



COMPLETE BLOOD COUNT, A MULTIFACETED INFLAMMATORY MARKER. CLINICAL AND BIOLOGICAL STUDY IN PNEUMONIA IN CHILDREN

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Abstract: Reactive C protein is traditionally considered the most used inflammatory marker. Although procalcitonin has a superior prognostic value, both can create difficulties in differentiating a bacterial infectious process from a viral one. Moreover, no marker is specific for etiology of infection. Complete blood count, investigation of first choice, can provide additional data in order to increase the accuracy of diagnosis. The paper aimed to study the way in which the markers in the complete blood count react during a bacterial or viral infectious process and emphasize their role in confirming diagnosis. The study included two groups, bacterial and viral pneumonia, and studied the following parameters: leukocytes count, percentage of neutrophils, percentage of lymphocytes, neutrophils/lymphocytes ratio, mean platelet volume/platelets ratio, platelets/lymphocytes ratio, mean platelet volume, erythrocyte distribution width, mean corpuscular volume. The study highlighted the usefulness of some of these markers, but only under the conditions of using broader reference values.

INTRODUCTION

Reactive C protein and erythrocyte sedimentation rate are traditionally considered the most used inflammatory markers. Often, in practice, there is a tendency to overuse inflammatory markers in order to increase the accuracy of the diagnosis. So, the list gets longer and longer: fibrinogen, ferritin, IL₆, IL₁₀, procalcitonin, soluble receptor of IL₂, tumour necrosis factor, neopterin, presepsin, and so on.(1) Newer, IL₁ and IL₁₈ tend to complete the list together with the experience accumulated over the pandemic SARS-CoV-2 with post-viral Kawasaki-like multiinflammatory syndrome in children.(2)

Although presepsin is recognized as having a superior sensitivity to the other parameters in sepsis, that neopterin is useful in assessing bacteriaemia, same as TNF in staphylococcal infection, none are specific in determining the etiology of the infection.(2) Furthermore, C reactive protein and procalcitonin may have elevated levels in viral infection as well (especially, in adenovirus) (3,4,5), and IL₆ and IL₈ have too short half-life to be used widely, to hospital screening.(6)

Nevertheless, literature data confirms the role of reactive C protein and procalcitonin in inflammation due to infection.(7) Complete blood count brings added value by leukocytes count and formula, as well as through by less conventional markers.

a. **Neutrophils lymphocytes ratio (NLR)** (in absolute values or percentages). It is a sensitive indicator of infection, with prognostic value, which involves the two sides of the immune system – innate one (by neutrophils) and adaptive (by lymphocytes).(8) It is also recognized as an indicator of “physiological stress”, the cut-off values still being debated. In pneumonia, it means short-term morbidity and mortality, the intensive care or rehospitalisation need. The normal values are 1-2, any value higher than 3 or lower than 0,7 being pathological. The gray area is located

between 3 and 6 (possibly due to extrainfectious pathology). The stress is mild considered between 6-9, moderate, between 9-18, and severe, above 18, just like in sepsis values between 5-10 means localized infection, 10-13, generalized one, 13-15, sepsis, and above 15, septic shock.(9)

- b. **Platelet count, mean platelet value (MPV), MPV/platelets ratio.** Platelets are essential in inflammation due to viral infection, its activation causing pulmonary injury. MPV is an indicator of platelet functionality and is negatively correlated with them in severely affected patients. At the onset of infection, the release of inflammatory cytokines stimulates thrombopoiesis, with efflux of young platelets in the circulatory stream and MPV increase as a result (in mononucleosis, the increase of MPV correlates with the impairment of liver function). Later, when oxidative stress cause the platelet destruction, megakaryocytes are stimulated, which also determines the MPV increase. However, there are studies that give a good prognosis when platelets grow, context in which also MPV should keep growing. MPV/platelets ratio becomes pathological at values higher than 3,71, with the meaning of an imminent sepsis.(10,11)
- c. **Platelet/lymphocytes ratio (PLR)** is considered, in turn, a severity predictor, the values bigger than 200 being considered pathological.(12)
- d. **Red cell distribution width (RDW)**, known as index of anisocytosis from anemias, also has a role of severity marker of a viral disease. The mechanism is still unclear (it is possible that viral infection changes erythropoiesis over time, and thus determine the RDW increase).(13)
- e. **Mean corpuscular volume (MCV).** It seems that this parameters has no value in sepsis (in sepsis, anemia is

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normocytic one). However, in combination with RDW, MCV becomes a marker of inflammation.(14)

AIM

As the main objective, the paper proposes to study the way the CBC markers react during the bacterial or viral infectious process, and emphasizing their role in confirming the diagnosis. Secondary, there is considered the correlation which smashes between reactive C protein and CBS inflammatory markers, during the same infectious process.

