RESEARCH PAPER

Comparative Evaluation of Blood Neutrophil Function Tests in Patients with Acute Exacerbation of COPD versus Newly Diagnosed COPD at a Tertiary Healthcare Hospital in Tamil Nadu

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ABSTRACT

Background

COPD is a major global health concern, with increasing prevalence and mortality. According to GOLD guidelines, COPD severity is classified based on spirometry criteria. Neutrophils play a key role in COPD pathogenesis, but their function in Acute Exacerbation of COPD (A/E COPD) is unclear. This study aimed to compare neutrophil function tests in A/E COPD and newly diagnosed COPD patients, stratified by GOLD severity criteria.

Methods

This analytical cross-sectional study included 150 COPD patients (40-80 years) attending Respiratory Medicine OPD/IPD at St. Peter's Medical College Hospital, Tamil Nadu, India. Neutrophil function tests (chemotaxis, phagocytosis, intracellular killing, and NBT test) were performed on each sample.

Results

Phagocytosis was lower in A/E COPD patients (1.86 ± 0.99) vs new COPD patients (2.78 ± 0.96, p=0.045). Bactericidal activity was also lower in A/E COPD patients (18.85 ± 3.27) vs new COPD patients (20.54 ± 2.79, p=0.028). Neutrophil count, NBT test, and chemotaxis showed no significant differences. Majority of patients (42.7%) were GOLD grade II (Moderate).

Conclusion

A/E COPD patients have impaired neutrophil function, particularly in phagocytosis and bactericidal activity, contributing to disease exacerbation.

Keywords: COPD, GOLD, Neutrophil function, Phagocytosis, Bactericidal activity, Acute Exacerbation

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Introduction

COPD is a major global health concern, ranking fourth in mortality worldwide and affecting 5% of India's adult population. By 2030, it's projected to be the third leading cause of death globally. COPD is a chronic lung airflow obstruction caused by inflammation from harmful particles and gases. Key risk factors include smoking, pollution, occupational exposure, HIV, and IV drug use, leading to lung imbalance and activating macrophages and neutrophils (1). AECOPD is a worsening of symptoms like breathlessness, cough, and sputum, leading to poor outcomes and increased mortality. About 50% of AECOPD cases are caused by bacteria or viral infections (2,3). Neutrophils play a key role in COPD, with increased counts and abnormal function. Lipopolysaccharides and inflammatory cytokines like GM-CSF prolong their lifespan, disrupting normal cell death (4). This leads to persistent neutrophils in the lungs, driving chronic inflammation and tissue damage (5).

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines use blood eosinophil counts for COPD management, but blood neutrophil counts (BNC) are underutilized. Elevated BNC indicates systemic inflammation, linked to COPD severity and comorbidities. Research shows correlations between disease severity and neutrophil activation markers, such as neutrophil elastase (NE) and myeloperoxidase, in sputum and bronchoalveolar lavage samples (6-8). Despite this, the mechanisms behind neutrophil migration and survival in COPD airways are unclear, highlighting a research gap (9). Hence, this study compares neutrophil function tests in patients with Acute Exacerbation of COPD versus newly diagnosed COPD at a tertiary healthcare hospital in Tamil Nadu, aiming to evaluate the correlation between neutrophil function and disease severity.

Objective

The main objective of this study is to evaluate the blood Neutrophil count and its functions test in COPD Patients visiting a tertiary health care.

Methods and Methodology

This analytical cross-sectional study was conducted at St. Peter's Medical College Hospital, Tamil Nadu, India, from October 2023 to October 2024. 150 COPD patients were selected based on consecutive sampling method

Inclusion criteria:

- Age above 40-80years.
- Both male and female
- Both Smokers & Ex smokers
- Patients who are with known COPD and newly diagnosed COPD cases.
- Patients with comorbidities such as CVD (cardiovascular disease), diabetes, hypertension and dyslipidaemia.

Exclusion criteria:

- Age < 40 and > 80 years.
- Patients with other Respiratory illnesses other than COPD (Acute infection, Autoimmune disease, Inflammatory diseases, Tumour, Haematological diseases)

Diagnostic criteria:

The diagnosis of COPD in this study is made using GOLD (Global Initiative for Obstructive Lung Disease) criteria.

