OPEN ACCESS

Middle East Research Journal of Biological Sciences
ISSN 2789-7710 (Print & ISSN 2958-2091 (Online)
Frequency: Bi-Monthly
DOI: 10.36348/merjbs.2024.v04i04.004

KUWAIT SCHOLARS PUBLISHER
Website: http://www.kspublisher.com/
Email: office@kspublisher.com

Identification of Microarn Biomarkers of Prognosis in Type 2 Diabetes Patients Positive for Sars-Cov-2 Variant Delta and Omicron in Nasopharyngeal Secretions in Pointe Noire

Freddy Saturnin Pouki[1,7,8,9*], Luc Magloire Anicet Boumba[1,2,3], Rebecca Frédérique Dussaud[1,3], Parode Ragive Takale[1,9], Charley Elenga-Bongo[1,4], Aladin Atandi Batchy[1,6,7], Christy Parfait Nganga[1,6,7], Ange Cerna Malonga Ngouma[1], Gainze Boyina[1], Jean Idrice Kélétéla[7], Cheick Ahmed Dabo Tidiane[7], Saar Abdoul Wahar[7], Siméon Nama[9], Ghislain Loubano Voumbi[1,5], Donatien Moukassa[1]

[1]Faculty of Health Sciences, Marien Ngouabi University, BP: 69 Brazzaville, Congo
[2]Pointe-Noire Research Zone, National Institute for Research in Health Sciences (IRSSA) Brazzaville, Congo
[3]Medical and morphological analysis laboratory, Hôpital Général Loandjili, Pointe-Noire, Congo
[4]Endocrinology and Metabolic Diseases Department, Hôpital Général Adolph Sicé, Pointe-Noire Congo
[5]Medical analysis laboratory, Dolisie General Hospital, Congo
[6]Laboratory department, Hôpital Général Adolph Sicé, Pointe-Noire, Congo
[7]Louise Michel private clinic, Pointe-Noire, Congo
[8]Tié Tié Referral Hospital, Pointe-Noire, Congo
[9]Guénin Medical Clinic, Pointe-Noire, Congo

Abstract: ***Introduction*:** The COVID-19 pandemic has affected the whole world, with a particularly high and severe incidence in patients with co-morbidities such as type 2 diabetes (T2DM). Detection of microRNAs in human samples may be an alternative to identify effective biomarkers for COVID-19 severity. ***Methods***: We recruited a total of 206 participants for this study. MicroRNA and variant analysis were performed on nasopharyngeal samples using qPCR. The study consisted of the detection of 17 microRNAs. ***Results*:** The majority of individuals with the Delta variant (68.18%, n=90) had severe symptoms while individuals with the Omicron variant had moderate symptoms (76.47%, n=13). The microRNAs hsa-miR-29a-5p (AUC 0.80; CI 0.68 to 0.89 and p<0.000), hsa-miR-203a-5p (AUC 0.80; CI 0.75 to 0.92 and p<0. 000), and hsa- hsa-miR-221-3p (AUC 0.74; CI 0.61 to 0.86 and p<0.000), hsa-miR-33b-5p (AUC 0.88; CI 0.75 to 0.98 and p<0. 000) and hsa-miR-30d-3p (AUC 0.82; CI 0.70 to 0.95 and p<0.000) have AUCs that indicate a good discriminatory ability to stratify patients at high risk of complications from COVID-19 variant omicron among T2DM. ***Conclusion*:** Our study shows that the microRNAs hsa-miR-29a-5p, hsa-miR-203a-5p, and hsa-miR-221-3p, hsa-miR-33b-5p and hsa-miR-30d-3p have better discrimination and could be used as prognostic biomarkers in nasopharyngeal secretions from COVID-19 variant omicron patients with type 2 diabetes. They could also serve as new therapeutic targets.

Keywords: Microrna, Sars-Cov-2 Variant Delta and Omicron, Type 2 Diabetes.

