



## A Case Study Of Leucocytes Morphological Changes In Peripheral Smear From Covid - 19 Positive Patients In Tertiary Care Hospital

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### Abstract

**Background:** Covid 19 is a pandemic disease caused by the coronavirus identified in 2019 December. The virus was recognized in Wuhan china, as the cause of the corona disease outbreak and has rapidly spread from china to various countries as the world's pandemic. Knowledge of the morphologic, physiologic, biochemical, and immunologic effects of viruses on cells is essential in understanding the pathophysiology of viral disease and in developing accurate diagnostic procedures and effective treatment. In our study, we described morphological changes in leukocytes of Covid 19 patients' peripheral blood smear.

**Aim Of The Study:** Leukocyte morphological analysis in peripheral blood in Covid 19 patients.

**Method:** This analytic study was done in covid -19 positive cases who were admitted at Government Thiruvarur medical college. Thiruvarur, Tamil Nadu India in the year 2020-2021, 200 cases were included in the study. The microscopic examination of the peripheral smear was done and the results were analyzed.

**Results:** In Neutrophils, a shift to the left is observed in 44% of COVID 19 (+) patients indicating associated secondary sepsis with COVID 19 pneumonia, Lymphopenia with plasmacytoid lymphocytes is seen in 30% of cases vacuolated monocytes observed in 20% of cases.

**Conclusion:** Patients which include APHA, ANNP, leucocytosis, neutrophilia, toxic granules, cytoplasmic vacuoles in neutrophils, neutrophil maturation series with the shift to left, lymphopenia, plasmacytoid lymphocytes, monocytes with cytoplasmic vacuoles and thrombocytopenia were observed. ANNP (Abnormal Neutrophilic Nuclear Projection) & APHA(Acquired Pelger Huet Anamoly) were more prominent features we encountered in the majority of COVID19 (+) cases.

**Keywords:** Peripheral smear, Leukocytes, Covid 19, Lymphopenia

### Introduction

Covid 19/Coronavirus disease is an ongoing pandemic disease that emerged from Wuhan in 2019 & spread as a pandemic throughout the world. The virus belongs to the RNA group of the virus, family in the order nidovirales (2-2)The mode of spread is from droplet infection & close contact.[1] Coronavirus caused severe acute respiratory disease syndrome (SARS/COV2), the immune-mediated inflammatory response plays an important role in

pathogenesis Apart from clinical presentation certain investigations plays important role in the diagnosis & severity of the disease. As they progress severely there are signs of lymphopenia, neutrophilia & eosinopenia.[2]In the peripheral blood film of covid 19 patients plasmacytoid lymphocytes, shift to the left of neutrophils, APHA (Acquired Pelger Huet Anamoly) & ANNP are commonly seen. Promyelocytes are larger than myeloblasts and have basophilic cytoplasm containing primary

(azurophilic) granules. A Golgi zone may be visible as a para nuclear hof or clearing. The nuclear chromatin is finely dispersed, and nucleoli may be visible. [3] Promyelocytes comprise approximately 2% of nucleated cells in the bone marrow and do not circulate in peripheral blood under normal conditions. [4] Reactive lymphocytes with immunoblast-like morphology are large cells with high nuclear-cytoplasmic ratios, condensed chromatin, and deeply basophilic cytoplasm. Another type of reactive lymphocyte has less condensed chromatin and abundant, pale blue cytoplasm that may appear to “hug” adjacent red blood cells. These cells are also called Downey type II cells. They can be seen in a variety of conditions, but are often increased in infectious mononucleosis due to EBV infection. The changes in cell morphology caused by infecting viruses are called cytopathic effects (CPE). Common examples are rounding of the infected cell, fusion with adjacent cells to form a syncytia (polykaryocytes), and the appearance of nuclear or cytoplasmic inclusion bodies. Inclusion bodies may represent either altered host cell structures or accumulations of viral components.[5] Many viruses inhibit the synthesis of host cell macromolecules, including DNA, RNA, and protein. Viruses may also change cellular transcriptional activity, and protein-protein interactions, promoting efficient production of progeny virus. For some viruses, specific cellular biochemical functions may be stimulated to enhance virus replication.[6]

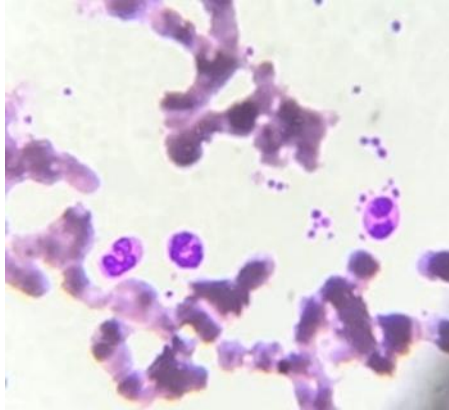
**Methods:** This analytic study was done in covid -19 positive cases who are admitted at Government Thiruvavur medical college. Thiruvavur, Tamil Nadu India in the year 2020-2021. 200 cases were included in the study. A microscopic examination of the peripheral smear was done and the results were analyzed. We analyzed the morphological abnormalities of leukocytes particularly neutrophils in covid 19 infected patients demonstrated by PS. For this study, PS was prepared from an EDTA sample, from covid 19, infected patients, and smear stained by a qualified technician with Leishman stain. PS examined under 10,40 &100X magnification. The morphological details & leukocytes were screened under 100X along with RBCs & platelets. Morphological variations in leukocytes were identified after viewing 100 leukocytes in each slide.