According to GOLD criteria FEV1/FVC ratio < 0.70% is classifies as COPD (10)**.** FEV1 ≥ 80% predicted is classified as GOLD-A or mild disease. 50% ≤ FEV1 < 80% predicted is classified as GOLD-B or moderate disease, 30% ≤FEV1<50% predicted is classified as GOLD-C or severe disease and FEV1<30% predicted is classified as GOLD-D or very severe disease.

Sample size:

From the previous study expected proportion of elevated neutrophil count is 34% (11)

$$n = \frac{Z\ 1\text{-}\alpha^2 \times pq}{d^2}$$

q = 1-p

p = 34% (proportion)

d = precision = 10%

z=2.576for99% confidence interval

$$= \frac{2.576 \times 2.576 \times 0.34 \times 0.66}{0.1 \times 0.1} = 148.90$$

(Sample adjusted to 150)

Institutional ethical committee approval and informed consent were obtained. Baseline data were collected using a proforma.10 ml of blood will be collected in two vacutainers containing ethylenediamine tetra acetic acid as anticoagulant under the guidance of trained staff nurse.

Methodology

After collecting the blood sample from the patients, each sample was processed for neutrophil function test which include nitroblue tetrazolium (NBT) test, phagocytosis, intracellular killing (candidacidal assay), and, chemotaxis.

Neutrophil function test

Nitroblue tetrazolium (NBT) test

Whole blood was used to analysis the NBT assay. Take 2 test tubes, 1 test tube for stimulated cells and another one for Unstimulated cells. In both test tubes add 100 µl of blood,200 µl HBSS, 50 µl of NBT (0.35 w/v prepared in 0.34% sucrose, diluted in PBS just before use). Along with this, in stimulated tube add 50 µl of endotoxin (prepared in house from Escherichia coli) whereas in unstimulated tube, endotoxin was replaced with additional 250 µl of HBSS. Then both tubes were incubated for 20minutes at 37°C and further 20 minutes at room temperature. Prepare the thin smear from the tubes and stained with Giema and focused under oil immersion (100 x). Count 200 neutrophils, among theses the observed cell which contain bluish black formazone granules was considered as stimulated by endotoxin. The ratio of stimulated and the unstimulated cell was expressed as percentage (12)

Purified WBC separation was needed for the remaining 3 assay

Process for WBC –separation

2ml of EDTA blood was mixed equal quantities of 1%Phosphate Buffered Saline (PBS) and 1% gelatin salutation. After mixing, the tube was kept upright for 45 minutes without distributing. At the end of 45 minutes, all the RBCs were settling down at the bottom of the tube, where as WBC and plasma were appearing at the upper layer which was collected and centrifuged for 3-5 min at 3000 RPM. The deposit which contain cells were washed for 3 times with 2ml of PBS with 7.2 PH to remove gelatin remnants. Finally the cell concentration was adjusted by adding 1 ml of HBSS. (13, 14)

Phagocytosis Function

Heat killed Candida albicans was used to test phagocytosis function. In one test tube, 250µl each of Hank's solution, 250µl plasma (pooled normal human serum), 250µl heat killed Candida suspension and 250µl of WBC suspension were added. In a second tube serum was replaced with 500 µl of Hanks solution which act as negative control. After thorough mixing, both tube was incubated at 37°C for 30 minutes followed by centrifuged the tubes at 3000 rpm for 5 min and the supernatant was discarded. Prepared a smear from the sediment and stained with Geimsa stain observed under microscope. Examine 200 neutrophil and count the number of Candida ingested per cell which was expressed as mean particle number (MPN). (15)

Candidacidal assay

This test help to assess the intracellular killing function. One tube considered as test which contain 250 µl of WBC suspension, 250 µl of Hanks solution, 250 µl of autologous plasma, and 250 µl of live Candida cells. Another tube considered as control which contain 250µl of WBC suspension, 250µl of live candida, 500 µl of Hanks solution. Both tubes were incubated at 37°C for 1 h with intermittent shaking. At the end of the incubation, 250 µl of 2.5% sodium deoxycholate was added to each tube and mixed well followed by 2 ml of 0.01% methylene blue, mixed and centrifuged at 2000 rpm for 10 min. Discard the supernatant and charged a small drop of deposited solution in the Neubauer counting chamber and at least 300 Candida cells were counted. Count the proportion of dead cells taken by methylene blue and appear blue color versus unstained cells (16)