Copyright © 2024 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

RESEARCH PAPER

***Corresponding Author:**
Freddy Saturnin Pouki
Faculty of Health Sciences, Marien Ngouabi University, BP: 69 Brazzaville, Congo

How to cite this paper:
Freddy Saturnin Pouki *et al* (2024). Identification of Microarn Biomarkers of Prognosis in Type 2 Diabetes Patients Positive for Sars-Cov-2 Variant Delta and Omicron in Nasopharyngeal Secretions in Pointe Noire. *Middle East Res J Biological Sci*, *4*(4): 121-126.

Article History:
| Submit: 28.06.2024 |
| Accepted: 29.07.2024 |
| Published: 31.07.2024 |

INTRODUCTION

Type 2 diabetes mellitus (T2DM), a metabolic disorder characterised by chronic hyperglycemia, is considered a major public health problem; its pathophysiology is complex and multiple factors are involved in the onset of T2DM. Its prevalence is currently increasing exponentially throughout the world, and particularly in Africa. (L. Guariguata *et al*., 2014).

Coronavirus 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the initial outbreak of which occurred in Wuhan, China. Unfortunately, this virus spread rapidly around the world, leading to the pandemic situation we have been experiencing since mid-March 2020. (Lu H, Stratton CW *et al.,* 2020). SARS-CoV-2 is an RNA virus belonging to the Betacoronavirus genus, which is transmitted by inhalation of droplets containing the virus or by manual contact with surfaces containing the virus and subsequent contamination via the hands, eyes, mouth and nose. Symptoms include fever and dry cough in most patients, which may be accompanied by breathing difficulties, loss of taste, nausea and diarrhea (Guan WJ

et al., 2020). Severe cases of COVID-19 can progress to severe acute respiratory syndrome (SARS) and a cytokine storm, in which the virus induces an intense inflammatory cascade (Coperchini F *et al*., 2020).

People with diabetes mellitus are associated with an increase in the severity and mortality of the infection compared with people without diabetes: 2.12 times mortality, 2.45 times severe COVID-19, 4.64 times ARDS and 3.33 times disease progression (J. Jing Yanga *et al.,* 2020). COVID-19 is 4 times more lethal in African T2DM patients (Ipouma *et al.,* 2021).

The biological connection between diabetes and COVID-19 is a crucial subject to study, as the two conditions are closely linked and can have serious consequences for the health of individuals. This correlation raises questions about the severity of COVID-19 infection in diabetic patients and highlights the need to study the biological interactions between these two conditions in greater depth in order to better understand and manage them. It would therefore seem useful to identify biomarker microRNAs to enable better stratification of D2T patients infected with SARS-CoV-2 at risk of developing serious consequences, thereby enabling the implementation of personalised healthcare.

MATERIAL AND METHOD

Study Population:

We conducted a descriptive cross-sectional study with prospective data collection. The study took place from September 2021 to August 2022, a period of 12 months. The study population consisted of T2DM patients with COVID-19 hospitalised at the Guenin and Louise Michel clinics and the Adolphe Sicé General Hospital in Pointe-Noire.

Clinical Survey: Data such as covid-19 symptoms and comorbidities were collected from medical records.

Biological Survey:

Laboratory analyses were carried out in the HDL Biomedical Analysis Laboratory of the Polyclinic Marie Madeleine Gombes Foundation in Pointe noire.

1- **Samples**

Nasopharyngeal swabs were taken by gently pushing the swab deep into the nostril (as far as the nasopharynx: approximately half the length from the nose to the ear) and detaching as many cells as possible by scraping the inside of the nostril using the virus collections and transport kit type citoswab (W/3ML VTM) supplied by CITOTEST LABWARE MANUFACTURING CO., LTD Haimen city 226100, China.

