Spotlight on hematology

Eosinophilia with 3-Part-Diff Hematology systems

MQZH 2019-1

Introduction

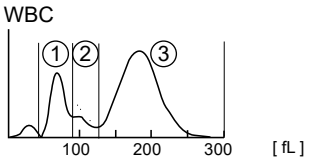
Measuring of the absolute eosinophilia number can be significant with specific tentative diagnoses or also over the course of disorders such as COPD. 3-Part-Diff Hematology systems that are often used in medical practices vary significantly in respect to the detection of eosinophilia of larger 5-Part-Diff Hematology systems that are used in hospitals and private laboratories. From this perspective, we want to take a closer look at the possibilities and limits of the eosinophilia measurement with these devices.
The preparation 2019-H3B derives from a patient with St. n. kidney transplantation and a minor, relative eosinophilia of 10.6%, whereas the absolute value of 0.37 G/L still being within the reference range.

Measurement techniques

The 3-Part-Diff Hematology systems count the total number of leucocyctes and divide the leucocytes based on their volume among three different subpopulations. For this, the blood sample is diluted and a (manufacturer specific) lysis reagent is added. As a result, the erythrocites are lysed to avoid any interferences. This causes either a loss of the cytoplasmic membrane (lymphocites, nucleus volume measurement) or a leakage of the cytoplasm, during which the residual membrane encloses the nucleus and existing cytoplasmatic granules.

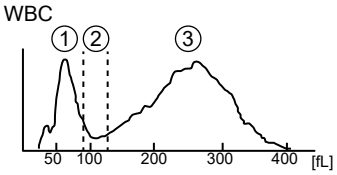
The impedance procedure is used for the measurement. When the cell passes the direct current at the measurement opening, an electrical impulse emerges that is proportional to the volume of the cell. The different cell volumes are applied to the WBC histogram, whereas the height of the curve corresponds with the cell number and the position of the population on the X-axis corresponds with the cell volume. This way, three subpopulations are formed: small cells, medium sized cells and large cells that are differentiated from each other depending on the manufacturer with fixed discriminators or, within given areas, with flexible discriminators.

Sysmex devices: pocH-100i, XP300



- Population 1, Lymphocytes
- Population 2, Monocytes, Eosinophils and Basophils
- Population 3, Neutrophils
- Discriminators flexible (are set by the device due to their curve characteristics, but can also be set manually)

Other devices: e.g. Orphée mythic, ABX Micros, Microsemi



- Population 1, Lymphocytes
- Population 2, Monocytes
- Population 3, Neutrophils, Eosinophils and Basophils
- Discriminators fixed

Warning signs (Flags)

If atypical or premature cells appear, these are also measured within the existing populations. In such cases, the shape of the curve is usually changed. Algorithms that are stored within the device software trigger warning signs (Flags) on suspicion of such cells. These Flags consist of letters and numbers that are displayed on the print in addition to the automatically collected differential blood count values. They usually do not trigger an acoustic device alarm and therefore may be the only indication of an atypical cell distribution.

For Sysmex devices, it is possible that the WBC differentiation is not performed, because the device does not recognize any valleys, for which the variable discriminators can be set. In this case, only the total number of leucocytes is specified.

Eosinophilic granulocytes

Eosinophils are cells occurring predominantly in the tissue and their functions are not entirely known.

The number of eosinophils in the blood or in the tissue may increase with a variety of disorders inter alia with allergies, infections, parasitic, inflammatory or neoplastic diseases.

Activated eosinophils release mediators and substances that may cause damage to various tissues / organs (inter alia cardiac, gastrointestinal, respiratory tract).

Effect of the lysing agent on leucocytes

The cells are treated with lysis reagent for leucocyte distribution.

The cytoplasm membrane of the leucocytes reacts to the lysing agent with a loss of cytoplasm content. The residual shell shrinks and wraps closer around the cell nucleus. How much the cells change under the influence of the lysis reagent depends on the cell type and the used lysis reagent.

