumor / normal cells, proinflammatory incl activation mast cells, neutralize heparin	22
Eosinophil-derived neurotoxin (EDN)	RNase 2, RNA cleavage	19	14	Matrix specific granules	Anti-respiratory virus infectivity, selective neuronal damage, bind to Toll-receptors	23
Eosinophil cationic protein (ECP)	RNase 3, RNA cleavage	21	14	Matrix specific granules	Anti-respiratory syncytial virus activity, affects coagulation factors, helmintotoxic	24
Eosinophil peroxidase (EPO)	Heme-peroxidase ~ 2/3 homologous myelo-peroxidase	66	17	Matrix specific granules	Generates reactive oxygen species, in particular O ₂ ⁻ and H ₂ O ₂ more efficacious than neutrophils, toxic for large microorganisms	25
Arylsulphatase B	Chondrotinsulphatase	60	5	Matrix small granules	Hydrolyse proteo- and glycosaminoglycans; deficient in mucopolysaccharidosis type VI	26
β-glucuronidase	Acid phosphatase 2	75 (unprocessed)	7	Matrix small granules	Hydrolyse glycosides, released in conc.-dependent response upon activation in inflammatory sites	27
Collagenase	Matrixmetallo-proteinase	70 - 90	11	Matrix specific granules	Degrading extracellular components, including bone and cartilage	28
Arachidonic acid	Polyunsaturad fatty acid	305 g/mol	-	Lipid bodies	Second messenger, precursor in production of eicosanoids, i.e. prostaglandins, leukotriens a.o.	29
Charcot-Leyden protein	Galectin 10	17	19	Primary granules	Interact in vitro and possibly inhibits lysophospholipase activity. Precise function is not known	17

Data from ref (1,3,12,21)

The eosinophil will respond in a – at least in some circumstances (27) – dose-dependent, and therefore controlled fashion to exterior stimuli. A large number of de novo generated mediators may in this way contribute, or influence, the response of the cell, in addition to the substances released by degranulation. It may be important to perceive the activity of eosinophils as regulated, and not just “all or none” – and in this way similar to the neutrophil granulocyte (30).

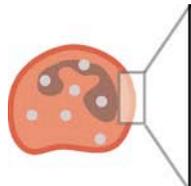
Table 2 gives an overview of small-molecule mediators and signalling substances which has been demonstrated to be generated and released from human eosinophils. Many of the substances do account for the harmful symptoms experienced in case of disorders involving eosinophilic hyperactivity, whereas some may actually seem to reduce an inflammatory response with eosinophil activity (12). The table is not extensive. They are all shortlived – possibly therefore mostly acting locally. Proteins with specific functions, but also potential deleterious effects on bystander cells and tissues were outlined in table 1.

Table 2.

Substance Abbreviation / full name	Chemical nature	Biological action
VIP / vasoactive intest peptide	neuropeptide	Broncho- and vasodilation diarrhoea
SP / substance P	neuropeptide	Vomiting, eczema, vasodilation
IL-3 / Interleukin-3	cytokine	Proinflammatory, reduce apoptosis
GM-CSF/gran.mono-col.stim.fact.	cytokine	Myeloid growth factor, priming
TGF-β1 / transform growth-factor	cytokine	T-cell / cytokine inhibition
O ₂ ⁻ superoxide, H ₂ O ₂ hydrogen peroxide, O ₂ [·] singlet oxygen	Oxygen metabolites	Toxic for microorganisms, all mammalian cells
PGE ₂ / Prostaglandin E2	lipid	Vasodilation, mucuous secretion, immunemodulation
PGD ₂ / Prostaglandin D2	lipid	Bronchoconstriction, plat aggregate
PGF _{2α} / Prostaglandin F2α	lipid	Bronchoconstriction , plat aggregate
TxA ₂ / Tromboxane A2	lipid	Broncho- and vasoconstriction
LTC ₄ / Leukotriene C4	lipid	Broncho- and vasoconstriction
PAF / platelet activating factor	lipid	Bronco- and vaso-constriction, plat aggregate, mucuous secretion, activate neutro-, eosinophils, mast cells

As illustrated in fig. 3, the eosinophil has a large number of surface signalling opportunities, and a (more recent) comprehensive list is given by Rothenberg and Hogan (1), which is reproduced here (fig. 4). In addition a link is given for the access electronically to an updated version of the CD-nomenclature and the ability to read details on every CD-molecule with regard to function, gene, molecular weight and production. Data have been obtained by flow cytometry and interpretations of observations in response to specific stimuli.

Figure 4.



Immunoglobulin receptors and members of the immunoglobulin superfamily				
CD4	CD47	CD58	CD101	
CD16	CD48	CD66	HLA class 1	
CD32	CD50	CD89	HLA-DR	
CD33	CD54	CD100	FcεR1	
Cytokine receptors				
CD25	CD120	CD131	TGFβR	
CD116	CD123	CD213		
CD117	CD124	IL-9R		
CD119	CD125	IL-13Rα1		
Adhesion molecules				
CD11a	CD18	CD49f	CD162	
CD11b	CD29	CD62L	CD174	
CD11c	CD44	CD156	αd integrin	
CD15	CD49d	CD162	β7 integrin	
Chemokine, complement, and other chemotactic factors				
CD35	CD191	PAFR	CystLT2R	
CD88	CD192	LTB ₄ R	fMLPR	
CD182	CD193	C3aR	CRTH2	
CD183	CD196	CystLT1R	Histamine 4R	
Enzymes				
CD13	CD46	PAR-2		
CD45	CD55			
CD45RB	CD59			
CD45RO	CD87			
Apoptosis, signaling, and others				
CD9	CD52	CD82	CD139	Siglec-10
CD17	CD53	CD86	CD148	LIR1
CD24	CD63	CD92	CD149	LIR2
CD28	CD65	CD95	CD151	LIR3
CD37	CD69	CD97	CD153	LIR7
CD39	CD71	CD98	CD161	TLR7
CD43	CD76	CD99	CD165	TLR8
CD48	CD81	CD137	Siglec-8	

<http://mpr.nci.nih.gov/prow/>

Fig. 4. Abbreviations and details. PAR Protease-Activated Receptor 2; CRTH2 G-protein coupled receptor 44 ~ CD294; fmlpR formyl-leucyl-methionyl receptor; siglec-8 and siglec-10 Sialic-Acid binding Ig-like lectin 8 and 10 ~ CD 328 and CD330 (?), both members of immunoglobulin superfamily expressed on the surface of cells of innate immunessystem; LIR leukocyte immunoglobulin-like receptors, no 1 ~ CD 85; TLR7 Toll-like receptor ~ CD 287 and TLR8 ~ CD288. You may also try to just “google” each CDnumber. It appears that there is no real CD-specificity for eosinophils, which therefore are difficult to identify by flow cytometry alone.

Activation of eosinophils may thus initiate a number of actions. Figure 5 illustrates an overview of these actions. It is important to note that eosinophils also may function as antigen presenting cells. Both viral and parasitic antigens may be processed and presented to T-cells which may be affected by cytokines, in addition to regulation, and in particular activation, of neutrophils and mast cells (1,31,32).

Figure 5.

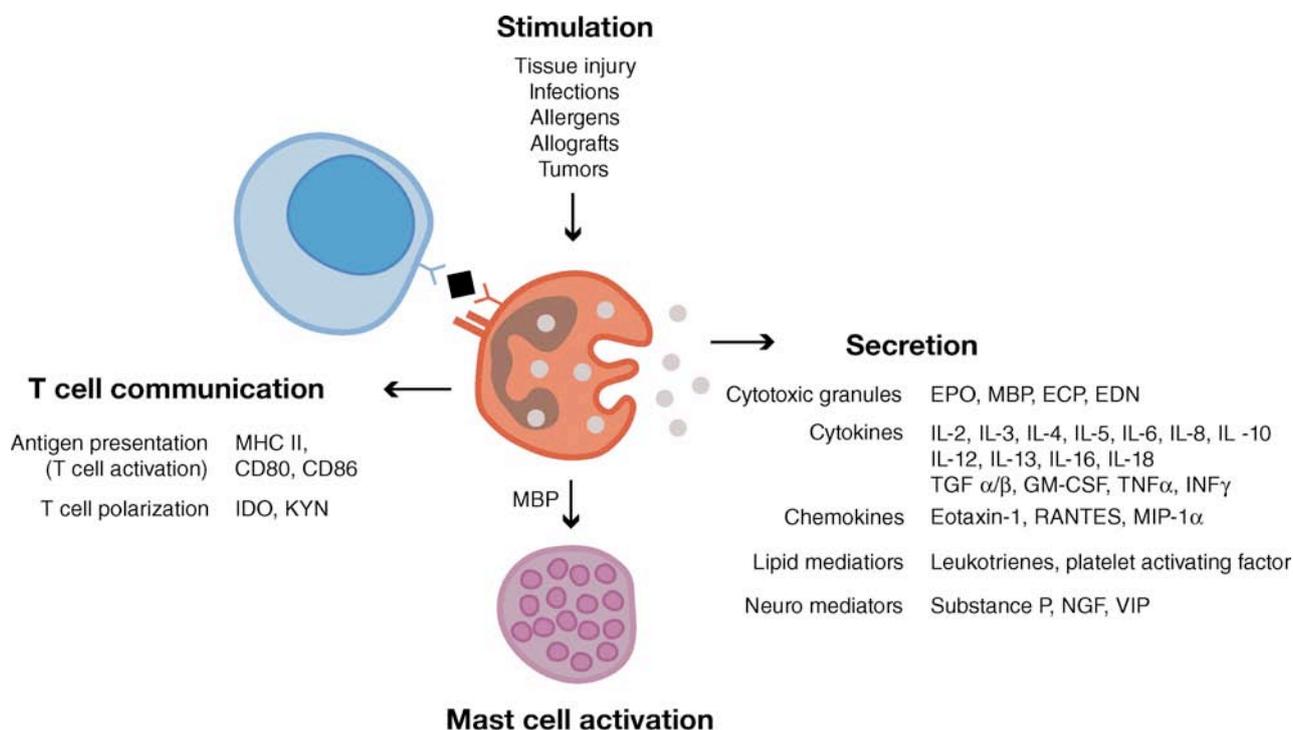


Fig. 5. Schematic diagram of an eosinophil and its multifunctional effects. Eosinophils are bilobed granulocytes with eosinophilic staining secondary granules. The secondary granules contain four primary cationic proteins, designated eosinophil peroxidase (EPO), major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN). All four proteins are cytotoxic molecules; in addition, ECP and EDN are ribonucleases. Eosinophils respond to diverse stimuli, including nonspecific tissue injury, infections, allografts, allergens, and tumors. In addition to releasing their preformed cationic proteins, eosinophils can also release a variety of cytokines, chemokines, lipid mediators, and neuromodulators. Eosinophils directly communicate with T cells and mast cells in a bidirectional manner. Eosinophils activate T cells by serving as APCs, and eosinophil-derived MBP is a mast cell secretagogue. Eosinophils can also regulate T cell polarization through synthesis of indoleamine 2,3-dioxygenase (IDO), an enzyme involved in oxidative metabolism of tryptophan, catalyzing the conversion of tryptophan to kynurenines (KYN), a regulator of Th1/Th2 balance (1).

