Quasi-species prevalence and clinical impact of evolving SARS-CoV-2 lineages in European COVID-19 cohorts, January 2020 to February 2022

Multicenter observational study (Jan 2020 – Feb 2022)
Italy, Netherlands, Spain, France
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Core research objectives

- To determine how the evolution of the SARS-COV-2 affects severity of COVID-19
- To describe prevalence of quasi-species (minority variants within individual patients)
- To assess the role of clades, mutations and quasi-species in driving COVID-19 severity

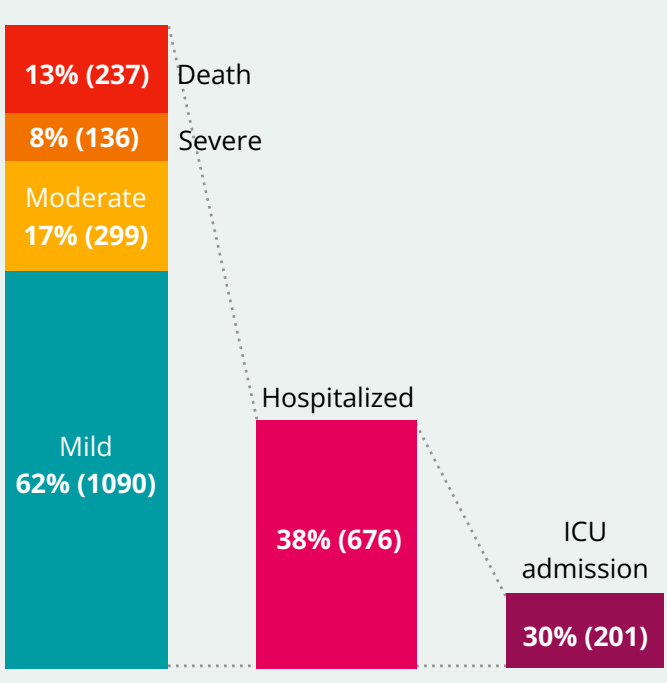
Sample collection and analysis

1,762 nasopharyngeal swabs from symptomatic patients

RNA extraction, RT-qPCR, whole-genome sequencing

Variant classification: Nextclade v2.2.0, Pangolin v1.9

Disease severity (WHO scale)



Mutation analysis

1,332 high-quality sequences analysed

187 mutations detected, **101** linked to disease severity

% of mutations

- **24.6%** in the **Spike** gene
- **12.4%** in the **nsp3** gene
- **12.0%** in the **Nucleocapsid** gene
- **5.6%** in the **polymerase** gene

What we learned

- Mutation rates sharply increased with the emergence of Variants of Concern (VOCs) especially in key viral regions
- Mutational variability across the viral genome highlights the dynamic nature of the virus
- Being immunocompromised, as well as 27 specific mutations in SARS-CoV-2 genome were linked to severe disease
- A small number of genomic hotspots were shared across all variants
- Intra-host viral diversity (quasi-species) was common among patients

Key findings on variants and severity

- 325 mutated positions, 426 unique substitutions across the genome
- Patients with Delta and Omicron showed higher viral loads compared to pre-Delta variants
- VOCs showed higher mutation rates, particularly affecting the S gene
- Quasi-species indicate SARS-CoV-2 evolves within individual patients
- Severe cases were linked to specific variants compared to 21J/Delta: 20A/EU2 (OR=2.80), Alpha (OR=2.41)
- Mutations occurred equally in base-paired and non-paired regions, suggesting structure did not constrain mutation rates

Public Health Implications

- The presence of quasi-species may affect associations between variants and disease severity, treatment, and vaccine effectiveness
- We recommend including minority variants in future molecular epidemiology and transmission studies
- We propose representing mutation probabilities within patient samples to capture minority subvariants

Read more at orchestra-cohort.eu

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