**Stastical Analysis:** All continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range [IQR]). Categorical data were expressed as percentages. using the independent sample t-test, the Mann-Whitney U test, or the chi-squared test, as appropriate. Correlations between variables were examined using Spearman's rank correlation analysis. Univariate and multivariate logistic regression analyses were performed to identify independent correlates.

**Result:** Our study highlights the abnormality of leukocyte neutrophils ANNP, APHA, shift to left, toxic granules & cytoplasmic vacuoles.

Conditions	Incidence %
ANNP (Abnormal Neutrophilic Nuclear Projection)	52%
APHA ( Acquired Pelger Huet Anamily)	64%
Leucocytosis	24%
Neutrophils with toxic granules	76%
Neutrophils with cytoplasmic vacuoles	44%
Neutrophils with the shift to left -	44%
Lymphopenia with plasmacytoid lymphocytes	30%
Vacuolated monocytes	20%
Thrombocytopenia	8%

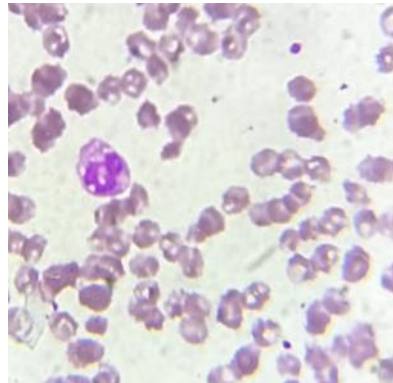
**Fig.1**



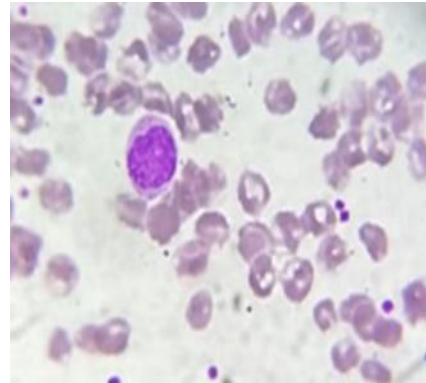
**Fig.2**



**Fig 3**



**Fig 4**



**Discussion:**

Our study demonstrates mainly the significant morphological changes in blood cells of covid-positive patients. In neutrophils various morphological changes are observed which are specific for covid- 19(+) patients. The changes include APHA-acquired pelger Huet anomaly with bilobed neutrophils (fig.1) – around 64% of cases show these features. Earlier in covid 19 pandemic, their changes were reported by Zini et al (1-12) Tai-sheng LI et al and supported by Gokula Kannan et al. This is an acquired morphologic feature of neutrophils that are infected by SAR S - COV 2 virus in COVID 19 disease, the hypothesis is unclear.

ANNP/ pseudo nuclear projection in neutrophils(fig.2) are observed in 52% of cases with covid-19(+). They are different from Barr bodies or drumsticks(1-13) as they are seen in females while ANNP was seen in both sexes with COVID positive taking steroid therapy.[7] Taran G, et al quoted that ANNP is augmented by steroid therapy. Furthermore,

post-COVID19 follow-up continued to demonstrate ANNP and tend to carry this abnormality even after recovering from the disease. Leucocytosis were observed in 24% of cases which was suggested as a significant factor for disease progression(2,2-12). Neutrophils presented with toxic granules and cytoplasmic vacuoles (fig.3) noted in 76% & 44% of COVID-19 cares respectively. [8]

In Neutrophils, a shift to the left was observed in 44% of COVID-19 (+) patients indicating associated secondary sepsis with COVID-19 pneumonia.(1,2,1-12) Lymphopenia with plasmacytoid lymphocytes(fig.4) is seen in 30% of cases observed by Zhou J, Li C et.al [9,10].

**Conclusion:** To summarize, in our study we observed certain features similar to that recorded in a few earlier studies in COVID-19 (+) patients which include APHA, ANNP, leucocytosis, neutrophilia, toxic granules, cytoplasmic vacuoles in neutrophils, neutrophil maturation series with the shift to left, lymphopenia, plasmacytoid lymphocytes, monocytes

with cytoplasmic vacuoles and thrombocytopenia. ANNP&APHA were more prominent features we encountered in the majority of COVID19 (+) cases.

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