CAT
Critically Appraised Topic

The basophil, futile or misunderstood?

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Clinical bottom line

The basophil has been described for a long time, yet only in the last 20 years has there been a large increase in knowledge. The reference method remains microscopic examination of 400 cells, although the lack of accuracy due to statistical error is acknowledged. Laboratories nowadays usually measure the basophils first on an automatic cell counter. Different measurement techniques are used to discriminate basophils from other white blood cells. These methods know their flaws, and their accuracy and correlation are moderate to quite good. A possible alternative for the reference method is flow cytometry. Several markers have been proposed, but a standardized protocol is lacking. However, at least two immunophenotypic markers may be needed for optimal accuracy.
Given the rather poor reliability of basophil counting, diagnostic use of basophilia as a stand-alone test is not certainly recommended. Nonetheless a basophil count of >0.45 x 10^9/L has a strong correlation with the diagnosis of an MPN. In line with the suggestion of mr. Smith, a basophil count of >0.40 x 10^9/L should trigger clinicians to examine the presence of an MPN. In absence of any such finding.
The current evidence for reactive basophilia, better "non-malignant basophilia", is weak. Often it is based on expert opinion and cross-references to mostly the same old studies. Most convincing evidence is available for smokers and lymphoid malignancies as well as in acute lung rejection. Reactive basophilia is therefore an exclusion diagnosis without any well-defined interval or strong diagnostic potential.
Further larger studies are required to assess the diagnostic power of basophilia in association with other laboratory parameters. BCR-ABL1, followed by JAK2 mutation (and on indication CALR, MPL mutation) analysis should be performed.

Introduction

The basophil was first morphologically described and named as such in 1879 by Paul Ehrlich after a description by Friedrich von Recklinghausen. Ehrlich had already described mast cells in his thesis in 1878. In the following years, this rare cell was mainly noted for its "explosive nature" to certain stimuli. It took until 1955 before a link was found with hypersensitivity, anaphylaxis and histamine release. This discovery led to the development of the 'basophil activation test', in which degranulation is assessed microscopically. In 1968, the immunoglobulin (Ig) E(rythema), the so-called 'serum factor', was named as the triggering factor for this explosive release. From 1990, knowledge about the function of the basophil gained momentum. This CAT provides an overview of current analysis, physiopathology and further diagnostics in basophilia[1,2].

The basophil develops from the hematopoietic stem cell via the common myeloid progenitor to eventually the granulocyte-monocyte progenitor or possibly to granulocyte progenitor. The prebasophil mast cell progenitor then matures in the bone marrow and finally in the spleen as a basophil mast cell progenitor. After subsequent maturation, it enters the bloodstream[1].

The cells play a role in innate immunity against ectoparasites (e.g., scabies, ticks), helminths and respiratory bacteria. Its function in protozoan infections is currently unknown. Furthermore, the basophil is involved in intermediate, late and delayed hypersensitivity reactions where it would maintain the T-helper 2 (see further) environment. In addition, it may have a role in the pathophysiology of lupus nephritis. Basophils are rapidly recruited from the blood into peripheral tissues exposed to antigens. The physiology therefore correlates rather weakly with the concentration of basophils in the blood. Apoptosis is induced by glucocorticoids, certain antihistamines and certain asthma medications.

The function is reflected in the actors that can lead to the 'activation' of basophils. Exposure to, among others, IgE, interleukin (IL)-3, IL-8 and C5a leads to degranulation. Secretory IgA, C3a, GM-CSF and IL-5 can also affect the activation, albeit after stimulation of a combination with other chemo-/cytokines. Subsequently, numerous chemo-/cytokines have the potential to lower the degranulation threshold. Finally, excitation is also possible by exogenous stimulators. These include bacterial cell wall components (formyl methionine phenylalanine from gram-negative bacteria, muramyl dipeptide from bacterial peptidoglycan), toll-like receptor ligands, as well as proteases (such as the Der p1 protein (allergen secreted by dermatophagoides, dust mites)). These can all lead to the release of pro-inflammatory mediators.

This activation leads to the release of preformed granules containing, inter alia, histamine leukotriene C4, numerous cytokines such as interleukin (IL)-4, -6 and -13, TNF-alpha and antimicrobial substances. Here, granule membrane molecules, such as CD63 and CD203c, reach the cell surface and can be analyzed by means of immunoflow cytometric techniques. Other basophilic mediators such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor may have an underestimated role in the pathogenesis of several reactive and neoplastic basophils[3]. Research into their role in, for example, angiogenesis in breast carcinoma is ongoing.

It can thus be argued that basophils have their role in innate immunity through the release of stimulating immune mediators[1].

Clinical/Diagnostic scenario

About 130 years after the first description, the reference method, defined by the Clinical Laboratory Standards Institute (CLSI) in the H20-A1, is still optical microscopic examination. Two individuals should count each 200 cells on two peripheral blood smears from the same sample. The International Council for Standardization in Hematology (ICSH) describes the basophil as follows:
“The basophil is 10-16 µm in diameter with pale blue cytoplasm, containing purple-black secondary granules. These granules are water soluble and can dissolve upon staining to create bright sites in the cytoplasm. The nucleus is segmented, but often hidden by the basophilic granules which can vary in number, size and shape.”

In 2021, peripheral blood smears are still performed on specific clinical or algorithmic-parametric indication (see appraisal) after analysis by an automated cell counter. Then, a white blood cell differentiation is determined via manual or automatic digital (e.g., Cellavision®) microscopy of 100-200 cells. Several methods of automatic cell counting of basophils have been developed, each with its own advantages and disadvantages. In general, automated cell counting has a higher precision and absent evaluator/interpretation variability. Furthermore, there is a decreased turn-around-time (TAT) due to the different pre-analytical processes (e.g., no staining) and analysis (microscopy vs. fully automatic analyses).

Over the past 30 years, both analytical capabilities and pathophysiological knowledge went through a big evolution. A basophil concentration is determined for each differentiation, but what is the diagnostic value of this? Its role in the diagnosis of chronic and acute myeloid leukemia is well known, but does it extend further? If basophilia is present, what is the necessary work-up and is follow-up always needed?