	Volume before lysing agent	Volume after lysing agent
Lymphocyte		
Neutrophilic granulocytes		
Monocyte		

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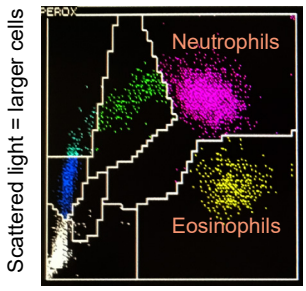
How does the ADVIA establish the eosinophils?

ADVIA 2120 - peroxidase coloring



The eosinophils are significantly stained and therefore appear on the extreme right-hand side. Although they are about the same size as the neutrophils, they are further down, because the strong coloring reduces the amount of the scattered light.

Conclusion

Although 3-Part-Diff analyzers cannot measure quantitative eosinophilia values, all three of the used devices either issued deviations from the reference range of the MXD population or a warning (Flag).

These findings must lead for all three devices to further clarification via a microscopic differential blood count when handled correctly by the examiner.

About
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Detection of eosinophilia in 3-Part-Diff

3-Part-Diff analyzers cannot disclose any quantitative eosinophilia values. However, an eosinophilia flag may be triggered based on the known cell volume with atypical high impulse counts in defined areas of the histogram. Such flags usually list several possibilities for this atypical finding such that a microscopic examination of a blood smear to evaluate the cause of the flag and other diagnostics are imperative.

The laboratory personnel should pay attention to such warnings and notify the contracting physician accordingly.

The electronic connection of hematology devices to a laboratory information system or a practice software is particularly sensitive. When introducing or changing the software, it should always be checked, if the warning messages (Flags) had also been transferred correctly when transmitting the results.

Comparison of 3-Part-Diff devices with a 5-Part-Diff device

We also measured the blood of MQ 2019-1 H3B with a 5-Part-Diff Hematology system that has additional measurement methods used to determine eosinophils and basophils. The ADVIA 2120 measured the following values from the sample:

Monocytes	0.20 G/l	5.8%
Eosinophils	0.37 G/l	10.6%
Basophils	0.04 G/l	1.2%
Neutrophils	1.89 G/l	54.1%

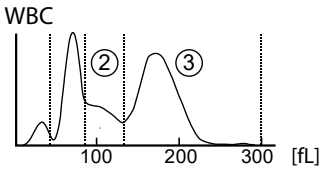
Calculated values:

MXD (M+E+B)	0.61 G/l	17.6%
Granulocytes (E+B+N)	2.3 G/l	65.9%

Sysmex - *XP300*

With this device, the increase of the eosinophilia values led to an increased value of 17.3 % for the «MXD» Population (medium sized cells). The increase is visible here despite the relatively low eosinophilia, which is due to the generally lower reference areas for the cell types that have been measured here (monocytes, basophils and eosinophils). A Flag has not been triggered. The sum of monocytes, eosinophils and basophils on the ADVIA is 17.6% and matches well with the XP300.

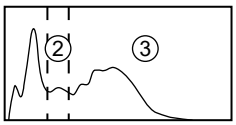
2	MXD	0.60 G/l	17.3%
3	Neutrophils	2.0 G/l	55.7%



Orphée Mythic

This device type measures eosinophils in the range of the large cells together with basophilic and neutrophilic granulocytes. The reference range for neutrophilic granulocytes is quite high; it is the largest population of leucocytes in the peripheral blood of the healthy individual on a percentage basis. A minor eosinophilia does not stand out in numbers in this area. The device triggered a relevant Flag «FL3», though.
The granulocytes are lower; the monocytes are higher than expected. Therefore, a part of the eosinophils was probably added to the monocytes.

2	Monocytes	0.4 G/l	11.1%
3	Granulocytes	2.3 G/l	59.7%



Microsemi

This analyzer also measures the eosinophils in the range of the neutrophilic and basophilic granulocytes such that there is no significant result here on a percentage basis, either. This device triggered a relevant Flag «G1/G2», though.
The granulocytes are lower; the monocytes are higher than expected such that part of the eosinophils were probably added to the monocytes.

2	Monocytes	0.3 G/l	9.8%
3	Granulocytes	2.0 G/l	61.4%