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Stepwise diagnostic procedure for the analysis of pathological changes of leukocytes

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Abstract: The automated analysis of changes of white blood cells and their differential has become a high-throughput laboratory investigation done by hematology analyzers. The subsequent step of manual review of a stained blood film by light microscopy by an experienced investigator is laborious and expensive and has insufficient reimbursement. However, this is the decisive step and basis for very expensive specialized diagnostics like cytogenetics and molecular genetics. The strategy for a stepwise diagnostic procedure plays an important role at this stage. A new proposal is presented here.

Keywords: blood film review; hematology analyzer; leukocyte differential; manual differential.

Introduction

The blood count and differential blood count are among the most commonly requested laboratory tests. Leukocytopenia and leukocytosis, as well as quantitative changes in granulocytes and lymphocytes, can have reactive causes, but also be the result of malignant changes in myelopoiesis or the lymphatic system.

The stepwise diagnostic procedure, proposed herein as the subject of discussion, focuses on the changes in leukocytes, without neglecting the relationship with the other cell systems. This stepwise diagnostic procedure is multifactorial – it depends on the type of changes, their degree and on time. The purpose of presenting stepwise diagnostics herein is to achieve a meaningful approach, rather than a complete list of all potential differential diagnoses.

The blood count is usually part of an admission profile or routine laboratory check, and is often requested regardless of the patient's clinical status. If changes in the blood count are suspected beforehand, or if the patient exhibits ambiguous symptoms, a differentiation of leukocytes (including assessment of the red blood cell count and platelets) and reticulocytes should always be ordered in addition to other lab tests – these tests have become a fixture in today's basic diagnostics [1].

The blood count, that is, a measurement of the concentration of the cellular blood components, including erythrocyte indices, can be performed almost in all cases by automated analyzers with a high degree of precision and accuracy.

The differential blood count, with a representation of the leukocyte subsets, can be realized correctly only partially by hematologic analyzers [2]. Generally, automated hematology systems can be used to classify and quantify inconspicuous cell populations (segmented neutrophil granulocytes, lymphocytes, monocytes, eosinophil granulocytes) to a high degree of precision. But cells that do not normally occur in blood are not detected reliably by automated systems, and thus not counted. They are, however, "flagged" if they occur in "larger" numbers. Flagged differential blood counts must be analyzed microscopically, or at least be checked. The most common flags are shown in Table 1, while Table 2 provides information on the performance of hematologic analysis systems.

A flag issued by a hematologic analyzer in the leukocyte differentiation of a blood sample means that there might be a cell population in the blood sample that normally does not occur. The sensitivity of detection of atypical populations, however, differs from one device to another and may be altered in part by the user.

As a rule,

– the more sensitive the device is in issuing warnings, the more pathological samples are recognized by it. The disadvantage of "sensitive" devices is that sometimes a warning is issued for inconspicuous

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Table 1: The most important warnings (flags) of hematologic analysis systems that warrant follow-up checks.

– Blasts
– Left-shifted granulopoiesis
– Atypical lymphocytes (includes both activated lymphoid cells as well lymphoma cells)
– Erythroblasts
– Platelet agglutinates

The flags are given different company-specific abbreviations, depending on the system used; these are not listed separately here.

Table 2: Performance of hematology analyzer systems.

Analyte	Assessment
– Leukocyte count	Very good
– Concentrations of neutrophils, lymphocytes, monocytes, eosinophils	Very good
– Concentration of basophils	Moderate
– Left-shifted cells, blasts, atypical lymphocytes	Possible flags, not certain
Plasma cells	Count and reliable classification not possible
– Erythrocyte indices (MCV, MCH, MCHC)	Very good
– Erythrocyte anisocytosis	Very good
– Erythroblasts (count)	Possible, often uncertain
– Erythrocyte morphology (see morphology checklist)	Not possible
– Platelet count	Very good
– Platelet agglutinates	Detection possible, not certain
– Platelet anisocytosis	Possible

samples and included in the smear. The device's high accuracy for normal cell populations is lost with manual microscopy, because only 100 or 200 cells are usually differentiated. It also adds extra time for the microscopic post-differentiation.

– the less sensitive a device is, the more pathological samples are not flagged and not included in the smear. The disadvantage is a more or less substantial proportion of pathological samples that are not recognized. This can put patients at risk, and it is the laboratory's responsibility.

Most laboratories work with this stepped approach and primarily use automated systems for differential blood counts. This is illustrated in Figure 1. The patient's blood sample is sent to the laboratory, with a lab request, where it is analyzed on an automated system. But this often leaves out the clinical question to be addressed (the left arrow leading away from the patient).