MATERIAL AND METHODS

The retrospective, observational study included hospitalized patients in Sibiu Pediatric Clinic with community-acquired pneumonia as diagnosis, bacterial or viral ones, during two years. The inclusion criteria were: pediatric age (0-18 years old), the diagnosis established by clinical criteria (functional respiratory syndrome and characteristic objective pulmonary changes), laboratory and imagistic criteria (lung radiography with bacterial or viral changes). There were excluded those who recently presented a similar episode (less than 30 days), those who were recently hospitalized (less than 30 days) for an infectious pathology, with another location, those who presented chronic hematologic pathology (which interferes with hematologic markers used in study). The patients were distributed in two groups, the group with bacterial pneumonia (A group), and the group with viral pneumonia (B group). From the point of view of laboratory changes, procalcitonin was the essential marker who finalized the study groups: the A group patients presented values of procalcitonin bigger than 0,5 ng/mL, while the B group patients presents only normal values of procalcitonin.

The laboratory parameters used in study were divided in three groups:

- a. conventional markers (reactive C protein, leukocytes count, neutrophils percentage, lymphocytes percentage)
- b. ratio markers (NLR, MPV/platelets ratio, PLR)
- c. unconventional markers (platelets count, MPV, RDW, MCV).

Procalcitonin was just a way of establishing the groups of study, no other approach.

The statistics included mean, standard deviation, odd ratio, Pearson correlation index.

RESULTS

A group contained 69 patients, and B group 66 patients.

1. Presentation of the results of conventional inflammatory markers

- a. Media and standard deviation (SD)

Table no. 1. Media and SD of conventional markers in the two study groups

Conventional markers	A group	B group
Reactive C protein (mg/L)	132,2 ± 89,7	10,15 ± 8,71
Leukocytes count (x 10 ³ /mmc)	20,46 ± 7,71	11,93 ± 4,78
Neutrophils percentage (%)	73,2 ± 14,15	50,6 ± 18,79
Lymphocytes percentage (%)	17,8 ± 11,75	37,1 ± 16,88

- b. Comparative analysis of conventional markers

Table no. 2. Odds ratio (OR) and significance level of conventional markers

Conventional markers	OR	p
Reactive C protein	0,001, 95%CI [0,0001-0,0185]	< 0,0000001
Leukocytes count	0,1005, 95%CI [0,036-0,280]	< 0,0001
Neutrophils count	0,137, 95%CI [0,0445-0,4270]	0,0006
Lymphocytes count	9,882, 95%CI [3,759-25,98]	< 0,001

The cut-off value for reactive C protein was 24,47 mg/L, for leukocytes count was 19,79 x 10³/mmc, for neutrophils percentage 81,51 %, and for lymphocytes percentage 37,16 %.

2. Presentation of the results of ratio markers

- a. Presentation of the results of NLR

Table no. 3. The results of NLR in the two study groups

Parameter	A group	B group
Normal values (n)	23	49
“0” values (n)	-	7
Mild stress (n)	10	-
Moderate stress (n)	15	3
Severe stress (n)	7	1
Gray area (n)	14	6
OR	0,173, 95%CI [0,082-0,365], p < 0,0001	

- b. Presentation of the results of MPV/platelets ratio

Table no. 4. The results of MPV/platelets ratio in the two study group

Parameter	A group	B group
Normal values (n)	44	43
Values > 3,71 (n)	25	23
OR	0,9414, 95%CI [0,465-1,9057], p = 0,866	
Media ± SD	3,47 ± 2,19	3,23 ± 1,66

- c. Presentation of the results of PLR

Table no. 5. The results of PLR in the two study groups

Parameter	A group	B group
Normal values (n)	55	50
Values > 200 (n)	14	16
OR	1,257, 95%CI [0,5575-2,8348], p = 0,581	
Media ± SD	158,95 ± 114,01	123,94 ± 83,18

3. Presentation of the results of unconventional inflammatory markers

- a. Media and SD

Table no. 6. Media and SD of the unconventional markers in the two study groups

Conventional markers	A group	B group
Platelets count (x 10 ³ /mmc)	389 ± 221,07	384,25 ± 161,24
MPV (fl)	9,97 ± 1,09	10,16 ± 1,02
RDW (%)	15,44 ± 3,16	14,92 ± 1,95
MCV (fl)	77,3 ± 7,12	81,7 ± 9,03

- b. Comparative analysis of unconventional markers

Table no. 7. Odds ratio and significance level of unconventional markers

Unconventional markers	OR	p
Platelets count	0,885, 95%CI [0,2814-2,7882]	0,835
MPV	1,928, 95%CI [0,537-6,9207]	0,314
RDW	0,762, 95%CI [0,2496-2,3297]	0,634
MCV	9,852, 95%CI [2,1555-45,0376]	0,0032

4. Correlation between reactive C protein and some of the other inflammatory markers

- a. In A group

Table no. 8. Pearson correlation index between reactive C protein and unconventional markers in A group