The eosinophil – activation.

It is in particular IL-5 which mobilizes eosinophils from the bone marrow to the blood. IL-5 exposure however also leads to IL-5R α down regulation on the mature eosinophil as in a negative feedback system (33). The receptor for the CC (C-C motif) chemokine eotaxin-1

(a small cytokine produced by epithelium and a powerful eosinophil chemo-attractant) CCR3 (CD193) is however, constitutively expressed on both CD34+ progenitors and on lineage-committed eosinophils, and the expression is further upregulated by inflammatory stimuli. Various adhesion molecules on endothelial cells and the corresponding receptor on eosinophil surface. P-selectin glycoprotein is responsible for a tethering of eosinophils to the endothelium, and the rolling process in addition to stimuli such as eotaxin, PAF and others prime the eosinophil more and cause adhesion (1,3). Eosinophils transmigrate out of the vessel wall towards chemokine-gradients, constituted in particular by eotaxins (34) and IL-5, but also by eotaxins, leukotrienes, anaphylatoxins and others (2,3). Chemotaxis is performed by actin filament contraction and continual cell cytoskeletal reorganization.

Figure 6.

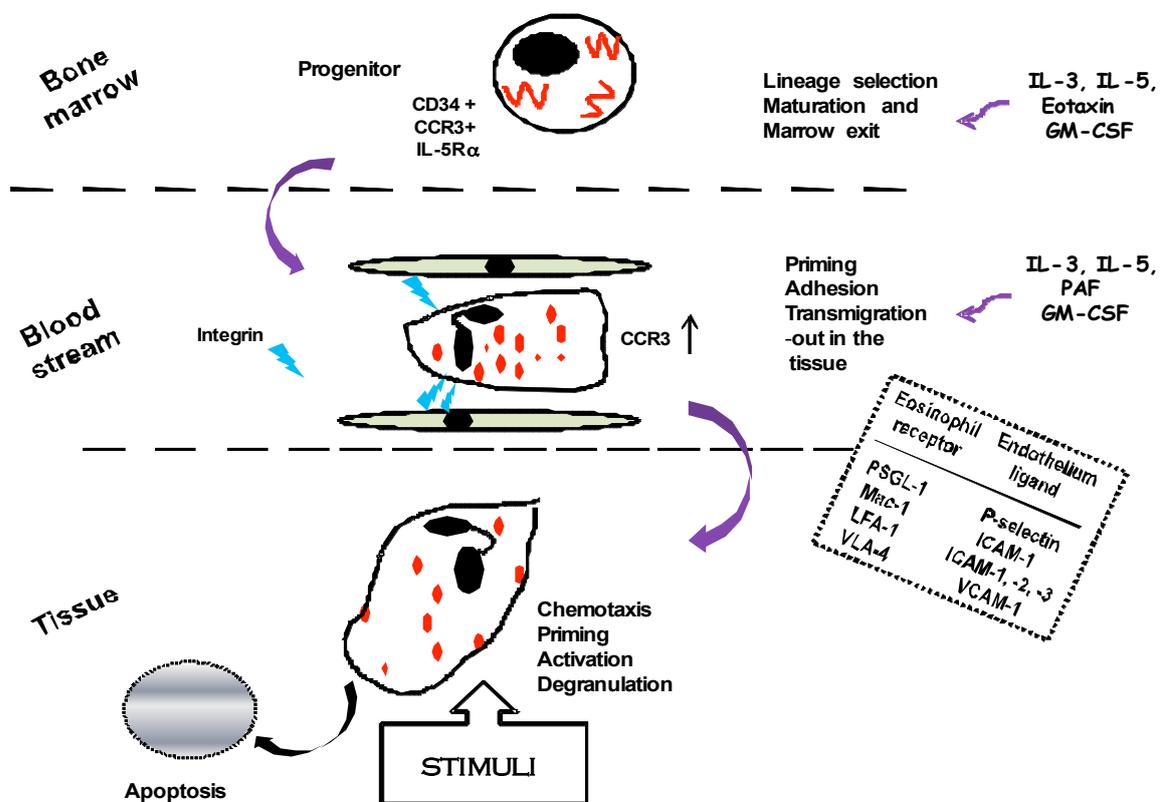


Fig 6. Illustration of selective eosinophil recruitment and extravasation. Eosinophilopoiesis is critically dependent on interleukin 5. Bone marrow progenitors (CD34+) display lineage commitment through up-regulation of the interleukin 5 receptor and can then undergo proliferation, maturation and differentiation. The upregulation of CCR3, particularly at the lamellipodium, facilitates chemotaxis into tissues in response to eotaxin. Furthermore, activation of several adhesion molecules on both the eosinophil and endothelium occurs in response to cytokines and allows a rapid and selective cell recruitment from the circulation. The primed eosinophil can now be fully activated and participate in host defense, immune modulation, and tissue repair (modified after (3)). IL interleukin; GM-CSF granulocyte-macrophage colony-stimulating factor; PAF platelet activating factor; CCR3 chemokine receptor 3; PSGL-1 P-selectin glycol-protein; LFA-1 lymphocyte function associated antigen; ICAM intercellular adhesion molecule; VLA-4 very late activation antigen-4; VCAM vascular cell adhesion molecule; Mac-1 macrophage -1 antigen or complement receptor 3 – an integrin. Fibronectin and laminin interacts with the cell surface in the tissue.

The processes involved in eosinophilia are multifactorial, although not more complex than other innate immune cells, like neutrophils. To summarize physiological stimuli for eosinophilia, these two illustrations (fig. 7 and 8) are useful (35).

Figure 7.

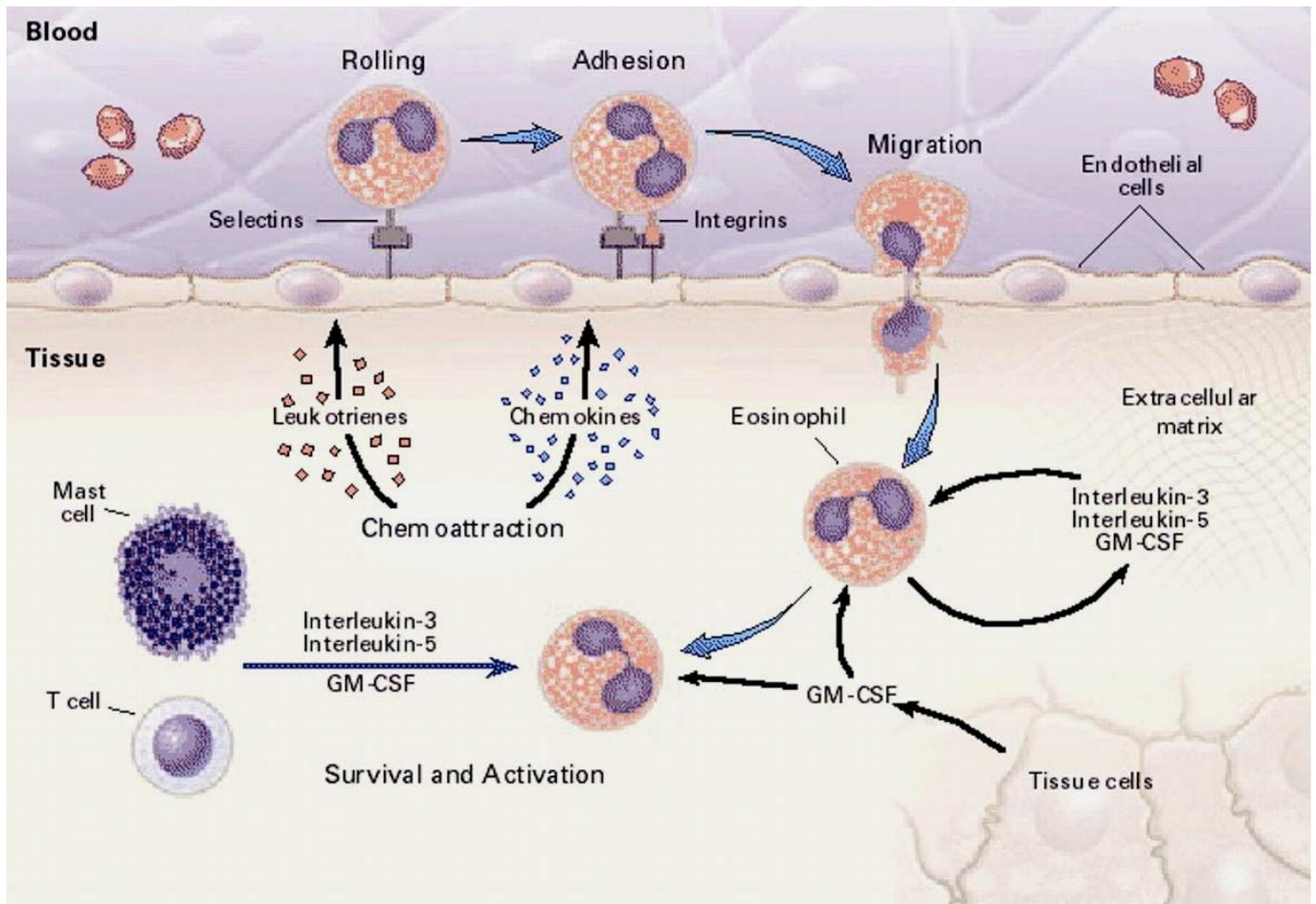


Fig. 7. Mature eosinophils in the peripheral blood adhere to endothelial cells through the interaction of selectins and integrins (CD18 and very late antigen 4) with endothelial receptors for these molecules. On exposure to chemoattractant mediators, eosinophils undergo diapedesis between endothelial cells and migrate into the tissues. The accumulation of eosinophils is regulated by the generation of survival and activation factors (interleukin-3, interleukin-5, and granulocyte–macrophage colony-stimulating factor [GM-CSF]) by T cells and probably mast cells. In response to extracellular matrix components, eosinophils themselves can also generate the cytokines that prolong their survival (35).

Figure 8.

