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Safety of COVID-19 vaccines administered in the EU: Should we be concerned?

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ABSTRACT

The COVID-19 pandemic has had an unprecedented and devastating impact on public health, society and economics around the world. As a result, the development of vaccines to protect individuals from symptomatic COVID-19 infections has represented the only feasible health tool to combat the spread of the disease. However, at the same time the development and regulatory assessment of different vaccines has challenged pharmaceutical industries and regulatory agencies as this process has occurred in the shorter time ever though. So far, two mRNA and two adenovirus-vectored vaccines have received a conditional marketing authorisation in the EU and other countries. This review summarized and discusses the assessment reports of the European Medicine Agency (EMA) concerning the safety of the 3 vaccines currently used in the EU (Pfizer, Moderna and Astra-Zeneca). A particular focus has been paid to safety information from pre-clinical (animal) and clinical (phase 3 trials) studies. Overall, the most frequent adverse effects reported after the administration of these vaccines consisted of local reactions at the injection site (sore arm and erythema) followed by non-specific systemic effects (myalgia, chills, fatigue, headache, and fever), which occurred soon after vaccination and resolved shortly. Rare cases of vaccine-induced immune thrombotic thrombocytopenia have been reported for Vaxzevria. Data on long-term studies, interaction with other vaccines, use in pregnancy/breast-feeding, use in immunocompromised subjects, and in subjects with comorbidities, autoimmune or inflammatory disorders are still missing for these vaccines. Therefore, careful follow-up and surveillance studies for continued vaccine safety monitoring will be needed to ascertain the potential risks of such adverse events or diseases. In conclusion, the benefits and risks of current COVID-19 vaccines must be weighed against the real possibility of contract the disease and develop complications and long-term sequels; all this on the basis of the available scientific evidence and in the absence of unmotivated biases.

1. Introduction

The COVID-19 pandemic is a global crisis with a devastating health, social and economic impact that represents a major burden on worldwide health systems, including the European Union (EU) [1,2]. The pandemic has had, and still has, a remarkable impact on the people's lives, both at individual and societal level, resulting in psychological problems and health behaviour changes [3]. The dysregulation of the immune system as a result of chemical exposures and modern lifestyles has been considered to play a role for the development and spread of COVID-19 and its complications [4]. The pandemic is changing rapidly and requires different strategies to maintain clinical preventive services, including immunization, in order to avoid overloading health systems and their eventual collapse [3,5,6].

Most COVID-19 patients have mild symptoms, including dry cough, sore throat, fever or chills, muscle or body aches, and spontaneous shortness of breath or difficulty breathing. However, symptoms may differ with severity of disease, age and presence of comorbidity. Comorbid adults and elderly patients experience multiple complications, including pneumonia, acute respiratory distress syndrome (ARDS),

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sepsis and septic shock, and acute kidney injury, which can be manifestations of a cytokine storm. Cardiomyopathy, arrhythmia, neurologic complications have also been reported as well as complications inherent to prolonged hospitalization [7,8]. Severe COVID infection has thus far resulted in almost 3 million deaths worldwide ([9] as of 10 April 2021).

The development and administration of vaccines for COVID-19 help to protect individuals from becoming ill; however, it is not known how long the immunity produced by vaccines lasts until more time passes since people were first vaccinated [10]. COVID-19 vaccines may be effective in reducing community spread and/or preventing disease in individuals, particularly serious forms of the disease [11].

Two types of Covid-19 vaccines are widely used: a) genetic, using messenger RNA (mRNA) to cause the body to produce viral proteins; and b) viral vectored, which uses genetically modified viruses, such as adenovirus to carry sections of coronavirus genetic material [11]. Both Pfizer/BioNTech's and Moderna's vaccines are mRNA-based that produce the synthesis of a version of the spike (S) protein from the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that causes COVID-19. mRNA vaccines elicit a potent immune response including antibodies and cytotoxic T-cells. The potency of these vaccines has been optimised by encapsulation of the mRNA into lipid nanoparticles (LNPs), which protects the mRNA from degradation by RNases [12]. However, these LNPs may also affect the distribution of the mRNA. Intramuscular administration of LNP-formulated mRNA vaccines results in transient local inflammation that drives recruitment of neutrophils and antigen presenting cells (APCs) to the site of delivery. These cells uptake LNPs and express the mRNA-encoded antigen which is further processed by the proteasome to generate peptide epitopes which are presented in the cell membrane. Subsequently, APC migrate to nearby draining lymph nodes where T-cell priming occurs [12,13]. Likewise, passive drainage of mRNA–LNPs through lymphatic vessels allows direct delivery of mRNA to the lymph nodes containing resident antigen-presenting cells and T-cells within them [14].

The use of mRNA vaccines against SARS-CoV-2 developed by Pfizer/BioNTech and Moderna has raised concerns in the mass media and the public regarding their safety as this is the first time mRNA vaccines are used at global scale. In addition, the latest thrombotic events associated with the AstraZeneca/Oxford University vaccine have added concerns to the safety of Covid-19 vaccines. Concerns raised on whether the efficacy of the current vaccines used worldwide to prevent COVID-19 transmission, complications and deaths outweigh the potential risk of unknown long-term adverse effects has triggered a passionate scientific and societal debate [11].

