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SEARCH FOR NEW BIOMARKERS OF VIRAL INFECTION: STUDY OF NEMO SCORE

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INDEX

1. ABSTRACT

Cell population data (CPD) are reported from Sysmex XN analyzers providing quantitative information on the morphological and functional characteristics of neutrophils, monocytes and lymphocytes. From the data of CPD a score is derived that could serve for the early diagnosis of viral infections, specifically, this refers to the NEMO score (neutrophils and monocytes), a score system formed by 5 hematological parameters derived from the Sysmex analyzer. The aim of this study was to assess whether NEMO score could serve as a biomarker of viral infection and to examine whether there were differences in its power of discrimination based on age and sex. For this reason, blood data from positive patients of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the respiratory syncytial virus (RSV), the influenza A virus (IAV) and influenza B virus (IBV) were collected and analyzed to find out the power of discrimination of the score. The results showed that the NEMO score had a remarkable discrimination power in positive patients in SARS-CoV-2 and RSV with respect to non-infected individuals, while in the IAV and IBV it was almost null. There were differences in the power on discrimination of the NEMO score between the control group and SARS-CoV-2-infected patients according to age and sex. In conclusion, the NEMO score could serve as a complementary tool for the diagnosis of viral infections although further studies are needed to understand the usefulness and applicability of NEMO score values in the diagnosis and prognosis of viral diseases.

Keywords: Biomarker, Cell Population Data (CPD), Sysmex analyzers, NEMO score, viral infections.

Català:

Els paràmetres de població cel·lular (CPD) reportats a partir dels analitzadors Sysmex XN aporten informació quantitativa sobre les característiques morfològiques i funcionals de neutròfils, monòcits i limfòcits. A partir de les dades de CPD es deriva una puntuació que podria servir pel diagnòstic precoç de les infeccions virals, concretament es parla de l'score NEMO (neutròfils i monòcits), un sistema de puntuació format per 5 paràmetres hematològics derivats de l'analitzador Sysmex. L'objectiu d'aquest estudi va ser avaluar si l'score NEMO podria servir com a biomarcador d'infecció viral i examinar si existien diferències en el seu poder de discriminació en funció de l'edat i el sexe. Per això es van recollir i analizar dades sanguinies de pacients positius en els virus del Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), el virus respiratori sincitial (RSV), el virus de la influença A (IAV) i el virus de la influença B (IBV) per tal d'esbrinar el poder de discriminació de l'score. Els resultats van mostrar que l'score NEMO tenia un poder de discriminació notable en pacients positius en SARS-CoV-2 I RSV mentre que en la influença B era gairebé nul. Hi va haver diferències en el poder de discriminació de l'score NEMO entre el grup control i els pacients infectats pel SARS-CoV-2 segons l'edat i el sexe. En conclusió l'score NEMO podria servir com a eina complementària pel diagnòstic d'infeccions virals, tot i que calen més estudis per entendre la utilitat i aplicabilitat dels valors de l'score NEMO en el diagnòstic i pronóstic de malalties víriques.

Paraules clau: Biomarcador, Paràmetres de població cel·lular (CPD), analitzadors Sysmex, score NEMO, infeccions virals.

2. INTRODUCTION

2.1. Viral infections

Acute respiratory tract infections usually manifest with mild symptoms that tend to resolve spontaneously. However, in some cases, they can get worse until they require hospitalization, which exerts a considerable influence on morbidity and mortality, especially among vulnerable populations such as children and the elderly.[1]

Respiratory syncytial virus (RSV) and several strains of the influenza virus, specifically influenza A, have historically been the main causes of these infections. However, the arrival of the COVID-19 pandemic at the beginning of 2020 meant a paradigm shift, in which SARS-CoV-2 assumed the dominant role in this spectrum of diseases, coinciding with a lower prevalence of previously prevalent viral agents.[1]

In 2022, the World Health Organization (WHO) reported almost 504 million cases of disease caused by the coronavirus 2019 (COVID-19), more than 6.2 million deaths related to COVID-19, and more than 11.30 billion vaccine doses administered globally.[2]

Even so, in 2022 there was a resurgence of influenza A (IAV) and RSV, causing increased hospitalizations in children and adults. There are many studies that have indicated a decrease in the risk posed by SARS-CoV-2 over time, possibly attributed to the predominance of less virulent variants, greater immunity derived from a previous infection or vaccination and improved management of patients with COVID-19.[1]

2.2. Polymerase Chain Reaction (PCR)

The SARS-CoV-2, influenza, and RSV viruses can cause infections that have a very similar symptomatology, and this hinders their clinical differentiation. It is important to prevent the transmission of these viruses, which is why a technique with a faster diagnostic capacity than conventional PCR is needed. The Xpert Xpress CoV-2/Flu/RSV plus test is an automated in vitro diagnostic test for the qualitative detection and simultaneous RNA differentiation of SARS-CoV-2, influenza A, influenza B and RSV viruses using reverse transcription PCR (RT-PCR) from nasopharyngeal swab samples or anterior nasal swab samples. A positive result indicates the presence of the identified virus; however, the negative results do not rule out infection by the mentioned viruses

and should not be used as a unique criterion on which to make decisions related to the treatment of patients or other decisions related to their care.[3]

It is for this reason that new mechanisms are needed for the early diagnosis of viral infections to avoid false negatives and future complications in patients. Although it is a rapid diagnostic test, lasting approximately 35 minutes, there are alternatives that can show greater speed and serve as a complementary tool in early diagnosis.

2.3. Hematology analyzers: Sysmex XN analyzer

The hemogram is one of the most requested laboratory tests, since it has the ability to provide useful information for the diagnostic orientation of both hematological and non-hematological diseases.[4]

The hemogram is based on obtaining the blood cell count (CBC) and the leukocyte differential. The CBC includes the count of leukocytes, erythrocytes and platelets and the concentration of hemoglobin, while the leukocyte differential allows the identification of the 5 leukocyte subpopulations (neutrophils, lymphocytes, monocytes, basophils and eosinophils). Modern hematological analyzers provide a precise and fast differential leukocyte count at a low cost.[4]

In recent decades, hematological analyzers have experienced rapid development thanks to technological progress. It does not only have an effect on automation and performance but also on increasing the number of parameters they report. The new technology allows to quickly examine a large number of cells to obtain complete hematological profiles. Hematological analyzers record electronic signals that evaluate cell morphology and characteristics. The use of sophisticated algorithms and software transforms this data into reportable results of cell counts and derived research parameters.[5]

Specifically, the Sysmex XN system (Sysmex Corporation. Kobe, Japan) is an automated blood analyzer that uses flow cytometry to measure individual cells. This technique allows analyzing the physiological and structural properties of cells while they flow through a very narrow flow cell. In the first place, aspirates a blood sample and adjusts the ratio to then be diluted according to a redefined ratio and then marked with a fluorescent marker that is specifically linked to nucleic acids.[6]

The sample is then transported to the flow cell. The sample is illuminated with a semiconductor laser beam that is able to separate cells using three different signals, each of which provides exclusive information. (**Figure 1**).