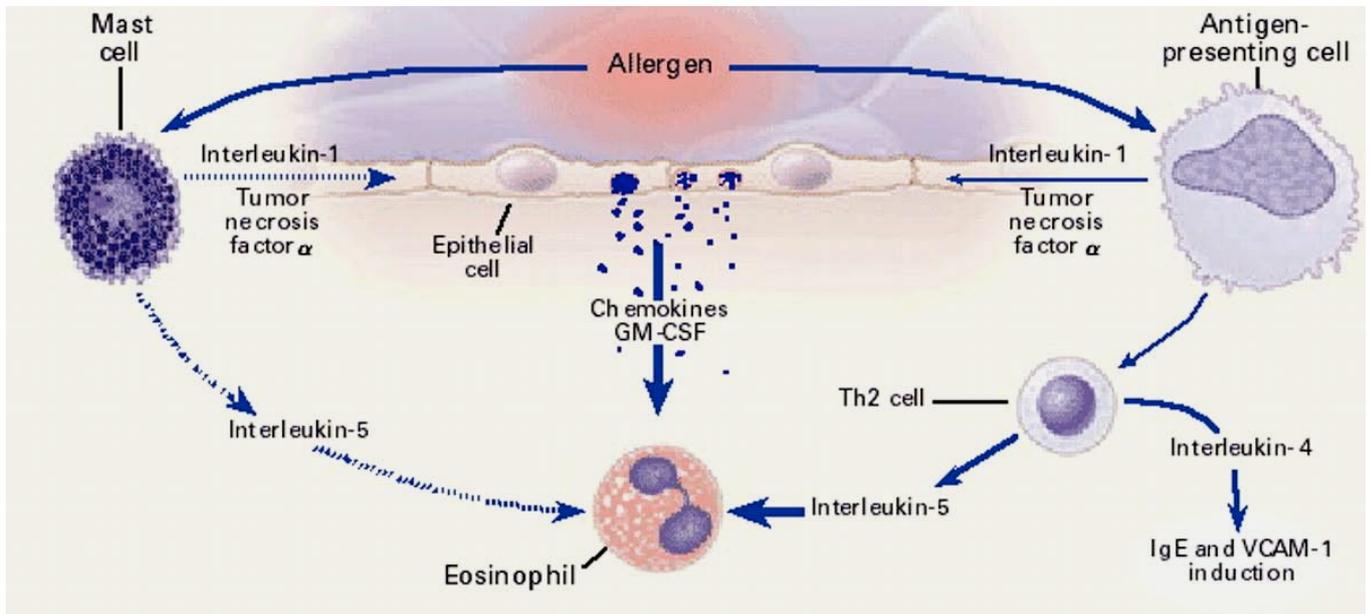
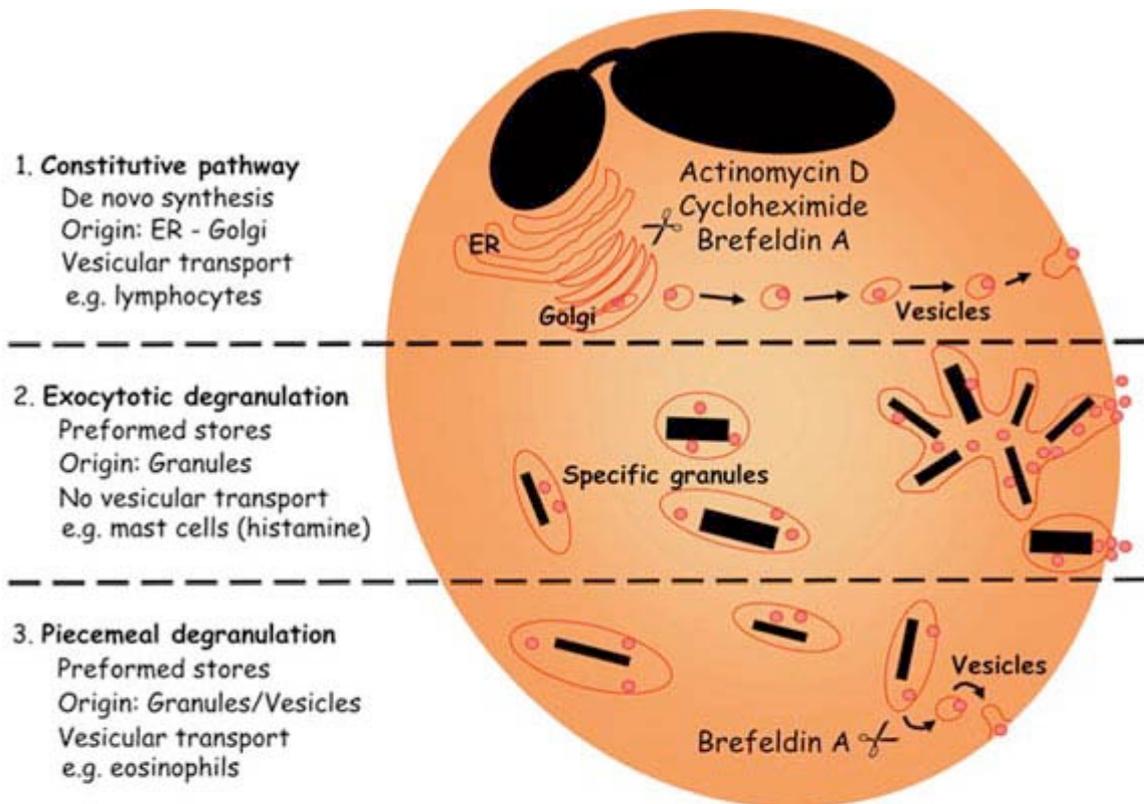


Fig. 8. After allergen exposure in sensitized subjects, two non-mutually exclusive pathways are thought to lead to the accumulation of eosinophils. In one pathway, allergen exposure results in the cross-linking of IgE receptors on mast cells and basophils and the immediate release of inflammatory mediators (histamine, prostaglandin, and leukotrienes). Mast cells then generate proinflammatory cytokines (e.g., interleukin-1 and tumor necrosis factor α) that induce respiratory epithelial cells to produce eosinophil-directed cytokines (e.g., granulocyte-macrophage colony-stimulating factor [GM-CSF]) and chemokines. In the other pathway, allergen is initially recognized by antigen-presenting cells such as dendritic cells and subsequently presented to type 2 helper T lymphocytes (Th2 cells). In contrast to mast cells, which do not appear to be required for the accumulation of eosinophils (indicated by the hatched arrows), Th2 cells are necessary for their accumulation (indicated by the solid arrows). These cells regulate allergic reactions by generating the eosinophil hematopoietin (interleukin-5) as well as interleukin-4, which induces IgE and vascular-cell adhesion molecule 1 (VCAM-1) (35).

The eosinophil – degranulation.

Eosinophil granulocytes has the ability to exocytosis in three different ways, each of them in a highly controlled fashion, and the ability separates eosinophils from neutrophils. Figure 9 illustrates the three ways, which are: 1. (top panel) classical exocytosis by vesicles, which may be blocked by actinomycin, cycloheximide or Brefeldin a, and which also occur in lymphocytes; 2. (middle panel) compound exocytosis, in which the granules intracellularly, in the cytoplasm, undergo granule-granule fusion to form a uniform membraneous compartment. In this way a very focused exocytosis will take place on the surface, which is considered to be particularly effective against heminths. This exocytosis is suitable for preformed constituents, like granula content; 3. (bottom panel) piecemeal degranulation, in which contents of specific granules are transferred into small vesicles, which fuse with the plasma membrane and exocytose the content (3,35).

Figure 9.



The “piecemeal degranulation” in fig. 9 permits eosinophils to a differential release of granula contents, and leave behind partially empty membrane bound compartments, and it appears to be the major secretory pathway in eosinophils. It is regulated by soluble N-ethylmaleimide-sensitive factors (SNAREs) (3,20,36). Brefeldin A is a lactine antibiotic ([link](#)) which interfere with anterograde proteintransport.

The eosinophil – apoptosis.

The lifespan in tissues is – like other granulocytes - not known, but estimates have been given for days to weeks (15,16). It has been reported that absence of various growth factors / eosinopietins are necessary for the cell to stay alive, but also to up-regulate Bcl-2 antiapoptotic activity or apoptosis may be induced by surface Siglec-8 (fig. 4, legend) (3). Recently, the transcriptome profile and functional properties of eosinophils was analyzed by gene expression. The results showed that it may be anticipated that large, active nucleoli in human eosinophils may represent marked activity of DNA repair systems, in

contrast to neutrophils. It may be that eosinophils have the propensity and functional property of non-terminally differentiated cells, such as monocytes (37).

The eosinophil – paraclinical.

Eosinophils have normal functions as outlined in the previous sections, and they may increase in numbers in blood or accumulate in tissues due to relevant stimuli, primarily infections. This hypereosinophilic state may thus be a physiological phenomenon and cause reactive or secondary eosinophilia. However, the number of eosinophils may also increase secondary or as a reaction to a benign or malignant, haematological or non-haematological disorder, primarily due to cytokine-driven eosinophilia. Autonomous clonal proliferations of eosinophils (neoplasms associated with rearrangements of platelet derived growth factor receptors, PDGFR, or fibroblasts growth factor receptors, FGFR1 or chronic eosinophilic leukaemia with other clonal markers) are very rare diseases. Finally, the cause of persisting symptomatic hypereosinophilia may remain unclear and then carries the name “true” idiopathic hypereosinophilic syndrome (HES). HES thus remains a diagnosis of exclusion, until more specific causes are reported or the possible clonal nature of disease is revealed. In this first section, the diagnostic work-up is described in detail.

Investigation of eosinophilia

Blood eosinophil count above the upper reference limit (in adults $\geq 0.5 \times 10^9/L$) is the hallmark of eosinophilia. Eosinophilia is regarded as mild if blood eosinophil count is $0.5 - 1.5 \times 10^9/L$, moderate if the count is $> 1.5 - 5.0 \times 10^9/L$ and severe if the count is $> 5.0 \times 10^9/L$.

The most common cause of eosinophilia in the western world is allergy and in the developing countries invasive parasite infections.

Eosinophilia can be divided in three different categories: (1) reactive eosinophilia, (2) clonal eosinophilia and (3) idiopathic hypereosinophilic syndrome (HES). The aim in the diagnostic work-up of eosinophilia is to (1) identify the clonal eosinophilias with PDGFRA or PDGFRB rearrangements (good response to tyrosine kinase inhibitors), (2) identify the 8p11 syndrome (dismal prognosis without allogeneic stem cell transplantation), (3) identify idiopathic HES before any major organ damage (especially irreversible cardiac fibrosis) and (4) consider the possibility of the several causes of reactive eosinophilia. The following review on differential diagnosis, diagnostic criteria, laboratory investigations and algorithms is based on various articles (38 – 49).

Reactive eosinophilia

Reactive eosinophilia is a nonclonal disorder where the production of eosinophils is increased as a response to exogenous stimuli (cytokines and growth factors like interleukin-5, interleukin-3 and granulocyte-macrophage colony stimulation factor mainly produced by T-helper cells). The causes of reactive eosinophilia are listed in table 3 and further illustrated in fig. 10 and fig. 12.

Table 3. Causes of reactive eosinophilia.

