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Scientific letter

Generalized use of Nirmatrelvir plus ritonavir (Paxlovid): Raising concerns

Uso generalizado de Nirmatrelvir más ritonavir (Paxlovid): Algunos planteamientos

Nirmatrelvir plus Ritonavir (Paxlovid) will be used in Spain soon.[1] Although the potential effect of the drug against COVID-19 infection has biochemical explanation and has been demonstrated in several in vitro studies,[2] final acceptance arrived from the Randomized Clinical Trial (RCT) supported by Pfizer and published by Hammond et al.,[3] which was carried out in patients who were neither vaccinated nor had previously had COVID-19 infection, with a mild COVID-19 infection of less than 5 days. Despite the spectacular results of this RCT, several concerns should be considered.

First, patients with at least one risk factor for severe COVID-19 disease were selected. These risk factors were, among others, being over 60 years of age, having a Body Mass Index (BMI) greater than 25 or having one of several relatively prevalent diseases such as high blood pressure, smoking, heart disease or lung disease. It is necessary to highlight that the proportion of people with at least one risk factor in our environment is very high. More than 40% of the Spanish population would have a BMI > 25[4] or almost 20% of the population is over 65 years old,[5] which could mean an excessive number of potentially treatable patients.

Another important point to keep in mind is that the composite variable "admission or death" was chosen as outcome. Thanks to this, the Number Needed to Treat (NNT) to avoid an outcome was 18 patients (event rate in the treatment group of 0.78% vs. 6.40% in the placebo group; absolute risk reduction of 5.62%). However, when analysing events, we found that death was 18.2% of events (12/66 cases). Looking only at deaths, risk reduction was 1.15% (0% vs 1.15%) with an NNT of 87 patients. Mortality could be more useful to take decisions since many admissions may be short and perfectly assumable nowadays. Thus, it is essential to deepen into the characteristics of patients with adverse outcomes, both in terms of their comorbidities as well as the implications of such admission (stay, clinical severity, maximum need for oxygen flow or admission to the ICU), the latter being missed from the study.

It should also be noted at this point that it seems that COVID-19 disease is beginning to behave like any other viral infection with the potential effect of producing community-acquired pneumonia (CAP). An unintended fact that emerges from the article is that mortality in unvaccinated patients with risk factors in the placebo group was only 1.15%. We must not lose sight of the fact that CAP has an incidence and hospitalization rate of 4.63 and 1.64 cases per 1000 people/year in Spain,[6,7] with a mortality of 4–18% in hospitalized patients, increasing dramatically in those with one or several comorbidities,[8] and that most of them are caused by viruses.[9]

Regarding the results, it seems clear that Paxlovid has a beneficial effect in preventing worsening of COVID-19 disease in selected mild patients with less than 5 days of symptoms, which is a promising milestone in the development of antiviral agents. Despite this, prudence is needed when regulating and using this medicine. When extrapolating the data to our daily clinical practice, and despite the fact that Pfizer is already carrying out another similar RCT in vaccinated patients (EPIC-Standard Risk [SR]; NCT05011513), it is important to be aware that at the moment there is only one published RCT and that it has been carried out in unvaccinated population that had not had COVID-19 disease. In Spain, at the end of March 2022, more than 80% of the population has been vaccinated and one in four people has already overcome the disease.[10] Thus, clinicians should perform optimal risk stratification before prescribing such drug so that Paxlovid does not become inappropriately used.

It may seem obvious, but we must not forget that the process of approving new drugs requires prudence, and although at the worst of the pandemic it was necessary to practice a medicine that was not strictly based on the evidence, fact that we all remember well, our situation today is no longer critical. That is why it is more necessary than ever to recover the scepticism and critical sense usually associated with scientific methodology, as well as the individual common sense of the art and science of medical practice.

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Double N501Y mutation hindering its detection by RT-PCR kit widely used at Clinical Microbiology laboratories

Doble mutación en N501Y que impide su identificación mediante un kit de RT-PCR de uso habitual en los laboratorios de Microbiología Clínica

Next-generation sequencing (NGS) techniques have become an indispensable tool for the study and control of the SARS-CoV-2 virus pandemic[1]. The sequencing of the complete viral genome has been vital not only to the development of vaccines and specific drugs, but also to the discovery and monitoring of the variants of interest which, since the appearance of what is now known as the alpha variant[2], have occurred and been related to the specific resurgences of the pandemic. However, and despite the efforts made by microbiology laboratories all over the world, there is insufficient sequencing capacity to characterise all SARS-CoV-2 positive samples with this technology. For this reason, different strategies have been developed for the characterisation of the virus by means of RT-PCR of known combinations of mutations[3–5]. However, the limitations involved in this type of study, which can lead to an erroneous variant assignment, must not be forgotten[6,7].

Currently, in our department, all the first samples of an episode with Cts under 32 are characterised using the Allplex™ Variants I&II (Seegene, Korea) panels, which study the most relevant spike mutations in the latest variants of interest (H69/V70-, W152C, K417N, K417T, L452R, E484K and N501Y). As of February 2022, in a scenario totally dominated by the Omicron variant, the samples that presented the H69/V70-, K417N and N501Y mutations were classified as Omicron by RT-PCR. If the deletion was not present, they were considered to belong to the BA.2 subvariant by RT-PCR.

Given the appearance of a sample with H69/V70- and K417N, but without N501Y, the decision to do sequencing was taken, since these results did not allow it to be assigned to any variant by RT-PCR. To do this, an adaptation of the ARTIC protocol for Oxford Nanopore was used by amplifying the entire genome with the VarSkip scheme[8]. The resulting library was processed in a MinION mk1c sequencer (Oxford Nanopore). The readings obtained were subsequently assembled using the ARTIC *pipeline* for data obtained from sequencers with Oxford Nanopore technology and analysed using the Nextstrain web app[9] with consistency verified using the Integrative Genomics Viewer (IGV) program[10].

The sequence obtained (deposited in GISAID as EPI_ISL_10968563) belongs to the 21K clade (Omicron) and to the BA.1.17 lineage and presented, among other characteristic spike mutations, the three mutations used by the Allplex Variants I&II scheme: H69/V70-, K417N and N501Y; although, the N501Y mutation, in addition to the usual A23063T change, also had T23065C. All mutations were found with sufficient coverage. In this case, T23065C accompanying A23063T produces a silent mutation since the codon they form continues to encode a tyrosine and maintains the N501Y mutation.

Therefore, this mutation is not expected to involve an evolutionary advantage for the virus or a fundamental change in its behaviour. However, it may pose a problem for characterisation schemes based on the study of specific mutations, such as the one we currently use in our laboratory; since if this change had occurred, for example, in a variant of the BA.2 lineage in the Allplex Variants I&II panels, only the K417N mutation would be positive.

In summary, we believe that this case is a further example of the enormous utility offered by NGS in Clinical Microbiology laboratories today, since it allows us not only to correctly assign circulating variants but also to monitor the evolution of the same variants.

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