Question(s)

1. **How is basophilia defined? What is the biological variation?**
2. **Analytics: What techniques exist currently for evaluating basophilia? What are there flaws?**
3. **Diagnostics: Basophilia and pathology**
    a. **What is reactive and what is malignant basophilia?**
    b. **What is the diagnostic value of basophilia?**

Search terms

1. MeSH Database (PubMed): MeSH term: “basophilia”
2. Pubmed (Medline; from 1966), SUMSearch (http://sumsearch.uthscsa.edu/), The National Institute for Clinical Excellence (http://www.nice.org.uk/), Cochrane (*http://www.update-software.com/cochrane*): “basophilia”. No results.
3. International organizations: e.g., National Committee for Clinical Laboratory Standards (NCCLS; http://www.nccls.org/), International Federation of Clinical Chemistry (IFCC; http://www.ifcc.org/ifcc.asp), American Diabetes Association (ADA; http://www.diabetes.org/home.jsp)
4. UpToDate Online

Relevant evidence/References

- ***Guidelines and Recommendations***

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Appraisal

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Definition of basophilia

Basophilia is an increased concentration of basophils in the blood. Historically, the reference values were 0.02-0.05x10^9/L[4] The CLSI recommends in the H20-A2 that the reference intervals should be statistically determined at the central 95% between the lower and upper reference limits of a presumed healthy reference population. The study of Ducrest et al. in Bern, Switzerland, 2005 immunoflow cytometric analysis of 95 presumed healthy individuals found these intervals were 0.014-0.087x10^9/L[5] In 2018, similar reference values were obtained in Barcelona, Spain, by analysis of 213 reference individuals on the XN-2000 (0.01-0.09x10^9/L)[6]. 0.1x 10^9/L is frequently considered the upper reference limit [3,7] Values outside this reference interval are, according to the argumentation above, therefore different from the normal, but of course not necessarily pathological. Valent et al. stated that basophilia between 0.1 and 1x10^9/L can be both reactive and neoplastic, but that persisting hyperbasophilia defined as an absolute basophilia of >1x10^9/L for at least 8 weeks is suggestive of underlying malignancy[3].

Both absolute and relative values for basophilia have been described. Is evaluation of both desirable? Several authors mention the latter as a corresponding percentage of the absolute number. Indeed, the focus in reviews and articles of chronic myeloid leukemia (CML), being the main differential diagnosis in persistently elevated basophilia, is on the absolute number of basophils and therefore seems to be preferred. A notable exception is the WHO (World Health Organization) criteria for the acceleration phase of CML that uses the percentage of basophils.

Pre-analytical consideration of basophil count

In accordance with the ICSH recommendation in 1993, peripheral blood samples are preferably taken in a tube containing dried K2EDTA [8]. A smear preparation for microscopic inspection is preferably made within 2-4h. When measuring with automatic cell counters, the absolute value of the differentiation is quite stable up to 48h of storage in room temperature. The only exception to this is the eosinophilia fraction of which a sharp decline is already observed after 12h, with a decrease of up to 78.8% after 24h. This reduction is not observed when stored at 4°C. A number of studies report an increase in basophils in long-term sample storage (>48h)[9–11].

Even though corticoid use in the neutrophils may cause an increase due to release of the marginal pool, a decrease in the number of basophils observed (Dunsky et al., 1979). Corticosteroids, like antihistamines, induce apoptosis of basophils. The effect of physical exertion remains unclear, with literature contradicting each other ranging from no effect to a limited increase that seems inexplicable by biological and analytical variation (see also below)[13–15].

An increased concentration of basophils was observed in newborns in general, newborns with Down's syndrome, as well as in tobacco-induced leukocytosis. These studies have not yet been reproduced[16,17].

Furthermore, there is a high biological intra- and interpersonal variation of the concentration of basophils. According to the EFLM, these are 12.4% (11.4-32.0) (28-05-2021) and 26.3% (22.1-33.9) (05-05-2020) respectively[18]. However, no difference is observed in reference values between sex[5].

Analytical techniques

1. Reference method: microscopy

According to the CLSI, the reference method remains microscopic examination of 400 cells by counting 100 cells per peripheral blood smears on 2 preparations of the same sample by two persons. Normal morphology has been defined by the ICSH (see previously: clinical diagnostic scenario). The reference method is susceptible to bias due to morphological misinterpretation and by bias due to the possibility of unequal distribution in the blood smear and statistical error given the low concentrations[19].

Interpretation

Incorrect morphological identification of basophils is limited when performed by experienced medical laboratory technologists on good quality peripheral smears. This is confirmed by the results of annual external quality controls for hematology carried out by Sciensano[20]. Almost all participating laboratories use May-Grünwald Giemsa or Wright-Giemsa who are sufficient for basophilic coloring[3,20].

One should be aware of aberrant morphology due to both pre-analytical and pathology.

- Lobulation may be lacking as a result of Pelger-Huët anomaly and cytoplasmic inclusions have been described in a May-Hegglin anomaly. Mast cells, by contrast, have a round nucleus.
- Various hereditary disorders, such as Chediak Higashi anomaly[21]have been linked to abnormal granules. Cells with mixed eosinophilic/basophilic granulation, on the other hand, have no relationship with the basophils, but are immature eosinophils[3].
- A decrease in granules can be an artifact due to the coloring process (the granules are water soluble). Degranulation is also observed in allergic reactions such as urticaria or anaphylactic shock and post-prandial hyperlipidemia
- Finally, this may be due to pathology such as myelodysplastic syndrome (MDS, possibly accompanied by nuclear condensation and signs of apoptosis)[3] and myeloproliferative disorders. In the latter, dysplasia is observed mainly in the acceleration phase of CML. Other dysplastic characteristics are abnormal size or staining, cytoplasmic vacuolization and hypersegmentation[22].
- Immature basophils (e.g., basophilic myelocytes) can be distinguished from immature mast cells by means of the nucleus and granules. Immature mast cells have a multilobulated nucleus with finer grains at certain early maturation stages.

The unique diagnostic value and accuracy of such abnormal morphology is not known. In clinical practice, this role is therefore limited to any additional argument in case of clinical suspicion.

