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METAGENOMIC ANALYSIS REVEALS INCREASED FUNGAL BURDEN IN THE RESPIRATORY TRACT OF COVID-19 PATIENTS WITH COMORBIDITIES

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Introduction/Objectives: SARS-CoV-2 infection can alter the microbial composition of the upper respiratory tract, favoring the growth of opportunistic fungi. In this context, understanding microbiome structure, with emphasis on the fungal community, is essential to evaluate possible clinical implications. Therefore, this study aimed to characterize the fungal diversity of the upper respiratory tract in individuals with COVID-19, comparing those with and without associated comorbidities.

Methods: Nasopharyngeal samples from COVID-19 patients underwent shotgun metagenomic sequencing on the Illumina NextSeq500 platform. Taxonomic classification was performed with Kraken2 using the PlusPFP-16 database, and abundances were aggregated at the family level. Diversity metrics (Shannon and Simpson indices) and richness (number of detected families) were estimated, comparing groups with and without comorbidities using the Wilcoxon test. Analyses and visualizations were conducted in R v. 4.5.1.

Results: A total of 226 samples were analyzed, of which 50 contained fungal reads, including 34 fungal families, with emphasis on Saccharomycetaceae, Aspergillaceae, Debaryomycetaceae, Cryptococcaceae, and Malasseziaceae. Total fungal abundance was significantly higher in patients with comorbidities ($p = 0.039$), including immunodeficiency, chronic heart disease, chronic lung disease, chronic kidney disease, and diabetes mellitus. Fungal richness was slightly higher in this group ($p = 0.26$), while Shannon ($p = 0.29$) and Simpson ($p = 0.29$) diversity indices showed no significant differences. The most abundant families varied between groups, with predominance of Aspergillaceae and Saccharomycetaceae in patients with comorbidities.

Conclusion: Metagenomic analysis revealed substantial fungal diversity in the upper respiratory tract of individuals with COVID-19, with higher total fungal burden in patients with clinical comorbidities. Despite the absence of significant differences in diversity indices, the higher abundance of opportunistic fungi in individuals with chronic conditions suggests an associated mycological imbalance. These findings highlight the relevance of monitoring the respiratory mycobiome in viral infection contexts, especially given the possibility of secondary fungal infections that may worsen the clinical course and negatively impact outcomes.

Keywords: Metagenomics, Respiratory Mycobiome, COVID-19.

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APPLICATION OF ARTIFICIAL INTELLIGENCE FOR PROSPECTIVE PREDICTION OF COVID-19 INCIDENCE DURING THE PANDEMIC

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Introduction: The COVID-19 pandemic posed major challenges for medicine not only in patient care, but also in preparing health responses and managing public and private resources. Artificial intelligence (AI) is a new technology capable of analyzing complex databases to generate, among other outputs, predictions of future data.

Objectives: We describe the development and prospective application of a machine learning model used to predict the number of SARS-CoV-2 infections using data from January 2021 to January 2022.

Methods: Development and use of an Artificial Neural Network (ANN) applied during the pandemic in a municipality in Serra Gaúcha-RS, after data cleaning, balancing, and segmentation into 74 neighborhoods/administrative regions according to the municipality's geographic division plan. Variables included clinical, demographic, and socio-spatial data, including SINAN data, provided by hospitals and the city government. Models used: Multiple Linear Regression, Decision Tree Regressor, Random Forest Regressor, and a Multilayer Perceptron (MLP) ANN. The study was approved by the Research Ethics Committee (Opinion 4.587.313).

Results: A total of 74,615 test results were used (24,147 in 2020 and 50,468 in 2021), with demographic, clinical, and socio-spatial variables. The MLP neural network had the best predictive performance among the tested models. The metric used to evaluate forecast accuracy was Mean Absolute Error (MAE). In August, the study showed 100% neighborhood coverage, with maximum errors of approximately 2.1 cases and values below 0.5 in central areas, demonstrating high predictive accuracy using 80% of the data for training and 20% for testing. Predictions were then made once per week, with the best results when forecasting incidence 30 days ahead.

