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Neutrophil lymphocyte ratio (NLR) is inversely proportional to cycle threshold value of orf1a gene in RTPCR may be used as biomarker in Covid19 patients

Arijit Chatterjee
Consultant Molecular Biologist (HOD - Molecular Biology), Department of Molecular Biology, Medinova Diagnostic Services Limited, Kolkata, West Bengal, India

Abstract
Corona virus disease (COVID-19) is an infectious disease caused by a newly discovered corona virus. The virus that causes COVID-19 is mainly transmitted through droplets generated when an infected person coughs, sneezes, or exhales. These droplets are too heavy to hang in the air, and quickly fall on floors or surfaces. A person can be infected by breathing in the virus if the person is within close proximity of someone who has COVID-19, or by touching a contaminated surface and then your eyes, nose or mouth.
This study was conducted to find the correlation between Neutrophil Lymphocyte Ratio (NLR) and Cycle Threshold Values of RTPCR in Covid-19 infected patients.
This study was conducted in a Diagnostic Centre, located in Kolkata. Data was collected from patients of Covid-19 between October'20to December'20, positive for Covid-19 Infection.
Basic demographic data was collected from Laboratory Information System (LIS) of the diagnostic centre. Whole blood EDTA sample were collected to analyze the features of WBC (Neutrophil and Lymphocyte)
Total 500 patients were studied for hematological parameters (NLR). Demographic data revealed age range from 10 years to 80 years. Males constituted 360(72%) and females 140(28%). Leukocyte Count showed Leucocytosis was observed in 60% of patients, high absolute neutrophil count in 5%, high Neutrophil Lymphocyte Ratio (NLR) in 55% patients.

Keywords: covid19, SARS CoV2, NLR, Ct Value, RTPCR

Introduction
Corona viruses (CoVs) are a large virus group belonging to the family - Coronaviridae, with a single-stranded RNA genome. The genome is surrounded by a helical capsid and a lipoprotein envelope containing several spikes of glycoprotein that together give the virus a crown appearance, hence the name "corona" which in Latin means crown. In December 2019, Covid-19 outbreak occurred in Wuhan, China, and has rapidly infected people all over the world presenting with clinical features of flu like symptoms to multi organ failure and death. Respiratory droplets and contact transmission are the main routes of spread of Covid-19 virus. On 11th, March 2020, WHO declared Covid-19 as Pandemic. India confirmed its first positive case on 30th January 2020 in Kerala. Currently, novel corona virus has affected millions of people all over world and near about 13 lakh deaths have been reported in the mid October 2020 as revealed by WHO's online dashboard. As complete blood count is the most common laboratory test performed and many of the hematological indices are varied in Covid-19 infected patients as revealed by the articles published by various countries, lymphocyte count as observed in severe/critically ill patients were reduced and Neutrophil Lymphocyte Ratio (NLR) has been suggested as a marker of systemic in inflammatory response in critically ill patients. In the present study we planned to analyze WBC parameters (NLR) in Covid-19 patients so that patients can be treated early with a limited resources available in an ongoing pandemic.

Aims and Objectives
This study was conducted to find the correlation between Neutrophil Lymphocyte Ratio (NLR) and Cycle
Threshold Values of RTPCR in Covid-19 infected patients.

Materials and methods
500 samples (Oropharyngeal and Nasopharyngeal Swabs) were collected from the patients and tested in Truelab
Quattro micro RTPCR system for COVID19.

Procedure
Equipment
- Name of the equipment: Truelab Quattro System
- Components of the equipment:

1. Trueprep AUTO/AUTO v2 Sample Prep Device.
2. Truelab Uno Dx/Truelab Duo/Truelab Quattro Real Time micro PCR Analyzer
3. Truelab micro PCR Printer.

- Location: Molecular Diagnostics section, Microbiology Department.
- Operating temperature: 18-25 °C
- Relative humidity: 30-80%

Primary Sample Collection
Sample Type for Analysis: Sputum, Nasopharyngeal (nasal) swab, Oropharyngeal (throat) swab, vaginal, anorectal, endocervical swab specimen

Sample collection and handling: The transport media is used as a medium for collection, decontamination and transport of various types of swabs specimens before proceeding for pre-treatment using Lysis buffer, ®extraction and purification of nucleic acids using Trueprep AUTO Universal ®Cartridge Based Sample Prep Kit and Trueprep AUTO Universal Cartridge Based Sample Prep Device.