Cell distribution

Cells are not equally distributed over a smear. As early as 1958, Davidson described that cell are unequally distributed over the smear preparation. Particularly with less quality smear preparations, larger immature cells are more peripherally located and a relative higher number of neutrophils are found in the tail of the preparation[23,24]. The so-called “battlement method” is used to overcome these distribution error. Additionally, standardization and automation of the smear and staining process, as well as the use of digital microscopy ensure that the contribution of cell distribution to the inaccurateness of microscopic cell differentiation is limited[19,22]. the argument of statistical error (cf. infra) puts this fact into perspective.

Statistical error

The most decisive factor is probably the statistical error associated with the low concentration basophils in the blood. On a cell count of 400 cells, only 4 basophils are counted in a healthy individual. However, in routine practice when counting only 100-200 cells are counted. In the latter, statistically speaking, the real percentage basophils are between 0-6% or vice versa and even if one counted 5% basophils on 100 cells, it is still theoretically-statistically possible that this individual contains a normal percentage of basophils given that the 95% confidence interval is 1-12%. Even if one follows the CLSI guidelines

∂	N = 100	N = 200	N = 500	N = 1000
0	0–2	0–2	0–1	0–1
1	0–6	0–4	0–3	0–2
2	0–8	0–6	0–4	1–4
3	0–9	1–7	1–5	2–5
4	1–10	1–8	2–7	2–6
5	1–12	2–10	3–8	3–7
6	2–13	3–11	4–9	4–8
7	2–14	3–12	4–10	5–9
8	3–16	4–13	5–11	6–10
9	4–17	5–14	6–12	7–11
10	4–18	6–16	7–13	8–13
15	8–24	10–21	11–19	12–18
20	12–30	14–27	16–24	17–23
25	16–35	19–32	21–30	22–28
30	21–40	23–37	26–35	27–33
35	25–46	28–43	30–40	32–39
40	30–51	33–48	35–45	36–44
45	35–56	37–53	40–50	41–49
50	39–61	42–58	45–55	46–54
75	65–84	68–81	70–79	72–78
100	96–100	98–100	99–100	99–100

Figure 1: Rümke table ∂ = proportion of specific white blood cell type; N= total number of cells counted.

and counts 400 cells, this statistical uncertainty persists. The CLSI acknowledges this difficulty and states that manual counting is less suitable as a reference method for cells that make up less than 5% of the total leukocyte count due to higher coefficients of variation[25]

Thus, although microscopy is the reference method, this method has an important inaccuracy due to misinterpretation, uneven distribution and primarily statistical error caused by the low concentration of basophils in blood.

2. Automated cell counting

General principle

Automatic cell counters differentiate white blood cell populations by at least partially the same properties of shape and color but are instead observed by electromagnetic signals. For example, the size of a cell is observed by impedance at the level of the aperture under a low frequency electromagnetic DC, but also by forward light scattering when passing along a laser beam. The complexity of the cell's internal structures (such as nucleus/cytoplasm ratio, nuclear density - i.e., chromatin structure and granularity) can be observed by conductivity (on radiofrequency), as well as by lateral light scattering. In addition, other methods are used that abandon the conventional morphological properties.

Over the years, differentiation on morphological properties was supplemented with or replaced by new parameters. Measurement of nucleic acids (RNA, DNA) – and thus immaturity or metabolic activity- is possible by analysis of fluorescence intensity after permeability with fluorescent dyes such as polymethine (Sysmex) or propidium iodide (Abbott). Neutrophils, eosinophils and monocytes have peroxidase activity that produces a black -slightly adsorbing- dye through reaction with a substrate such as 4-chloro-1-naphtol in which the intensity of the activity can be plotted. Furthermore, eosinophils have the unique property of being able to polarize light. This can be differentiated by measurements with orthogonal polarized and depolarized laser beams in the Abbott (Cell Dyn) instruments.
Nonetheless, basophils are not well discriminated from other populations.

Basophil counting

In 1974, basophils were automatically counted by the Technicon company for the first time with the Hemalog D continuous flow hematology analyzer. Cells were colored with alcian blue with subsequent analysis of the intensity of staining. Although accurate, the method was abandoned as it was not compatible with the efficiency (speed) of the new analyzers.
Today, basophils are determined firstly on of their resistance of their cytoplasm to acidic lysis and secondly on their light scattering at different angles in combination with depolarization and fluorescence as described above.

The first technique is used by Sysmex (Kobe, Japan), ADVIA (Siemens Healthcare Diagnostics, Deerfield IL) and Horiba (Horiba medical, Kyoto, Japan).
For example, the Sysmex XE-2100 had/has a '4-diff' channel in which lymphocytes, monocytes, eosinophils and neutro + basophils are separated. The basophilic population is subsequently determined in a baso channel that suppresses granulation. Newer generation devices perform a staining after lysis in a so-called WNR channel[26].

- The presence of acid-resistant cells can cause a falsely increased basophil count, creating "pseudobasophilia". In the study of Jacomo et al. a correlation (Pearson correlation 0.728 $p<0.001$) with atypical lymphocytes was described, but not with microscopic concentration of basophils[27]. Pseudobasophilia is indeed caused by atypical lymphocytes such as lymphoma cells, plasma cells, but also reactive lymphocytes (mainly virocytes), immature granulocytes and blasts[28]. Vani therefore proposed in 2013 that it be an independent marker for the "flag" "atypical lymphocytosis?"[29]. Furthermore, in the context of the rare mucopolysaccaridosis type VI, misidentification because of abnormal granulation of the neutrophils is described. Finally, pseudobasophilia has been described in heparin use as anticoagulant[26].
- Underestimation by this method is also possible after lysis of the basophils.

The correlation with immunophenotypically counted basophils, microscopically confirmed after cell sorting, is therefore only 0.64 (see further: "flowcytometry by immunophenotype")[30]. Comparison of the newer generations of automated cellcounters with immunophenotyping have not been re-conducted for the time being. Validations of novel generation devices, such as the Sysmex XN series show a lower incidence of pseudobasophilia[31].

