19-21 GIUGNO ROMA
UNIVERSITA' CATTOLICA DEL SACRO CUORE
CENTRO CONGRESSI EUROPA
RESEARCH and CARE: FROM BENCH, TO BEDSIDE, TO COMMUNITY
Promosso da SIMIT
16° CONGRESSO NAZIONALE ICAR
Italian Conference on AIDS and Antiviral Research
P 197
POSTER 4 YOU ICAR 2024

Lactoferrin assumption in vaccinated subjects infected by SARS-CoV-2 influenced time length of negativization

G. Costanza ,T. Cosio, M. Ciotti , C. Lanna, L. Rosa, F. Velluso, C. Galluzzi, R. Gaziano, S. Grelli, M.P. Valenti, L. Bianchi, E. Campione.

Introduction

- The **SARS-CoV-2** pandemic brought about significant changes from all perspectives. Measures such as community lockdowns, social distancing, and quarantine slowed down the spread of COVID-19. In Italy, vaccination campaigns have represented a crucial strategy to improve this scenario (WHO, Ministero della Salute). **Lactoferrin (Lf)**, a glycoprotein belonging to the transferrin family, has been explored as an alternative remedy against **COVID-19.**
- **Lf** is typically found in mucous membranes and neutrophil granules. Specific lactoferrin receptors are present in lymphocytes, monocytes, and macrophages in the human body, and it plays a role in regulating the activity of Natural Killer (NK) cells.
- Regarding the physiological antiviral activity,**Lf** binds free iron in body fluids and areas of inflammation, with a capacity twice that of transferrin, protecting the body from toxic radicals of oxygen and decreasing the availability of ferric ions for the microorganisms that invade the host, thanks to its cationic structure, tends to bind with the glycosaminoglycans of the plasma membrane, mechanically protecting the host cell from the entry of viruses.
- Based on positive findings from previous studies in **Tor Vergata University Hospital**, we conducted a randomized, double-blind intervention trial using **oral liposomal apolactoferrin supplements** on positive **SARS-CoV-2** asymptomatic and paucisymptomatic patients during the pandemic's peak, aiming to assess Lf's effects on the vaccinated population.

Study Design

- **The study population** includes 120 paucisymptomatic **COVID-19** subjects treated according to the standard of care, and asymptomatic untreated subjects, who will be randomized into two arms of 60 subjects each: the experimental lactoferrin arm and the placebo arm.
- **The inclusion criteria** comprised individuals aged 20 years and older who test positive for **SARS-CoV-2** by **rRT-PCR**. This includes paucisymptomatic individuals who are either treated according to the standard of care or asymptomatic and untreated. Additionally, individuals presenting symptoms must have a body temperature exceeding 37.5° C, along with symptoms such as cough, headache, asthenia, diarrhea, myasthenia, SpO2 > 93%, or PaO2/FiO2 > 300 mmHg without oxygen inhalation.
- **Exclusion criteria:** pregnant and lactating women, individuals reporting allergies to milk proteins, those with a history of bronchial hyperactivity, and individuals suffering from pre-existing respiratory diseases.

Methods

- **Primary Endpoint:** Assessment of the mean time required to achieve a negative rRT-PCR for SARS-CoV-2 between November 2021 and March 2022. The time to negativization was calculated from the first positive rRT-PCR test result until three consecutive negative rRT-PCR test results, taking into account vaccination history in two arms (placebo and Lf).
- **Secondary Endpoint:** The objective was to evaluate the differences in negativization time between asymptomatic and mild-to-moderate patients, considering sex, age, and comorbidity between the two arms (placebo and Lf). A questionnaire was administered at T0 (baseline) and T1 (10 days).
- **Statistical Analysis:** Descriptive and inferential statistical analyses were performed. All results were expressed as the arithmetic mean±SEM. Univariate analysis of the relationship between clinic-pathological variables in the enrolled subject cohort was performed using the t-test. Correlation analyses were performed using the chi-square or McNemar test. Differences were considered statistically significant with $p < 0.05$. The SPSS 20.0 software program was used for statistical analysis.

Results

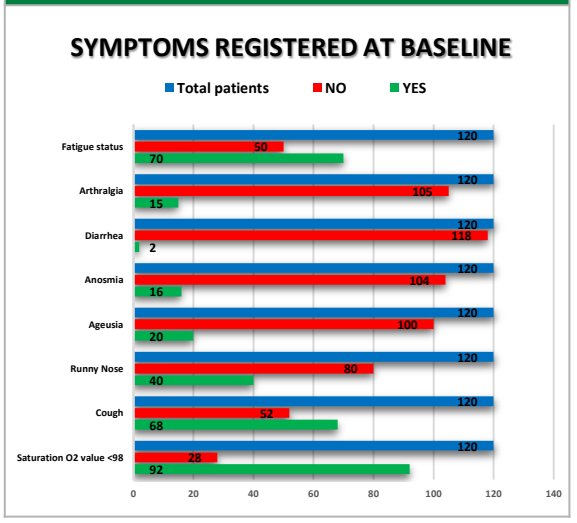
- Between November 2021 and March 2022, a total of **120** subjects were enrolled in this study. 92% of the enrolled subjects were vaccinated. The negativization time for **SARS-CoV-2** was shorter in subjects who received **Lf** compared to the placebo arm.
- **68** patients were male and **52** were female, with an average age of **53** years.
- **56** patients were asymptomatic, while **64** presented mild-to-moderate disease.
- **57** patients presented comorbidities, while **63** did not have any comorbidities.
- **55** patients were under pharmacological regimen, while **65** were not following a therapy.