Chemotactic activity

On a clean glass slide (25 mm × 75 mm), 5 ml of 1.2% agarose solution containing MEM medium, pooled human serum, and sodium bicarbonate were poured and allowed to solidify. Series of 3 wells were cut at the diameter of 3mm at a distance of 3mm. The central well was loaded with Formyl-Methionyl-Leucyl-Phenylalanine (FMLP) a known chemoattractant and the peripheral wells contained WBC cells (test samples). In a control set up where chemoattractant is replaced with subject's serum. The slides were incubated at 37°C for 2 h and then it was immersed in methanol for 30 min followed by fixation with formalin for 30 min. After the removal of the agarose layer the slide was stained with Giemsa. And measure the linear distance travelled by the cells under the microscope using an oculometer. Spontaneous migration toward the well containing buffer also noted to calculate chemotactic index. (16)

Statistical Test

SPSS version 16 was used to analysis the data for this study, Categorical data was expressed as frequency and percentage. Neutrophil function test was compared with personal habit of COPD patients and between A/E COPD & New COPD patients by One way ANOVA and student t test respectively. p value less than 0.05 was considered statistically significant

Results

Table 1: Distribution of COPD patients by GOLD grade (Spirometry Criteria)

GOLD Grade	Severity	FEV1 % Range	No. of. Patients	Percentage
I	Mild	≥ 80	35	23.3
II	Moderate	50–79	64	42.7

III	**Severe**	**30–49**	21	14.0
IV	**Very Severe**	**< 30**	30	20.0
Total			**150**	**100**

Table 1 shows the distribution of COPD patients by GOLD grade, based on spirometry criteria. The majority of patients that is 42.7% had moderate disease, 23.3% had mild disease, 20% had very severe disease and 14% had severe disease. This indicates a significant proportion of this study patients have moderate COPD, highlighting the need for early intervention and management.

Table 2: Demographic Characteristics of COPD Patients Stratified by GOLD Severity Criteria

Demographic Characteristics		**GOLD Severity Criteria**				
		Mild N =35	**Moderate N = 65**	**Severe N =21**	**Very Severe N = 30**	**Total N =150**
Age group (years)	**<50**	6	0	0	2	8
	50–59	4	2	2	2	10
	60–69	17	50	12	18	97
	≥70	8	12	7	8	35
Gender	**Male**	20	47	16	21	104
	Female	15	17	5	9	46

Table 2 depicts the demographic characteristics of COPD patients. Most patients (64.7%) are between 60-69 of age, with a significant proportion of males (69.3%). The majority of patients with moderate COPD are in the 60-69 age group, while those with very severe COPD are distributed across all age groups. Males outnumber females across all grades, representing a potential gender disparity in COPD prevalence or diagnosis.

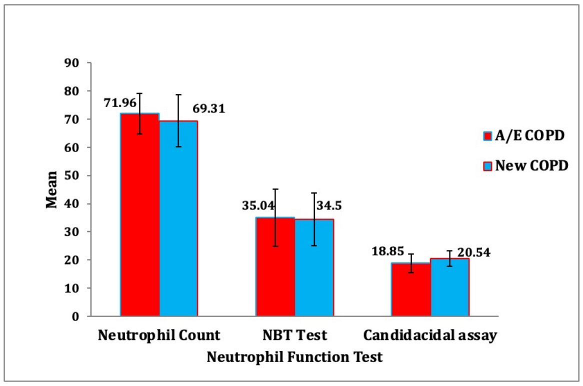
Table 3: Comparison of Neutrophil Functions Test by personal habit among COPD Patients

Neutrophil Functions Test	**Smoker Mean ± SD**	**Non-Smoker Mean ± SD**	**Betel Chewers Mean ± SD**	**p-value**
Neutrophil Count (%)	72.5± 8.13	70.8± 7.51	71.9 ± 7.63	0.326
NBT Test (% Cells)	36.03± 10.63	33.67± 9.02	30.5± 9.07	0.156
Phagocytosis (Mean Particle Number)	2.110± 1.04	2.07± 0.88	1.92± 0.83	0.447
Chemotaxis (mm)	0.42± 0.15	0.41± 0.14	0.44± 0.13	0.524
Candidacidal assay (% of Dead Candida)	19.46± 6.02	19.48± 4.02	18.7± 5.4	0.289