Abbott devices, such as the CELL-DYN Sapphire and Alinity HQ, characterize basophils based on light scattering at different angles, depolarized light and fluorescence without prior acid lysis. Therefore, there is no phenomenon of pseudobasophilia. In the above-mentioned study of Amundsen, the measurement on the CELL-DYN Sapphire correlated better with the immunoflow cytometric count ($r=0.81$), but instead had a higher intrarun variability of +/- 50%, despite the higher number of white blood cells counted (20,000)[30]

Finally, the Beckman Coulter DxH 800 analyzer uses the combination of impedance, conductivity and light scattering at 5 different angles. As described above, this can be a differentiation based on internal structures such as granules and lobes. [26]

When using the common white blood cell canal as with Abbott and Coulter, degranulated neutrophils, core RBC and platelet aggregates may be the cause of incorrect measurements. Conversely, basophils can be incorrectly counted in the neutrophil population.

Performance of basophil count in automatic cell counters

- Regardless of the method used, the correlation is 'weak' to 'quite good' when the automated basophil count is compared with the reference method (see table on the right). Hoffman's study published in December 2020 showed a correlation coefficient with the reference method of up to 0.72. The reference method also has limited accuracy[26].
- However, automated cell counters show a moderate correlation among each other with a coefficient ranging from minimally weak (0.01) for older generation devices to a maximum of 0.818 (good) for the Sysmex XN series.
- This performance is despite the much larger number of cells counted (5000-20000) compared to the reference method[26,30].

Note: analytical variation

- The European Federation of Clinical Chemistry published as specification desired imprecision, inaccurateness and total error respectively 6.2%, 7.3 and 17.5%[18]. The intrarun variation differs whether quality control material (QC material) or fresh human samples is used.
- Intrarun variation is limited to 5-10% when using QC samples. The fixation needed for stabilization of cells would change the cellular characteristics.
- The desired specifications, on the other hand, are not always achieved with fresh human blood, even in the newer generations of devices[11,32]. Vis et al. argue that a coefficient of variation (CV) of 20% is a pursuable target for "within-run" and "between-batch" precision[33].

Hematology analyzer	n	Coefficient of correlation
Abbott CELL-DYN Sapphire	200	0.265
	292	0.17
	125	0.159
	272	0.478
	156	0.30
Abbott Alinity h	346	0.38
	314	0.53
ABX Pentra	174	0.23
Beckman Coulter DxH-800	292	0.26
	125	0.321
	272	0.581
	132	0.58
	102	0.444
	249	0.039
Mindray BC-6800	134	0.56
	186	0.719
Siemens Advia 120 or 2120	292	0.34
	200	0.485
	444	0.23
Sysmex XE-2100	200	0.616
	663	0.63
	101	0.238
Sysmex XN series	390	0.70
	292	0.30
	122	0.21
	102	0.466
	261	0.08
	346	0.585

Figure 2: Correlation of different automated cell counters with the reference method (microscopy). Table from Hofman et al, Clin. Chem. Lab. Med. 2021.

Automated cell counters allow rapid differentiation of white blood cells. Accurate counting of basophils was and remains a technical challenge. Roughly speaking, two techniques are used: firstly, based on acid lysis and secondly merely on light scattering at different angles/types of light. In acidic lysis, pseudobasophilia should be considered.
Regardless of the technique, the correlation with the reference method showed a correlation coefficient up to 0.72, however the correlation varies from 0.01-0.818 between different cell counters.

3. Flow cytometry by immunophenotype.

Markers of differentiation:

- Basophils have a lower lateral light scattering than myeloid cells and are therefore located just above (increased lateral light scattering) the lymphocytes towards the monocytes but have a lower CD45 expression than these latter populations.
- Once isolated based on the lateral light scattering and CD45 the most specific differentiation markers for basophil counting are CD123 and CD193.
  - CD123 is an interleukin 3 receptor that is only highly expressed in basophils and plasmacytoid dendritic cells. The latter is a type 1 interferon secreting cell that releases large amounts of interferon after exposure to viral antigens to its toll-like receptor 7 and 9. This cell makes up only 0.4% of peripheral white blood cells and expresses CD4 and HLA-DR[34] Mast cells are, unlike basophils, CD117/KIT positive and CD123 negative, allowing discrimination based on these markers[3] The combination of light scattering, CD123 positivity and HLA-DR negativity is sufficient for the identification of basophils[5]
  - CD193 is an eotaxine receptor CCR3. The expression of this is rare in certain T-helper cells and can therefore be filtered out by CD3 negativity[19].
- They are also positive for myeloid markers CD13 and CD33. Furthermore, they have expression of CD9, CD22 and CD25 (weak), CD3 and also FcεRI/IgE and CD 294 (= T helper 2 cell marker, prostaglandin D2, CRTH2)[19,35].
- Cd203c is an ectoenzyme with ATPase activity and is located in the granules. It is expressed to a higher extent after degranulation and is therefore a marker for basophilic activation.

Accuracy and standardization

Ducrest et al. compared two markers for immunophenotypic differentiation of basophils using CD123 and CD193 in 95 healthy individuals, which showed a strong correlation ($R^2 = 0.9139$). The correlation with the routine automatic cell counters ranged from 0.24 to a maximum of 0.81. Amundsen et al. came to similar findings and was able to indicate the accuracy of these markers via cell sorting followed by May-Grunwald Giemsa staining[5,30].

The CLSI recognizes the diagnostic usefulness and accuracy of flow cytometry and calls it a 'potential alternative reference method'. However, standardization for an international flow cytometric method has not yet been completed[19].

Preferably, two instead of one, immunophenotypic markers (e.g. CD123, HLA-DR) are used to distinguish populations (cf. above) with due certainty and to prevent pseudobasophilia[28].

Flow cytometry is a potentially alternative reference method for the determination of basophilia. The use of two immunophenotypic markers (e.g. CD123/HLA-DR or CD193/CD3) in combination *with CD45 and lateral scattering is preferable.*

4. Conclusion analytical techniques

- Manual microscopic counting is the reference method for determining basophil concentration in the blood. It has limited accuracy, mainly due to statistical error.
- Immunophenotypic flow cytometry based on at least two immunophenotypic markers is the most accurate method for determining basophil concentration in the blood and is therefore preferable in specific situations e.g. in studies.
- Automated cell counters score significantly less well. The correlation with the reference method, microscopy, is rather weak with correlations ranging from 0.08 to 0.719. Furthermore, there is only a weak correlation between and within the different measurement techniques ranging from 0.01 to 0.818, where the newer generations of devices have a better correlation between them. Finally, there is a large analytical variation that comes on top of the aforementioned biological variation.
- Few accurate provisions may prevent solid substantiated research into the diagnostic usefulness of basopenia and basophilia.