The three signals it generates are:[6]

- Forward Scatter (FSC): cell volume
- Side Scatter (SSC): cell content such as nucleus and granules
- Side Fluorescence Light (SFL): quantity of nucleic acids and cellular organelles.

These three signals are used to count and differentiate leukocytes, nucleated RBCs (NRBC), reticulocytes and platelets, as well as to detect immature and abnormal cells from unique algorithms. [6]

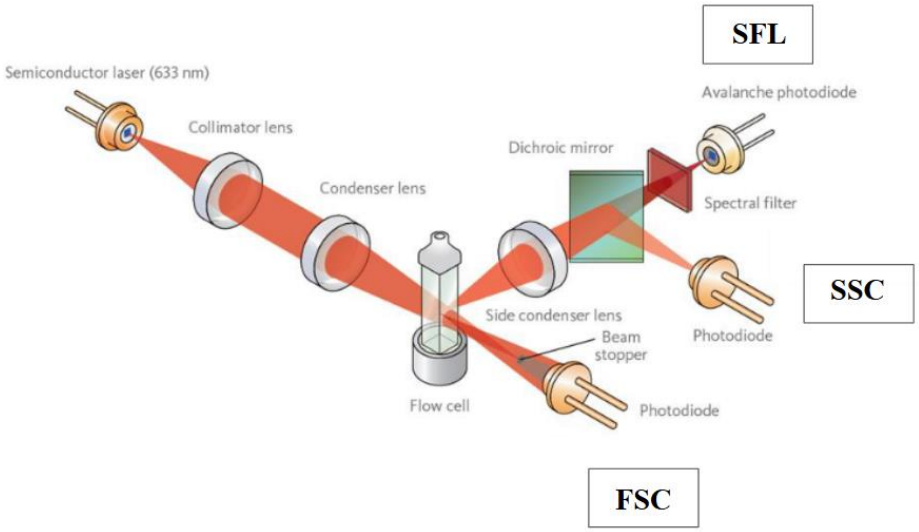


Figure 1. Illustrative diagram of the operation of a flow cytometer. Adapted from Sysmex [6]
(FSC, forward scatter; SFL, side fluorescence light; SSC, side scatter)

These optical signals of the leukocyte differential are represented in the three axes of the white blood cell differential fluorescence channel dispersion diagram (WDF)[7] (**Figure 2**).

Optical signals along the X-axis (SSC) are related to internal complexity, i.e. cellular granularity; fluorescence along the Y-axis (SFL) represents the content of nucleic acids, while the Z-axis (FSC) is proportional to the volume and cell form.[5,7]

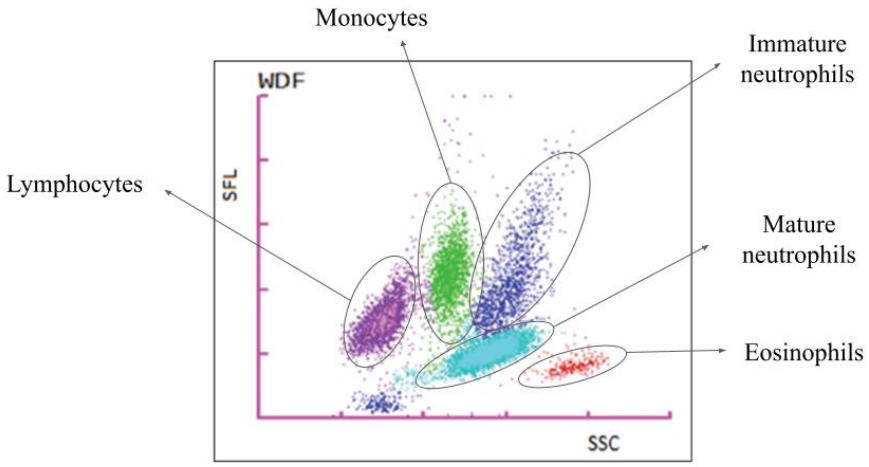


Figure 2. The WDF channel differentiates lymphocytes (purple), monocytes (green), eosinophils (red), mature neutrophils (light blue) and immature neutrophils (dark blue) and has the ability to detect abnormal cells such as immature leukocytes and atypical lymphocytes. Marking of blood cells with fluorescent dyes after perforation of the cell membrane with specific lysates. Adapted from Urrechaga et *al.*[8]

However, Sysmex analyzers are not the only ones that report leukocyte morphological parameters but Coulter hematological analyzers (Beckam Coulter Inc., Miami, FL, USA) are also able to evaluate the morphology of leukocytes using VCS technology [5], which combines three physical principles: electrical impedance by volume determination (V), an electromagnetic probe to obtain conductivity (C) and laser dispersion (S) to obtain complexity, providing a standard average and deviation for each analyzed population.[4]

2.4. Cell Population Data (CPD)

The company Sysmex, thanks to the use of technology based on flow cytometry, can include, together with the basic hemogram, cell population data (CPD) parameters, which offer quantitative information on the morphological and functional characteristics of neutrophils, monocytes and lymphocytes. Basically, CPDs specifically reflect variations in blood cell morphology in response to different clinical situations. All this allows a deeper study of cells and any morphological variation in the face of various stimuli, such as infections, and provides important data on the state of cellular activation.