If the blood count is not flagged (the green flag in Figure 1), the blood count results will be shared with the submitter, without there having been a microscopic check. The age- and gender-dependent reference ranges, which were recently updated, are generally included in the laboratory printouts [3]. Assessment and comparison against the clinical symptoms are done by the submitter.

If the blood count is flagged (the red flag in Figure 1), the EDTA blood is streaked out and manually assessed under the microscope; this requires, of course, an optimal staining of the smear [4]. The results of the blood count, the percentage-based and absolute readings of the leukocyte subset, as well as any additional changes to the leukocytes, erythrocytes and platelets detected microscopically, will be returned to the submitter without any interpretation.

The problem with this approach is that automated hematologic analyzers cannot reliably detect pathological cell populations, which means that there is no warning. This is why there are many additional algorithms (rules) [5] that, based on combinations of blood count readings, suggest that there should be conspicuous cell populations even if the device does not flag them. Such blood counts are flagged, streaked out and analyzed under the microscope by means of the algorithm applied. For example, blood counts with granulocytopenia are streaked out for fear that dysplasias or blasts might be overlooked in the case of a myelodysplastic syndrome (MDS). This creates a considerable amount of work, especially because it is usually non-hematological diseases that cause granulocytopenia (e.g. status after chemotherapy or viral infection). Even new methods that include immunocytological criteria require a high rate of post-processing [6]. A quick look through the microscope might be a compromise when it comes to workflow decisions [7].

This is where our "new" proposal sets in. The results of differential blood counts without the clinical question and without being flagged by the device are sent to the submitter, without any additional algorithms influencing the decision tree, because these rules are incomplete. If the treating physician cannot connect the changes in the blood count to the clinical symptoms, a question is created. The question (to remain with this example) of "ambiguous granulocytopenia" and "with suspected MDS" is communicated to the laboratory via the request form. The process in the laboratory is modified accordingly (shown by the right arrow in the figure and the information contained in the lab request). In this case, a manual differential blood count is always done. The submitter receives the results of the blood count and of the differential blood count, and the question of the submitter is also answered. Such a clear