This review summarized and discusses the assessment reports of the European Medicine Agency (EMA) concerning the safety of the 3 vaccines currently used in the EU (Fig. 1). A particular focus has been paid to safety information from pre-clinical (animal) and clinical (phase 2/3 trials) studies; however, efficacy issues of these vaccines are beyond the scope of this review. The Janssen's COVID-19 vaccine has not been considered in this review because the European Commission granted a conditional marketing authorisation very recently and vaccination campaigns will not start until mid-April 2021 at best. Other COVID-19 vaccine candidates are currently in various stages of development to determine safety and efficacy: 184 in preclinical development and 85 in clinical development (detailed information on the landscape of candidate vaccines is compiled by the World Health Organisation (WHO) and can be accessed at its webpage https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines). In particular, two vaccine candidates based on adenovirus expressing the full-length S protein have completed their phase 3 clinical trials, one developed in China, based on human adenovirus type 5 (Ad5) [15] and other in Russia, which combines recombinant human Ad26 and Ad5 in a prime–boost vaccination regimen [16]. For more details on other vaccines, see Dai and Gao [17] and Kyriakidis et al. [18].

2. COVID-19 vaccines currently authorised and used in the European Union

2.1. Pfizer BioNTech COVID-19 mRNA vaccine (Comirnaty)

On December 21st, 2020, the EMA Committee for Medicinal Products for Human Use (CHMP) issued a positive opinion for granting a conditional marketing authorisation to Pfizer/BioNTech Comirnaty vaccine.

According to the publicly available EMA CHMP assessment report [19], three pivotal (preclinical) experimental toxicology studies were conducted in Wistar rats. Two of them were repeat-dose toxicity studies, one with 30 μg of the clinically relevant variant and other with 100 μg of another variant, and the third one was a developmental and reproductive toxicity (DART) fertility-embryo foetal development (EFD) study, wherein the human clinical dose (30 μg RNA/dose) was administered to female rats twice before the start of mating and twice during gestation. The main results of these studies are shown in Table 1. The liver effects were considered as non-relevant and attributed to the LNP where the mRNA was encapsulated. No vaccine-related effects were observed on

Vaccine	Technology	EU Approval	Efficacy	Doses	Storage
• BNT162b2 • Pfizer/Biontech	• mRNA	• Conditional marketing approval	• 95 %	• Two doses	• -90-(-60)° C
• mRNA1273 • Moderna	• mRNA	• Conditional marketing approval	• 94,1%	• Two doses	• -25-(-15)° C
• ChAdOx1 nCoV-19 • AstraZeneca/Oxford	• Viral vector Adenovirus	• Conditional marketing approval	• 66%	• Single dose	• 2 – 8 ° C

Fig. 1. Diagram with the three vaccines against SARS-CoV-2 used currently used in vaccination campaigns in the EU.

Table 1

Pivotal non-clinical experimental toxicology studies conducted by applicants on the three vaccines under consideration.

	Pfizer/BioNTech	Moderna	Astra-Zeneca
Other names	Comirnaty	mRNA-1273	ChAdOx1-S [recombinant] AZD1222, Vaxzevria
Type of vaccine	mRNA-based	mRNA-based	Chimpanzee adenovirus-vectored (5×10^{10} vp)
Adjuvants or novel excipients	Lipid nanoparticles (LNPs) Novel excipient ALC-0159 containing an acetamide moiety	Lipid nanoparticles (LNP) Polyethylene glycol-lipid conjugate (PEG2000 DMG) SM-102 (an ionisable lipid)	None used
Animal	Wistar rats	Sprague Dawley rats	Mice
Repeat-dose toxicity studies	2 pivotal studies: (30 μg of variant 8 and 100 μg of clinically relevant variant 9) x 3 doses/group (3 weekly administrations over 17 days)	1 non-GLP-compliant study (of mRNA-1273) 6 GLP-compliant studies (of mRNA others than 1273) (9 to 150 μg mRNA/dose)	3 GLP compliant studies (using 2 platforms: (ChAdOx1 and AdCh63) 2 doses of each vector over a 14-day interval 13-day observation period 1 pivotal study: 3 doses/group (3.7×10^{10} vp/dose) on days 1, 22, 43
	3- week recovery period	x 2-4 doses/group 2-week recovery period	28-day recovery period
	Local effects: Oedema and erythema at the injection site Histopathology: inflammation	Local effects: Oedema and erythema at the injection site	Local effects: Effects other than pharmacological immune-related effects were not reported
	General systemic effects: ↑ acute phase proteins ↓ albumin-globulin ratio	General systemic effects: ↑ body temperature ↓ albumin, ↑ globulin	General systemic effects: ↑ body temperature
	↑ fibrinogen	↓ albumin/ globulin ratio	↓ albumin
	↓ haematological parameters (erythrocytes, haemoglobin, haematocrit, and reticulocytes)	↑ cytokines ↑ fibrinogen ↑ APTT	↑ globulin levels
	↑ immune cells (large unstained cells, neutrophils, eosinophils, basophils)	↑ potassium (plasma) ↑ white blood cells ↑ neutrophils ↑eosinophils ↓ lymphocytes	= haematology parameter ↑ monocytes
	Enlargement of spleen and inguinal lymph nodes	Enlargement of spleen and lymph nodes ↑ organ weight (spleen, liver, and adrenal gland) Skin thickening and mixed cell inflammation at the injection site	
	↑ cellularity in bone marrow	↑ cellularity and mixed cell inflammation (lymph nodes)	