The composition of the activated cell membrane differs from inactive cells due to the expression of signaling molecules and receptors in response to activation. These activated cell membranes are more susceptible to perforation by reagents and can therefore

penetrate a greater amount of fluorescent dye and bind to cytoplasm organelles and nucleic acids.[8]

CDPs offer several advantages, since these are generated during the complete hemogram without additional samples and analysis and are always available. Being numerical, CDPs are more objective and accurate than manual differential counts, as thousands of leukocytes (WBC) are automatically evaluated and represent volume, granularity and cellular complexity.[5]

In infections caused by SARS-CoV-2, immune deregulation occurs and leads to certain characteristics in the differential count of white blood cells and morphological anomalies that could be useful for early recognition of the infection (**Figure 3**).[9]

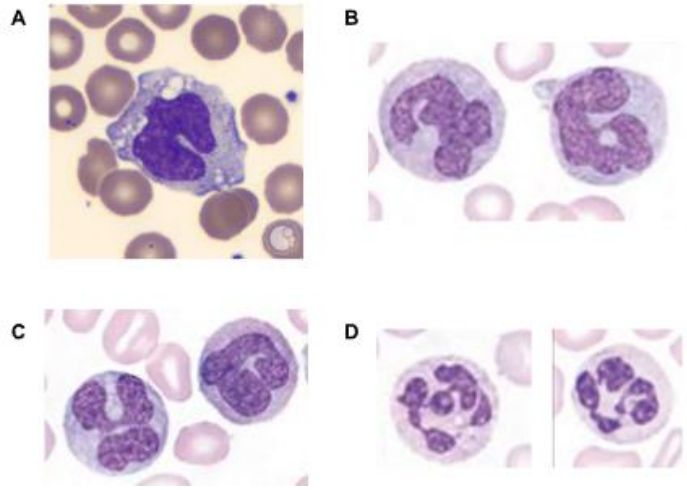


Figure 3. Images of monocytes in peripheral blood. Photograph A shows monocytes in basal form, presenting a blue-grey cytoplasm with fine cytoplasmic granules; the nucleus is irregular, with carvings or in the form of a kidney. While atypical monocytes are observed, a larger than normal (B) nucleus-cytoplasm relationship with nuclear forms and abnormal chromatin patterns can be seen. You can even see twisted and disconnected nuclei (C). Dysplasia can also be observed in granulocytes, characterized by hypersegmentation as well as hypergranularity and presence of small vacuoles (D). Adapted from Lusky and Scordino [10,11]

These anomalies in monocytes were detected in a study carried out in 2021, in which significant morphological changes were observed in the white blood cells associated with SARS-CoV-2 infection. These results suggest that the study of monocyte morphology can be a very useful tool for diagnosing viral infections.[11]

2.5. Application of CPD in viral infections: score NEMO (neutrophils and monocytes)

The NEMO score is based on the combination of 5 parameters derived from the complete blood count (CBC) and the cell population data (CPD) obtained through Sysmex hematological analyzers. The 5 parameters are: NEUT# (absolute neutrophil count), LYMPH# (absolute lymphocyte count), IG% (granulocyte percentage), NE-SFL (neutrophil fluorescence intensity) and MO-X (monocyte complexity). These parameters can be obtained from hemograms routinely.

The NEMO score is currently used for the early diagnosis of sepsis, since the identification and early treatment of this condition are essential for the patient's prognosis. In fact, it was in 2018 that Urrechaga et *al.* created this scoring system based on the CPD parameters generated by the Sysmex analyzers discussed above.[8]

The NEMO score includes CPD values for neutrophils and monocytes (NE-SFL and MO-X, respectively), which characterize the early innate immune response. The activation of this first line of defense occurs within minutes of the stimulus. These act as ideal biomarkers for early detection of the disease and provide real-time information on morphological and functional changes in leukocytes.[12]

As indicated in Figure 3, the change in morphology in monocytes occurs in the presence of infection, so this score is able to measure this change from the MO-X parameter. This parameter increases in the presence of a greater number of granules, vacuoles and other cytoplasmic inclusions and decreases in the presence of lower cell complexity.[13] An increase in MO-X may be due to greater activation or structural alteration of monocytes, making it a good marker of immune activity.

NE-SFL is related to the content of nucleic acids within the cell and reflects the maturity and activation of neutrophils, so this parameter increases in proportion to the amount of DNA and cellular RNA.[13] In general, SFL values mainly show the type and quantity of nucleic acids and organelles in cells.[14]

The complexity of monocytes (MO-X) is indicated in the X-axis of the diagram generated by the flow cytometer, while the fluorescence intensity of neutrophils (NE-SFL) is indicated in the Y-axis of the diagram.

Depending on the value that each parameter adopts (MO-X, NE-SFL, NEUT#, LYMPH# or IG%), a stratification scale of the risk of sepsis is created. The values of this score range from 0 to 13 points and, depending on the score obtained, patients are classified into 3 large groups according to the risk of suffering sepsis:[5]

- NEMO 0-3: low probability
- NEMO 4-5: avarege probability
- NEMO ≥ 6: high probability

3. HYPOTHESIS

The NEMO score has proven to be a fast and accessible prognostic tool, since it can be calculated from parameters derived from a routine hemogram. Until now, this score has been only applied for the early detection of sepsis, but it is reasonable to speculate that NEMO score could be also used as a biomarker of viral infection by evaluating the changes that are induced in the cellular counting parameters.

Therefore, the hypothesis of this work, is that the NEMO score is significantly higher in viral infected patients than in non-infected patients and the power of discrimination of this score may be influenced by sex and age.

4. OBJECTIVES

To confirm this hypothesis, the objectives of this work were:

- To evaluate whether NEMO score can be used in SARS-CoV-2, RSV, IAV and IBV as a biomarker of viral infection.
- To define a cutting point of the NEMO score to differentiate healthy patients from patients with viral infection.
- To analyze whether there are significant differences in the discrimination power of NEMO score between control and infected patients, depending on sex and age.

5. MATERIALS AND METHODS

5.1. Study design

Data were collected from a total of 252 patients with a confirmed diagnosis of RSV, IAV, IBV or SARS-CoV-2 at Hospital Sant Pau i Santa Tecla (Tarragona) between September 1, 2024, and February 7, 2025. This study included patients aged 18 years or older, of both male and female sex. The control group was obtained from healthy preoperative patients, ensuring that none of them presented hematological manifestations of viral infection such as leukocytosis, monocytosis or neutrophilia.