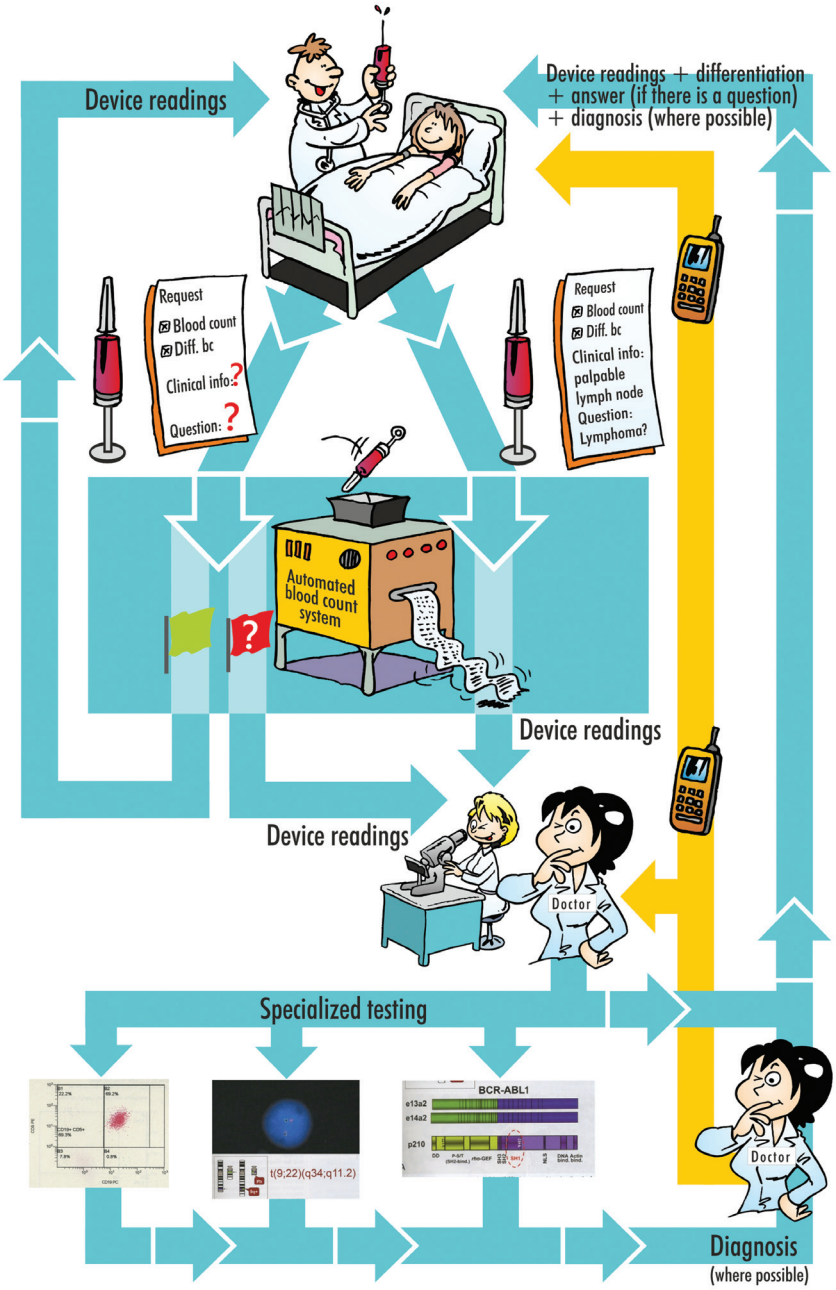

Figure 1: Schematic representation of the recommended laboratory workflow.

question (rather than a specious one) can be answered via a report, text modules or text-based abbreviations. Table 3 illustrates some examples.

The "new" proposal does not allow (anymore) for a "manual" differential blood count to be requested in connection with a normal blood count, without flags and without a question. A manual blood count is time-consuming and only justified when the submitter specified a question that requires a microscopic differentiation and that an analyzer device cannot perform (see Table 2).

To make sure that nothing is overlooked under the microscope, a check list is used. It contains the items that should be considered with every microscopic analysis (see Table 4). This check list is particularly useful in hematological stepwise diagnostics. The changes in the blood count are thus summarized as "bullet points" and yield a pattern. For example, the description leukocytosis, granulocytosis, left shift to the blasts, basophilia, inconspicuous red blood count and thrombocytosis with anisocytosis and suspected chronic myeloid leukemia (CML)

Table 3: Examples of a clinical question and possible answer from the laboratory.

Question	Answer
Unclear anemia	Normocytic, hypo-regenerative anemia Morphologically, no conspicuous erythrocytes
Unclear thrombocytopenia	Platelet agglutinates +++ Suspected pseudothrombocytopenia
Unclear lymphadenopathy. Lymphoma?	10% atypical lymphocytes, presumably neoplastic
Unclear cytopenia – MDS?	Pancytopenia with individual blasts and dysplasias. Suspected MDS or acute leukemia
Unclear fever. Unclear splenomegaly	"Atypical lymphocytes, presumably reactive" Suspected viral infection

suggests that the molecular-genetic test of *BCR-ABL1* can be confirmed or discarded.

Hematological stepwise diagnostics

The numerical blood count, the differential blood count and determination of reticulocytes are the basis of hematological stepwise diagnostics. If the clinician suspects a blood disease, additional methods are often necessary that go beyond basic hematological diagnostics.

In order to develop from the basic diagnostics a suspected diagnosis/diagnosis, the physician will usually also require information about the patient's condition, his/her previous history and any medication previously administered or taken. This synopsis serves primarily as orientation as to whether a reactive or neoplastic event is involved and whether there is an acute need for action to order further, urgent testing.

In most cases, the clinical information, routine laboratory tests and basic hematological diagnostics are sufficient to explain reactive changes to the leukocytes adequately. If a blood disease is suspected, further specialized testing will usually be required. The starting point prior to (expensive) specialized testing on peripheral blood generally involves sufficient clinical data and questions, the blood count, reticulocytes and a manual differential blood count, the combination of these findings, identification of a pattern, as well as formulation of one or several working hypotheses. The question of "unclear leukocytosis" would be better phrased as "persistent unclear leukocytosis for three months without fever, with normal lymph node status and splenomegaly" or "persistent leukopenia following a flu-like infection six months ago" or "progressive cervical lymphadenopathy for two weeks", that is, the duration, clinical symptoms

Table 4: Check list (for adults) so as not to overlook anything under the microscope.

– Leukocyte count	Leukopenia (<3.8 g/L) – normal – leukocytosis (>10 g/L)
– Granulocyte count	Granulocytopenia (<1.8 g/L) – normal – granulocytosis (>8 g/L)
– Lymphocyte count	Lymphocytopenia (<1.0 g/L) – normal – lymphocytosis (>3.0 g/L)
– Monocyte count	Monocytopenia (<0.1 g/L) – normal – monocytosis (>1.0 g/L)
– Eosinophil count	Normal – eosinophilia (>0.5 g/L)
– Basophil count	Normal – basophilia (>0.1 g/L)
– Left shift	How far (unsegmented? Meta? Myelo? Promyelo? Blast?)
– Blasts	Myeloid? Granules? Auer rods?...?
– Dysplasias	Pale granulocytes? Pseudo Pelger forms? Clearing up of the core?
– Atypical lymphocytes	Probably reactive? presumably neoplastic? unclear dignity?
– Inclusions	Phagocytosis? toxic granules? Döhle bodies?
– Hb level	Anemia (<11/12/13.5 g/dL (by age and sex)) – normal – polycythemia (>16/17.5 g/L (by sex))
– Anisocytosis	Microcytes (<6 μm) – normocytes – macrocytes (>10 μm)
– Poikilocytosis	Teardrop shape? Fragmentocytes?...?
– Polychromasia	?
– Inclusions	Malaria? Jolly bodies? Basophilic stippling?...?
– Hb distribution	Anulocytes? Hb distribution disorders? Spherocytes?...?
– Storage of erythrocytes	Clumping? Rouleaux?
– Erythroblasts	?
– Platelet count	Thrombocytopenia (<140 g/L) – normal – thrombocytosis (>300 g/L)
– Platelet anisocytosis	Macro platelets?
– Platelet agglutinates	?
– Platelet poikilocytosis	?