Table 1 (*continued*)

	Pfizer/BioNTech	Moderna	Astra-Zeneca
	Reversible functional hepatic and/or biliary effects (↑GGT, ↑AST, ↑ALP)	↑ cellularity in bone marrow Hepatocyte vacuolation Kupffer cell hypertrophy Centrilobular degeneration ↑AST, ↑ALP, ↑bilirubin, ↑triglyceride, ↑cholesterol, ↑BUN	
DART-EFD	30 μg RNA/dose (x 4 doses) No effects observed	Human clinical dose (x 4 doses) Non adverse effects observed (rib nodules and wavy ribs, ↓pregnancy index) No dose administered during early organogenesis.	One preliminary study (2 doses) No adverse effects observed Anti-S glycoprotein antibody transferred via placenta and lactation. The main and definitive study is ongoing (mice)
Genotoxicity	Not performed (no expected genotoxic effects)	Final vaccine formulation Novel excipient (LNP) No relevant genotoxic risk	Not performed (no required for viral vaccines)

APTT: activated partial thromboplastin time.
GGT: Gamma-glutamyl transferase.
ALT: Alanine aminotransferase.
ALP: alkaline phosphatase.
BUN: blood urea nitrogen.

female fertility, gestation, embryo-foetal or offspring development up to weaning. No genotoxicity studies were performed as the components of the vaccine formulation (LNPs and mRNA) are not expected to elicit genotoxic effects [19]. In particular, the delivered mRNA is active in the cytoplasm of a cell and it does not enter the nucleus or interact with the genome, such that it is non-replicating [20].

The safety evaluation conducted in humans consisted of one ongoing phase 2/3 clinical trial which included 21,720 subjects who received two doses of the vaccine (30 μg of mRNA) and 21,728 individuals in the placebo group, who were followed-up over 2 months (Table 2). The most frequent adverse events were local reactions, consisting of pain at the injection side, less frequently redness and swelling, and very rarely lymphadenopathy. These were considered most often vaccine typical reactions, which appeared 1−3 days after vaccination and persisted for 1−2 days. The systemic reactions more frequently reported were fatigue and headache, followed by myalgia and chills, arthralgia and fever (<15 %) (Table 2). The frequency of headache, fatigue and fever was higher after the second dose in both age groups (16−55 and >55 years). These events were reported within 7 days after the first or second dose and were largely attributable to reactogenicity events. None immediate adverse event, occurring within 30 min after dosing and indicative of an allergic reaction to vaccine, was reported. However, three cases of anaphylaxis were reported during vaccination campaigns by the time the EMA assessment report was written [19].

Serious adverse events and deaths occurred in a small number of subjects and were balanced in both study arms and in the two age groups. However, none of the deaths were considered related to study intervention. Both local and systemic reactions were transient, of mild to moderate intensity, and resolved within a few days after vaccination.

Table 2

Safety evaluation in humans conducted in randomized clinical trials by applicants on the three vaccines under consideration.

	Pfizer BioNTech	Moderna	Astra-Zeneca
Dose	30 µg of mRNA x 2 doses	100 µg of mRNA x 2 doses	5×10^{10} vp x 2 doses
No. subjects	21,720 (vaccine group) 21,728 (placebo)	15,185 (vaccine group) 15,166 (placebo)	12,021 (vaccine group) 11,724 (placebo)
Age groups	16-55 and >55 years	≥18 years	18-55, 56-69, ≥70 year
Follow-up period (median after 2nd dose)	≥ 2 months (50.6% of individuals) (median, 50.6% after 2nd dose)	63 days (median after 2nd dose)	55.6 days (median after 2nd dose)
Local effects	Injection site pain (~80%)	Injection site pain (83-88%)	Tenderness (75%)
	Redness and swelling (<10%)	Erythema (<9%)	Pain (54%)
	Lymphadenopathy (0.4%)	Swelling/induration (<12%)	Redness (<3%)
(Reactogenicity in the vaccine group)		Lymphadenopathy (<14%)	Warmth (<18%)
			Itch (<7%)
			Swelling or induration (<3%)
			Lymphadenopathy (0.3%)
Onset after vaccination	1-3 days	1-2 days	<7 days
Duration	1-2 days (median)	1-3 days	3.3 days (mean)
Local effects persisting > 7 days		3.8% (vaccine group) 1.3% (placebo) Lymphadenopathy, pain	
Systemic reactions (overall data)		Vaccine group (84.1%) Placebo group (53.5%) (after any dose)	Vaccine group (86%) Placebo group (71.7%) (for any local and systemic adverse events, after any dose)
Systemic adverse reactions (Reactogenicity in the vaccine group)	Fatigue (<60%)	Fatigue (<66%)	Fatigue (62%)
	Headache (<50%)	Headache (<59%)	Headache (57%)
	Myalgia and chills (30%)	Myalgia (<58%)	Muscle pain (49%)
	Arthralgia (20%)	Arthragia (<43%)	
	Fever (≤16%)	Fever (<16%)	Feverishness (9%)
	Nausea (0.8%)	Nausea/vomiting (<19%)	Nausea (22%) and vomiting (2%)
		Chills (<45%)	Malaise (44%)
Onset after vaccination	7 days after 1st or 2nd dose	1-2 days after any dose	≤ 7 days of any dose
Duration		1-2 days (median)	2.8 days (mean)
Any adverse events (overall data)	Vaccine group (26.7%) Placebo group (12.2%)		
Adverse events (imbalanced)	Appendicitis (8/4, vaccine/placebo)	Hepatobiliary disorders (15/3, vaccine/placebo) (mainly cholelithiasis and cholecystitis)	Nervous System Disorders (headache, lethargy, migraines, somnolence, dizziness) • Vaccine group (<12%) • Placebo group (<8%)
	Biliary events (14/5, vaccine/placebo)		
	None vaccine-related	None vaccine-related (chance)	
	Vaccine group (0.6%, 56 cases)	Vaccine group (16 cases)	Vaccine group (0.8 %)