The study was conducted retrospectively, and through the LabSuite software of Hospital Sant Pau i Santa Tecla, it was possible to collect the necessary patient data. Specifically, the age, sex, and hemogram request number of each patient were gathered in order to obtain the parameters required for calculating the NEMO score. The application of specific filters within the software made it possible to extract patients who tested positive for each of the viruses of interest.

The diagnosis of SARS-CoV-2, RSV, IAV or IBV infection was performed by the chain reaction of reverse transcriptase polymerase (RT-PCR). RT-PCR tests were performed with nasopharyngeal swab or nasal swab, using the Xpert ® Xpress CoV-2/Flu/RSV plus (Cepheid, Sunnyvale, CA, USA).

In addition, one of the inclusion criteria used was to have an associated hemogram the same day, the day before or the day after the PCR was performed to identify the viral infection.

5.2. Blood analysis

Blood samples to measure the NEMO score were collected in vacutainer tubes with EDTA anticoagulant (ethylenediaminotetraacetic acid) in order to make the hemogram. It should be noted that it was not necessary to take additional blood to calculate its value, these tubes were obtained routinely by the cell count. The hemogram of the patients was performed in a Sysmex hematological analyzer (Kobe, Japan). The parameters collected in the Sysmex analyzer were: MO-X, NE-SFL NEUT#, IG%, LYMPH#.

5.3. Calculation of NEMO score

Table 1 shows how the calculation of the NEMO score was performed. Each parameter was associated with a score based on the value obtained, after the assignment of the individual score the sum of the set was made to end up giving the final result of the score.[8]

Table 1. Scores associated with each NEMO score parameter

NEMO SCORE PARAMETERS	UNITS	SCORE
MO-X	ch	≥ 118 → 2 < 118 → 0
NE-SFL	FI	≥ 52.9 → 3 < 52.9 → 0
NEUT#	$1x10^3$ µL	≥ 5.4 → 3 < 5.4 → 0
LYMPH#	$1x10^3$ µL	≥ 1.2 → 3 < 1.2 → 0
IG	%	≥ 0.4 → 2 < 0.4 → 0

MO-X, monocyte complexity; NE-SFL, fluorescence intensity of neutrophils; NEUT#, absolute neutrophil count; LYMPH#, absolute lymphocyte count; IG%, percentage of immature granulocytes; ch, lateral dispersion channels; FI, fluorescence units.

5.4. Statistical analysis

The statistical study was carried out using the MedCalc software version 23.2.1 (MedCalc Software Ltd., Ostend, Belgium). The median and interquartile range (IQR) of each group was calculated. The Shapiro-Wilk test was carried out to know what type of distribution the data followed. To calculate the significance between each group, control group against individual viruses and between viruses, the nonparametric Kruskal-Wallis test was applied together with the Conover post-hoc test after checking that the data followed a nonparametric distribution. Finally, the ROC curves were made determining the AUC and the Youden index to assess the best point of such as well as the associated sensitivity and specificity. If the biomarker has presented an AUC value equal to 0.5 [12], it has been considered that it has no discriminative capacity and an AUC value of 0.7 has been considered that it has discriminating potential.[15]

6. RESULTS AND DISCUSSION

6.1. Descriptive analysis

The study was carried out with a total of 252 patients where 194 were positive for any of the viruses studied, including 54 positives in SARS-CoV-2, 35 in RSV, 86 in IAV and 19 in IBV, the rest of the patients were part of the control group. In addition, the age of patients was between 18 and 102 years. **Table 2** shows the characteristics of each group as well as the parameters that are part of the NEMO score.

Table 2. Descriptive table informing the characteristics of each group studied.

	Control	SARS-CoV-2	RSV	IAV	IBV
N	58	54	35	86	19
NEMO score (IQR)	5 (5-5)	7 (5-7)	7 (5-10)	5 (4-7)	5 (2.5-6.5)
Age, years	52.70	75.07	76.51	63.38	47.85
Women, N (%)	32 (55.17%)	20 (37.04%)	20 (57.14%)	40 (57.14%)	10 (52.63%)
MO-X, ch	119.2 (118.3-120.7)	120.8 (119.5-122.4)	121.5 (119.9-122.95)	122.2 (120.7-123.8)	121.8 (120.53-122.88)
NE-SFL, FI	45.7 (43.9-47.3)	44.85 (43.5-46.4)	44.5 (43.05-46.45)	44.9 (43-47.5)	46.4 (45.28-47.13)
NEUT, $1x10^3$ µL	3.57 (3.09-4.21)	5.67 (4.01-7.53)	7.84 (5.35-10.81)	5.21 (3.83-8.13)	4.93 (2.81-7.04)
LYMPH, $1x10^3$ µL	2.26 (1.8-2.59)	1.12 (0.7-1.77)	1.15 (0.76-1.44)	0.76 (0.49-1.24)	1.26 (0.67-1.69)
IG, %	0.2 (0.2-0.3)	0.4 (0.3-0.7)	0.5 (0.3-0.8)	0.5 (0.3-0.7)	0.3 (0.2-0.38)

The data are presented with median (IQR).

RSV, syncitial respiratory virus; IAV, influenza A virus; IBV, influenza B virus; IQR, interquartile range; MO-X, monocyte complexity; NE-SFL, intensity of neutrophil fluorescence; NEUT, absolute neutrophil count; LYMPH, absolute lymphocyte count; IG (%), percentage of immature granulocytes.

The p-value obtained in the Kruskal-Wallis test was 0.000013, so the null hypothesis (H0) was discarded, and the results were statistically significant. Therefore, the post-hoc Conover correction was performed.

Figure 4 showed that there were statistically significant differences between the control group and the positive groups in SARS-CoV-2, RSV and IAV, while the IBV group only showed significance with the SARS-CoV-2 and RSV group. Viral groups have also showed statistical differences between them.