2-**Molecular analysis**:

a) Extractions

We extracted RNA from nasopharyngeal secretions using the Total RNA Purification Insert PI12200-37 kit, Norgen Biotek Corp (CANADA), in accordance with the manufacturer's recommendations.

b) Amplifications

b-1- For variant analysis:

Extracted RNA was subjected to PCR using the SARS-CoV-2 E Spike Delta/Omicron TaqMan Typing MDx 40-0813-96 kit, TIB MOLBIOL, Germany.

- **Procedure:**

First step: Mix preparation

- 4µl molecular biology water (nuclease free water)
- 1µl primers and probes (PSR)
- 10µl RT polymerase
- 5µl total RNA

Second step: programming the Mic (thermocycler): 42 min39 seconds

RT-PCR Cycle	Step	Temperature	Duration
Cycle 1	Step 1 Step 2	55°C 95°c	3min 1min
Cycle 1 (40x)	Step 1 Step	95°C 63°C	3sec 10 sec

Choice of fluorochromes and targets:

- FAM (Omicron: Spike ins214EPE)
- HEX (Delta: Spike del157/158)
- ROX (SARS-CoV-2: SARS E-gene)
- Cy5 (UBC Human gene)

b-2- For microArn Expression

The SYBR Green qPCR Master Mix Universal amplification kit (MedChemExpress USA) was used. We also used a set of 17 primer pairs encoding microRNAs supplied by NeoBiotech (Table I).

- **Procedure:**

First step: Mix preparation

- 10 µL SYBR Master Mix (Universal)
- 0.4 µL of sense primers
- 0.4 µL anti-sense primers
- 5 µL total RNA
- 4.2µL ddH2O

Second stage: programming the Mic (thermocycler):

After choosing the "sybr green" intercalating dye mode, the amplification parameters were as follows: Initial denaturation at 95°C for 30 seconds followed by 40 cycles of denaturation at 95°C for 10 seconds and 30 seconds of hybridization at 60°C. We then performed a melting curve with one cycle at 95°C for 15 seconds, followed by 60 seconds at 60°C and 95°C for 15 seconds. Total time 1 hour 10 minutes 05 seconds.

The expression of each microRNA was carried out in duplicate in each sample and the level normalised to beta-2 globulin.We assessed this expression using the Livak method with the formula Rq = 2^-(ΔΔCt)

(Schmittgen TD and Livak KJ 2008). A positive value of relative quantification (Rq) corresponds to overexpression and a negative value to underexpression.

Table I: Primer pairs used

hsa-miR-9-5p	Forward: TCT TTG GTT ATC TAG CTG TAT GA Reverse: TCA TAC AGC TAG ATA ACC AAA GA
hsa-miR-15b-3p	Forward: TAG CAG CAC ATC ATG GTT TAC A Reverse: TGT AAA CCA TGA TGT GCT GCT A
hsa-miR-21-3p	Forward: CAA CAC CAG TCG ATG GGC TGT Reverse: ACA GCC CAT CGA CTG GTG TTG
hsa-miR-29a-5p	Forward: ACT GAT TTC TTT TGG TGT TCA G Reverse: GTG AAC ACC AAA AGA AAT CAG T
hsa-miR-30d-3p	Forward: CTTTCAGTCAGATGTTTGCTGC Reverse: GCA GCA AAC ATC TGA CTG AAA G
hsa-miR-33a-5p	Forward: GTG CAT TGT AGT TGC ATT GCA Reverse: TGC AAT GCA ACT ACA ATG CAC
hsa-miR-33b-5p	Forward: GTG CAT TGT AGT TGC ATT GCA Reverse: TGC AAT GCA ACT ACA ATG CAC
hsa-miR-122-3p	Forward: AAC GCC ATT ATC ACA CTA AAT A Reverse: TAT TTA GTG TGA TAA TGG CGT T
hsa-miR-126-5p	Forward: CAT TAT TAC TTT TGG TAC GCG Reverse: CGC GTA CCA AAA GTA ATA ATG
hsa-miR-130a-5p	Forward: GCT CTT TTC ACA TTG TGC TAC T Reverse: AGT AGC ACA ATG TGA AAA GAG C
hsa-miR-141-3p	Forward: TAA CAC TGT CTG GTA AAG ATG G Reverse: CCA TCT TTA CCA GAC AGT GTT A
hsa-miR-486-5p	Forward: TCCTGTACTGAGCTGCCCCGAG Reverse: CTC GGG GCA GTC CAG TAC AGG A
hsa-miR-203a-5p	Forward: GTG AAA TGT TTA GGA CCA CTA G Reverse: CTA GTG GTC CTA AAC ATT TCA C
hsa-miR-221-3p	Forward: AGC TAC ATT GTC TGC TGG GTT TC Reverse: GAA ACC CAG CAG ACA ATG TAG CT
has-miR-223-5p	Forward: CGT GTA TTT GAC AAG CTG AGT T Reverse: AAC TCA GCT TGT CAA ATA CAC G
has-miR-375-5p	Forward: GCG ACG AGC CCC TCG CAC AAA CC Reverse: GGT TTG TGC GAG GGG CTC GTC GC
hsa-let 7a-5p	Forward: GA GGT AGG TAG GTT GTA TAG TT Reverse: AAC TAT ACA ACC TAC TAC CTC A
beta 2 globuline	Forward: TCGCAACCTCAGGAACAGAC Reverse: CAGGAAAGGGGGCTTAGTGG