Table 2 (*continued*)

	Pfizer BioNTech	Moderna	Astra-Zeneca
Serious adverse events (overall data)	Placebo group (0.6%, 53 cases)	Placebo group (10 cases)	Placebo group (1.1 %)
	Bell's paralysis	Bell's paralysis • Vaccine group (n = 3) • Placebo group (n = 1)	Bell's paralysis • Vaccine group (n = 3) • Placebo group (n = 3)
Serious adverse events	• Vaccine group (n = 4) • Placebo group (n = 0)	Cerebrovascular accidents Embolic strokes Transient ischaemic attack Deep vein thrombosis • Vaccine group (n = 3, 2, 1, 2) • Placebo group (n = 1, 0, 0, 0)	Paraesthesia, hypoesthesia and muscular weakness (not imbalanced): • Vaccine group (0.47%) • Placebo group (0.65%)
Anaphylaxis	Vaccine group (n = 2) Placebo group (n = 1) (not treatment-related)	Vaccine group (n = 1) Placebo group (n = 1) (without temporal relation with injection)	Vaccine group (n = 1) (not treatment-related)
Deaths	Vaccine group (n = 2) Placebo group (n = 4) (none treatment-related)	Vaccine group (n = 6) Placebo group (n = 7) (none treatment-related)	Vaccine group (n = 2) Placebo group (n = 4) (none treatment-related)
Ongoing pivotal studies (long-term events)	2-year follow-up	2-year follow-up	1-year follow-up

They were milder and of slightly lower frequency among subjects >55 years of age. They were attributable to reactogenicity to the vaccine as the reactogenicity profile was similar to that of any authorised vaccine [19].

2.2. Moderna COVID-19 vaccine (mRNA-1273)

On January, 6th 2021, the EMA CHMP issued a positive opinion for granting a conditional marketing authorisation to Moderna COVID-19 vaccine [21].

The results of the preclinical (regulatory toxicology) studies are shown in Table 1. Overall, these are roughly similar to those reported for the Pfizer/BioNTech vaccine. Six repeated-dose GLP-compliant studies were performed in Sprague Dawley rats, with a dose range from 9 to 150 µg mRNA/dose (Table 1). The results were quite similar among the studies, supporting that observed toxicities were not product specific, but rather caused by the immunologic responses towards the translated antigens, and potentially by a contribution of the novel LNP formulation. A local inflammatory response was not only noted in the direct vicinity of the injection site, but also in adjacent tissues and/or organs [21].

The genotoxic potential of the final vaccine formulation and a novel excipient (one of the four lipids that protect and carry the mRNA in LNP) was evaluated. The test used consisted of a bacterial reverse mutation test, in vitro mammalian cell micronucleus test in human peripheral blood lymphocytes, and in vivo mammalian bone marrow erythrocyte micronucleus assay in the rat. The results indicated that a relevant genotoxic risk was not expected for mRNA-1273. A GLP-compliant DART study was conducted in female Sprague Dawley CD rats. No mRNA-1273-related effects were observed on female fertility, embryo-foetal or post-natal survival, growth or development in the offspring [21].

Concerning the human studies on clinical safety, 15,185 subjects were enrolled in the vaccine group of the pivotal phase 3 trial and 15,166 in the placebo group, with a median follow-up of 63 days after the second injection (Table 2). Overall, qualitative findings were roughly similar to those observed by Pfizer-BioNTech, with minor differences in the frequency of some local and systemic adverse reactions (reactogenicity). A higher incidence of lymphadenopathy, chills, arthralgia and nausea was reported with Moderna's vaccine, particularly after the second dose [21]. Remarkably, delayed local reactions at the injection-site characterised by erythema, induration, and tenderness have been reported by in 244 participants (0.8 %) after the first dose and in 68 participants (0.2 %) after the second dose of the phase 3 clinical trial [22].

Regarding more serious adverse events of the nervous system, 3 cerebrovascular accidents, 2 embolic strokes and 1 transient ischaemic attack were reported in the vaccine arm, whereas the placebo group showed 1/0/0, respectively. Furthermore, there were 2 reports of deep vein thrombosis in the vaccine group and none in the placebo. Detailed background information for stroke and transient ischaemic attack indicated a significant medical history or increased risk for these events [21]. Conversely, no similar events were reported for the Pfizer/BioNTech vaccine [19].

2.3. Astra-Zeneca COVID-19 vaccine (Vaxzevria - AZD1222)

On January, 29th 2021, the EMA CHMP issued a positive opinion for granting a conditional marketing authorisation to Astra-Zeneca. COVID-19 vaccine AZD1222 is a monovalent vaccine composed of a single recombinant, replication-deficient chimpanzee adenovirus (ChAdOx1) vector that has been modified to contain the gene encoding the Spike (S) glycoprotein of SARS CoV-2 [23].