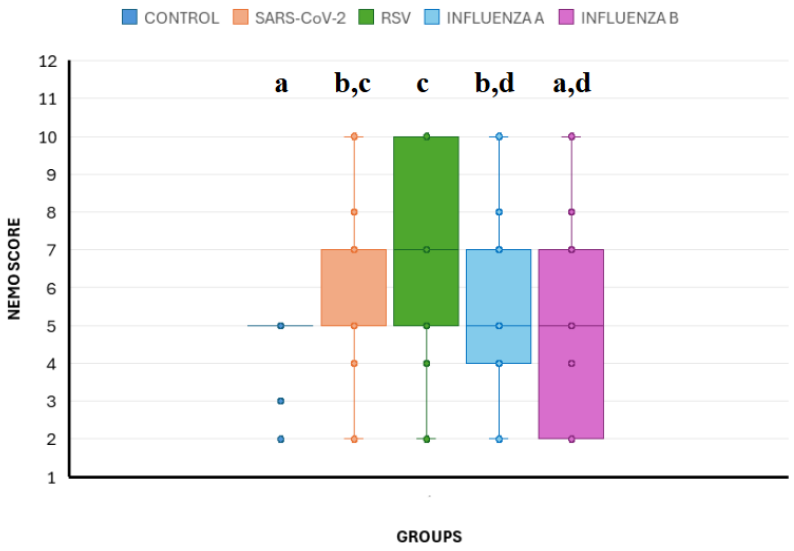


Figure 4. Distribution of NEMO score in the different groups studied. The boxes show the interquartile ranges (Q1 and Q3), while the horizontal line inside the box represents the median. The ends of the bars (mustaches) represent the maximums and minimums that the NEMO score has reached in each of the groups and the points that are distributed along the mustaches are the outliers of the score. The letters a, b, c and d indicate $p<0.05$ using Kruskal-Wallis test and Conover post-hoc test.

The distribution of the NEMO score was shown to be unequal in the different groups studied and all groups presented outliers. The control group showed a low median with a very narrow interquartile range because the dispersion of its values was similar although it included some outliers. Still, the distribution of the values was more compact than in the rest of groups. The group with the highest value dispersion was the RSV with a high score median and a wide IQR, thus reflecting higher NEMO score values; the IBV group showed similar scattering. The SARS-CoV group reported more moderate scores, with a higher median than the control and narrower IQR.

6.2. ROC curves

6.2.1. Global analysis

ROC curves were generated for each group studied (**Figure 5** and **Table 3**). In the case of RSV infection, an AUC of 0.77 was observed, with a cut-off point established at a value greater than 5. A sensitivity of 62.86% and a specificity of 100% were obtained for its diagnosis. The SARS-CoV group had similar results, with an associated AUC of 0.726 and a sensitivity and specificity of 53.7% and 100%, respectively.

The performance to detect IAV was more discreet, with an AUC of 0.652 and a sensitivity of 48.84%, while in IBV, AUC of 0.517 and a p-value of 0.8496 were obtained, so the score had almost no discriminative capacity and the AUC was not statistically significant. These results suggested that that the NEMO score would not be useful to discriminate against patients infected with IBV and non-infected patients.

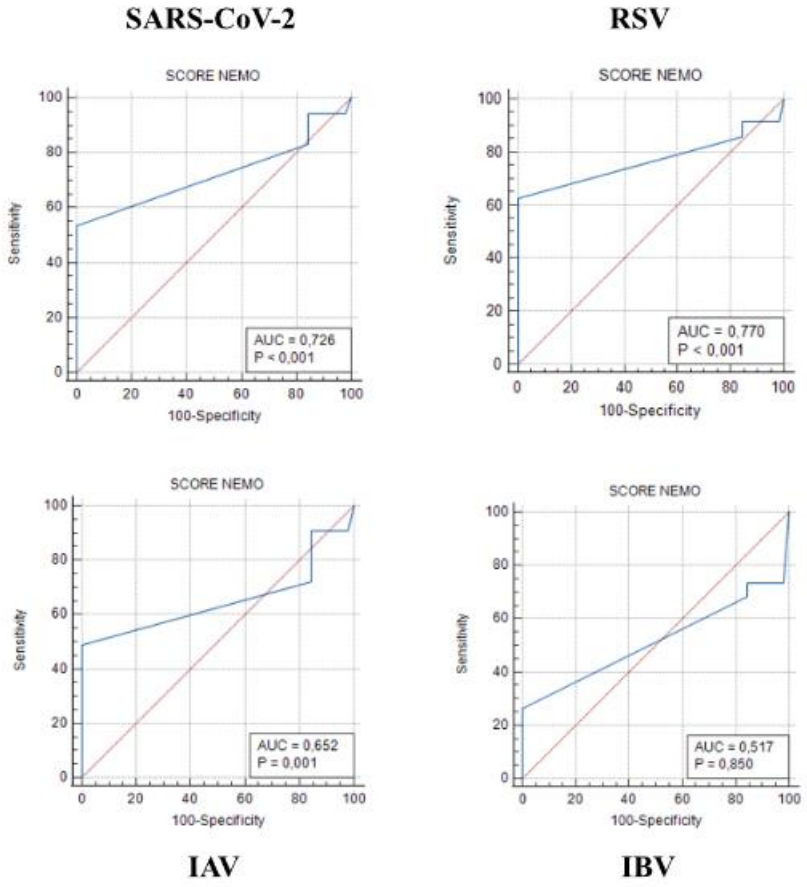


Figure 5. Analysis by ROC curves of the value of the NEMO core in the SARS-CoV-2, RSV, IAV and IBV with the AUC and the corresponding p-value.

Table 3. Summary table showing AUC, p-value, Youden index, associated criterion, sensitivity and specificity for each group type studied.

	SARS-CoV-2	**RSV**	**IAV**	**IBV**
AUC	0.726	0.770	0.652	0.517
P-value	<0.0001	<0.0001	0.0005	0.8496
Younden index	0.5370	0.6286	0.4884	0.2632
Associated criterion	>5	>5	>5	>5
Sensitivity	53.7	62.86	48.84	26.32
Specificity	100	100	100	100

IAV, influenza A virus; IBV, influenza B virus

The NEMO score could serve as a diagnostic marker in the case of positive patients in RSV and SARS-CoV-2, has a limited diagnostic capacity for IAV and it would not be suitable to discriminate against the presence of IBV. This score has a specificity of 100% in all cases, so it detects all true negatives and avoids false positives but, consequently, presents a lower sensitivity, which can give false negatives.