Diagnostic elaboration of basophilia

1. Pseudobasophilia

An increased basophil concentration measured via automated cell counters always deserves a microscopic check to confirm basophilia.

- The International Society of Laboratory Hematology (ISLH) recommends making a smear preparation for a first basophilia >0.5 $x10^9/L$[36]. Depending on the analytical technique used (acidic lysis vs. common white blood cell channel), pseudobasophilia by reactive lymphocytes, plasma/lymphoma cells/blasts or by degranulated neutrophils, nucleated red blood cells or platelet aggregates in the second method should be excluded. Note that only the absolute number of basophils are mentioned and that the value on which a smear test is indicated is roughly five times the upper limit of the normal.

- In the case of basophilia < 0.5 $x10^9/L$, a microscopic assessment only needs to be performed on indication.
    - A first indication is the presence of cells that can cause falsely elevated basophilia such as atypical lymphocytosis and/ or platelet aggregates.
    - A second indication is if basophilia is associated with other malignant hematological characteristics. For example, in the case of an increased number of immature granulocytes in combination with neutrophilia, eosinophilia and thrombocytosis; or the presence of a leuko-erythroblastic formula in the context of chronic myeloid leukemia or primary myelofibrosis respectively. Such samples are often already marked for microscopy even without the presence of basophilia.

- In hyperbasophilia (>$1x10^9$ basophils/L), neoplasia (type of chronic myeloid leukemia)should always[3].

Causes pseudobasophilia
- Preanalytics (long-term steel storage)
- Statistical error and high variation.
- Analytic
    - Acid lysis?
      Plasma cells, lymphoma cells, virocytes, blasts
    - Common WBC channel
      Degranulated neutrophils, nucleated RBC, platelet aggregates..

2. Reactive basophilia?

Reactive basophilia is described as a basophil concentration between 0.1-1 x10^9/L which is not due to hematological malignancy. Often it is associated with eosinophilia. Here we describe the physiology of the allergic spectrum and the parasitic infections and supplement this with additional literature.

Allergy

Hypersensitivity reactions are characterized by an inappropriate severe reaction of the innate and adaptive immune system to antigens that are not or almost not harmful. The type I hypersensitivity reaction, allergy, is characterized by production of IgE antibodies against antigens. Often, these are (glyco)proteins with many epitopes, sometimes with an enzyme activity such as proteases. In this reaction, a T helper 2 (Th2) environment is created that is also observed in parasitic infections. This environment is supported by the innate immune system and by stimulated by the eosinophil, mast cell and basophil. The latter produces, among other things, IL-4, IL-13 (see also introduction) which supports and strengthens this Th2 environment. Degranulation with histamine release leads to vasodilation and extravasation of fluid that can be observed superficially in urticaria. Numerous other chemo- and cytokines, leukotrienes and prostaglandins are involved.
This causes the typical clinic that ranges from a limited local response to life-threatening condition.

Despite this clear physiological role in allergic spectrum, the concentration of basophils has no diagnostically significant role. The cause of this is multifactorial. Firstly, there is large analytical and biological variation of basophil counting which may impede correlation studies. A second aspect is the hypothesis that basophils are recruited locally, which sometimes would generate basopenia instead. Finally, corticosteroid/antihistamine therapy provoke basophil apoptosis. It should be noted that non-iatrogenic (= pathophysiological) causes of increased corticoid levels, such as chronic inflammation (e.g., autoimmunity), also may lead to basopenia.

Chronic spontaneous urticaria is characterized by recurrent urticaria for six weeks or more, with or without angioedema and without a unique identifiable physical cause. The condition is associated with the allergic spectrum, autoimmune disorders and thyroid. Diagnosis is mainly clinical-anamnestic. Eosinopenia has been observed in 10% of cases and is linked to basopenia. The combination is predictive of a weak response to second generation antihistamines. Basopenia alone is more common in more severe forms[37,38].

About 10-30% of the population suffers from allergic rhinitis that is accompanied by sneezing, nasal run and obstruction, chronic cough, itchy eyes and nose... Although several studies have shown that in these patients there is an increased basophilia with or without eosinophilia and correlates with the severity of the symptoms around sampling, basophil count has no diagnostic or therapeutic implications[28,39]. Depending on the case, skin tests and/or total and specific IgE determination may be appropriate. Local mast cell activity as in allergic rhinitis is not correlated with increased tryptase[40].

Finally, we discuss acute IgE mediated sensitivity reactions. The severity varies from local itching complaints to life-threatening conditions such as anaphylaxis. The latter is characterized by sudden deterioration of two or more systems (cardiovascular, respiratory, gastrointestinal, mucocutaneous) and may lead to hypotension with cardiovascular collapse, bronchoconstriction/laryngeal edema, vomiting and/ or diarrhea, with or without dermatological symptoms (urticaria, itching, swelling of skin/mucous membranes. Diagnostic symptom scores for anaphylaxis have a sensitivity of 96.7% and specificity of 82.4%. In +/- 5% of cases, the course is biphasic with a late response after an average of 7h. It orders of prevalence it may be caused by nutrition, medication and insects. On time treatment with adrenaline and subsequent administration of corticosteroids and antihistamines is lifesaving.
Underlying mechanism is mainly based on mast cell degranulation. Diagnostics therefore consists of tryptase determination (serum or plasma) 1(to 4) hours after the onset of symptoms and after 24 hours in order to determine a basal level[40] An increase of more than 2 + 1.2 times the basal level (in µg/L) is significant if the result is a normal value (<11.4µg/L) during the event. A good correlation has been demonstrated with hypotension[41]. Skin and specific IgE tests can confirm the suspected antigenic sensitization. Histamine release can also be measured, but has a shorter half-life[42]. So, though the diagnosis is mainly clinical, tryptase measurement can be done to support the diagnosis.