-
1. Infections
 - a. parasites, especially tissue invasive parasites, like filariasis, ascariasis, strongyloidiasis, trichinosis, toxocarisis, schistosomiasis, hookworm (*Achylostoma*, *Necator*)
 - b. recovery from a bacterial infection
 2. Allergy
 - a. atopic diseases: asthma bronciale, allergic rhinitis, atopic eczema, urticaria
 - b. food allergy
 3. Drugs
any drug, but especially seen with antibiotics, sulphonamides, antirheumatics, anticonvulsants and allopurinol, DRESS syndrome (see also page 27)
 4. Lung diseases
 - a. acute and chronic idiopathic eosinophilic pneumonia (Loefflers diasese see page 27)
 - b. Churg-Strauss syndrome (tissue eosinophilia, vasculitis and granulomas), see page 27
 - c. allergic bronchopulmonary aspergillosis
 5. Eosinophil-associated gastrointestinal disorders
 - a. primary or secondary eosinophilic esophagitis
 - b. primary or secondary gastroenteritis, including celiac disease
 - c. primary or secondary colitis, including inflammatory bowel disease
 6. Other causes of autoimmune, inflammatory or toxic origin
 - a. connective tissue diseases (scleroderma, polyarteritis nodosa, LED etc.)
 - b. eosinophilic fasciitis
 - c. Kimura disease (follicular hyperplasia, eosinophilic infiltrates, proliferation of venules)
 - d. sarcoidosis
 - e. chronic pancreatitis
 - f. eosinophilia-myalgia syndrome
 - g. toxic oil syndrome
 7. Malignant diseases
 - a. lymphoproliferative diseases where eosinophils are not part of the malignant clone (Hodgkins disease, non-Hodgkin lymphomas especially T-cell lymphomas)
 - b. carcinomas (especially metastatic diseases)
 8. Clonal expansion of immunophenotypically aberrant T cells without overt lymphoproliferative disease (T-cell hypereosinophilic syndrome i.e. T-HES, see also table 9)

9. Endocrine hypofunctions (i.e. Addison disease)

Idiopathic hypereosinophilic syndrome

The traditional criteria for idiopathic hypereosinophilic syndrome consist of persistent eosinophilia ($\geq 1.5 \times 10^9/L$ for > 6 months) and target organ damage. The current WHO-criteria for chronic eosinophilic leukaemia and idiopathic hypereosinophilic syndrome are shown in table 4 (38).

Table 4. Diagnosis of chronic eosinophilic leukaemia (CEL) and idiopathic hypereosinophilic syndrome (HES), modified from WHO-criteria (2008)

Required: Persistent eosinophilia $\geq 1.5 \times 10^9/L$ in blood, increased numbers of bone marrow eosinophilia, and myeloblasts $< 20\%$ in blood or marrow.

1. Exclude all causes of reactive eosinophilia secondary to:
 - a. Allergy
 - b. Parasitic disease
 - c. Infectious disease
 - d. Pulmonary diseases (hypersensitivity pneumonitis, Loeffler's etc.)
 - e. Collagen vascular disease
2. Exclude all neoplastic disorders with secondary, reactive eosinophilia:
 - a. T cell lymphomas, including mycosis fungoides, Sezary syndrome
 - b. Hodgkin lymphoma
 - c. Acute lymphoblastic leukaemia/lymphoma
3. Exclude other neoplastic disorders in which eosinophils are part of the neoplastic clone:
 - a. Chronic myelogenous leukaemia (Ph chromosome or *BCR/ABL* fusion gene positive) and other myeloproliferative neoplasms or myelodysplastic/myeloproliferative neoplasms
 - b. Neoplasms with *t(5;12)(q31-35;p13)* or other rearrangements of *PDGFRB*
 - c. Neoplasms with *FIP1L1-PDGFR* fusion gene or other rearrangements of *PDGFRA*
 - d. Neoplasms with rearrangements of *FGFR1*
 - e. Acute myeloid leukaemia, including those with *inv(16)(p13q22)*, *t(16;16)(p13;q22)*
4. Exclude T cell population with aberrant phenotype and abnormal cytokine production
5. If there is a clonal cytogenetic or molecular genetic abnormality, or blast cells are more than 2% in the peripheral blood ($>2\%$) or more than 5% in the bone marrow, diagnose chronic eosinophilic leukaemia, not otherwise specified (CEL, NOS).*
6. If there is no demonstrable disease that could cause eosinophilia, no abnormal T-cell population, and no evidence of a clonal myeloid disorder, diagnose idiopathic hypereosinophilic syndrome (when organ-involvement) or idiopathic hypereosinophilia (without organ dysfunction)

* The ending NOS excludes clonal eosinophils with recurrent gene rearrangements.

Clonal eosinophilia

Eosinophilia is regarded as a clonal disease when there is a positive test for clonality (usually cytogenetic or molecular genetic marker) or it is very likely that eosinophils are part of otherwise diagnosed myeloid malignancy. The improved methods to reveal the clonal origin of hypereosinophilia have shifted the balance towards chronic eosinophilic leukaemia and decreased the diagnoses of idiopathic hypereosinophilic syndrome. Moreover, the new 2008 World Health Organization criteria for the diagnosis and classification of myeloproliferative neoplasms have moved towards predominantly genetic classification system with disease specific molecular markers. Thus, myeloid neoplasms with molecularly characterized eosinophilia (i.e. FIP1L1-PDGFRFA fusion gene) previously classified under CEL/HES are now assembled into a new category of their own. The myeloid disorders associated with eosinophilia can according to these guidelines be divided to molecularly defined and clinicopathologically defined diseases as shown in table 5 (38). The classification is further discussed in fig. 11 (page 28) and fig.12 (page 30).

Table 5. Classification of myeloid neoplasms that can be associated with eosinophilia

1. Acute myeloid leukaemia
 2. Chronic myeloid disorders
 - a. Molecularly defined
 - i. BCR/ABL+ chronic myeloid leukaemia
 - ii. PDGFRA-rearranged eosinophilic disorder
 - iii. PDGFRB-rearranged eosinophilic disorder
 - iv. KIT-mutated systemic mastocytosis
 - v. 8p11 syndrome (FGFR1 rearrangements)
 - b. Clinicopathologically assigned
 - i. Chronic myeloproliferative neoplasms (including chronic eosinophilic leukaemia not otherwise specified (NOS) and mastocytosis)
 - ii. Myelodysplastic syndromes
 - iii. Myelodysplastic / myeloproliferative syndromes
-

Laboratory investigations and imaging studies in unexplained persistent eosinophilia

The diagnostic work-up of unexplained persistent eosinophilia relies on clinical history (especially allergy, drugs, and travel history) as well as symptoms and signs which may point to a reactive eosinophilia or a specific organ related eosinophilic syndrome. The investigations that are indicated are listed in table 6 and can be focused on the basis of clinical suspicion.

Table 6. Investigations in unexplained persistent hypereosinophilia.

-
1. Blood counts and morphology to assayed for
 - a. severity of eosinophilia and
 - b. abnormalities in other blood cells which might point to clonal eosinophilia
 2. Serum total immunoglobulin E, and specific tests for allergy (skin prick tests and allergen specific IgE tests) if indicated.
 3. Investigation of parasitic infections
 - a. stool parasites
 - b. serological tests for suspected parasitic infections like schistosomiasis, filariasis, toxocariasis ect.
 - c. specific studies according to focal findings (imaging studies, spinal fluid, blood smear, tissue biopsy ect.)
 4. Bone marrow aspiration and biopsy
 5. Cytogenetic analysis on bone marrow aspirate
 6. Molecular analysis on peripheral blood cells for *FIP1L1-PDGFR* fusion gene
 7. Serum tryptase, serum erythropoietin and JAK2 mutation analysis
 8. Investigation of blood T-cells (immunophenotyping or molecular analysis) for possible cytokine-driven eosinophilia (T-HES)
 9. Imaging studies (CT scan, ultrasound) of chest and abdomen for underlying lymphoma or non-haematological malignancy.
 10. Serum troponin and ECG / eccocardiogram
 11. Pulmonary function tests and broncoalveolar lavage if clinically indicated
 12. Serum interleukin 5 concentration (if available)
-

The diagnostic work-up of unexplained eosinophilia can be divided in two categories: (1) the definitive tests to diagnose clonal eosinophilia which should be performed directly if the suspicion of primary haematological disease is high and the risk of organ failure is imminent and (2) investigation of reactive causes of eosinophilia (with follow-up to confirm persistency).

The definitive tests for clonal eosinophilia include:

1. **Full blood count.** Diagnosis of persistent hypereosinophilia and suspicion of chronic eosinophilic leukaemia arises from the full blood counts including white cell differential. Absolute eosinophil count should be $\geq 1.5 \times 10^9/L$. In otherwise unexplained cases follow the counts for 6 months to confirm the persistence of eosinophilia.
2. **Blood cell morphology.** Examine the blood film for morphological abnormalities that may indicate other haematological diseases, like increase in monocyte count seen in chronic myelomonocytic leukaemia with eosinophilia, circulating blasts seen in acute leukaemia, dysplastic changes in neutrophils seen in myelodysplastic syndrome, atypic chronic myeloid leukaemia or chronic myelomonocytic leukaemia, abnormal lymphocytes or raised amount of lymphocytes seen in chronic lymphoproliferative diseases, leuko-erythroblastic changes seen in myelofibrosis or disorders with bone marrow infiltration etc. Abnormalities in the morphology of eosinophils have been described in hypereosinophilic syndrome and chronic eosinophilic leukaemia, like enlarged cell size, sparse granulation with clear areas of cytoplasm and nuclear hypo- or hypersegmentation, but they may also be seen in reactive conditions.
3. **Bone marrow aspiration and biopsy.** Examine bone marrow morphology to confirm excess of eosinophils and to exclude other haematological disorder or bone marrow infiltration, which may be associated with eosinophilia. If the proportion of myeloid blasts is $>20\%$, proceed with the differential diagnostics of acute leukaemia. In case of less prominent increase of blasts (5 – 19%), proceed with differential diagnostics of myeloproliferative and myelodysplastic disorders. Bone marrow biopsy should be stain for reticulin fibers (myelofibrosis) and tryptase (mast cell disorders, where also CD117 staining or analysis by flow cytometry may be helpful). Immunocytochemistry for lymphoid malignancies should be analyzed when indicated by the morphological findings.
4. **Cytogenetics on bone marrow aspirates.** Examine the karyotype on bone marrow aspirates (G-banding of at least 20 bone marrow metaphases). The translocations between chromosome 5q33 (PDGFRB) and one of its several partner chromosomes (38c, 41) as well as chromosome 8p11 (FGRFR1) and one of its partners can be detected by conventional cytogenetics and can be confirmed with relevant FISH-probes. Intrachromosomal deletion of chromosome 4 resulting in FIP1L1-PDGFRB fusion gene is cytogenetically occult, but can be demonstrated by interphase FISH with probes flanking the deleted part of chromosome 4 as well as upstream and downstream sequences. Samples should be tested for FIP1L1-PDGFRB fusion gene either with FISH or with molecular methods (see below).
5. **Molecular analysis for FIP1L1-PDGFRB fusion gene.** Peripheral blood sample is suitable for RT-PCR analysis of FIP1L1-PDGFRB fusion gene. The advantage of RT-PCR over FISH is the greater sensitivity of the method which allows the

detection of the fusion gene even if the proportion of positive cells is rather low. RT-PCR can also be used for the detection of minimal residual disease during treatment with kinase inhibitors.