Various types specimen must be collected as per standard procedures using a swab TM sample specific swab.

Extraction Procedure By Trueprep Auto Universal Cartridge Based Sample Prep Device

1. Put on a fresh pair of latex gloves.
2. Before using the AUTO Universal Cartridge Based Sample Prep Trueprep device for the first time, plug-in the bottles from the reagent pack into the back of the device. Ensure that the cap colour of the bottles matches the caps attached to the tubing.
3. Take a new cartridge from the AUTO Universal Cartridge based ®T rueprep sample prep kit, label the patient ID, date, in the space provided on the Cartridge label and place the cartridge in the cartridge stand provided with the ®Trueprep AUTO Universal Cartridge based sample prep device.
4. Perform sample pre-treatment as per sample type (refer disease specific TMT ruenat pack insert) and transfer the entire content of the bottle with pretreated sample using disposable transfer pipette (graduated-3ml) into the sample chamber of the cartridge.
5. Switch ON the AUTO Universal Cartridge Based Sample Prep Device ®T rueprep and press EJECT button.
6. Place the cartridge into the device, gently close the door and press START.
7. The device performs all the steps automatically.
8. Meanwhile, mention the patient ID, date, in the space provided on the Elute collection tube (ECT) label provided in the Cartridge pouch and affix the label to the Elute collection tube, present in the Cartridge pouch.
9. The process is concluded by a beep sound from the device and automatic ejection of cartridge. Take out the cartridge and place it on the cartridge stand.
10. Pierce the seal of elute chamber and aspirate out the entire elute into Elute collection tube (ECT) using disposable transfer pipette provided in the cartridge pack.
11. Dispose cartridge as mentioned under "Disposal & Destruction".
12. 12. Inspect the replaceable tray in the cartridge holder. If there are liquids spilled in the tray, take out the tray and discard as per the instructions in Section 8.3 of user manual of AUTO Universal Cartridge based Sample Prep device. ®T rueprep
13. Switch off AUTO Universal Cartridge Based Sample Prep Device. ®T rueprep
14. Clean any liquid spills, if any, according to section "Cleaning and decontamination".
15. Proceed to analysis (refer Truelab™ Uno Dx/Duo/Quattro manual).

Test Procedure of Truelab Quattro Real Time micro PCR Analyzer

1. Switch on the Truelab Analyzer.
2. Select User and enter password.
3. For Truelab Uno Dx, select the test profile for "SARS CoV-2" to be run from the Profiles Screen on the analyzer screen. For Truelab Duo/Quattro, select the Bay (Idle1/2) for Duo and (Idle1/2/3/4) for Quattro from the Status Screen to view the Profiles Screen. Select the test profile for "SARS CoV-2" to be run from the Profiles Screen on the Analyzer screen.
4. Enter the patient details as prompted in the Truelab Analyzer screen.
5. Press Start Reaction.
6. For Truelab Quattro, the chip tray opens automatically on tapping the "Start Reaction" button.
7. Open a pouch of Truenat™ SARS CoV-2 and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.
8. Label the chip and the tube with the patient ID using a marker pen at the space provided on the back side of the chip and the space on the micro tube label.
9. Place the Truenat™ SARS CoV-2 chip on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the Analyzer. Gently press the chip to ensure that it has seated in the chip tray properly.
10. 10. Place the microtube containing freeze dried RT PCR reagents in the microtube ®stand provided along with the Truelab Real Time micro PCR workstation after ensuring that white pellet of dried PCR reagents remains at the bottom of the microtube. Remove the microtube cap and dispose it off as per the section on "Disposal and Destruction" (Section 16). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified RNA from the Elute Collected Tube into the microtube. Allow it to stand for 30-60 seconds to get a clear solution. Do not mix it by tapping, shaking or by reverse pipetting. Using the same filter barrier tip, pipette out six (6) µL of this clear solution and dispense into the centre of the white reaction well of the Truenat™ SARS CoV-2 chip. Take care not to scratch the internal well surface and not to spill elute on the outside of the well. Dispose off the micro tip as per the section on "Disposal and Destruction".
11. For Truelab Quattro, select "YES" at the Please load Sample prompt. Chip tray will close automatically and the reaction will start.
12. Read the result from the screen.
13. After the reaction is completed, for Truelab Quattro, tap the "Open/Close Tray" button to eject the chip tray.
14. Take out the Truenat™ SARS CoV-2 chip-based Real Time PCR test at end of the test and dispose it off as per the section on "Disposal and Destruction".
15. Turn on Truelab micro PCR printer and select print on the screen for printing out hard copy of the results. Test results are automatically stored and can be retrieved any time later.
16. Switch off the Truelab Analyzer.