If the patient does not obtain a normalization of the tryptase after anaphylaxis, a third sample should be analyzed a few days later to consider any underlying mastocytosis (incidence 1/10,000) or mast cell activation syndrome. Serum tryptase is usually > 20 µg/L. In 80% of patients there is a presence of the typical urticaria pigmentosa. In case of increased basal serum tryptase after anaphylaxis and/or presence of urticaria pigmentosa, the c-KIT mutation should be determined and/or an extracutaneous biopsy (e.g. from the bone marrow) should be taken to demonstrate/ exclude any underlying system mastocytosis on the basis of the WHO criteria. In 2/3 cases we speak of an indolent system mastocytosis that is accompanied by episodic complaints. Tryptase is also a prognostic marker in the latter. Maintenance therapy with antihistaminic drugs is then recommended as well as vigilance when taking medication[43,44].

Parasitic infections
In recent years, the role of basophils in parasite infections has become increasingly clear. In animal studies, there are clear correlations with basophilia. As with allergy, there might be a local accumulation, not associated with a rise in blood basophil count[45,46]. At present, no study has been able to detect peripheral basophilia in parasitic infections in humans.

Autoimmunity
Despite the suspicion that basophils also contribute to the pathogenesis of Morbus Crohn, lupus nephritis and bullous pemphigoid[47] literature research could not provide studies showing peripheral basophilia in this pathology. In ulcerative colitis no disease linked basophilia could be found[48].
Nevertheless, declares Smith et al. (see further) that basophilia could be assigned due to autoimmunity in five patients[49].

Immunological/infectious: other
A limited number of studies indicate an increased basophilia in immunological non-Th2 related or autoimmune pathology. In 2001, Tikkanen et al. published a study noting absolute and relative basophilia in the context of acute rejection after (heart-)lung transplantation. Although they themselves report mild basophilia, according to the criteria of Valent et al. (see earlier) in the first month post-transplantation, there is an average hyperbasophilia in the 'organ rejection population[50].

In several infections, an increased basophil count is reported. Minderjahn et al. states that phlegmonous appendicitis is associated with basophilia[51] Although the study's conclusion is statistically significant, the difference ($0.031x10^9$/L vs $0.028x10^9$/L) is smaller than the intrapersonal variability (see earlier) of basophilia and is therefore not clinically applicable.

Chronic conditions
Feriel et al. state that increased basophilia was observed in diabetics in a study of Xu et al. The difference with the control group is statistically significant but is less than the analytical variation

of current cell counters. Moreover, with a median of only $0.1x10^9$/L one can discuss if this is in fact basophilia. In diabetic keto-acidosis, a static and 'analytical' significant difference is observed with a median of $0.2x10^9$cells/L[28,52]. A link to hypothyroidism was debunked in a study in 1993.

Other

The recent review of Feriel et al. reports that basophilia is also observed for tuberculosis, wind pox, variolapox, cirrhosis and after exposure to foreign proteins. In the 1959 study to which they refer, the link with cirrhosis and exposure to foreign proteins is debunked and the link with the others called into question[53] The study published 1984, which would claim that there is mild basophilia in patients with iron deficiency anemia, states that they cannot make such association. [28,54]

Higher clozapine variations were observed by Jakobsen et al. after-age correction in chronic basophilia, among others. The study had only one patient with basophilia... One study reports a higher relative basophilic number when exposed to mercury, but does not provide information on the strength of this correlation[55].

Smith et al. found that basophilia was half of the ‘non-myeloproliferative basophilia’ was due to (lymphoid) malignancies and tobacco use. Other less frequent causes included use of corticosteroids, infections, previous splenectomy, monoclonal gammopathy and inflammatory states. The assignment is based on expert opinion, without further citation[49]

The level evidence of reactive basophilia is weak and often based on expert opinion. In fact, it is usually an exclusion diagnosis, of which the diagnostic added value is limited.

A reactive state (allergy, parasitic infections...) is more likely to be accompanied by basophilic activation, then a strong systemic increase. Although basophils and mast cells play an important role in type I sensitivity reactions, basophil concentration in the peripheral blood has no diagnostic role. Their role is therefore rather supportive and prognostic in this setting.

In diabetic ketoacidosis and acute rejection after lung transplantation, mild and hyperbasophilia is observed on average.

After exclusion any malignant basophilia (see below) reactive basophilia should not be further elaborated. Follow-up after a minimum of eight weeks is sufficient.

3. Malignant basophilia

Myeloid neoplasms

Diagnostics

Any basophilia > 0.48 $x10^9/L$ should be considered neoplastic[49]. This contradicts a previous study that states that only with a hyperbasophilia that persisting for more than 8 weeks, >1x10^9 basophils/L, one should work out a myeloid neoplasm. [3] and is based on a retrospective study of 382 leukocytosis patients (>=9.7x10^9/L) and basophilia (> 0.09x10^9/L) for which a BCR-ABL1 mutation analysis was requested. In the population with the latter criteria, a myeloid neoplasm was detected in 50%, and increased to 100% when basophilia was >0.48x10^9/L. Malignant basophilia was cause by CML in about half of the cases, followed by essential thrombocytosis, polycythemia vera, primary myelofibrosis (PMF) and myeloid neoplasms not otherwise specified each accounting for about one tenth of the causes. A small part was attributed to chronic myelomonocytic leukemia, acute myeloid leukemia, chronic neutrophilia leukemia and myelodysplastic syndrome(MDS)[49]. In other words, around 90% of the causes of basophilia are linked to CML or a JAK2 V617F associated condition. However, it should be born in mind that there is a strong bias in the patient population, which therefore does not allow to extrapolate this statement to the entire population. It is thus merely indicative.

Keynote: A persistent leukocytosis with basophilia should therefore be elaborated with a BCR-ABL1 and in negativity JAK2V617F mutation analysis.

Prognosis and staging

The usefulness of basophilia extends beyond diagnostics alone. In CML, PMF, MDS, basophilia is a negative prognostic marker. Braga et al. stated in 1996, long before the arrival of tyrosine kinase inhibitors, that peripheral basophilia in combination with bone marrow blasts had the strongest predictive relationship with survival in CML[56]. The absolute fraction of basophils is particularly important. Compared to white blood cell count or eosinophilia, it has a better correlation with the percentage of metaphases with Philadelphia chromosome[57]. Basophilia is therefore used in CML scoring systems such as the Sokal, Hasford and EUTOS score[28,58].By contrast, the new ETLS score that has a superior prognostic predictive value does not include the basophil count[59].