6. **Molecular analysis for *Wilms tumor (WT)* gene.** RT-PCR on bone marrow or peripheral blood for WT1 has recently been reported to discriminate secondary or reactive eosinophilia from idiopathic hypereosinophilia (HES) and CEL, both of which shows significantly higher levels. The transcript amount in bone marrow correlated with measurements in blood, and was representative for response during treatment of HES and CEL. However, cases with HES were diagnosed according to the WHO 2001 criteria. In the novel 2008 classification, no clonal abnormality must be demonstrated in idiopathic HES.
7. **Additional tests.** Serum markers for chronic myeloproliferative disorders include elevated tryptase and decreased erythropoetin as well as demonstration of JAK2 mutation in blood cells.

Table 7. Examples of chromosomal rearrangements and fusion genes reported with PDGFRB (right) and FGRFR1 (left column) in conditions with eosinophilia.

Cytogenetics	Fusion gene	Cytogenetics	Fusion gene
t(1;3;5)(p36;p21;q33)	WDR48-PDGFRB	t(8;13)(p11;q12)	ZNF198-FGFR1
der(1) or (5) t(1;5)	GPIAP1-PDGFRB	t(8;9)(p11;q33)	CEP110-FGFR1
t(1;5)(q21;q33)	TPM3-PDGFRB	t(6;8)(q27;p11-12)	FGFR1OP1-FGFR1
t(1;5)(q23;q33)	PDE4DIP-PDGFRB	t(8;22)(p11;q11)	BCR-FGFR1
t(5;10)(q33;q21)	CCDC6-PDGFRB	t(7;8)(q34;p11)	TRIM24-FGFR1
t(5;12)(q31-33;q24)	GIT2-PDGFRB	t(8;17)(p11;q23)	MYO18A-FGFR1
t(5;14)(q33;q32)	KIAA1509-PDGFRB	t(8;19)(p12;q13.3)	HERVK-FGFR1
t(5;15)(q33;q22)	TP53BP1-PDGFRB	ins(12;8)(p11;p11p22)	FGFR1OP2-FGFR1

Data from (48c, 41)

Tests that should be performed to diagnose (or exclude) reactive eosinophilia and / or demonstrate target organ dysfunction

1. **Tests for allergy.** As allergic conditions are the most common cause of reactive eosinophilia, examine serum total IgE. If there is any suspicion of specific allergic condition, examine skin prick tests and/or allergen specific IgE-tests.
2. **Tests for parasitic infections.** Examine repeated stool specimen for the diagnostics of parasite infections. Specimen of duodenal aspirate, sputum, spinal fluid, urine, blood film and tissue biopsy may also be examined if clinically indicated.

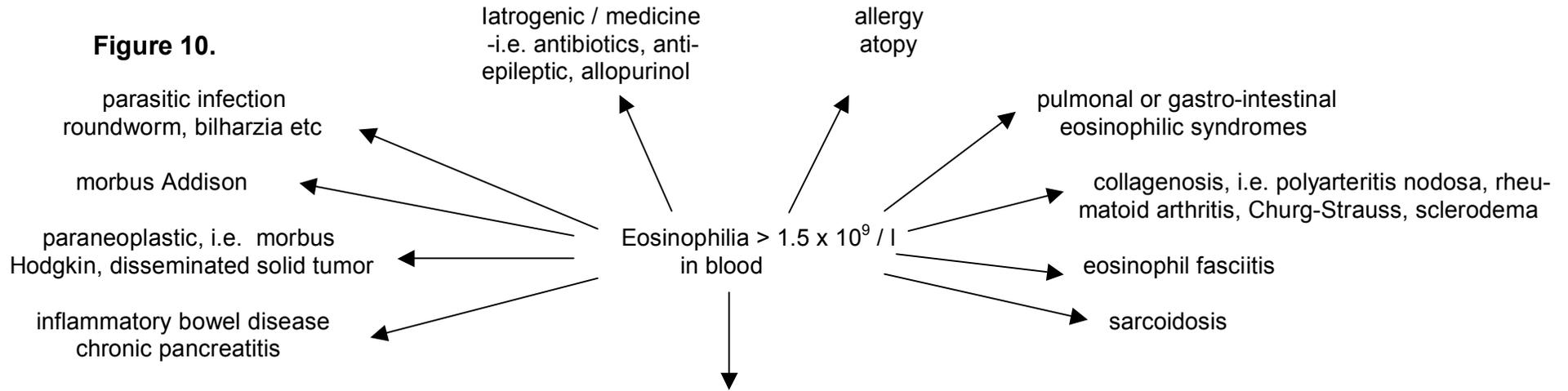
For suspected parasitic infections like schistosomiasis, filariasis, toxocariasis etc. examine serological blood tests.

3. **Tests for abnormal T cells in peripheral blood.** Consider the possibility of abnormal T cells as the cause of reactive eosinophilia (condition which is sometimes called T-HES). Analyse the immunophenotype of blood T-cells with multiparameter flow cytometry. T cells with aberrant phenotype (CD3+/4-/8- or CD3-/4+) indicates reactive eosinophilia (T-HES). These aberrant T cells may or may not be clonal and can be further characterised by molecular methods (rearrangement of T cell receptor gene). Serum IL-5 measurement can also be helpful and is recommended if it is available.
4. **Tests for eosinophilia-mediated organ damage.** The evaluation of persistent eosinophilia should include tests for eosinophil-mediated organ damage, especially cardiac and pulmonary problems. These investigations include ECG, echocardiogram, serum troponin concentration, chest X-ray, pulmonary function tests. Also bronchoalveolar lavage may be performed, if clinically indicated.
5. **Imaging studies.** Imaging studies (CT scan, ultrasound) of chest and abdomen should be performed for possible underlying lymphoma or non-haematological malignancy.

Handling of patients with eosinophilia, irrespective of the degree of eosinophilia – although more urgent the higher the count – therefore imply a classic clinical approach. Obtaining a sufficient and thorough anamnesis, focusing on travelling, infectious symptoms, autoimmune disease, drugs, itching and eczema or systemic symptoms like nightsweats or weight loss may be clues to the diagnosis. Some clinical observations like splenomegaly or lymphoma, type of rash, affection of organfunction in respiration, circulation or neurology may contribute to a possible diagnosis or in a combined fashion give a rational examination by relevant tests (above).

The diagnostic / clinical algorithm when meeting the patient with eosinophilia may be illustrated in fig. 10. This algorithm for diagnostic work-up of persistent eosinophilia is modified from (38, 43) and combined with every other differential diagnosis in eosinophilia given in this guideline. In addition therapy is briefly stated for eosinophilia due to clonal bone marrow disorders and hypereosinophilia (for details, see treatment section, page 30). The use of monoclonal antibody, against interleukin-5 or CD52, in the treatment of eosinophilia is not settled until more clinical experience (page 33).

Figure 10.



If none of the differential-diagnosis above is demonstrated following anamnesis, clinical examination and diagnostic tests (microbiological, bloodsamples, tissuebiopsies, imaging) then measure s-tryptase and perform bone marrow examination including morphology, FISH, RT-PCR, flow cytometry and / or karyotype for clonality and examine for

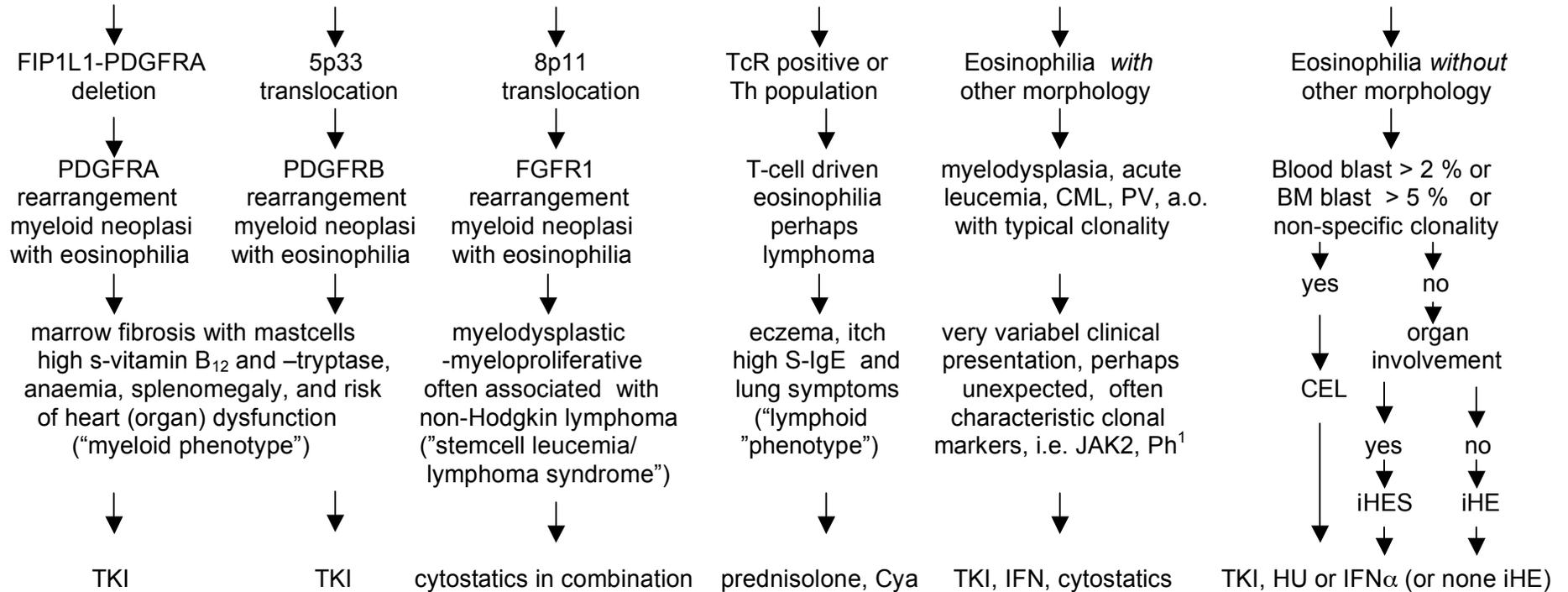


Fig. 10: Algorithm for eosinophilia. abbreviations and comments. BM bone marrow; CEL chronic eosinophilic leukaemia; CML chronic myeloid leukaemia; CyA cyclosporine A; FGFR fibroblast growth factor receptor; iHE idiopathic hypereosinophilia; iHES idiopathic hypereosinophilic syndrome; HU hydroxyurea; IFN α interferon- α (2a or 2b); PB peripheral blood; PDGFR platelet derived growth factor A or B; PV polycythemia vera; TKI tyrosine kinase inhibitor; s serum. When blasts exceeds 20 % in blood or bone marrow: acute leukaemia.