Results

In the present study, out of 500 patients, age of the patients ranged from 10 years to 80 years. Male patients constituted 360(72%) and females 140(28%). Male to female ratio was 18:7. Peak number 76(22.4%) of patients were in the age group of 51-60 yrs.

Table 1: Reveals age and gender wise distribution of Covid-19 patients.

Age	Male	Female	Total
1 - 10	5	1	6
11 - 20	20	7	27
21 - 30	45	11	56
31 - 40	56	16	72
41 - 50	59	36	95
51 - 60	85	46	131
61 - 70	47	10	57
71 - 80	43	13	56
Total	360	140	500

Table 1: Age and Sex wise distribution of COVID19 patients

Total WBC count of the patients ranged from 2,800-29,600/cumm of blood. Out of 500 patients, 277 (55.4%) patients showed leucocytosis (>11,000/cumm of blood).Males constituted 183 cases and females 94. Leucopenia (<4,000/cumm of blood) was observed in 92 patients. High ANC (>7,000/cumm of blood) was observed in 233 (84%) patients. Among 233 patients, 198 showed leucocytosis and 35 patients showed normal WBC count. Males constituted 196 and females 37 cases.

Low ALC (<1,000/cumm of blood) was observed in 57 (11.4%) patients. Males were 34 and 23 female patients.

High NLR (>3.47) was observed in 57(11.4%) patients and 34 patients were male and 23 were female.

Now, these 57 patients showed decreased Ct values of Orf1A gene in COVID19 RTPCR i.e. viral load was highly increased in those patients.

Discussion

High Neutrophil Lymphocyte ratio (NLR) was observed in 57(11.4%) patients. In a study done by Yan X *et al* [6] high NLR was observed in non survivors compared to survivor group. Neutrophils are hyper activated in sepsis and delayed apoptosis being the common events in Covid-19 [2]. NLR is a Biomarker of disease severity such as sepsis and bacteremia. NLR appears to be an efficient indicator of Mortality in Covid-19 patients [2, 6. 8].

Conclusion

This study clearly indicates that patients with increased Neutrophil Lymphocyte Ratio (NLR) may be indicative of Decreased Cycle Threshold Values of Orf1A gene in RTPCR of COVID19. That means NLR is inversely proportional to Ct value of Orf1A gene of RTPCR in COVID19 patients which may be Used as a biomarker to identify the disease as well as its severity.