Results of 2

SYMPTOMS REGISTERED AT BASELINE IN ENROLLED SUBJECTS

- No differences between two arms (**Lf and Placebo**) has been reported considering all features analyzed (randomized and double-blinding study). Differences of **COVID-19** symptoms were registrated compared to first pandemic period (diarrhea cases present, conjunctivitis absent).

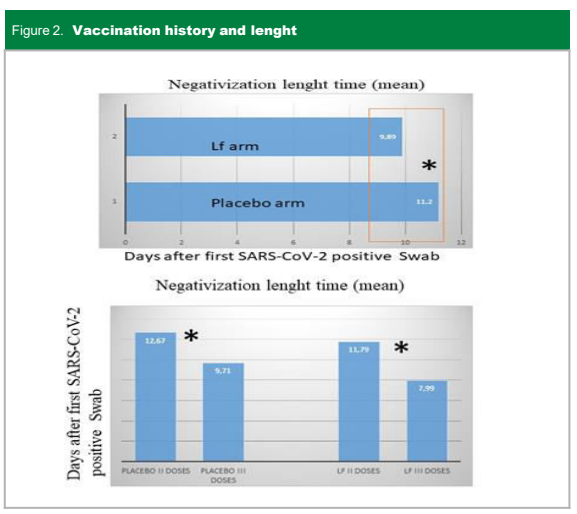
Figure 1. **Symptoms registered at baseline**



VACCINATION HISTORY AND LENGHT

- The time for SARS-CoV-2 to become undetectable was shorter in individuals who received lactoferrin compared to those in the placebo arm, specifically 9.89 days versus 11.2 days, respectively.

Figure 2. **Vaccination history and lenght**



Results of 3

CORRELATION BEETWEEN NEGATIVIZATION AND FEATURES OF SUBJECT LF TREATED

- Observing the arm of patients treated with Lf (n=60) and taking into account the variables: Age, sex, patient status and comorbidities we found what is indicated in the Table 1.

Table 1 **Correlation between negativization (avg time in days) and features of subject Lf treated (n=60)**

	Time of negativization (days)		P-value
AGE	<9.89	>9.89	
<53.3	23	15	
>53.4	12	10	0,20
SEX			
Female	16	10	
Male	22	12	0,06
PATIENT STATUS			
Asymptomatic	17	11	
Mild-to Moderate	11	21	0,03
COMORBIDITIES			
Present	8	19	
Absence	20	13	0,01

Safety

- **Lactoferrin** is a versatile protein found in breast milk and various biological fluids, recognized for its antimicrobial and immune regulating properties. Recent research indicates that **Lf** could help in preventing **COVID-19**, providing potential health advantages without significant side effects.
- Lactoferrin is known for its ability to bind to a wide range of pathogens, including viruses such as coronaviruses and respiratory virus
- Preliminary research suggests that **Lf** may interfere with the binding of the **SARS-CoV-2** virus to host cells and reduce its ability to replicate
- Studies conducted in vitro and in vivo have underscored the potential of lactoferrin to diminish the severity of COVID-19 symptoms and hasten the recovery process.
- Lactoferrin might also enhance the immune response against the virus.
- There have been no reports of serious side effects associated with taking lactoferrin in recommended dosages.

Conclusion

- The intake of Lf in pauci-symptomatic SARS-CoV-2 positive subjects with two doses of vaccine reduced the time of negativization.
- The use of Lf as a supplement could be considered as part of an integrated approach to COVID-19 prevention.
- The correlation between COVID-19 and Lf highlights the importance of further studies to fully understand the role of this protein in the prevention and treatment of the disease.
- Further studies are needed to determine the precise mechanisms through Lf impacts the pathogenesis of COVID-19 and to identify potential therapeutic strategies for the future applications.

References

1. Campione E, Lanna C, Cosio T, Rosa L, Conte MP, Iacovelli F, et al. Lactoferrin Against SARS-CoV-2: *In Vitro* and *In Silico* Evidences. Front Pharmacol. 2021 Jun 17;12:666600. doi: 10.3389/fphar.2021.666600. PMID: 34220505; PMCID: PMC8242182.
2. Iacovelli F, Costanza G, Romeo A, Cosio T, Lanna Cet al. Interaction of *Pelargonium sidoides* Compounds with Lactoferrin and SARS-CoV-2: Insights from Molecular Simulations. Int J Environ Res Public Health. 2022 Apr 26;19(9):5254. doi: 10.3390/ijerph19095254. PMID: 35564648; PMCID: PMC9101775.
3. Cutone A, Rosa L, Bonaccorsi di Patti MC, Iacovelli F, et al. Lactoferrin Binding to SARS-CoV-2 Spike Glycoprotein Blocks Pseudoviral Entry and Relieves Iron Protein Dysregulation in Several In Vitro Models. Pharmaceutics. 2022 Oct 3;14(10):2111. doi: 10.3390/pharmaceutics14102111. PMID: 36297546; PMCID: PMC9612385.