Regarding non-clinical data to address the concerns raised for the purpose of granting a conditional marketing authorisation, three GLP compliant repeated-dose studies were performed in mice using the same (ChAdOx1) or a closely related platform to support the safety of the vaccine. Mice were considered a relevant species for toxicity assessment as they develop an immune response to the vaccine antigen. Two doses of the respective vectors were administered intramuscularly with a 14 day-interval, followed by a 13-day observation period. In addition, a pivotal repeated-dose toxicity study was conducted in mice by administering control or test item on days 1, 22 and 43, followed by a recovery period of 28 days (Table 1). The local effects observed in the toxicity study were considered non-adverse and related to the pharmacological immune-related effects of the vector administration. The minor changes reported for haematology and clinical chemistry parameters were consistent with the expected pharmacology effects and disappeared after the recovery period [23].

No genotoxicity and carcinogenicity studies were carried out as these studies are normally not required for viral vaccines according to relevant guidelines, and no adjuvants or novel excipients are used in this vaccine. A preliminary DART study failed to observe adverse effects related to the vaccine on female reproduction, foetal or pup survival and no foetal visceral or skeletal findings were observed either. The results indicated sufficient transfer of anti-S glycoprotein antibody via placenta and lactation. The main and definitive DART study in mice is ongoing and the results will be provided to EMA. Adenovirus infections are prevalent worldwide and wild-type adenoviral infection in pregnant women is generally not associated with congenital anomalies [23].

The clinical safety of AZD1222 is based on the interim analysis of the results from four individual studies pooled comprising 12,021 subjects receiving any dose of the vaccine and 11,724 the placebo. The median of duration of follow-up was 55.6 days after the second dose. Local and systemic adverse events were generally milder and reported less frequently after the second dose than after the first dose. Adverse events were less frequently reported in adults aged ≥ 65 than adults aged 18–65 [23].

Local and systemic adverse events are shown in Table 2. The majority of events was of mild to moderate severity, occurred within ≤ 7 days of any dose and were less frequently observed after the second dose. In relation to adverse events of special interest, their overall incidence was low: 0.8 % in the vaccine group and 1.1 % in the placebo. Less than 0.1 % participants reported serious adverse events considered treatment related, 3 in the vaccine group (fever, increased C-reactive protein, and myelitis transverse) and 2 in the control group (autoimmune haemolytic anaemia and myelitis). One case of multiple sclerosis was considered unrelated to the vaccine as new and pre-existent brain lesions were observed in the Magnetic Resonance Imaging. Other neurological serious adverse events in both the vaccine group and the placebo were facial spasm, migraine, ischaemic stroke, presyncope, syncope, serotonin syndrome, subarachnoid haemorrhage and transient ischaemic attack. However, only facial spasm and migraine may be potentially related to the intervention [23].

3. Thrombosis associated with thrombocytopenia in subjects vaccinated with Vaxzevria (COVID-19 vaccine AstraZeneca)

Overall, the number of cases of thrombosis reported after vaccination with Vaxzevria in relation to the number of people vaccinated is less than the number of these events that occur in the general population, thereby the administration of this vaccine is not considered to be associated with an increased risk of thrombotic events. However, very rare cases of thrombosis combined with thrombocytopenia, and sometimes bleeding, have been reported [24]. These events included cerebral venous sinus thrombosis (CVST) and splanchnic vein thrombosis (SVT) and have been referred to as vaccine-induced immune thrombotic thrombocytopenia (VITT) [25,26].

According to the EMA's Pharmacovigilance Risk Assessment Committee (PRAC), from the 34 million people that received the vaccine in the European Economic Area (EEA) and the UK as of April 4th 2021, 169 cases of CVST and 53 cases of SVT were reported to EudraVigilance, with most of them occurring within the first two weeks of vaccination in women <60 year. The estimated incidence of this rare adverse effect is around 1 in 100,000 people under the age of 60 [24]. The comparison of the number of reported cases of VITT with their incidence rate in the general population has shown higher numbers in the pool of all vaccinated people, but not in older people. However, with the available evidence it has not been possible to identify specific risk factors. While a causal link with the vaccine has not been proven at this time, this condition deserves further analysis as it might be possible. Accordingly, it should be listed as very rare side effects of Vaxzevria [24].

Since VITT clinically resembles autoimmune heparin-induced thrombocytopenia (HIT, a rare clotting disorder affecting 1–2% of patients treated with heparin), an immune response has been suggested as potential pathomechanism. HIT is caused by antibodies that recognize complexes formed when negatively charged carbohydrates present in heparin molecules bind to a positively charged platelet protein (platelet factor 4, PF4), which plays an important for clotting [27]. Two recent case-series studies have shown that the serum of patients with VITT has high levels of antibodies to PF4–polyanion complexes [25,26]. It has been suggested that the vaccination might induce the formation of autoantibodies against PF4 as part of the inflammatory reaction and immune stimulation of vaccination. These antibodies would then cause massive platelet activation via the Fc receptor as also occurs with HIT [25,28]. Despite DNA and RNA can form multimolecular complexes with PF4 [25], VITT has been reported thus far only with adenovirus-vectored vaccines (Vaxzevria and Janssen vaccine) but not with mRNA vaccines.