The fact that there are no significant statistical differences between the control group and the IBV group may be because the group has a limited sample size (n =19) and this may have conditioned the results, since they may be insufficient to get to see the real magnitude of the difference between the two groups. In addition, in this group there is a quite large dispersion in the values, so all this set of factors may have influenced the power of the statistical tests used

6.2.2. Analysis based on sex

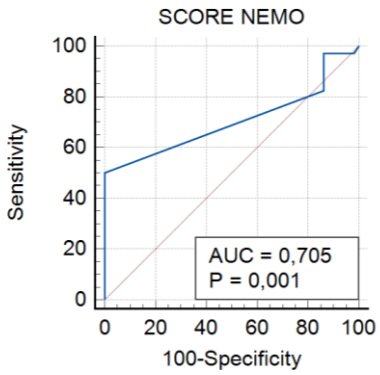
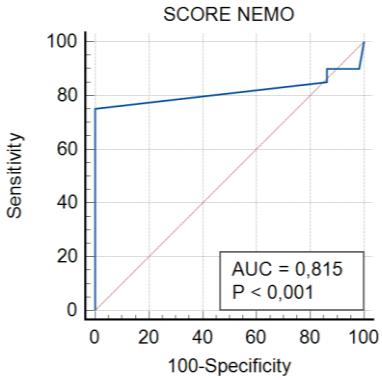
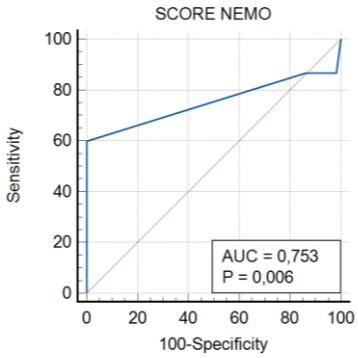
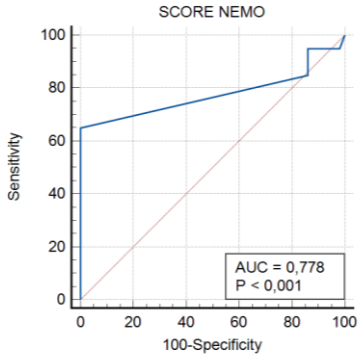
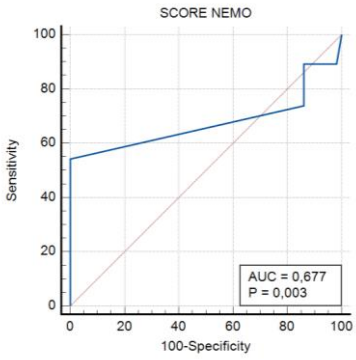
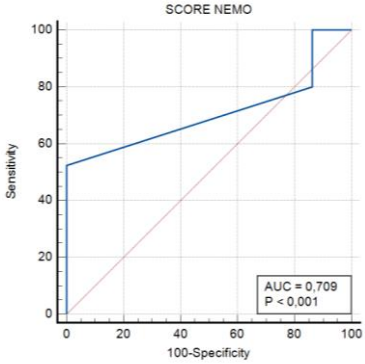
Subsequently, the each group on infected-patients was separated according to sex to investigate whether there was higher discrimination effect depending on sex.

ROC curves were made for each population studied (**Figure 6** and **Table 4**). The female group corresponding to SARS-CoV-2 presented an AUC of 0.815 and a cut-off point of 5 with an associated sensitivity and specificity of 75% and 100%, respectively and a high Youden index, specifically 0.750. The male and female groups in RSV also showed a high AUC, specifically 0.753 in the male sex and 0.778 in the female sex. The group corresponding to IAV showed similar AUCs between the male and female groups but in female group the AUC was 0.709 compared to the male group with an AUC of 0.677, so the biomarker had greater power of discrimination in women in comparison to IAV-infected men. The IBV group showed an AUC of 0.586 in the female group with a sensitivity of 40% and a specificity of 100%, and in the male group showed an AUC of 0.570 with a sensitivity of 33.33% and a specificity of 96.15%.

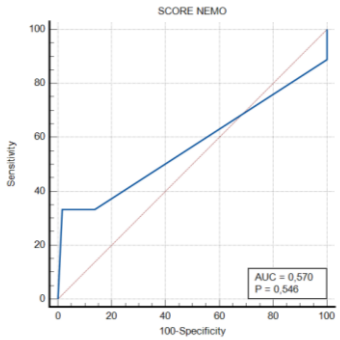
Separating the analysis by sex shows an improvement in the diagnostic capacity of the NEMO score for the SARS-CoV-2, especially in women, since it has a specificity of 100% and a quite high sensitivity (80%), causing the false negatives to decrease. This more exhaustive analysis shows again that IBV is the virus with the worst diagnostic

performance, especially in men. This suggests that sex can act as a diagnostic performance-modifying factor for NEMO score.

In the study carried out in 2018 by Urrechaga et *al.* on the application of NEMO score in sepsis[8] no analysis of the population by sex was made, so it would be interesting to be able to do this segregation, since the preliminary results obtained in this study indicate that there could be an effect of sex on the discrimination power of NEMO score.

	MEN	**WOMEN**
SAR-CoV-2		
RSV		
IVA		

IVB



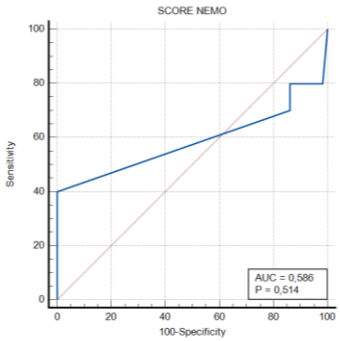


Figure 6. Analysis by ROC curves of the value of the NEMO score in the SARS-CoV-2, VRS, IVA and IVB with the AUC and the corresponding p-value according to sex

Table 4. Summary table showing AUC, p-value, Youden index, associated criterion, sensitivity and specificity for each type of group studied according to sex.

	SARS-CoV-2		**RSV**		**IVA**		**IVB**	
	♂	♀	♂	♀	♂	♀	♂	♀
AUC	0.705	0.815	0.753	0.778	0.677	0.709	0.570	0.586
P-value	0.0006	0.0001	0.0063	0.0003	0.0029	0.0002	0.5465	0.5136
Youden Index	0.5000	0.7500	0.6000	0.0063	0.5435	0.5250	0.3161	0.4000
Associated criterion	>5	>5	>5	>5	>5	>5	≤2	>5
Sensitivity	50	75	60	65	54.35	52.5	33.33	40
Specificity	100	100	100	100	100	100	96.15	100

♂, men; ♀, women; IAV, influenza A virus; IBV, influenza B virus

In general, NEMO score tends to show higher AUCs in women and, mainly, has a greater discriminative capacity in the female group of SARS-CoV-2. This could be due to a difference in the immune response between male and female. In fact, women present greater activation of the immune system compared to men. The activation in the male sex is more reduced and, therefore, results in a slower elimination of the virus. There are other factors that may have an influence on the difference in immune response between men and women such as sex hormones, since progesterone and estrogen enhance immune response activity or immune cell activity, which is higher in women than in men.[16] On the other hand, it has been previously reported that SARS-CoV-2 generates morphological changes in monocytes, and these morphological anomalies could be useful for the early diagnosis of viral infections[11]. In this sense, it would be interesting to investigate whether these changes observed in monocytes are also detected according to age or are more accentuated in the female or male sex.