Ethical Considerations

This study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved under number 125/CERS/FMMG-2021/PNR by the Health Research Ethics Committee (CERS) of the Marie Madeleine Gombes Foundation in Pointe Noire.

Statistical Analysis

The categorical data are expressed in numbers (percentage). To compare categorical data, we used the $\chi 2$ test, and to calculate the area under the curve (AUC), we performed the ROC analysis. A P value of less than 0.05 was considered to indicate statistical significance. All analyses were conducted using SPSS software (version 26.0; IBM)

RESULTS

Table II shows the distribution of variants according to some clinical parameters. There was a significant difference in the distribution of variants in relation to the severity of COVID-19 symptoms ($p < 0.001$). The majority of individuals with the Delta variant (68.18%, n=90) and the other variants (77.19%, n=44) had severe symptoms while individuals with the Omicron variant had moderate symptoms (76.47%, n=13).

Table II: Clinical characteristics

	Variante Delta		Variante Omicron		Other variants		p
Variables	**Number 132**	**Frequency (%) 64,08**	**Number 17**	**Frequency (%) 8,25**	**Number 57**	**Frequency (%) 27,67**	
Gravity							
Severe	90	68,18	04	23,53	44	77,19	<0,001
Moderate	42	31,82	13	76,47	13	22,81	
Comorbidity							
No comorbidity	47	35,6	5	29,4	35	61,4	<0,001
wiyh comorbidity	85	64,4	12	70,58	22	38,59	

Table III shows that only four (04) hsa-miR 33a-5p, hsa-miR 33b-5p, hsa-miR 203a-5p, hsa-miR223-5p were significantly up-regulated and thirteen (13) hsa-mir 9-5p, hsa-mir 15b-3p, hsa-mir 21 hsa-mir 29a-5p, hsa-mir 30d-3p, hsa-miR 122-3p, hsa-miR126-5p, hsa-miR130a-5p, hsa-miR141-3p, hsa-miR221-3p, hsa-let7a-5p, hsa-miR375-5p and hsa-miR486-5p were significantly down-regulated irrespective of the clinical and variant context.