with signs of inflammation and the condition of the lymphatic system and/or the extramedullary hematopoiesis shift the tolerance limits for changes in the blood count. A good example is the upper limit of lymphocytes and their morphological variations, which are heavily dependent on age and clinical symptoms [8].

The role of image recognition systems (computer-aided microscopy) is difficult to assess. These systems work with automatically generated smears and staining, and analyze the leukocytes by means of digital image processing [9]. The photographed cells are preclassified and must then be approved by lab staff for the final report. The time advantage of digital image processing comes with the downside of having to work in front of a screen and of the emphasis being on the leukocytes, while erythrocyte and platelet morphology is neglected to some degree (e.g. fragmentocytes [10]). In addition, fine, low-contrast structures, such as fine LGL granules, are not detected, or the treatment of core shadows is not yet taken into account [11]. Digital image processing systems, however, are continually being improved, which means a definitive conclusion or verdict is not possible at this point.

The specialized tests are represented in the figure at the bottom with respect to peripheral blood (from left to right): immunocytology, cytogenetic testing incl. FISH analysis and molecular genetics. When analyzing bone marrow, it is important to differentiate between aspiration and histology, and when analyzing the lymph node, between fine needle aspiration and histology. The articles in this issue deal with these methods in greater detail. At this point, one ought to reference hematology textbooks [12, 13].

The hematological diagnostics described so far – with the synopsis of patient findings to date, therapies, clinical symptoms, clinical question, results from the blood count, differential blood count (under the microscope) and reticulocytes, as well as other laboratory results – will produce a hypothesis of one or several diagnoses. There simply cannot be a "simple" or "easy" algorithm for further stepwise diagnostics and the meaningful use of specialized testing. The variety of possible diagnoses and changes is simply too great. Only 30–40 years ago, hematological diagnostics was (almost) nothing more than an analysis under the microscope, performed by the treating physician himself or herself. In some countries, this is still true today. Modern-day hematological diagnostics with immunocytometry, cytogenetics and molecular genetics can no longer be performed by the clinician himself or herself for the most part. This work is done by specialist laboratories instead. This means that the findings are invariably interpreted by people who do not know the patient. But the time-honored approach, "the diagnosis is made at the bedside", still holds true today. This dilemma can be solved only if clinicians and laboratories cooperate and focus on the patient. Thus, such efficient cooperation can help with the choice of specialized testing, the kind that can be performed in real time and that will reliably confirm or discard the working diagnosis. This diagnostic cycle ends at the point where a definitive therapy has been chosen for the patient.

Summary

What is "new" about this proposal for stepwise diagnostics to clarify pathological changes in leukocytes is that it is the clinician's question that controls the processes. It can be illustrated as follows:

- Differential blood counts from an automated hematological analyzer without flags are no longer streaked out, and the findings are shared with the submitter without further commentary.
- Differential blood counts from an automated hematological analyzer with flags are streaked out, and the findings are verified in combination with the results of the analyzer and microscopy. The findings are then returned to the submitter, and comments are added only if further clarification is urgently recommended.
- Blood counts based on a question are always streaked out and analyzed under the microscope, and the submitter's question is "answered".
- When it comes to clarifying hematological questions, any specialized testing should be preceded by basic hematological diagnostics (including reticulocytes and microscopic differential blood count), the findings of which should be available to the laboratory performing the specialized tests.
- Then, the necessary specialized tests should have a rational foundation that consists of the clinical questions and the significance of testing methods.
- The diagnostics should be continued between the clinicians and specialist laboratories until the treatment of the patient has been decided and/or follow-up checks of the patient have been completed.

The proposal presented herein creates clarity on both sides, for the clinician and the laboratory, as to the diagnostics and its implementation. It also renders unnecessary complex sets of rules that contain controversial limits and algorithms. The procedure taken at this point has a far-reaching effect on the outcome for a patient with changes in the blood count.

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