The fact that the NEMO score has a greater diagnostic capacity in women can have important clinical implications. The NEMO score is derived from the hemogram, a simple test, very fast and available in most clinical laboratories, so it could be a tool for early diagnosis or triage support in the case of having limited resources in hospitals. On the other hand, in emergency services, this biomarker could help to prioritize confirmatory diagnostic tests, such as PCR, in women who presented a high NEMO score value. Likewise, it would be interesting to carry out a study on how the immune response to viral infections varies according to sex.

6.2.3. Analysis based on age

Finally, a separation of groups was carried out according to age to investigate whether if there were significant differences depending on the age of patients (**Figure 7** and **Table 5**).

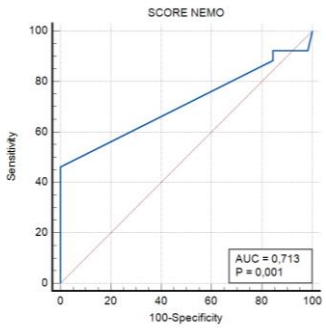
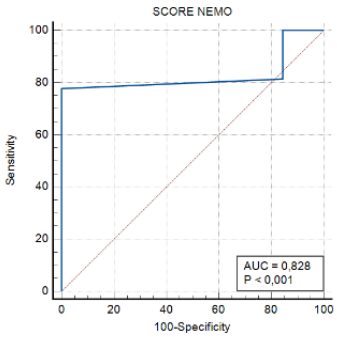
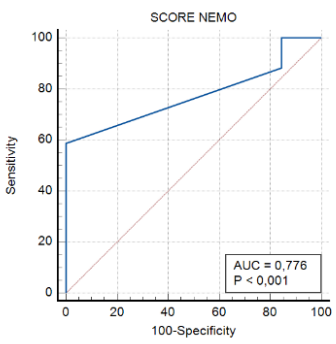
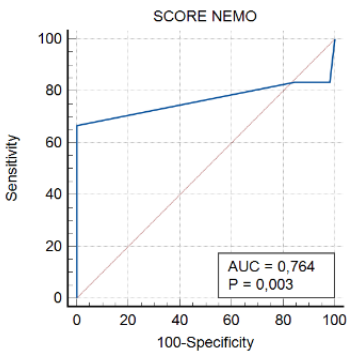
The age cut for each group of infected patients was made specifically, taking into account that the same n remained in each subgroup. The ROC curves were made for each subgroup were obtained by the SARS-CoV-2 group with an age greater than 76 years, an AUC of 0.828, with a cut point >5, sensitivity of 77.78%, specificity of 100% and a fairly high Youden index (0.778). On the other hand, the group with an age greater than 76 years presented an AUC of 0.713, a sensitivity of 46.15% and a specificity of 100% with cut point >5.

In the RSV group with an age ≤79 and >79, showed very similar AUCs, specifically 0.776 and 0.764, respectively, the group >79 showed greater sensitivity (66.67%), so the diagnostic capability of the NEMO score was similar in both groups and showed moderate discrimination power.

Subgroups referring to IAV had very similar AUC (0.663 and 0.699), so the score's diagnostic capacity for this virus was more limited. On the other hand, the diagnostic criterion for IBV remained very low for any group, since the AUC values did not reach statistical significance in either group.

Thus, the NEMO score showed a greater diagnostic capacity in the SARS-CoV-2 group with an age greater than 76 years.

At a clinical level, this could lead to an improvement in the early diagnosis of SARS-CoV-2 in the geriatric population. Patients over the age of 76 have a higher risk of complications in the event of infection, so a biomarker with a high capacity for discrimination in viral infection can be very useful. In addition, this can be obtained routinely when making a hemogram, so it can detect the infection before patients have obvious symptoms and a periodic control with serial hemograms can be established to detect infections caused by SARS-CoV-2. It could also help detect infections mainly in residences without the need for PCR and in this way prioritize more tests or isolations to avoid contagion, since contracting viral infections in geriatric populations can lead to complications and even death of the patient, so it is very important an early diagnosis of viral infections.