Notwithstanding the above, the EMA's PRAC statement issued on April 7th 2021 considered that the overall benefits of Vaxzevria in preventing COVID-19 disease outweigh the risks of side effects and recommended healthcare professionals to warn people receiving the vaccine to seek medical attention if they develop symptoms suggestive

of these rare conditions [24].

Notably, no signal has been identified so far from monitoring for other COVID-19 vaccines, but close safety monitoring is warranted. However, a number of thrombocytopenia (particularly immune thrombocytopenia, ITP) events have been reported after administration of mRNA vaccines in the US. In a series of 20 patients recently published [29], nine received the Pfizer-BioNTech vaccine and 11 the Moderna vaccine. The question of whether these cases are actually primary ITP coinciding with the administration of the vaccine or ITP secondary to vaccination remains unanswered. If the latter is true, this means an incidence of 1 case in a million vaccinated persons in the US. Comparatively, approximately 50,000 adults are diagnosed with ITP in the US each year and 1 case in 40,000 children develops secondary ITP after receiving measles-mumps-rubella vaccine [29].

It should be noted that a distinctive characteristic of SARS-CoV-2 infection is vascular damage, leading to severe endothelial injury, increased vascular permeability, microvascular occlusion and coagulopathy, which can manifest as venous and arterial thrombotic events. In the context of hyperinflammation, a serious malfunction of cytokine-controlled coagulation can occur resulting in thromboembolic events associated with higher mortality rate in COVID-19 patients [30,31]. Importantly, a systematic review has reported a very high incidence of thrombotic complications in 34 % of COVID-19 patients admitted to ICU despite anticoagulant thromboprophylaxis [32]. Although these events represent a well-known complication of any serious infection, additional mechanisms such as endothelial damage, increased vascular permeability and microvascular occlusion may be implicated in COVID-19.

4. General discussion

Two mRNA and two adenovirus-vectored vaccines have received a conditional marketing authorisation in the EU and other countries. This authorisation has been granted in the interest of public health because the benefit of immediate availability outweighs the risk from less comprehensive data than normally required. In clinical trials, these vaccines have shown very high levels of protection against symptomatic infections with COVID-19 [33].

The S protein of SARS-CoV-2 is a major target of virus neutralizing antibodies. Indeed, it has become a key antigen for vaccine development as this protein is intended to trigger a strong and relatively long-lasting production of high affinity virus neutralizing antibodies, which block the S-protein and its receptor-binding domain (RBD) interaction [34]. However, these vaccines are also expected to elicit a concomitant T-cell response of the Th1 type, supporting the B-cells responsible for the production of S-specific antibodies and cytotoxic T-cells that kill virus infected cells [19].

4.1. Non clinical (animal) studies

According to the EMA's assessment reports, the results of the non-clinical (animal) studies conducted by applicants for the three vaccines considered in this review failed to reveal any major or special hazard for humans regarding conventional studies of repeated dose toxicity, genotoxicity and reproductive and developmental toxicity. However, it should be noted that the absence of dose-response designs in the studies increases the difficulty to interpret the effects. In addition, since no long-term animal studies were conducted due to the need of urgent development of the vaccines, the authorization issued was restricted to emergency use.

4.2. Adverse reactions

Overall, the most frequent adverse effects reported after the administration of the three vaccines considered in this review consisted of local reactions at the injection site (sore arm and erythema) followed by non-specific systemic effects (myalgia, chills, fatigue, headache, and

fever). These adverse reactions are considered as the result of the immune system's reaction that occurs soon after vaccination (also referred to as reactogenicity) and resolves shortly [35]. Given the scale-up of mass vaccination campaigns across the world, severe adverse events may occur and will likely generate concerns among patients and requests for evaluation.

Severe allergic reactions due to anaphylaxis represent a very rare side effect associated with most vaccines, including mRNA vaccines. For this reason, it has been recommended that subjects with a previous history of severe allergic reactions to any vaccine ingredient should not receive it [36]. This safety concern is currently followed-up via routine pharmacovigilance.

Long-term safety data, interaction with other vaccines, data on use in pregnancy/breastfeeding and other vulnerable subgroups (e.g., patients with co-morbidities such as chronic obstructive pulmonary disease, diabetes, chronic neurological disease, or cardiovascular disorders), immunocompromised patients and subjects with autoimmune or inflammatory disorders were missing. However, such information will be collected in the post-authorisation safety studies and in ongoing pivotal studies where the studied population will be followed-up for up to 2 years, as this is a specific obligation of a conditional marketing authorization. These studies will also address potential risks specific to vaccination for COVID-19 (e.g. vaccine-associated enhanced respiratory disease) [19,21,23].

The phase 3 clinical trials of all COVID-19 vaccines conducted in several countries with different numbers of volunteers mainly focused on immunogenicity and safety, and primarily reported the side effects and adverse events that arose shortly after vaccination. Despite the limitations of those trials (neither all population subgroups nor long-term adverse events are covered), they have the advantage of using a vaccine arm and a control arm, which enables to assess whether there is a numerical imbalance between the vaccine group compared with the placebo group. Further information about the current status of ongoing/approved clinical trials of COVID-19 vaccines can be accessed at https://clinicaltrials.gov/.

Since potential long-term adverse effects (mediated by immune or non-immune mechanisms) cannot be ruled out with the three COVID-19 vaccines mentioned above, careful follow-up and surveillance studies for continued vaccine safety monitoring will be needed to ascertain the potential risks of such adverse events or diseases [37].