Beside these prognostic meaning, basophilia it also used to be staging of CML. A percentage of basophils in peripheral blood of more than 20% is a criterion for the acceleration phase according to the WHO.

Likewise in PMF, peripheral basophilia is associated with decreased survival as an independent prognostic marker (HR: 4.79, $p<0.001$)[60]. Dobrowolski et al. note in their retrospective study that more than 3 months of persistent absolute basophilia primary myelofibrosis is associated with higher incidence on progression to acute myeloid leukemia. This statement can be viewed with some reserve as it is a retrospective study and patient selection may show a strong bias. Only 64/623 patients were enrolled[61].

Basophilia after/ as a result of initiation of therapy pomalidomide, on the other hand, has been associated with a better response, i.e. a lower incidence of anemia[62].

Finally, for MDS basophilia is also a negative prognostic marker. MDS with >1% basophils in bone marrow studies is more commonly associated with chromosomal abnormalities and complex karyotypes with a higher incidence of progression to acute leukemia and significantly lower survival[63]. This was confirmed in a large retrospective cohort study in 2010 by stating that basophilia (>0.25 x10^9/L)[64].

Basophilic leukemia

This leukemia comprises less than 1% of acute myeloid leukemias. A clear distinction should be made between basophilic leukemia and myeloid neoplasms associated with a reactive polyclonal increase in basophilic numbers. For example, an MDS or ALL may be associated with a -sometimes substantial- hyperbasophilia without monoclonality of the latter population.

Hyperbasophilia should be present which also exceeds 40% of the white blood cell count. This population should belong to a malignant clone. This can be supported by, firstly, the immature morphology of the basophils (see above), secondly, the type of underlying neoplasm and thirdly the presence of a clonal (molecular or cytogenetic) marker[3].

In case of suspicion of basophilic leukemia, a conventional karyotyping should be carried out after the exclusion of other types of AML and an analysis of the presence of the Philadelphia chromosome, BCR-ABL1 and JAK2V617F. Unique karyotypical abnormalities are limited to the translocation (X;6) (p11q23) that leads to expression of the MYB-GATA1 fusion gene in immature basophil precursors. Furthermore, the t (3;6) (q21; p21) is reported and abnormalities by 12p. Depending on the other findings, additional molecular or cytogenetic research may be carried out.

The diagnosis of chronic or acute basophilic leukemia is accompanied by a poor prognosis ranging from 2 to 36 months. Stem cell transplantation may be considered. Depending on the associated neoplasm (e.g., CML), targeted therapy is initiated (e.g., tyrosine kinase inhibitors), often independent of basophilia. One should be aware of histamine-related symptoms and, if necessary, start prophylactic antihistamines[3].

Addendum: Role of tryptase in hemato-oncology

Mast cells and immature (mainly neoplastic -hypogranular) basophils in the bone marrow produce tryptase. This is increased in certain myeloid neoplasm such as BCR-ABL positive CML and has a prognostic value[3]. An increased tryptase (>15ng/mL) when diagnosed with CML chronic phase was associated with a to 3x higher risk of progression[65]. In 2009 it was proposed to use tryptase (as well as histamine/its metabolites) as a potential marker of minimal residual disease[66] Prognostic, it is a better marker than basophils, possibly due to underestimation of the immature (degranulated) basophils in microscopy[67]. Nevertheless, the European, Belgian and British do not use tryptase in the follow-up to CML, perhaps due to the accessible follow-up of the robust BCR-ABL marker.

conclusion

- Basophilia is associated with myeloproliferative diseases such as CML, PV, ET, PMF.
- There is an association with poor prognosis in CML, PMF and MDS.
- Basophilic leukemia is a rare acute myeloïd leukemia with evidence of a malignant clone.

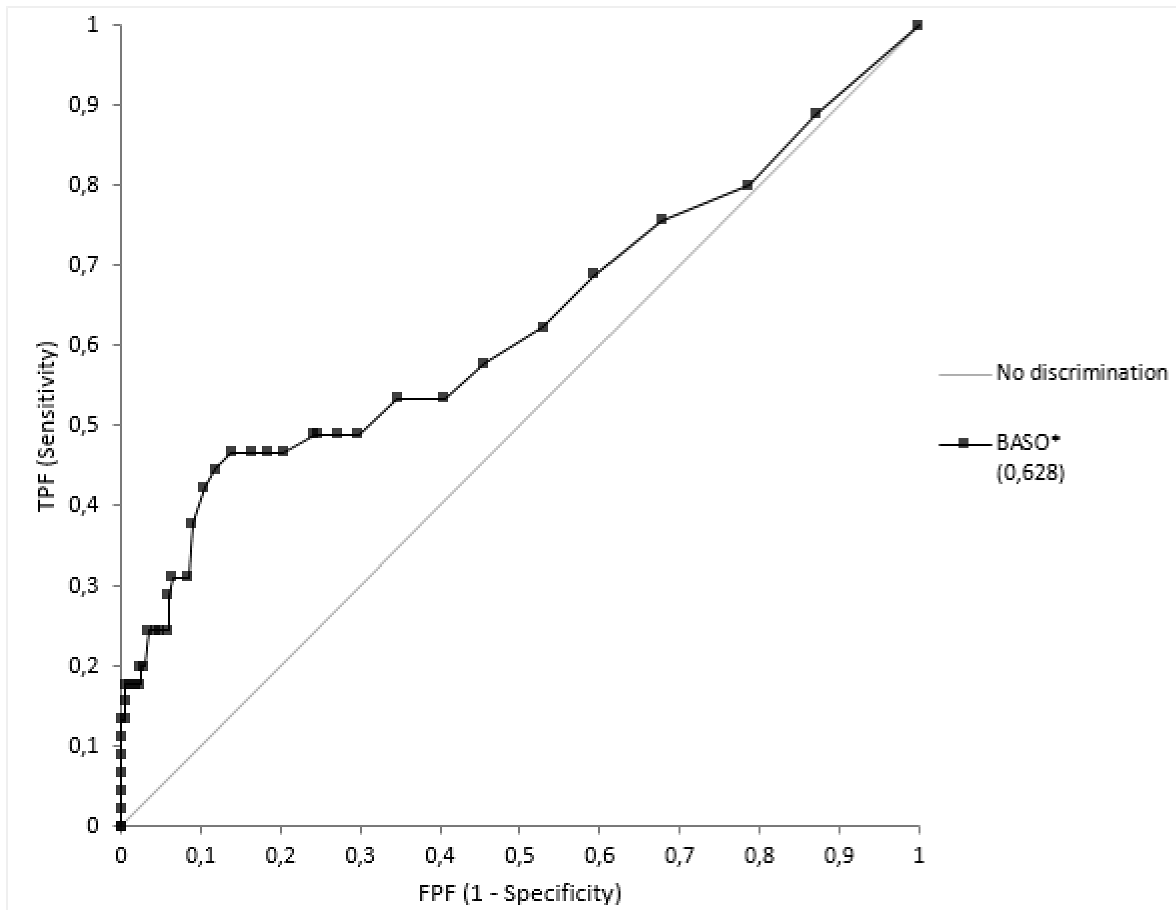
What is the diagnostic power of basophilia?