	≤76	>76
SARS-CoV-2		
	≤79	**>79**
RSV		
	≤70	**>70**

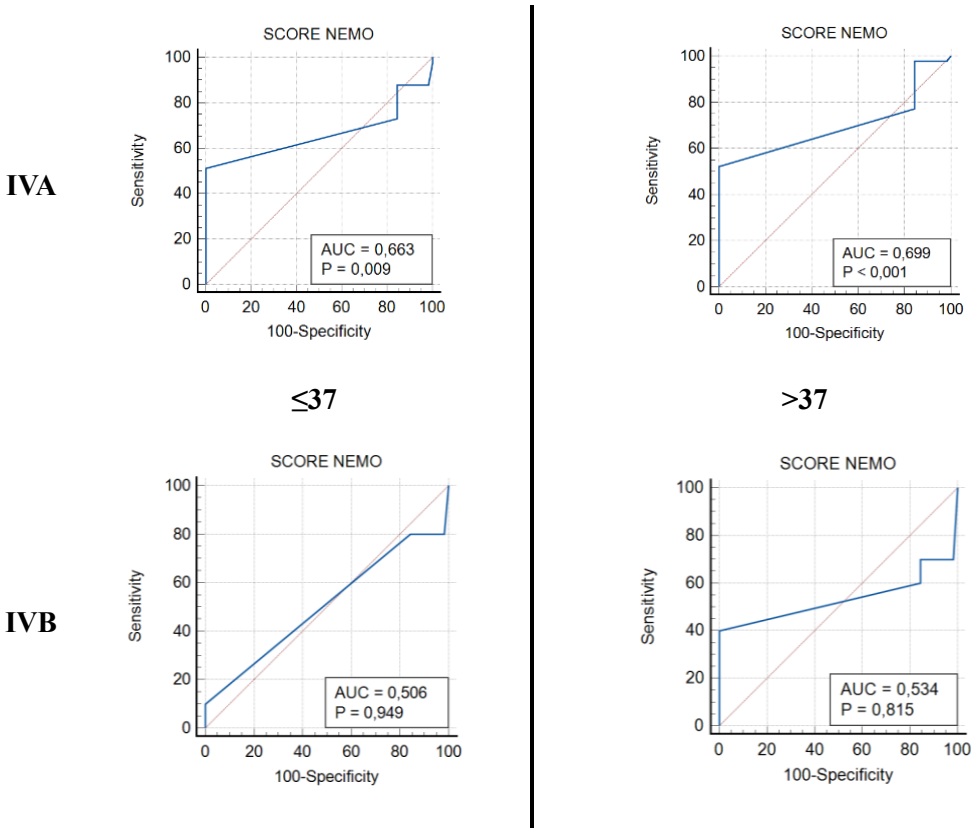


Figure 7. Analysis by ROC curves of the value of the NEMO score in the SARS-CoV-2, RSV, IAV and IBV with the AUC and the corresponding p-value according to age.

Table 5. Summary table showing AUC, p-value, Youden index, associated criterion, sensitivity and specificity for each type of group studied according to age.

	SARS-CoV-2		**RSV**		**IAV**		**IBV**	
	≤76	**>76**	**≤79**	**>79**	**≤70**	**>70**	**≤37**	**>37**
AUC	0.713	0.828	0.776	0.764	0.663	0.699	0.506	0.534
P-value	0.0008	<0.0001	0.0002	0.0034	0.0093	0.0003	0.9490	0.8148
Youden index	0.4615	0.7778	0.5882	0.6667	0.5122	0.5227	0.1828	0.4000
Associated criterion	>5	>5	>5	>5	>5	>5	>2	>5
Sensitivity	46.15	77.78	58.82	66.67	51.22	52.27	80	40
Specificity	100	100	100	100	100	100	1.72	100

IAV, influenza A virus; IBV, influenza B virus

After having analyzed the results at a global and categorical level (age and sex), it was observed that NEMO score could serve as a good diagnostic biomarker in positive patients in SARS-CoV-2 and RSV, while at the level of sex and age it was seen that score

had a greater power of discrimination in women infected for SARS-CoV-2 and in individuals with an age greater than 76 years also infected for SARS-CoV-2.

In all the cases studied, the positive group for IVB has shown very low AUC values and, therefore, a very limited diagnostic capacity and with a very reduced performance. This result may be due to several factors such as the presence of a small number of positive patients due to IBV, as mentioned above. Another possible factor could be that the activation of the immune system is insufficient compared to the rest of the viruses studied or even that it has been able to generate a delayed response.[16] For this reason, the NEMO score is not able to reflect the power of the immune response, either because the taking of the blood sample has been done too soon or too late with respect to the onset of symptoms. Likewise, it would be interesting to take a sample of positive patients for this higher virus, to detect whether the low discrimination of the score is due to the sample or is related to the activation of the immune system.

Some limitations of this study are that it has not been possible to bias almost the population, that is, as control patients, healthy preoperative patients who did not present neutrophilia, monocytosis or leukocytosis have been taken. But it has not been ruled out that they could present other associated diseases such as hemopathies or neoplastic diseases. An “arbitrary” selection of these patients has been made, so that, possibly, many of them have presented an increase in the NEMO score due to certain unidentified pathologies.

Therefore, a priori, it could be concluded that this score alone would not be enough to discriminate positive patients from negatives due to the low sensitivity it presents, since it could lead to false negatives. However, the NEMO score could serve as a support tool for conventional PCR to detect viral infections, since it gives us an idea of how active the host's immune system is. In addition, this score could serve, together with the patient's clinical history, to know the complications that could develop depending on the value obtained.

It should be noted that obtaining the NEMO score is faster than performing a PCR, since obtaining a complete hemogram takes a few minutes while a PCR requires more time, this offers an advantage when obtaining the results quickly and thus making an early diagnosis to the patient.

To improve the study and obtain more robust results, it would be convenient to consult the clinical history of patients, this would allow to rule out both hematological and non-hematological diseases that could interfere with the results. In addition, it would also be advisable to increase the sample size to reinforce the statistical validity.

It should be noted, during the last decade there has been an increase in the interest of researchers in the clinical implementation of the CPD parameters of leukocytes. Currently, the lack of standardization is a major disadvantage in this diagnostic field, since there are only two technologies in use (Coulter and Sysmex) that report these CPD parameters.[17] In the future, all laboratories would need to have these technologies available in order to be able to promote the use of CPD parameters.

This study is only the beginning of a very long path, the application of NEMO score in viral infections is innovative and a deeper and tighter study should be carried out, since this score has only been applied in patients at risk of suffering sepsis. Likewise, WDF dispersion diagrams and CPD parameters provide a non-invasive and continuous method for monitoring the intracellular characteristics of leukocytes, such as the content of nucleic acids, structural complexity and heterogeneity. These parameters are a very easy and simple tool to obtain with a simple routine hemogram, it does not involve an additional sample taking or an added cost, which makes them very practical in the clinical field.[18] CPDs provide us with very valuable information on the morphology of leukocytes, but many more studies on the application of these parameters for the diagnosis of viral infections are required.

7. CONCLUSIONS

- The NEMO score can serve as a diagnostic marker in the case of positive patients in RSV and SARS-CoV-2, has a limited diagnostic capacity for IAV and it is not appropriate to discriminate against the presence of IBV.
- Cut-off point greater than 5 is the value that differentiates healthy patients from those infected.
- There are differences in the power of discrimination of the NEMO score between the control group and SARS-CoV-2-infected patients according to age and sex. No changes were observed in the other groups of infected patients.

NEMO score can become a very powerful tool for the detection of viral infections, so it can be concluded that it can be used as a complementary tool to PCR for its diagnosis. It is necessary to do a more in-depth study on this score and emphasize the use of CPDs, since they are a very useful tool, without added costs and easy to obtain from routine hemograms.

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