Discussion: The forecasts led to an estimated savings of R$118,996.80 for the state treasury in purchasing diagnostic

tests. In addition, they helped managers adapt hospital sectors to create new Intensive Care Units (ICUs). A hospital with 2 ICUs prepared up to 8 ICUs during the pandemic.

Conclusion: The use of AI demonstrated not only predictive accuracy for COVID-19 cases during the pandemic, but also supported hospital management, rational resource use, and important public health measures for an effective response to the COVID-19 pandemic emergency.

Keywords: Artificial Intelligence, Machine Learning, Covid-19, Public Health.

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ASSOCIATION OF INFLAMMATORY CYTOKINES AS PREDICTORS OF SEVERITY IN COVID-19 PATIENTS

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Introduction: SARS-CoV-2 infection can result in a wide variety of clinical outcomes, from asymptomatic cases to severe pneumonia and death, largely due to differences in patients' immune responses. During infection, cytokines and chemokines are produced simultaneously and interact within a complex network that can favor or inhibit disease progression. We investigated the immunological profile of hospitalized COVID-19 patients to identify immune signatures associated with disease severity.

Methods: This cross-sectional study analyzed serum samples from 76 hospitalized, unvaccinated COVID-19 patients from the state of Pernambuco, classified into two groups: Critical/deaths (n = 43) and severe (n = 33). For analysis of pro-inflammatory cytokines, patients were subdivided into four groups: critical/deaths, severe, deaths, and recovered. Quantification of 80 soluble mediators was performed using the Human Immune Response ProcartaPlex 80-plex panel with the MAGPIX System (Merck, USA), based on Luminex xMAP technology. Data normality was assessed (Shapiro-Wilk test) and cytokine levels were compared between groups using the Mann-Whitney test. Analyses were performed in GraphPad Prism 8.0.1, with significance set at $p < 0.05$. To evaluate differential regulation between groups, log2 fold change of medians was calculated (upregulated/downregulated).

Results: Twelve inflammatory cytokines showed significant differences. MDC, CCL17, CCL11, and CCL8 were elevated in the severe and survivor groups, suggesting a more regulated immune profile in moderate cases. HGF, CXCL9, CXCL13, PTX3, CCL19, CCL1, IL2R, and M-CSF were elevated in critical cases, indicating possible immune hyperactivation associated with disease severity.

Conclusion: The chemokines MDC, CCL17, CCL11, and CCL8 may be considered possible markers for moderate disease, whereas cytokines/chemokines/growth factors HGF, CXCL9, CXCL13, PTX3, CCL19, CCL1, IL2, and M-CSF may serve as markers for more critical disease and COVID-19-related death. These findings reinforce the importance of dysregulated inflammation in the progression of SARS-CoV-2 infection. Identifying these biomarkers may help stratify patients, monitor severity, and guide therapeutic strategies.

Keywords: SARS-CoV-2, COVID-19, Cytokines.

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ASSOCIATION OF THE RS179008 POLYMORPHISM IN THE TLR7 GENE WITH COVID-19 SEVERITY IN PATIENTS FROM SOUTHERN BRAZIL: A POTENTIAL GENETIC RISK MARKER IN WOMEN

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Introduction/Objectives: COVID-19, caused by SARS-CoV-2, is a global disease with profound impact on public health systems and the economy. It shows wide clinical variability, from asymptomatic to critical cases, with heterogeneity influenced by factors such as age, comorbidities, and genetic predisposition. The TLR7 receptor, from the Toll-like family, recognizes pathogen-associated molecular patterns, activating innate immunity. Encoded on the X chromosome, it is highly expressed in the lungs and spleen. The rs179008 polymorphism in the TLR7 gene affects receptor functionality, altering cytokine and type I interferon production, which may influence the response to viral infection. This study evaluated the association between the polymorphism and COVID-19 severity in patients from Southern Brazil.

Methods: The study evaluated 315 patients with confirmed COVID-19 diagnosis: 191 non-hospitalized and 124 hospitalized. The study was approved by the Research Ethics Committee for Human Subjects. The rs179008 polymorphism was genotyped by real-time PCR. Genotype frequencies were evaluated for Hardy-Weinberg equilibrium. Variables with normal distribution were assessed using t-test, Mann-Whitney, or chi-square tests depending on the variable type and