To examine the diagnostic value of basophils, a retrospective study was performed on all patients with basophilia in 2018 and 2019 at the Imelda hospital in Bonheiden, Belgium. Basophilia was defined as >0.2 x10^9 basophils/L. Given the limited clinical significance in a pediatric population, children below the age of 12 were excluded from the analysis. In the case of the presence of multiple samples from one patient only the first sample was retained for analysis. By this criteria 247 unique samples were included, of which 45 (18.2%) had a myeloproliferative disease or MDS/MPN syndrome. Futhermore the study population included 6 cases of AML and 5 cases of MDS. 10/45 patients had a diagnosis within 30 days of the date of sampling.

ROC analysis was performed with an area under the curve (AUC) of 0.628 The optimal cutoff for the diagnosis of an MPN, MPN/MDS overlap or AML disease with is 0.36 x 10^9 basophils/L, with a positive and negative likelihood ratio (LR) of 3.37 and 0.62. Given the biased -artificially high-incidence of the disorders in our study population, the use of an demonstrative value for malignant basophilia based on a robust positive likelihood ratio (>10) is therefore preferred. A basophilia >0.54 x 10^9/L has a positive and negative LR of 17.96 and 0.83, respectively.

- 247 unique patients after exclusion
- 45 with MPN + 6 AML + 4 MDS
  - CML 8
  - CMML* 6
  - ET 11*
  - MDS/MPN 1 (MDS/MPN RS-t)
  - MPN 1
  - MF 7
  - PV 13*

  1 patient had both ET/CMML, 1 ET/PV
- 10 new diagnoses + 1 AML
  - 2 ET
  - 3 PV
  - 4 CML
  - 1CMML



	1	0	Total
MPN	45	202	247

	AUC	95% CI	SE
BASO*	0,628	0,523 to 0,733	0,0538

Figure 3: ROC analysis for basophil concentration vs. the presence or absence of an MPN in all samples of all patients >12 years of age in 2018-2019 in Imelda Hospitals. Analysis-it, Excel.

When performing the analysis on samples of within 30 days of diagnosis an AUC of 0.804 (95% CI 0.631-0.976) was found with a same optimal cut-off of 0.36 (LR 5.05; LR- 0.35) and a LR+ >10 at a basophil count of 0.45 x10^9/L.

These findings were confirmed by independent data from the first semester of 2021. Respectively 142 of 81 patients had a basophilia of >0.2. 9 patients had a basophilia >/=0.54 x 10^9/L. 6/9 samples had a myeloproliferative disorder, the other being a hairy cell leukemia, CLL, and AML. By lowering this cut-off tot 0.45, 3 and 2 more cases of MPN and CLL respectively are added.

Given that the study population included only 15 new diagnoses, it was not possible to define the predictive value of each MPN with their individual cell line. In other words, larger studies are needed to ascertain the diagnostic value integration of basophilia together with the concentration of a characteristic cell line (e.g. platelet count in essential thrombocytosis).

To conclude, this data shows that basophilia alone is a weak diagnostic marker for MPN. The optimal cut-off value is associated with a slight effect on the post-test probability. However with increasing basophil count, the probability of having an MPN rises as well. Therefore every basophil count > 0.64 x 10^9/L should be investigated for the presence of an MPN.

Conclusion (Clinical bottom line)

The basophil has been described for a long time, yet only in the last 20 years has there been a large increase in knowledge. The reference method remains microscopic examination of 400 cells, although the lack of accuracy due to statistical error is acknowledged. Laboratories nowadays usually measure the basophils first on an automatic cell counter. Different measurement techniques are used to discriminate basophils from other white blood cells. These methods know their flaws, and their accuracy and correlation are moderate to quite good. A possible alternative for the reference method is flow cytometry. Several markers have been proposed, but a standardized protocol is lacking. However, at least two immunophenotypic markers may be needed for optimal accuracy.

Given the rather poor reliability of basophil counting, diagnostic use of basophilia as a stand-alone test is not certainly recommended. Nonetheless a basophil count of >0.45 x 10^9/L has a strong correlation with the diagnosis of an MPN. In line with the suggestion of mr. Smith, a basophil count of >0.40 x 10^9/L should trigger clinicians to examine the presence of an MPN. In absence of any such finding.

The current evidence for reactive basophilia, better "non-malignant basophilia", is weak. Often it is based on expert opinion and cross-references to mostly the same old studies. Most convincing evidence is available for smokers and lymphoid malignancies as well as in acute lung rejection. Reactive basophilia is therefore an exclusion diagnosis without any well-defined interval or strong diagnostic potential.

Further larger studies are required to assess the diagnostic power of basophilia in association with other laboratory parameters. BCR-ABL1, followed by JAK2 mutation (and on indication CALR, MPL mutation) analysis should be performed.

To do/Actions

1) Feedback of clinicians.
2) Implementation of the study findings in daily laboratory practice.
3) Inform clinicians concerning the findings of a basophilia >0.45.