Inflammatory markers	Direction	Intensity	p
Platelet count	negative	weak	0,206
MPV	positive	insignificant	0,531
RDW	negative	insignificant	0,567
MCV	positive	weak	0,194

- b. In B group

Table no. 9. Pearson correlation index between reactive C protein and unconventional markers in B group

Inflammatory markers	Direction	Intensity	p
Platelet count	positive	weak	0,164
MPV	negative	insignificant	0,898
RDW	positive	insignificant	0,603
MCV	positive	moderate	0,00045

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DISCUSSIONS

From the presentation of the laboratory data, the following can be found:

1. The study groups are numerically homogeneous.
2. The mean and standard deviation for conventional inflammatory markers are in accordance with the literature data. The greatest statistical significance is found in the case of reactive C protein (0,001, 95%CI [0,0001-0,0185], $p < 0,0000001$), follows the leukocytes count (0,1005, 95%CI [0,036-0,280], $p < 0,0001$), neutrophils percentage (0,137, 95%CI [0,0445-0,4270], $p = 0,0006$), and lymphocytes percentage (9,882, 95%CI [3,759-25,98], $p < 0,001$). The fact that the cut-off values seem higher than the estimated ones, can be explained in their variability: the reactive C protein can show increased values in viral infectious pathology, and the adaptive immune system can be triggered differently over time.
3. The ratio markers presented the following results:
 - a. NLR presented a statistically significant difference between the study groups in the sense that patients from A group who presented “physiological stress” were significantly more numerous than patients from B group (0,173, 95%CI [0,082-0,365], $p < 0,0001$). The statistical significance is also maintained in the stress intensity:
 - i. mild (23.4706, 95%CI [1.3461-409.2450], $p = 0.0305$)
 - ii. moderate (5.8333, 95%CI [1.6029-21.2295], $p = 0.007$). No statistically significant difference was found for severe stress (7.3387, 95%CI [0.8773-61.3861], $p = 0.0659$), and for the gray area and “0” values, no clinical correspondence was found. Oxidative stress develops homogeneously, both in bacterial and viral infectious pathology.
 - b. For the MPV/platelets ratio (0,9414, 95%CI [0,465-1,9057], $p = 0,866$), and PLR (1,257, 95%CI [0,5575-2,8348], $p = 0,581$) no statistically significant differences were found, although their average was higher for patients from A group.
4. Non-conventional inflammatory markers “behaved” in a less expected manner:
 - a. although literature attests to the presence of reactive thrombocytosis during the infectious process, the mean and standard deviation, both for A and B groups, remained within normal limits (0,885, 95%CI [0,2814-2,7882], $p = 0,835$); it is possible that reactive thrombocytosis develops during the infectious process and not from its onset
 - b. the mean MPV fell within the normal limits, in both groups, with a slightly higher value, but statistically insignificant for the patients from B group (1,928, 95%CI [0,537-6,9207], $p = 0,314$); although the trend is consistent with literature data (increased values of MPV in viral infection), the results remain inconsistent
 - c. no agreement was found between the platelets count and MPV values; it is possible that this concordance is not obvious from the onset of the condition
 - d. the average RDW exceeded the upper limit of normal for the patients from A group (in discord with the literature data), but the difference between the groups is not statistically significant (0,762, 95%CI [0,2496-2,3297], $p = 0,634$)
 - e. the results obtained for MCV are somewhat surprising; it is the only unconventional inflammatory marker whose average showed statistical significance between the groups (9,852, 95%CI [2,1555-45,0376], $p = 0,0032$); in other words, patients from A group are significantly more numerous than those from B group, for a lower value of 89.01 fl.
5. The Pearson index revealed only one significant correlation:
 - a. the relationship between reactive C protein and MCV in B group was positive, moderate, with a significance level of 0.00045.
 - b. all other relations, from both groups, were insignificant, the parameters not changing at the intensity of the C reactive protein.
6. Conventional inflammatory markers proved statistically significant, in favour of A group, but at higher than expected cut-off values (24.41 mg/L, for reactive C protein; $19,79 \times 10^3/\text{mmc}$, for the leukocytes count; 81,51%, for the percentage of neutrophils).
7. Among the relative inflammatory markers, only NLR showed statistical significance, in favour of A group, for mild and moderate stress (values between 6-18).
8. The only unconventional inflammatory marker, MCV, proved statistical significance, in favour of A group, but at values lower than 89.01 fl.

CONCLUSIONS

The study proved that the complete blood count remains an investigation of first choice, for all human pathology. In infectious pathology, and in children, in addition to simplicity and safety, the complete blood count brings more arguments than the conventional ones. However, in order to differentiate between bacterial and viral infectious pathology, it is necessary to establish other reference values for the inflammatory markers in the complete blood count, conventional or not, compared to the predetermined ones.

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