NBT- Nitroblue tetrazoliumTest

Table 3 demonstrates the comparison of neutrophil functions in COPD patients based on smoking status. This results indicates that there was no significant differences in neutrophil count, NBT test, phagocytosis, chemotaxis, and bactericidal activity among smokers, non-smokers, and betel chewers. This suggests that smoking status may not significantly impact neutrophil function in COPD patients.

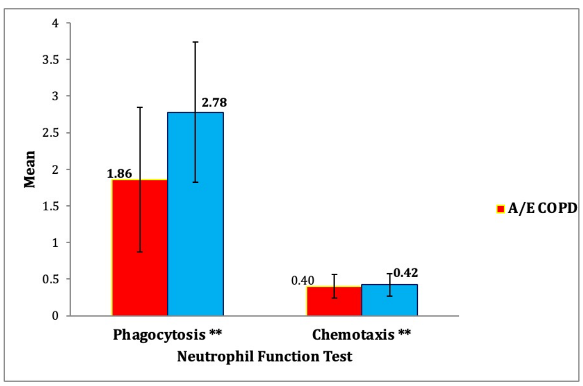
Figure 1: Comparison of Neutrophil Functions Test of Neutrophil count, NBT test and Candidacidal assay between A/E COPD & New COPD Patients



NBT- Nitroblue tetrazoliumTest

Figure 1 shows a comparison of neutrophil functions in patients with Acute Exacerbation of COPD (A/E COPD) and newly diagnosed COPD. Neutrophil count, NBT test, and chemotaxis, didn't show any significant difference. This suggests impaired neutrophil function in A/E COPD patients, potentially contributing to disease exacerbation.

Figure 2: Comparison of Neutrophil Functions Test of Phagocytosis and chemotaxis assay between A/E COPD & New COPD Patients



Note- ** significant

Figure 2 depicts phagocytosis and bactericidal activity between A/E COPD and new COPD. We have noticed a significant difference between the two groups ($p < 0.05$), with lower phagocytosis and bactericidal activity in A/E COPD patients.

Discussion

Chronic obstructive pulmonary disease is the third commonest cause of death globally. COPD caused 3.84 million deaths in 2019, and the number is expected to increase 4.4 million annually by 2040. Even though COPD is a considerable problem universally, China and India accounts for more than 50% of all cases of global COPD (17).

Airway inflammation includes a complex network of interactions connecting various immune-related cells, comprising neutrophils and lymphocytes, which can lead to persistent respiratory tissue injury and damage (18). Neutrophils are crucial players in the inflammatory response in COPD, often collecting in the airways and contributing to tissue damage. Raised neutrophil counts are commonly noticed in COPD patients, signifying their participation in disease pathogenesis.

In this present study, 42.7% had moderate disease, with FEV1% ranging from 50-79. 23.3% of patients had mild, 20% with very severe disease and 14% had severe disease. The distribution indicates a significant proportion of patients had moderate COPD, highlighting the need for targeted interventions to prevent disease progression.

Hogendoorn et al conducted a review of 37 similar studies, have reported that COPD exacerbation rates increases with disease severity. They have found that global exacerbation occurrences were 0.82 for mild, 1.17 for moderate, 1.61 for severe, and 2.10 for very severe COPD, indicating a strong link between airflow limitation and exacerbation risk (19).

In the present study, majority of patients that is 64.7% were in the 60-69 age group, indicating a high burden of COPD in this age range. Notably, the moderate COPD group had the highest number of patients, with 50 of them in the 60-69 age group. The very severe COPD group had 30 patients, with an even distribution across age groups. The gender inequality was evident in this study, with males accounting for approximately 69% of the study population, suggesting potential differences in COPD prevalence or diagnosis between genders.

Morena D et al found a prominent age difference between individuals with and without COPD. The COPD population had a mean age of 73 years that is significantly higher than those without COPD and their mean age was 40 years. This highlights the strong link between increasing age and COPD prevalence (20).