It is important to note that vaccination campaigns are carried out in a different context than phase 3 clinical trials, in the sense that a control group (i.e., placebo) is not available. Thereby, any new serious adverse event that may occur shortly after vaccination does not necessarily have to be considered as secondary to vaccination, but may actually be a primary event (or disease) coincident with the administration of the vaccine. A thorough assessment of each case should be conducted in relation to the subjects' medical history, current and past treatments, and investigation of the genetic background, if appropriate.

According to the 4th Pharmacovigilance Report on COVID-19 Vaccines of the Spanish Agency for Medicines and Health Products (AEMPS), at the time of the latest data cut-off (March 21st, 2021) a total of 6,125,119 doses of COVID-19 vaccines had been administered in Spain since the beginning of the vaccination campaign (December 27th, 2020). A total of 4,136,963 people were vaccinated of which 67 % were women. Seventy nine percent of the administered doses corresponded to Comirnaty, 16 % to Vaxzevria and 5% to Moderna vaccine, and 11,182 adverse events were reported, 93 % in people between 18 and 65 years old (93 %) and mostly women (82 %) [38]. The most frequently reported events continue to be general disorders (fever, pain at the injection site), events of the central nervous system (headache, dizziness) and musculoskeletal (myalgia and arthralgia) (Table 3). The report concluded that, after reviewing the available data, no hitherto unknown adverse reactions have been identified that may give cause for concern [38]. However, no information is available so far on potential long-term adverse events.

Table 3

List of the 10 most reported adverse events of the 3 COVID-19 vaccines that are currently being administered in Spain.

	Vaccine		
	Comirnaty	Moderna	Vaxzevria
Adverse Event	4,834,876*	304,715*	985.528*
Pyrexia	41 %	50 %	66 %
Headache	30 %	31 %	44 %
Myalgia	22 %	23 %	31 %
Pain at the injection site	14 %	15 %	13 %
Malaise	13 %	10 %	15 %
Nausea	9%	11 %	12 %
Chills	8%	12 %	19 %
Arthralgia	8%	8%	9%
Fatiga	8%	8%	14 %
Lymphadenopathy	7%	7%	–
Dizziness	–	–	11 %

* Number of doses administered until March 21st, 2021.

Source: 4th Pharmacovigilance Report of the AEMPS [38]

According to the UK Medicines and Healthcare products Regulatory Agency, more than 30 Million doses of vaccines had been administered as of 28 March 2021 (10.9 and 19.5 million first doses of the Pfizer/BioNTech and AstraZeneca vaccines, respectively). A total of 43,491 Yellow Cards were received and analysed for the Pfizer/BioNTech vaccine and 116,162 for the AstraZeneca vaccine [33]. These reports included 124,371 and 440,871 suspected reactions, respectively (although a single report may contain more than one symptom). Table 4 summarised the main adverse effects reported.

In contrast to the limited evidence of serious adverse effects of COVID-19 vaccines mentioned above, there is growing evidence that health consequences of COVID-19 extend far beyond acute infection (the so-called persistent COVID-19, post-acute COVID-19 syndrome or late squeals of the disease) as reported in patients followed-up for 9 months after illness. Approximately 30 % reported persistent symptoms, despite undergoing mild illness [39]. Two categories have been reported: a) subacute or ongoing symptomatic COVID-19, which extent from 4 to 12 weeks beyond acute COVID-19; and b) chronic or post-COVID-19 syndrome, which extent beyond 12 weeks of the onset of acute COVID-19 and are not attributable to alternative diagnoses. The main symptoms

Table 4

List of the most common suspected reactions reported for the two COVID-19 vaccines that are currently being administered in UK (as of March 28th, 2021).

	Comirnaty [a]		Vaxzevria [b]	
Suspected reactions	Total	Fatal	Total	Fatal
Blood Disorders	3698	1	3030	2
Cardiac Disorders	1567	43	4325	57
Ear Disorders	1235	0	3261	0
Eye disorders	1897	0	5521	0
Gastrointestinal disorder	13,387	15	46,391	6
General disorders	36,796	127	151,940	205
Immune system disorders	689	1	1314	1
Infections	2864	55	8705	52
Metabolic disorders	777	1	5353	3
Muscle and tissue disorders	16,718	0	54,853	0
Nervous system disorders	23,534	21	95,234	63
Psychiatric disorders	1942	0	8047	1
Renal and urinary disorders	311	2	1248	1
Reproductive and breast disorders	672	0	1136	0
Respiratory disorders	5200	25	12,660	52
Skin disorders	8974	1	24,605	1
Vascular disorders	1601	4	4400	24

[a] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/966631/COVID-19_mRNA_Pfizer-_BioNTech_Vaccine_Analysis_Print_2_.pdf.

[b] https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/966183/COVID-19_AstraZeneca_Vaccine_Analysis_Print.pdf.

and abnormalities correspond to respiratory, haemetological, cardiovascular, neuropsychiatric, renal, endocrine, gastrointestinal, hepatobiliary, and dermatologic alterations [40].

EMA and competent authorities of EU Member States have developed a safety monitoring plan for COVID-19 vaccines (i.e., a guidance on good pharmacovigilance practices) with the aim of collecting and rapidly reviewing new information emerging from the outcome of the COVID-19 vaccination campaigns currently in place. This plan will allow the EU Medicines Regulatory Network to assess safety data arising from spontaneous reports, observational studies, etc. Thus, any potential safety concerns identified will be addressed by implementing appropriate regulatory measures to protect individual and public health under a transparent and timely manner [41].