The eosinophil – clinical.

There are no valid data on the frequency of eosinophilia. It must also be very different in different parts of the world due to the many different causes (table 3,4,5 and fig. 10). Furthermore, the incidence of eosinophilia must be anticipated to be very different and depend upon individual hospitals and departments, routine in using differential counts etc.

Even though the incidence of eosinophilia *per se* may not be that interesting, it is probably most common that a laboratory results – the differential count or examination of a slide of peripheral blood – attract attention to the presence of an increased number of eosinophils, than it is the clinical symptoms primarily. Hypereosinophilia may then be an important diagnostic clue. However, the combination of eosinophilia and symptoms caused by eosinophils is very important to relate and realize, in order to make the correct diagnostic work-up (last section) and give the proper treatment (next section). It is generally accepted that there is no strict correlation between the degree of eosinophilia and the risk of organ-involvement (tables 1 & 2). Some clinical entities have been recognized for many years and named as specific conditions, and they will briefly be described below. The technical progress in diagnostic tools, in particular in genetic analysis, has increased the number of specific, clonal haemopoietic diseases where eosinophilia has a specific cause ((table 5 and fig. 10). These disorders are very important to identify because of the availability of targeted therapy.

Clinical manifestations of eosinophilia differ very much between patients. In patients with reactive eosinophilia (table 3), the primary disease or cause also may contribute to the clinical presentation. In patients with primary, clonal haematological disorders, some patients may be asymptomatic and the clinical presentation otherwise very heterogeneously – and any comorbidity may also interact irrespective of the cause of eosinophilia. Most organ-specific symptoms may be caused by the eosinophilia, however the frequency in each specific disease is difficult to state due to the limited patient-material. More than one organ may be involved, including the bone marrow affection in primary eosinophilia. Some organs, however are more frequently affected in hypereosinophilic conditions, and the involvement is not possible to differentiate from other, much more common causes for insufficiency or symptoms (table 8). Sometimes, tissue biopsies must be performed to demonstrate infiltration of eosinophils. The tissues most vulnerable and most frequently affected by eosinophil products or penetration are the heart (~ 60 %), the nervous system, the skin and the respiratory and gastrointestinal tract (~ 20 %) in that order. The symptoms may be life-threatening and are major sources of morbidity in eosinophilia. Any symptom may be experienced in eosinophilia, not just the one more common stated. The definition

of persistent eosinophilia in 6 months in HES is only rarely acceptable to await due to the risk of precipitation of symptoms, and not at all in case of symptoms already elicited by eosinophils. This “working diagnosis over time” is therefore not realistic to use anymore. In a few cases historical laboratory data obtained in another relation previously, may reveal a longer lasting condition.

Table 8. Clinical manifestations due to hypereosinophilia, irrespective of primary cause.

Organ	Symptoms	Ref.
Heart	Myocardial necrosis (weeks), valvular involvement, thrombosis (months later) and fibrosis (end stage) (Loeffler’s endocarditis and myocardial fibrosis in late stages) manifesting in congestive cardiac insufficiency, hypertrophy, dilation, arrhythmias, and pericardial effusion.	49, 50
Nervous system	Cerebral thrombosis – mostly arterial, transient ischemia, embolic or local thrombus formation. Encephalopathy, in particular cognitive and / or upper neuron paresis. Peripheral neuropathies, symmetric or not, sensory or motoric or both.	49, 51
Skin	Urticaria, angioedema, pruritus, papulous or nodulous lesions, mucocutaneous ulcera.	49, 52
Pulmonary	Chronic, generally non-productive cough. Bronchial hyperactivity may be present in some, and some may have pulmonary symptoms secondary to heart affection.	49, 53
Gastrointestinal	Diarrhoea, intermittent or persistent, but various abdominal symptoms may be experienced, also depending on a more selective localization in the gastrointestinal tract	49, 54
Rheumatological	Artralgia, mostly major joints, arthritis and myalgia. Raynaud’s phenomenon. Autoimmune phenomena mostly develop in rheumatic disorders with eosinophilia,	49, 55

Eosinophilia in some non-haematological conditions.

Some clinical conditions with eosinophilia may demonstrate selective organ manifestations of chronic nature – in particular abdominal (56,57) and pulmonal (58,59). These patients may be referred to specialists in gastroenterology or lung diseases for further evaluation and treatment by colleagues in other specialties or in collaboration, using principles from treatment of eosinophilia in haematological disorders. Likewise, haematological patients with pronounced organ-related symptoms should be considered to be conferred with specialists in that particular problem.

Some clinical conditions shows eosinophilia as part of other disorders (reactive or secondary eosinophilia), and three syndromes are described briefly here for clarification.

- **DRESS syndrome:** Drug Rash (or Reaction) with Eosinophilia and Systemic Symptoms. A serious condition developing one week to two months after drug-exposure. Allopurinol, antiepileptics and antibiotics, but also imatinib and many other drugs have been associated with DRESS (41, 60, 61). The systemic symptoms may present as fever and involvement of one or more internal organs. Patients will often have fever, malaise, extensive exanthema, lymph node enlargement and pharyngitis. The patients may have signs of hepatitis, nephritis, arthritis or pneumonitis. Cessation of the given medication, immunosuppression and (intensive) symptomatic therapy is indicated.
- **Churg-Strauss syndrome:** a small-vessel necrotizing vasculitis, which may be defined by different criteria, but is characterized by marked eosinophilia, asthma, mononeuropathy or polyneuropathy, migrating pulmonary infiltrates, paranasal sinus abnormality and /or extravascular eosinophils in biopsies or samples (at least four of six criteria present in American College of Rheumatology Criteria) (41, 62, 63). Up to ½ patients have antineuro-trophil cytoplasmic antibodies present, and in most of these other autoantibodies may be detected, i.e. anti-myeloperoxidase. It is a chronic disease, with a risk of vasculitis symptoms in all organs, and treated by immunosuppressive agents, sometime alkylation or antibody-therapy. It may in some cases be difficult to rule out a haematological disorder without specific tests, and thus differentiate a vasculitis and a clonal blood disorder.
- **Loeffler syndrome:** originally a parasitic induced eosinophil pneumonia, but now also referred to in drug induced or self-limiting acute pneumonitis, with transient pulmonary infiltrates, glucocorticoid sensitive and with variable lung manifestations (64).

Eosinophilia in haematologic bone marrow diseases.

The symptoms in primary eosinophilia, due to a clonal bone marrow disorder (table 4, 5 and fig. 10), may be asymptomatic or have any of the symptoms given in table 8, in addition to any degree of constituent symptoms – fatigue, weight loss and night sweats due to a hypercatabolic state in any degree. Some discomfort may be noted due to a – mostly moderate - enlarged spleen, if present. Some symptoms may be related to anaemia, or haemorrhagic diathesis due to thrombocytopenia.

An increasing number and variety of cytogenetic aberrations have been reported in clonal eosinophilia by banding technique, involving translocations, additions, insertions, deletions, other abnormalities and complex karyotypes in the last 20 years (41, 42, 45, 65, 66) and associated with CEL. Therefore, classic karyotypes must be performed (table 5 and 6). In addition, some specific cytogenetic abnormalities have long been associated with acute myeloid leukaemia, i.e. inv(16), t(5;16), t(8;21) and others (67).

The clonal aspect may in female patients also be demonstrated by X-chromosome inactivation, HUMARA test (68) and the WT1 in both male and female patients (48, 69).

A closer approach to the molecular pathogenesis has been revealed by demonstration of some specific tyrosin kinases, many related to the described cytogenetic aberrations, in myeloproliferative neoplasias in general (70) and in eosinophilia-associated chronic myeloproliferative disorders (45,46).

Figure 11.

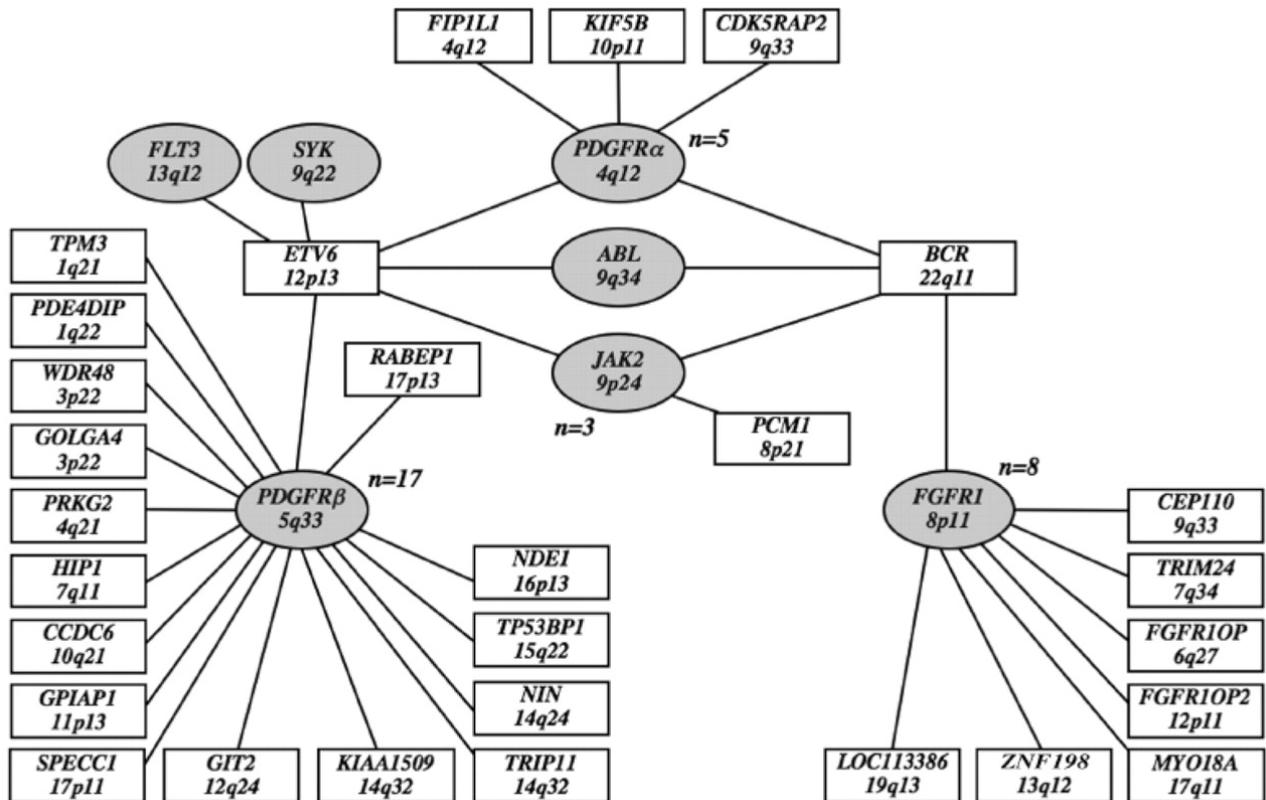


Fig. 11. Network of tyrosine fusion genes in eosinophilic myeloproliferative disorders and related diseases (46). FGFR1: fibroblast growth factor receptor 1.

