Enfermedades Infecciosas y Microbiología Clínica 41 (2023) 127–136

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

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.

References

1. https://www.sanidad.gob.es/gabinete/notasPrensa.do?id=5694
2. Reina J, Iglesias C. Nirmatrelvir más ritonavir (Paxlovid) una potente combinación inhibidora de la proteasa 3CLpro del SARS-CoV-2 [Nirmatrelvir plus ritonavir (Paxlovid) a potent SARS-CoV-2 3CLpro protease inhibitor combination]. Rev Esp Quimioter. 2022, http://dx.doi.org/10.37201/req/002.2022, reina21feb2022. Spanish. Epub ahead of print. PMID: 35183067.
3. Hammond J, Leister-Tebbe H, Gardner A, Abreu P, Bao W, Wisemandle W, et al. Oral nirmatrelvir for high-risk, nonhospitalized adults with Covid-19. N Engl J Med. 2022, http://dx.doi.org/10.1056/NEJMoa2118542. NEJMoa2118542. Epub ahead of print. PMID: 35172054; PMCID: PMC8908851.
4. Basterra-Gortari FJ, Bes-Rastrollo M, Ruiz-Canela M, Gea A, Martinez-Gonzalez MÁ. Prevalence of obesity and diabetes in Spanish adults 1987–2012. Med Clin (Barc). 2017;148:250–6, http://dx.doi.org/10.1016/j.medcli.2016.11.022. English, Spanish. Epub 2017 Jan 9. PMID: 28081903.
5. https://www.ine.es/jaxiT3/Datos.htm?t=1488
6. Rivero-Calle I, Pardo-Seco J, Aldaz P, Vargas DA, Mascarós E, Redondo E, et al. Incidence and risk factor prevalence of community-acquired pneumonia in adults in primary care in Spain (NEUMO-ES-RISK project). BMC Infect Dis. 2016;16.
7. De Miguel-Díez J, Jiménez-García R, Hernández-Barrera V, Jiménez-Trujillo I, De Miguel-Yanes JM, Méndez-Bailón M, et al. Trends in hospitalizations for community-acquired pneumonia in Spain: 2004–2013. Eur J Intern Med. 2017;40:64–71.
8. Torres A, Cilloniz C, Niederman MS, Menéndez R, Chalmers JD, Wunderink RG, et al. Pneumonia. Nat Rev Dis Primers. 2021;7:25, http://dx.doi.org/10.1038/s41572-021-00259-0. PMID: 33833230.
9. Wunderink RG, Waterer G. Advances in the causes and management of community acquired pneumonia in adults. BMJ. 2017;358:j2471, http://dx.doi.org/10.1136/bmj.j2471. PMID: 28694251.
10. https://www.sanidad.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov/situacionActual.htm

David Sánchez Fabra [a,*], Tina Herrero Jordán [b]

[a] F.E.A (Facultativo Especialistas de Área) de Medicina Interna, Servicio de Medicina Interna del Hospital Reina Sofía de Tudela, Tudela, Navarra, Spain
[b] F.E.A (Facultativo Especialista de Área) de Neumología, Servicio de Medicina Interna del Hospital Reina Sofía de Tudela, Tudela, Navarra, Spain

* Corresponding author.
E-mail address: davidsanchezfabra@gmail.com (D. Sánchez Fabra).

https://doi.org/10.1016/j.eimc.2022.04.007

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

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

Las nuevas tecnologías de secuenciación (NGS, del inglés *Next Generation Sequencing*) se han convertido en una herramienta indispensable para el estudio y control de la pandemia causada por el virus SARS-CoV-2[1]. La secuenciación del genoma completo viral ha sido vital no solo para el desarrollo de vacunas y fármacos específicos, sino también para el descubrimiento y la monitorización de las variantes de interés que, desde la aparición de la ahora conocida como variante alpha[2], han ido sucediéndose y relacionándose con los recrudecimientos puntuales de la pandemia. No obstante, y pese a los esfuerzos realizados por los laboratorios de Microbiología en todo el mundo, no se dispone de la capacidad de secuenciación suficiente para caracterizar todas las muestras positivas a SARS-CoV-2 con esta tecnología. Por ello se han desarrollado diferentes estrategias para la caracterización del virus mediante RT-PCR de combinaciones conocidas de mutaciones[3–5]; sin embargo, no debemos olvidar las limitaciones que este tipo de estudios presentan y que pueden llevar a una asignación de variante errónea[6,7].

En la actualidad, en nuestro servicio se caracterizan todas las primeras muestras de un episodio con Cts por debajo de 32 mediante los paneles Allplex™ Variants I&II (Seegene, Corea) que estudian las mutaciones de la espícula más relevantes en las últimas variantes de interés (H69/V70-, W152C, K417N, K417T, L452R, E484K y N501Y). A partir de febrero de 2022, en un escenario totalmente dominado por la variante ómicron, aquellas muestras que presentaron las mutaciones H69/V70-, K417N y N501Y fueron clasificadas como variante ómicron mediante RT-PCR. En caso de no presentar la deleción se consideró que pertenecían a la subvariante BA.2 mediante RT-PCR.