4.3. Non-pharmacological measures during the vaccination campaigns: possible scenarios for the evolution of the COVID-19 pandemic

A recent study presented a mathematical model structured according to age, adapted to a series of epidemiological data from the United Kingdom, to estimate the dynamics of the SARS-CoV-2 virus in 2021, based on scenarios of relaxation of non-pharmacological interventions and characteristics vaccines [42]. Another study estimated that vaccination is insufficient to control the outbreak caused by COVID-19. Elimination of all non-pharmacological interventions after the end of the vaccination program will lead to 21,400 deaths due to the virus, in the case of a vaccine that prevents 85 % of infections, and the number increases to 96,700 deaths if the vaccine prevents only 60 % of infections (estimated by January 2024) [43].

However, although vaccination of the most vulnerable groups will allow some relaxation of control measures, this must be done gradually in order to mitigate the large-scale consequences for public health [11]. For the prediction of the number of daily deaths mentioned previously, the specialists used data from the United Kingdom (a death caused by COVID-19 was considered as the death that occurred within 28 days of a positive SARS-CoV-2 test). Experts point out that the actual number of deaths could be much higher. Prioritizing older groups and vulnerable people would lead to the greatest reduction in deaths.

The high efficacy of vaccines is a long-term key element. Although mass vaccination will significantly reduce the spread of the disease, other measures will be needed, such as periodic testing, disease progression monitoring and isolation strategies.

At the same time, for longer periods, there is a likelihood of a decrease in the degree of immunization of patients. As a result, the need to implement seasonal vaccination programs against SARS-CoV-2 is anticipated, mainly protecting the most vulnerable, similar to seasonal influenza vaccination [44].

Some medical experts believe that the SARS-CoV-2 virus can become a seasonal flu-like infection, while others believe it is more likely to circulate at low levels for years, with the potential for recurrence of outbreaks, which could be complicated by new viral strains. The end of the pandemic would be to stop the threat of increasing the number of infections and severe forms of the disease, which put a lot of pressure on health systems. The timing of pandemic control and a gradual return to normalcy depends on how quickly people around the world are vaccinated. For example, through a combination of mass vaccination and acquired immunity from infection, the US and Europe can aim for a "return to normal" with some minimal disease control measures. But this could take longer in Asia, where low rates of infection in some places mean that populations need to rely on a high vaccination rate to achieve collective immunity (when at least two-thirds of the population is immune to the virus). At that point, the virus will likely continue to circulate for many years, but will not pose a major threat to health systems [45].

Experts believe that the end of the pandemic will be less defined than its beginning, as countries will be able to control the transmission of the virus at different times. But they agree that although vaccines have been

developed in record time, the virus will continue to exist [46].

The health and economic burden of COVID-19 has resulted in a high prevalence of the disease and socioeconomic costs. The huge amount of economic resources invested in scientific research has accelerated the development of COVID-19 vaccine candidates at a rate never seen before. This expense has been worth because of the impact of vaccines on individual and public health, health costs and the wider economy. A recent study has modelled the COVID-19 disease transmission and its economic impact to gain insight on potential future post-vaccination scenarios in particular how to achieve a best-case health and economic scenario [47]. According to this study, a safe and effective COVID-19 vaccine not only will save lives and reduce costs of the health sector but also will mitigate the impact on the economy by avoiding physical distancing and the consequences thereof.

5. Conclusion

The three COVID-19 vaccines considered in this review did not reveal any major hazard based on conventional animal studies of repeated dose toxicity, genotoxicity and reproductive and developmental toxicity; however, no long-term studies have been conducted because of the urgent development of the vaccines. In phase 3 clinical trials, the most frequent adverse effects reported consisted of local reactions at the injection site and, to a lesser extent, non-specific systemic effects. These common and expected adverse reactions (reactogenicity) occur shortly after vaccination and resolve in a few days, and represent the early reaction of the immune system after vaccination. Conversely, serious adverse events involving different organs/systems are quite uncommon and mostly balanced between vaccine and placebo groups. However, data on long-term safety data, interaction with other vaccines, data on use in pregnant or breastfeeding women, immunocompromised patients and other vulnerable subgroups are still missing and therefore pivotal studies are still ongoing to cover a follow-up period of 2 years. Although the administration of the Vaxzevria vaccine does not lead to an increased overall risk of thrombotic events, very rare cases of cerebral venous sinus thrombosis and splanchnic vein thrombosis accompanied by low platelet levels have been reported, which are currently under investigation and close surveillance. The benefits and risks of current COVID-19 vaccines must be weighed against the real possibility of contracting the disease and developing complications and long-term sequels. Given the high public interest and social alarm that this information generates when it appears in the mass media, health professionals and health authorities must adequately and timely inform the population about a sensitive issue such as COVID-19.

Authors' statement

- Antonio F. Hernández: conceptualization, data curation, visualization, writing- original draft preparation, writing- reviewing and editing
- Daniela Calina: writing- original draft preparation, writing- reviewing and editing
- Konstantinos Poulas: data curation, writing- original draft preparation, writing- reviewing and editing
- Anca O. Docea: writing- original draft preparation, writing- reviewing and editing
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Declaration of Competing Interest

The authors report no declarations of interest.

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