The Platelet-Derived Growth Factor Receptor (PDGFR) A and B has been identified as a partner-gene in eosinophilia (fig. 11) (39, 41, 42, 43, 44, 45, 46). In particular, a dys-regulated tyrosine kinase originating from a interstitial deletion on chromosome 4 where PDGFRA fuse with FIP1-like1 (FIP1L1) gene has been described in detail (71, 72, 73, 74, 75), and the fusion gene cooperates with IL-5 to induce a CEL-like disease in mouse models (76) and the severity of disease seems to be associated with polymorphic variations at the IL5R α locus (77).

In recent years two phenotypes of eosinophilia have been described in primary, clonal eosinophilia – a myeloid and a lymphoid or T-variant (44, 78, 79), with individual variations in manifestations. The “myeloid phenotype” may have a male preponderance, the “lymphoid” seems to show a higher incidence among females.

Table 9. Clinical and diagnostic differences between (so-called) “m- and I-HES.”

Myeloid “m-HES”	Lymphoid “I- or T-HES”
Splenomegaly and hepatomegaly	Increased IL-5 production
Leukocytosis, immature forms	Increase S-IgE
Increase serum vitamin B ₁₂ & tryptase conc.	Polyclonal hypergammaglobulinemia
Anemia and trombocytopenia	Itching, eczema
Cardiac complications	Urticaria, angioedema
Less glucocorticoid sensitive	Pulmonary symptoms
More aggressive clinical phenotype	Glucocorticoid sensitive
Association with systemic mastocytosis SM	Approximately 25 % of HES patients
PDFGR disorders	T-cell phenotype subsets

The T-cell clone may be detected by TcReceptor analysis as described in the section on diagnostic work-up or analysis for aberrant T-cell phenotypes (CD3+/4-/8- or CD3-/4+) (80, 81, 82), associated with eosinophilia by IL-5 production.

Incidences of the various subtypes of clonal, primary eosinophilia have been reported to be some 25 % of I- or T-HES (78, 79), while a significant spread of incidences has been reported in FIP1L1- PDGFRA derived eosinophilia, varying from 4 to more than 80 % (41, 42, 83, 84). Probably the lower values are the most representative in unselected patient-populations. The precise incidences of various primary eosinophilia are not known.

Eosinophilia thus represents a vary heterogeneous clinical spectrum, and may be caused by another disease or the eosinophilic granulocyte is the representative of a clonal disorder (35, 39 – 49, 85) or so-called iHES (idiopathic hypereosinophilic syndrome) when clonality is not demonstrated, but organ dysfunction is demonstrated (heart, lung etc), or (simply) idiopathic hypereosinophilia (iHE) when the patient shows no organ involvement (fig. 10) (38). Another clinical-biological approach than given in fig. 10, is shown in fig. 12 (85), with the additional point that here idiopathic hypereosinophilic syndrome represents non-clonal eosinophilia. Both fig. 10 and fig. 12 demonstrates the crucial importance of correct diagnosis for eosinophilia, in order to choose the right treatment.

Figure 12.

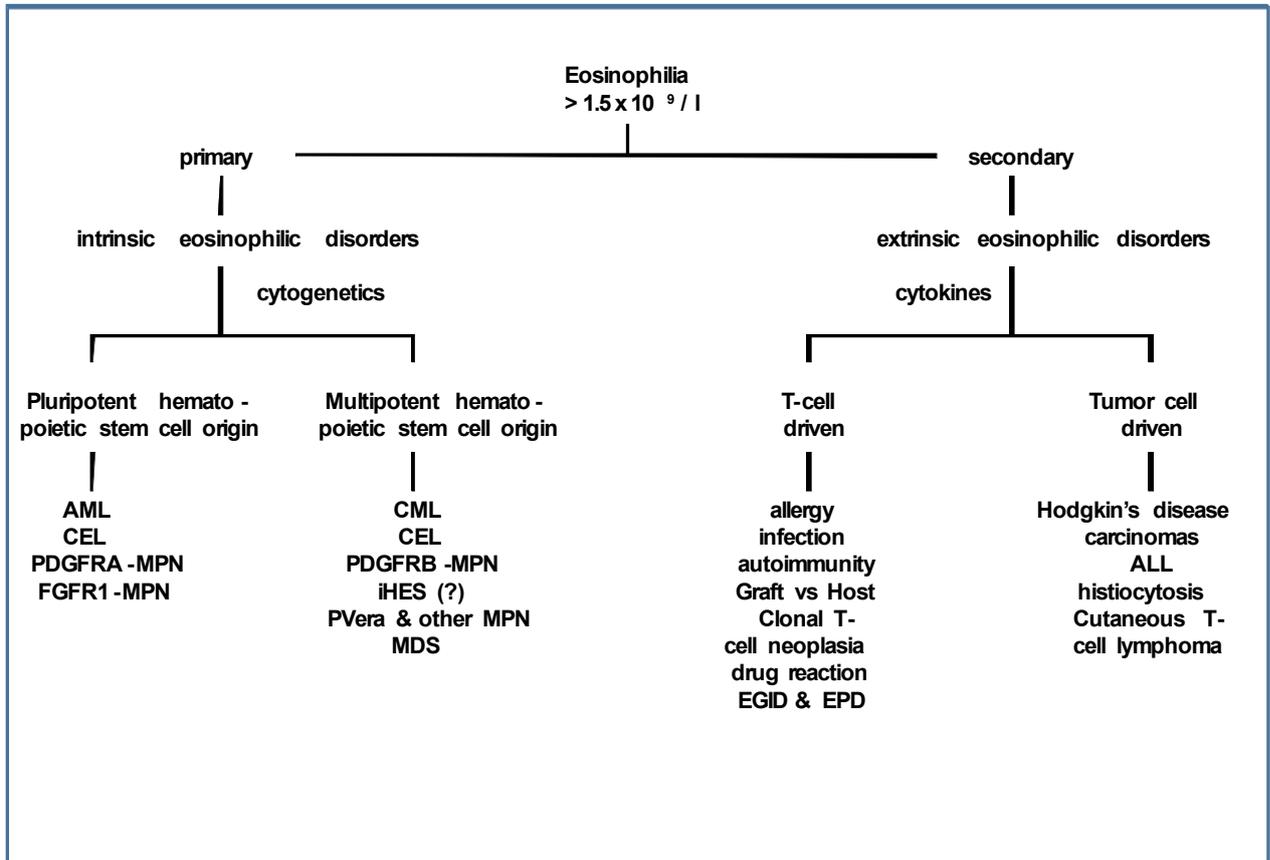


Fig.12. Classification of eosinophilic disorders. Eosinophilia is either mediated by cytokines (in particular IL-5) or a consequence of mutations, translocations or other cytogenetic abnormality in hematopoietic stem cells leading to predominant eosinophil differentiation. Modified from (38, 54, 57, 58, 59, 85). AML: acute myeloid leukemia; CEL chronic eosinophilic leukemia; CML chronic myeloid leukemia; MPN myeloproliferative neoplasm; MDS myelodysplastic syndrome; PDGFRA/B platelet derived growth factor A/B, PVera polycythemia vera; EGID eosinophilic gastrointestinal disorders; EPD eosinophilic pulmonary disorders; ALL acute lymphocytic leukemia.

Treatment of eosinophilia.

Several review articles have recently been published in this field (39, 41, 44, 46, 86) and including secondary / reactive causes, where anti-infective, immunosuppressive and symptomatic therapy is effective. The following thoughts, recommendations and even wording have been influenced by the reports of Roufosse et al. and Klion et al. in eosinophilia due to a haematological (non-reactive) disease (87, 88) – although it may be difficult to interpret clonality in many previous, older reports. In the following

hypereosinophilia therefore refers to conditions with clonal eosinophilia or possibly iHES and iHE.

This section focus on eosinophilic, haematological disorders, as depicted in fig. 10 lower half, fig. 12 left half, when all other causes or reactive eosinophilia have been eliminated, and a specific / clonal disorder with eosinophilia been identified, and includes the iHES and iHE (table 4).

Conditions with clonal eosinophilia are chronic disorders in which the toxicity of the treatment has to be carefully considered. Corticosteroids and hydroxyurea have been the standard treatment (49), together with interferon alpha (IFN- α) (90). With the recent discovery of the FIP1L1-PDGFR α fusion with constitutive tyrosine kinase activity in a subgroup of patients, and presence of increased IL-5 production by abnormal T cells in others (91), the treatment recommendations have changed.

Currently the treatment of hypereosinophilia should be based on disease severity and eventual detection of pathogenic variants. For FIP1L1-PDGFR α positive patients, imatinib is the first line therapy. For others, corticosteroids are generally recommended. Hydroxyurea, INF- α , and imatinib are used for corticosteroid-resistant cases, as well as for corticosteroid-sparing purposes. Recent data suggest that mepolizumab, an anti-IL-5 antibody, is an effective corticosteroid-sparing agent for F/P-negative patients.

The relationship between the absolute eosinophil count and organ damage is not always consistent (92, 93). Other markers of disease progression have been proposed, but none has been validated. Nevertheless, it might be of value to monitor the therapeutic response in FIP1L1-PDGFR α positive hypereosinophilia using RT-PCR for the transcript levels (71, 88). In I-HES (T-cell driven eosinophilia) the numbers of phenotypically aberrant lymphocytes can be evaluated by FACS (80, 90). However, in most cases the response to treatment are monitored by clinical symptoms and eosinophil counts.

The specific therapeutic spectrum includes:

- Corticosteroids
- Myelosuppressive agents
- Immunomodulatory therapy
- Monoclonal antibodies
- Tyrosine kinase inhibitors
- Bone marrow transplantation

Corticosteroids

Corticosteroids are first-line treatment for most patients with hypereosinophilia, except the FIP1L1-PDGFR α positive eosinophilias. Corticosteroids are also indicated, together with imatinib, in patients with FIP1L1-PDGFR α -positive eosinophilia and signs of myocarditis (94). For FIP1L1-negative patients, the usual starting dose of corticosteroid dose is $\frac{1}{2}$ -1 mg prednisone/kg body weight/day. Most patients will respond to this treatment and the

dose can be slowly tapered. Prophylaxis against osteopenia and opportunistic infection should be considered for patients requiring maintenance treatment.

A history of angioedema, a profound and rapid eosinopenic response to challenge with prednisone, high serum IgE levels, and no hepatosplenomegaly are favourable predictors of long-term response to corticosteroid treatment (49). However, corticosteroid toxicity is common and steroid sparing alternatives are usually needed.