Table III: MicroRNA expression profile

Expression	**Variante delta**		**Variante Omicron**	
	DT2-covid-19(+)		**DT2-covid-19(+)**	
	Moderate severity	**severity**	**Moderate severity**	**severity**
Over expression	hsa-miR 33a-5p	hsa-miR 33a-5p	hsa-miR 33a-5p	hsa-miR 33a-5p
	hsa-miR 33b-5p	hsa-miR 33b-5p	hsa-miR 33b-5p	hsa-miR 33b-5p
	hsa-miR 203a-5p	hsa-miR 203a-5p	hsa-miR 203a-5p	hsa-miR 203a-5p
	hsa-miR 223-5p	hsa-miR 223-5p	hsa-miR 223-5p	hsa-miR 223-5p
Under-expression	hsa-miR 9-5p	hsa-miR 9-5p	hsa-miR 9-5p	hsa-miR 9-5p
	hsa-miR 15b-3p	hsa-miR 15b-3p	hsa-miR 15b-3p	hsa-miR 15b-3p
	hsa-miR 21-3p	hsa-miR 21-3p	hsa-miR 21-3p	hsa-miR 21-3p
	hsa-miR 29a-5p	hsa-miR 29a-5p	hsa-miR 29a-5p	hsa-miR 29a-5p
	hsa-miR 30d-3p	hsa-miR 30d-3p	hsa-miR 30d-3p	hsa-miR 30d-3p
	hsa-miR 122-3p	hsa-miR 122-3p	hsa-miR 122-3p	hsa-miR 122-3p
	hsa-miR 126-5p	hsa-miR 126-5p	hsa-miR 126-5p	hsa-miR 126-5p
	hsa-miR 130a-5p	hsa-miR 130a-5p	hsa-miR 130a-5p	hsa-miR 130a-5p
	hsa-miR 141-3p	hsa-miR 141-3p	hsa-miR 141-3p	hsa-miR 141-3p
	hsa-miR 221-3p	hsa-miR 221-3p	hsa-miR 221-3p	hsa-miR 221-3p
	hsa-miR 221-3p	hsa-miR 221-3p	hsa-miR 221-3p	hsa-miR 221-3p
	hsa-miR 375-5p	hsa-miR 375-5p	hsa-miR 375-5p	hsa-miR 375-5p
	hsa-miR 486-5p	hsa-miR 486-5p	hsa-miR 486-5p	hsa-miR 486-5p
	hsa-let7a-5p	hsa-let 7a-5p	hsa-let 7a-5p	hsa-let 7a-5p

Table IV: Area under the curve (AUC) ROC was calculated to analyze the prognostic performance of microRNAs associated with severity of signs in a context of T2DM infected with SARS-COV-2 variants delta and omicron.

Table IV: Area under the ROC curve for microRNAs according to severity of signs

microARN	**variante delta**		**variante Omicron**	
	T2DM-covid-19(+)		**T2DM-covid-19(+)**	
	Moderate severity	**severity**	**Moderate severity**	**severity**
hsa-miR-9-5p	0,45	0,56	0,53	0,68
hsa-miR-15b-3p	0,34	0,4	0,6	0,33
hsa-miR-21-3p	0,48	0,47	0,6	0,66
hsa-miR-29a-5p	0,47	0,53	0,34	0,80
hsa-miR-30d-3p	0,44	0,4	0,57	0,82
hsa-miR-33a-5p	0,51	0,48	0,52	0,27
hsa-miR-33b-5p	0,52	0,49	0,58	0,88
hsa-miR-122-3p	0,34	0,57	0,7	0,51
hsa-miR-126-5p	0,49	0,44	0,48	0,32
hsa-miR-130a-5p	0,38	0,46	0,58	0,26

microARN	variante delta		variante Omicron	
	T2DM-covid-19(+)		T2DM-covid-19(+)	
	Moderate severity	severity	Moderate severity	severity
hsa-miR-141-3p	0,38	0,37	0,44	0,24
hsa-miR-486-5p	0,64	0,58	0,60	0,58
hsa-miR-203a-5p	0,41	0,51	0,4	0,80
hsa-miR-221-3p	0,64	0,52	0,44	0,74
has-miR-223-5p	0,49	0,44	0,59	0,25
has-miR-375-5p	0,54	0,49	0,44	0,42
hsa-let 7a-5p	0,32	0,53	0,56	0,16

DISCUSSION

The aim of this study was to identify microRNA biomarkers of prognosis in type 2 diabetic patients with different severities of COVID-19 delta and omicron variants in nasopharyngeal secretions in Congo.