Ante la aparición de una muestra con H69/V70- y K417N, pero sin N501Y, se optó por secuenciar esta muestra ya que estos resultados no permitían asignarla a ninguna variante mediante RT-PCR. Para ello se utilizó una adaptación del protocolo ARTIC para Oxford Nanopore mediante la ampliación del genoma completo con el esquema VarSkip[8]. La librería resultante se procesó en un secuenciador MinION mk1c (Oxford Nanopore). Las lecturas obtenidas fueron posteriormente ensambladas mediante el *pipeline* de ARTIC para datos obtenidos de secuenciadores con tecnología de Oxford Nanopore y se analizaron mediante la webApp de Nextstrain[9] comprobando su consistencia mediante el programa Integrative Genomics Viewer (IGV)[10].

La secuencia obtenida (depositada en GISAID como EPI_ISL_10968563) pertenece al clado 21K (ómicron) y al linaje BA.1.17 y presentaba entre otras mutaciones características en la espícula las tres mutaciones empleadas por el esquema de Allplex Variants I&II: H69/V70-, K417N y N501Y; si bien, la mutación N501Y, además del cambio habitual A23063T, presentaba también T23065C. Todas las mutaciones se encontraron con suficiente cobertura. En este caso T23065C acompañando a A23063T produce una mutación silente ya que el codón que conforman sigue codificando una tirosina y mantiene la mutación N501Y.

Por lo tanto, es de esperar que esta mutación no suponga una ventaja evolutiva para el virus ni un cambio fundamental para su comportamiento. No obstante, sí que puede suponer un problema para los esquemas de caracterización que se basen en el estudio de mutaciones concretas, como el que empleamos en nuestro laboratorio en la actualidad; ya que, si este cambio se hubiese dado, por ejemplo, en una variante del linaje BA.2 en los paneles Allplex Variants I&II solo sería positiva la mutación K417N.

En resumen, creemos que este caso se trata de un ejemplo más de la enorme utilidad que presentan las NGS en los laboratorios de Microbiología Clínica en la actualidad, ya que nos permiten no solo la correcta asignación de las variantes circulantes, sino también la monitorización de la evolución de esas mismas variantes.

Bibliografía

1. Oude Munnink BB, Nieuwenhuijse DF, Stein M, O'Toole Á, Haverkate M, Mollers M, et al. Rapid SARS-CoV-2 whole-genome sequencing and analysis for informed public health decision-making in the Netherlands. Nat Med. 2020;26:1405–10.
2. Rambaut A, Loman N, Pybus OG, Barclay W, Barrett J, Carabelli A, et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations - SARS-CoV-2 coronavirus /nCoV-2019 Genomic Epidemiology [Internet]. Virological. 2020 [consultado 17 Mar 2022]. Disponible en: https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563
3. Ong DSY, Koeleman JGM, Vaessen N, Breijer S, Paltansing S, de Man P. Rapid screening method for the detection of SARS-CoV-2 variants of concern. J Clin Virol. 2021;141:104903.
4. Nörz D, Grunwald M, Olearo F, Fischer N, Aepfelbacher M, Pfefferle S, et al. Evaluation of a fully automated high-throughput SARS-CoV-2 multiplex qPCR assay with built-in screening functionality for del-HV69/70- and N501Y variants such as B.1.1.7. J Clin Virol. 2021;141:104894.
5. Kami W, Kinjo T, Arakaki W, Oki H, Motooka D, Nakamura S, et al. Rapid and simultaneous identification of three mutations by the Novaplex™ SARS-CoV-2 variants I assay kit. J Clin Virol. 2021;141:104877.
6. Alejo-Cancho I, Gual-de-Torrella A, Gallego M, Urrutikoetxea-Gutierrez M, Lejarraga C, López de Goikoetxea MJ. Misidentification of the SARS-CoV-2 Mu variant using commercial mutation screening assays. Arch Virol [Internet]. 2022 [consultado 17 Mar 2022]. Disponible en: https://link.springer.com/10.1007/s00705-022-05395-w
7. Camp JV, Buchta C, Jovanovic J, Puchhammer-Stöckl E, Benka B, Griesmacher A, et al. RT-PCR based SARS-CoV-2 variant screening assays require careful quality control. J Clin Virol. 2021;141:104905.
8. VarSkip multiplex PCR designs for SARS-CoV-2 sequencing [Internet]. New England Biolabs Inc. 2021 [consultado 17 Mar 2022]. Disponible en: https://github.com/nebiolabs/VarSkip
9. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics. 2018;34:4121–3.
10. Integrative genomics viewer | Nature Biotechnology [Internet]. [consultado 6 Jun 2021]. Disponible en: https://www.nature.com/articles/nbt.1754