Myelosuppressive agents

Hydroxyurea

Hydroxyurea (1-3 g/day) is the myelosuppressive drug that is preferably used to lower the eosinophil count, and it acts synergistically with IFN- α . This combination has been used with success in several cases with eosinophilia (95). Also, a combination of hydroxyurea and imatinib has been reported to be effective. A response to treatment with hydroxyurea is commonly seen within 2 weeks and it is not effective in cases where a rapid decrease in eosinophil count is needed.

Side effects: myelosuppression, gastrointestinal toxicity, leg ulcers, skin rash.

Vincristine

Vincristine can be used for rapid lowering of the eosinophils in patients with extremely high eosinophil counts ($> 100 \times 10^9/L$). It is rarely used for long-term management of eosinophilia. However, it has been used in some cases (87, 96). The recommended dose for adults is 1–2 mg intravenously.

Side effects: neurotoxicity

Combination regimens

A small series of patients with hypereosinophilia has been treated from 1999-2001 with a combination of 2-chlorodeoxyadenosine and cytarabine, and some 55 % obtained a complete remission, with a median overall survival of 44 mo. Dosage was 1 g / m² of cytarabine and 12 mg / m² for cladribine (97).

Side effects: febrile neutropenia, bone marrow insufficiency.

Immunomodulatory therapy

Interferon- α

Low doses of IFN- α (1-2 million U/m²/d) are often effective but the response usually become evident after several weeks of treatment (95). Low-dose hydroxyurea (500 mg daily) potentiates the effect of IFN- α (98). Monotherapy with IFN- α should be avoided in I-HES; *in vitro* data have demonstrated an inhibitory effect of IFN- α on spontaneous apoptosis of clonal CD3⁻CD4⁺ T cells (99). In this setting a corticosteroid should be added because of its proapoptotic effect on the clonal T cells. PEG-IFN- α 2b have been used effectively in a few patients with eosinophilia (100). IFN- α treatment may be used in pregnancy (101).

Side effects: myelosuppression, flu-like symptoms, depression or other mental symptoms, fatigue, increased liver transaminases, gastrointestinal discomfort, thyroid affection, etc.

Cyclosporine A

A case report has recently been published demonstrating a maintenance effect of cyclosporine A therapy in an adult patient with I-HES and T-cell receptor rearrangement (102). This is well explained by an inhibitory effect on the production of IL-5 (8-10).

Side effects: hypertension, renal insufficiency, tremor, headache, hyperlipidemia, gingival hyperplasia, muscle cramps, hypertrichosis, etc.

Monoclonal antibodies

Two different humanized, monoclonal anti-IL-5 antibodies, SCH55700 (Schering-Plough) and mepolizumab (Bosatria®) (GlaxoSmithKline), can markedly decrease the eosinophil count in hyper-eosinophilia, regardless of the underlying cause (103 - 105). These responses were in some patients sustained for up to a year, after multiple infusions of anti-IL-5. The therapy appears well tolerated (106). However, these substances are currently only available in clinical trials or for compassionate use, also in each of the Nordic countries. The routine clinical use in treatment algorithms (fig. 10) is not settled, but antibody treatment may be valuable in several primary and secondary causes (fig. 12). The monoclonal anti-CD52 antibody (Mabcampath®; alemtuzumab) has been used successfully in some cases with hypereosinophilia. It might be an alternative treatment for patients with HES refractory to other therapies, as the other antibodies mentioned (10, 107, 108).

Side effects: difficult to evaluate, but may be minor depending on dosages.

Tyrosine kinase inhibitors

Imatinib mesylate

Imatinib mesylate is active against several receptor tyrosine kinases, including the fusion kinase originating from the FIP1L1-PDGFR α mutation. A number of studies have shown a striking potency of imatinib in patients with FIP1L1-PDGFR α -positive hypereosinophilia, and no case of primary resistance to imatinib has been reported (39, 71, 109, 110). There is a general consensus for the use of imatinib as first-line therapy in patients with the FIP1L1-PDGFR α fusion gene and in cases with clinical and laboratory signs of this subtype of eosinophilia, e.g. tissue fibrosis, increased serum B12 and increased serum tryptase levels, and often male sex. The imatinib response rates in FIP1L1-PDGFR α -positive patients is close to 100%, with very few cases acquired imatinib. The T674I substitution in the ATP-binding domain of PDGFR α (71, 109 - 111) is associated with imatinib resistance, similar to the T315I mutation observed in patients with CML. *In vitro* data suggest that tyrosine kinase inhibitors under development are effective even in the presence of the T674I mutation (112).

The responses to imatinib in FIP1L1-PDGFR α -positive patients are rapid, and eosinophil counts are normalized within 1 week of treatment. The clinical manifestations usually disappear within 1 month. The exception is cardiac involvement, which is irreversible unless treatment is begun before fibrosis leads to permanent damages (110). The side effects of imatinib therapy are generally mild and rarely requires to discontinuation of treatment. However, acute cardiac failure has been seen and has led to the recommendation that patients with evidence of cardiac involvement (e.g. increased s-troponin levels) should be pretreated with corticosteroids (94).

The dose required to induce and maintain remission is generally lower (100 mg/day) than for patients with CML (\geq 400 mg) (110). Influence of imatinib on clinical manifestations related to heart involvement are variable, and endomyocardial fibrosis appears to be irreversible (72, 110). Reversal of bone marrow pathology and molecular remission can be achieved in most patients with the FIP1L1-PDGFR α fusion gene (110, 113). It has been recommended that the imatinib dose should be adjusted to ensure molecular remission, in order to prevent the development of acquired resistance (87).

Imatinib has become first-line therapy for patients with FIP1L1-PDGFR α -associated eosinophilia, but the overall follow-up is short, and prospective randomized trials are limited (114). It is unclear if imatinib can be curative for clonal eosinophilia, through eradication of the leukaemic clone. It has been reported that interruption of imatinib in FIP1L1-PDGFR α -positive patients in molecular remission, is followed by recurrence of the disease within months (113, 115), making maintenance therapy with imatinib necessary (116).

Durable responses have been obtained in patients with PDGFR β fusion genes and eosinophilia, but reports are still based on low number of patients (117) (table 7). The effect of imatinib therapy in PDGFR-negative eosinophilia is unclear, although responses have been seen in some patients. Currently, there are no markers that can help identify PDGFR-negative patients with imatinib-sensitive disease. A short course of imatinib 400

mg daily has been recommended to patients with clinical and biological findings typically seen in m-HES and those resistant to therapy with corticosteroids. A rapid haematological response support continuation of imatinib treatment. In a recent review, it was suggested that presence of splenomegaly or lung disease could be associated with a higher probability (89% and 96% respectively) of complete haematological response to imatinib (118). Imatinib is not useful in patients with I-HES.

Second generation TKI

Several alternative tyrosine kinase inhibitors have been tested *in vitro* and *in vivo* (animal models) for effects on FIP1L1-PDGFR α activity. Nilotinib (Tasigna®; AMN107), is able to inhibit kinase activity of wild-type FIP1L1-PDGFR α (119). PKC412 (112), and sorafenib (120), are able to inhibit kinase activity of both wild-type FIP1L1-PDGFR α and the imatinib-resistant T674I mutant form. Emerging data on Dasatinib (Sprycel®; BMS-354825) in these Ph¹ negative myeloproliferative disorders indicate the need for larger clinical studies (121).

Side effects: fluid retention, muscle cramps, diarrhea, skin rash and elevated liver enzymes.

Bone marrow transplantation

Nonmyeloablative allogeneic bone marrow transplantation has been used successfully in HES (122), but the transplantation related toxicity still remain a major problem. This treatment can be considered for patients with FIP1L1-PDGFR α -positive patients, resistant or intolerant to imatinib therapy or FIP1L1-PDGFR α -negative patients, for instance FGFR1-positive eosinophilia (38c), with progressive end-organ damage when standard therapies or any experimental therapy have been exhausted.

Table 10. Present treatment options for eosinophilia due to a clonal haematological disorder, or iHES and iHE.

Medication and administration	Indications	Dose	Comments
Corticosteroids Oral, or i.v.	First-line treatment unless FIP1L1-PDGFR α positive	Initial dose ≥ 40 mg prednisone q.d	Side effects in higher dosage or prolonged therapy
Hydroxyurea oral	Second-line treatment	1-3 g / day	Slow onset of action
Cladribine & cytarabine i.v.	Second-line treatment	2-Cda 12 mg /m ² & Ara-C 1 g / m ² / 5 d	Patient-population not characterized by clonality
Vincristine i.v.	Consider for counts $>100,000/\text{mm}^3$,	1-2 mg i.v.	For rapid reduction of eosinophil count
IFN- α s.c.	Second-line therapy	1-2 mU / m ² q.d.	Slow onset of action
Cyclosporine A oral	Lymphocytic variant	100 mg maintenance / d	Induction therapy includes corticosteroids and hydroxyl-urea
Imatinib mesylate oral	First-line treatment for FIP1L1-PDGFR α positive. Consider for other refractory cases	100 - 400 mg q.d.	Together with corticosteroids if cardiac involvement

Treatment of eosinophilic-induced organ dysfunction is symptomatic according to the manifestations of in particular cardiac, pulmonary, skin etc. It may involve evaluation and assistance from other specialists in internal medicine.

Closing statements.

Meeting a patient with eosinophilia represents a challenge – diagnostic and therapeutically, and the encounter will in most cases result in a multidisciplinary approach. Optimal diagnostic repertoire is important to give the right treatment, and possibly to monitor the patient. It may be considered to centralize the patients without an obvious other cause for eosinophilia to haematologic departments.

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The list of references is not exhaustive, but may be considered representative.

List of abbreviations.

Many abbreviations are also given where used in legends to figures and tables, and they are not repeated here if only used once.

CD: cluster of differentiation
CEL: chronic eosinophilic leukemia
CML: chronic myeloid leukemia
CT: computerized tomography
DRESS: drug rash (or reaction) with eosinophilia and systemic symptoms.
ECG: electro cardiogram
ECP: eosinophilic cationic protein
END: eosinophil-derived neurotoxin
EPO: eosinophil-derived peroxidase
FACS: fluorescence activated cell sorter
FGFR: fibroblast growth factor receptor
FISH: fluorescence in-situ hybridization
GM-CSF: granulocyte-macrophage colony stimulating factor
HES: hypereosinophilic syndrome, divided in idiopathic HES (iHES) or idiopathic hypereosinophilia (iHE)
HUMARA: human androgen receptor X-chromosome inactivation assay
IFN: interferon alpha
IL: interleukin
LED: lupus erythematosus disseminatus
PCR: polymerase chain reaction
PDGFR: platelet derived growth factor (A or B)
Ph: Philadelphia chromosome
RT-PCR: reverse transcriptase – polymerase chain reaction
TcR: T-cell receptor
TKI: tyrosine kinase inhibitor
WT1: Wilms tumor antigen