Our results showed that the Delta variant 132 (64.08%) was the most commonly detected in our population, followed by the other variants 57 (27.67%) and the Omicron variant 17 (8.25%). Interestingly, the distribution of variants was significantly related to symptom severity and other health variables ($p<0.005$). The prevalence of symptoms characteristic of omicron infection differed from that of the SARS-CoV-2 delta variant, with a lower incidence of severe symptoms (23.53%) and no deaths. These results are consistent with the existing literature. Several studies have shown that the delta variant can cause more severe symptoms than other variants of the virus (Kirsebom. 2022; Lopez Bernal and al., 2021). Similarly, work by Pulliam and al. 2022 and Menni *et al*., 2022 suggests that the Omicron variant is associated with less severe disease.

The results of microRNA quantification (table III) in our population show that four (04) hsa-miR-33a-5p, hsa-miR-33b-5p, hsa-miR-203-5p, hsa-miR-223-5p were significantly up-regulated and thirteen (13) hsa-miR-9-5p, hsa-miR-15b-3p, hsa-miR-21-3p hsa-miR-29a-5p , hsa-miR-30d-3p, hsa-miR-122-3p, hsa-miR-126-5p, hsa-miR-130-5p, hsa-miR-141-3p, hsa-miR-221-3p, hsa-miR-375-5p and hsa-let 7a-5p were significantly down-regulated regardless of clinical outcome and regardless of the sars-cov-2 variant involved. MicroRNAs can regulate nearly a third of the human genome and are widely implicated in multiple pathways, such as cell proliferation, cell death, stress resistance and fat metabolism. Furthermore, evidence suggests that a gain or loss of function of one or more microRNAs is associated with the diagnosis, progression and prognosis of several diseases. Therefore, deregulated microRNAs, in addition to serving as biomarkers of disease (Nicoletti, Ad and al., 2022), may represent potential therapeutic targets for a better understanding of the signalling pathways involved and the pathogenesis of disease.

The value of microRNAs as predictive biomarkers of severity in nasopharyngeal secretions was determined using the AUC of ROC curves. According to Hosmer and Lemeshow, 2013 AUC: "0.5 = no discrimination; 0.5 to 0.7 = poor discrimination; 0.7 to 0.8 = acceptable discrimination; 0.8 to 0.9 = excellent discrimination; and > 0.9 = exceptional discrimination". In our study, no microRNA showed AUC or acceptable discrimination for the delta variant. However, for the omicron variant, we found that hsa-miR-29a-5p (AUC 0.80; CI 0.68 to 0.89 and $p<0.000$) under-expressed, hsa-miR-203a-5p (AUC 0.80; CI 0.75 to 0.92 and $p<0.000$) over-expressed and hsa-miR-221-3p (AUC 0. 74; CI 0.61 to 0.86 and $p<0.000$) showed acceptable discrimination for use as a predictive biomarker of severe severity in T2DM infected with the sars-cov-2 omicron variant. And the over-expression of hsa-miR-33b-5p (AUC 0.88; CI 0.75 to 0.98 and $p<0.000$) and the under-expression of hsa-miR-30d-3p (AUC 0.82; CI 0.70 to 0.95 and $p<0.000$), in nasopharyngeal secretions have a high probability of correctly classifying type 2 diabetic patients positive for the COVID-19 omicron variant as severe compared with non-severe.

MicroRNAs therefore appear to be biomarkers of interest for many pathologies (Mustapha Zendjabil and al.; 2017). However, these applications face several challenges, as there are currently considerable differences between sample processing procedures, detection methods and, above all, strategies for standardising results (Abed and al., 2023).