Studies by Christopher et al. (21) and Sinha et al. (22) reported different COPD prevalence rates. Christopher et al. found 4.6% prevalence among 1015 individuals, with females slightly higher (4.9%) than males (4.1%). Sinha et al. reported 10.1% prevalence among 1203 individuals, with males higher (12.2%) than females (7.8%). These variable values indicates COPD's heterogeneity across populations and settings, highlighting the need for localized data to guide targeted interventions.

The present study compared neutrophil functions in COPD patients based on smoking status, including smokers, non-smokers, and betel chewers. The results showed no significant differences in neutrophil count, NBT test, phagocytosis, chemotaxis, and bactericidal activity among the three groups ($p > 0.05$ for all). The mean values for these neutrophil functions were comparable across smokers, non-smokers, and betel chewers, indicating that smoking status may not have a significant impact on neutrophil function in COPD patients. This suggests that other factors may be contributing to the altered neutrophil function observed in COPD.

Unlike our study, Van der et.al, reported that acute smoking augmented circulating neutrophil counts, signifying neutrophils were mobilized into the bloodstream, possibly driving inflammation in smoking-related diseases like COPD (23).

Neutrophils play a key role in killing bacteria, mainly through phagocytosis. These cells kill bacteria by phagocytosis, releasing reactive oxygen species (ROS), lactoferrin, and proteinases, or producing neutrophil extracellular traps (NETs). In COPD, disease advancement continues even though, neutrophils are abundant in the lungs, due to infectious exacerbations. This suggested that neutrophil function may be impaired or overwhelmed in COPD, contributing to disease pathology (24).

In our study, we compared neutrophil functions in patients with Acute Exacerbation of COPD (A/E COPD) and newly diagnosed COPD. The results showed significant differences in phagocytosis and bactericidal activity between the two groups. Phagocytosis was lower in A/E COPD patients (1.86 ± 0.99) compared to new COPD patients (2.78 ± 0.96), with a p-value of 0.045. Similarly, bactericidal activity was lower in A/E COPD patients (18.85 ± 3.27) compared to new COPD patients (20.54 ± 2.79), with a p-value of 0.028. However, neutrophil count, NBT test, and chemotaxis showed no significant differences between the two groups. These findings suggest that A/E COPD patients have impaired neutrophil function, particularly in phagocytosis and bactericidal activity, which may contribute to disease exacerbation.

Conclusion

This comparative evaluation of neutrophil function tests in patients with Acute Exacerbation of COPD versus newly diagnosed COPD at a tertiary healthcare hospital in Tamil Nadu reveals impaired neutrophil function in A/E COPD patients, particularly in phagocytosis and bactericidal activity. These findings suggest that targeting neutrophil dysfunction could be a potential therapeutic strategy for managing COPD exacerbations, ultimately improving patient outcomes in this population.

References

1. Shanmugam L, Ravinder SS, Johnson P, Padmavathi R, Rajagopalan B, Kindo AJ. Assessment of phagocytic activity of neutrophils in chronic obstructive pulmonary disease. Lung India. 2015 Sep-Oct;32(5):437-40. doi: 10.4103/0970-2113.164159. PMID: 26628755; PMCID: PMC4586995

2. Dhooria S, Agarwal R, Sehgal IS, Prasad KT, Garg M, Bal A, et al. Spectrum of interstitial lung diseases at a tertiary center in a developing country: A study of 803 subjects. PLoS One. 2018;13(2):e0191938

3. Sharma BB, Singh S, Sharma KK, Sharma AK, Suraj KP, Mahmood T et al; SWORD study group. Proportionate clinical burden of respiratory diseases in Indian outdoor services and its relationship with seasonal transitions and risk factors: The results of SWORD survey. PLoS One. 2022 Aug 18;17(8):e0268216.

4. John CR, Douglas RG. Apoptosis. Physiological Pathology. Cambridge: Cambridge University Press; 2011. p. 355

5. Plataki M, Tzortzaki E, Rytila P, Demosthenes M, Koutsopoulos A, Siafakas NM. Apoptotic mechanisms in the pathogenesis of COPD. Int J Chron Obstruct Pulmon Dis 2006; 1: 161-71.