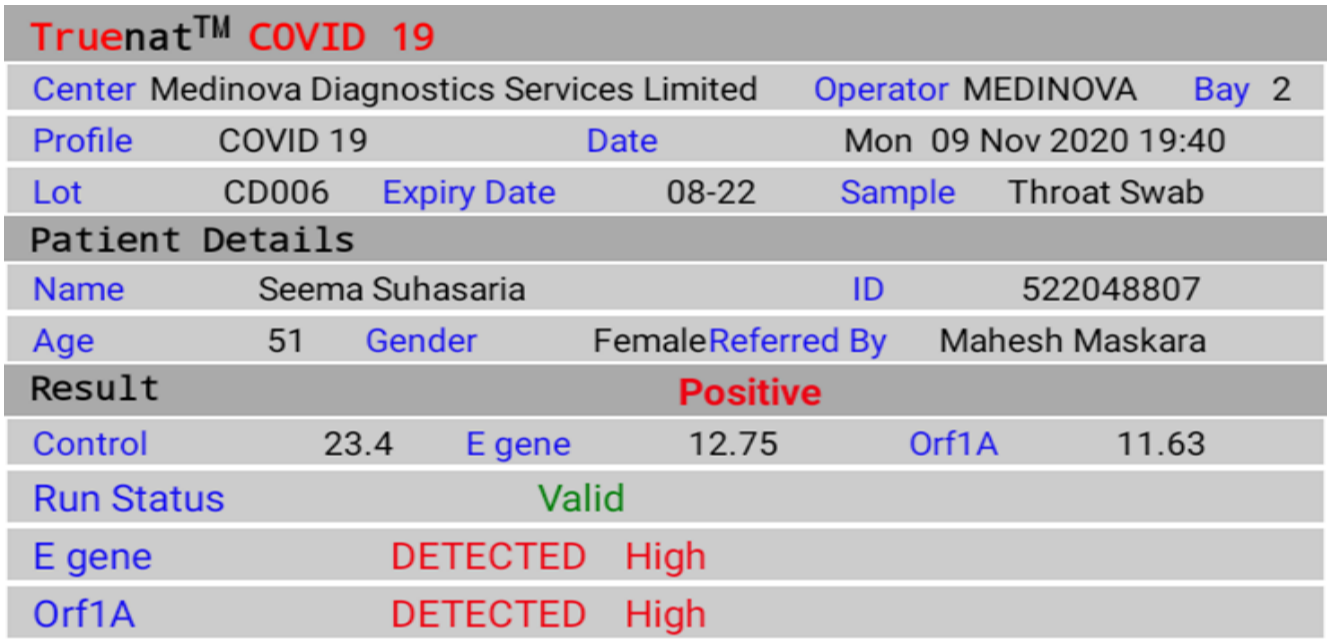

Fig 1

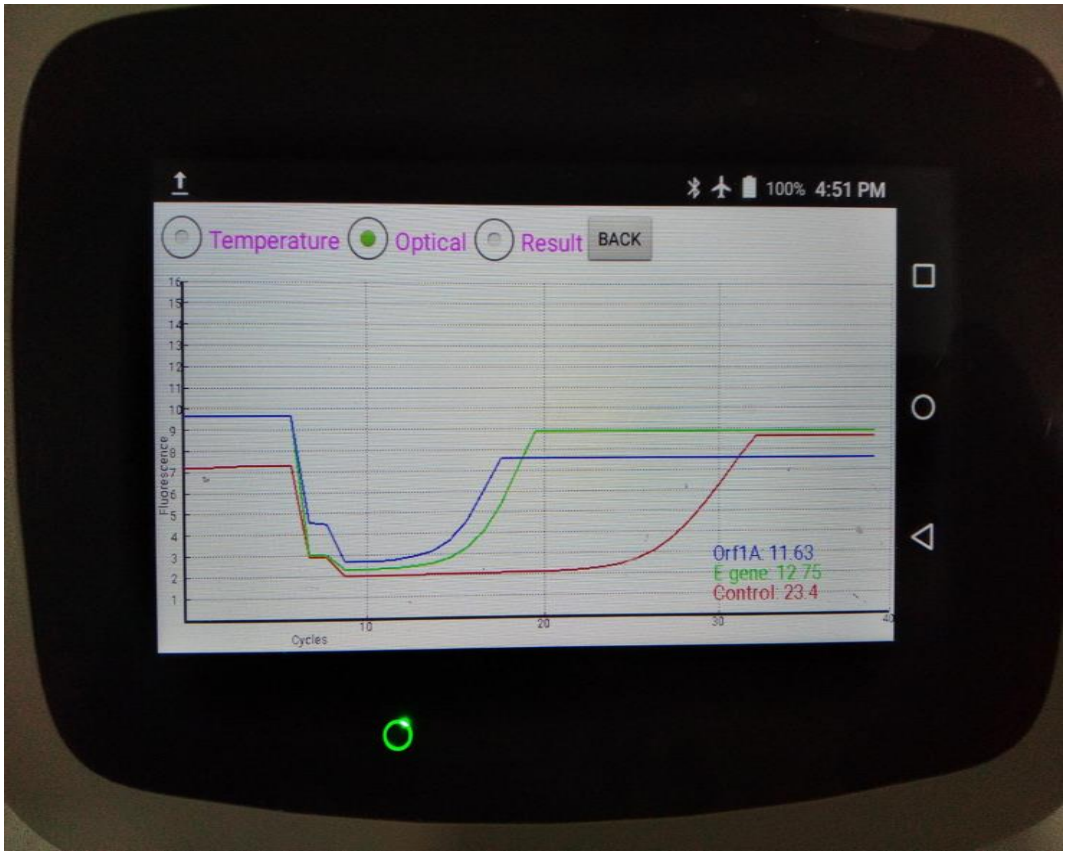

Fig 2

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