CONCLUSION

Our study shows that the microRNAs hsa-miR-29a-5p, hsa-miR-203a-5p, and hsa-miR-221-3p, hsa-miR-33b-5p and hsa-miR-30d-3p are good indicators for identifying patients at high risk of COVID-19 omicron variant complications among Congolese T2DM patients. These prognostic biomarkers can therefore be used to target specific interventions, monitor high-risk patients closely and make informed decisions about care. However, careful use and large-scale clinical validation are required.

REFERENCES

- Abed, R. M., Abdulmalek, H. W., & Yaaqoob, L. A. (2023). The role of miRNA20a and miRNA320 in Iraqi patients with COVID-19: a case–control

study. *Egyptian Journal of Medical Human Genetics*, *24*(1), 68.

- Coperchini, F., Chiovato, L., Croce, L., Magri, F., & Rotondi, M. (2020). The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine & growth factor reviews*, *53*, 25-32.
- Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., ... & Zhong, N. S. (2020). Clinical characteristics of coronavirus disease 2019 in China. *New England journal of medicine*, *382*(18), 1708-1720.
- Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U., & Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes research and clinical practice*, *103*(2), 137-149.
- Hosmer Jr, D. W., Lemeshow, S., & Sturdivant, R. X. (2013). *Applied logistic regression*. John Wiley & Sons.
- Ipouma, M. N. (2020). Rapport OMS: COVID-19 and diabetes in Africa: a lethal combination. *Lancet Diabetes Endocrinol*, *8*, 813–22 November 29, 2021 https://doi.org/10.1016/ S2213-8587(21)00315-6.
- Kirsebom, F. C., Andrews, N., Stowe, J., Toffa, S., Sachdeva, R., Gallagher, E., ... & Bernal, J. L. (2022). COVID-19 vaccine effectiveness against the omicron (BA. 2) variant in England. *The Lancet Infectious Diseases*, *22*(7), 931-933.
- Lopez Bernal, J., Andrews, N., Gower, C., Gallagher, E., Simmons, R., Thelwall, S., ... & Ramsay, M. (2021). Effectiveness of Covid-19 vaccines against the B. 1.617. 2 (Delta) variant. *New England Journal of Medicine*, *385*(7), 585-594.
- Lu, H., Stratton, C. W., & Tang, Y. W. (2020). Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. *Journal of medical virology*, *92*(4), 401.
- Menni, C., Valdes, A. M., Polidori, L., Antonelli, M., Penamakuri, S., Nogal, A., ... & Spector, T. D. (2022). Symptom prevalence, duration, and risk of hospital admission in individuals infected with SARS-CoV-2 during periods of omicron and delta variant dominance: a prospective observational study from the ZOE COVID Study. *The Lancet*, *399*(10335), 1618-1624.
- Nicoletti, A. D. S., Visacri, M. B., da Ronda, C. R. D. S. C., Vasconcelos, P. E. D. N. S., Quintanilha, J. C. F., de Souza, R. N., ... & Pincinato, E. D. C. (2022). Differentially expressed plasmatic microRNAs in Brazilian patients with Coronavirus disease 2019 (COVID-19): preliminary results. *Molecular biology reports*, *49*(7), 6931-6943.
- Pulliam, J. R., van Schalkwyk, C., Govender, N., von Gottberg, A., Cohen, C., Groome, M. J., ... & Moultrie, H. (2022). Increased risk of SARS-CoV-2 reinfection associated with emergence of Omicron in South Africa. *Science*, *376*(6593), eabn4947.
- Schmittgen, T. D., & Livak, K. J. (2008). Analyse des données Pcr en temps reel par la méthode CT. *Protocole Nat3*, 1101-1108.
- Yang, J., Zheng, Y. A., Gou, X., Pu, K., Chen, Z., Guo, Q., ... & Zhou, Y. (2020). Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *International journal of infectious diseases*, *94*, 91-95.
- Zendjabil, M., Favard, S., Tse, C., Abbou, O., & Hainque, B. (2017). The microRNAs as biomarkers: what prospects?. *Comptes rendus biologies*, *340*(2), 114-131.