6. Dicker AJ, Crichton ML, Pumphrey EG, et al. Neutrophil extracellular traps are associated with disease severity and microbiota diversity in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2018;141(1):117–127. doi: 10.1016/j.jaci.2017.04.022.

7. Cockayne DA, Cheng DT, Waschki B, et al. Systemic biomarkers of neutrophilic inflammation, tissue injury and repair in COPD patients with differing levels of disease severity. PLoS One. 2012;7(6):e38629. doi: 10.1371/journal.pone.0038629.

8. Wouters EF, Groenewegen KH, Dentener MA, Vernooy JH. Systemic inflammation in chronic obstructive pulmonary disease: the role of exacerbations. Proc Am Thorac Soc. 2007;4(8):626–634. doi: 10.1513/pats.200706-071TH.

9. . Rahman I. Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. J Biochem Mol Biol 2003;36:95–109.

10. Celli BR, MacNee W and ATS/ERS Committee Members. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J 2004; 23

11. Mike Lonergan ,Alison J.Dicker Blood neutrophil count are associated with exacerbation frequency and mortality in COPD 2020; 21:166.

12. Gentle TA, Thompson RA. Neutrophil function tests in clinical immunology. In: Gooi HC, Chapel H, editors. Clinical Immunology: Apractical Approach. Oxford, New York: Oxford University Press; 1990. p. 56-65.

13. Pattan SS, Bhat KG, Kumbar VM, Kugaji MS, Pattar G. Defining the normal range for screening panel of neutrophil function tests in healthy adult Indian participants. Indian J Health Sci Biomed Res 2021; 14:141-5.

14. Perutelli P, Catellani S, Scarso L, Cornaglia-Ferraris P, Dini G. Processing of human cord blood by three different procedures for red blood cell depletion and mononuclear cell recovery. Vox Sang 1999; 76:237-40

15. Wilkinson PC. Neutrophil leucocyte function tests. In: ThompsonRA, editor. Techniques in Clinical Immunology. 2nd ed. Oxford, London: Blackwell Scientific Publications; 1981. p. 273-93.

16. Metcalf JA, Gallin JI, Nauseef WM, Root RK. Laboratory Manual of Neutrophil Function. New York: Raven Press; 1986. p. 60-4

17. Quaderi S, Hurst J. The unmet global burden of COPD. ***Glob Health Epidemiol Genom***. 2018;3.

18. Nathan C. Points of control in inflammation. ***Nature***. 2002;420(6917):846-52.

19. Hogendoorn M, Feenstra TL, Hoogenveen RT, Al M, Mölken MR. Association between lung function and exacerbation frequency in patients with COPD. Int J Chron Obstruct Pulmon Dis. 2010;5:435–444. doi: 10.2147/COPD.S13826.

20. Morena D, Izquierdo JL, Rodríguez J, Cuesta J, Benavent M, Perralejo A, Rodríguez JM. The Clinical Profile of Patients with COPD Is Conditioned by Age. J Clin Med. 2023 Dec 9;12(24):7595. doi: 10.3390/jcm12247595. PMID: 38137664; PMCID: PMC10743861.

21. Christopher DJ, Oommen AM, George K, Shankar D, Agrawal A, Thangakunam B. Prevalence of airflow obstruction as measured by spirometry, in rural Southern Indian Adults. COPD. 2020;17:128–35. doi: 10.1080/15412555.2020.1723074.

22. Sinha B, Vibha, Singla R, Chowdhury R. An epidemiological profile of chronic obstructive pulmonary disease: A community-based study in Delhi. J Postgrad Med. 2017;63:29–35. doi: 10.4103/0022-3859.194200.

23. Van der Vaart H, Postma DS, Timens W, ten Hacken NH. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. Thorax. 2004;59(8):713–721. doi: 10.1136/thx.2003.012468.

24. Lonergan M, Dicker AJ, Crichton ML, Keir HR, Van Dyke MK, Mullerova H, Miller BE, Tal-Singer R, Chalmers JD. Blood neutrophil counts are associated with exacerbation frequency and mortality in COPD. Respir Res. 2020 Jul 1;21(1):166. doi: 10.1186/s12931-020-01436-7. PMID: 32611352; PMCID: PMC7329438.