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Vacunas

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Vaccination strategies

Adenoviral vector-based COVID-19 vaccines-associated cerebral venous sinus thromboses: Are those adverse events related to the formation of neutrophil extracellular traps?

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ABSTRACT

In March and April 2021 several countries temporarily suspended vaccinations with adenoviral vector-based COVID-19 vaccines. Concerns of national regulators particularly regarded very rare cases of cerebral venous sinus thrombosis after immunization with this type of vaccine. Until now, these adverse events were interpreted as standard hypercoagulable events, but their clinical characteristics suggest that they may actually represent unique thrombotic disorders referred to as immunothrombosis. In this paper it is speculated that it is possible that immunothrombosis after this type of vaccine results from formation of neutrophil extracellular traps (NETs) in veins affected by stagnant blood flow. Such a stasis occurs in individuals with anatomical variants of cerebral venous outflow, which may explain why these events are primarily seen in the cerebral veins. It has already been found that SARS-CoV-2 spike protein can evoke release of NETs. There is also a question if thrombotic events after adenoviral vector-based COVID-19 vaccines could be avoided. These vaccines will still be needed to curb COVID-19 worldwide, since they do not require transportation and storage at very low temperatures. Perhaps, vaccinations with these vaccines should be performed in combination with prophylactic administration of dipyridamole, which is an inexpensive pharmaceutical agent reducing the release of NETs.

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Asociación de las vacunas frente a la COVID-19 basadas en vectores de adenovirus y trombosis del seno venoso cerebral: ¿guardan relación estos episodios con la formación de trampas extracelulares de neutrófilos?

Palabras clave:
COVID-19
Trampas extracelulares de

RESUMEN

En marzo y abril de 2021 diversos países suspendieron temporalmente la vacunación con vacunas frente a COVID-19 basadas en vectores adenovirales. Las preocupaciones de los reguladores nacionales contemplaban en particular los casos raros de trombosis del seno

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neutrófilos
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Trombosis
Vacuna

venoso cerebral tras la inmunización con este tipo de vacunas. Hasta la fecha dichos episodios han sido interpretados como eventos estándar de hipercoagulación, aunque sus características clínicas sugieren que realmente pueden representar trastornos trombóticos únicos denominados inmunotrombosis. En este documento se especula la posibilidad de que la inmunotrombosis tras la administración de este tipo de vacuna se derive de la formación de trampas extracelulares de neutrófilos (NETs) en las venas afectadas por un flujo sanguíneo estancado. Dicha estasis se produce en individuos con variantes anatómicas de flujo venoso cerebral saliente, lo cual puede explicar por qué estos episodios se aprecian primeramente en las venas cerebrales. Se ha comprobado que la proteína pico de SARS-CoV-2 puede evocar la liberación de NETs. También está la cuestión de si pueden evitarse los episodios trombóticos tras la administración de las vacunas frente a COVID-19 basadas en vectores adenovirales. Dichas vacunas seguirán siendo necesarias si queremos doblegar la COVID-19 a nivel mundial, ya que no requieren transporte ni almacenamiento a muy bajas temperaturas. Quizás la vacunación con estas vacunas debería realizarse en combinación con la administración profiláctica de dipiridamol, que es un agente farmacéutico económico que reduce la liberación de NETs.

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In March and April 2021 several countries temporarily suspended vaccinations with the adenoviral vector-based COVID-19 vaccines: the Vaxzevria ChAdOx1 nCoV-19 vaccine (AstraZeneca, Södertälje, Sweden) and the Janssen COVID-19 (Ad.26.COV2.S) vaccine (Janssen Biotech, Inc., New Brunswick, NJ, USA). The concerns of national regulators particularly regarded very rare cases of cerebral venous sinus thrombosis (CVST). Importantly, fatality associated with CVST after these vaccinations was very high, 22.6% in the group of 62 patients who developed this complication after Vaxzevria vaccine.[1–4] Although there were also some cases of CVST after vaccinations with mRNA-based vaccines, such events were not more frequent than in general population and their clinical course was rather benign.[5] Even if health authorities stated that benefits of vaccination with adenoviral vector-based vaccines (AVBV) continued to outweigh associated risks, some countries decided to restrict vaccinations with these vaccines,[6,7] albeit such decisions were later either reversed or AVBVs were dedicated to selected groups of people.[2]

Nonetheless, it could be suspected that in the near future in the developed countries, as soon as a majority of the population will be vaccinated, immunizations against COVID-19 will probably be performed primarily with mRNA-based products. There is, however, an important logistic problem associated with such vaccines. They require transportation and storage at very low temperatures: −90 to −60 °C (−130 to −76 °F) in the case of the Pfizer vaccine and − 50 to −15 °C (−58 to 5 °F) in the case of the Moderna vaccine), which in many countries is not manageable. By contrast, AVBVs require refrigerators that maintain temperature between 2 to 8 °C (35.6 to 46.4 °F), which is feasible even in remote locations. Therefore, these vaccines will still be needed to curb COVID-19 worldwide.

In this context, taking into account public awareness of these very rare yet dangerous thrombotic complications, the question how vaccination with AVBV can trigger CVST, and whether the incidence of these adverse events could be reduced, seems extremely important. Currently experts think that although causal relationship between these vaccines and CVST has not been unequivocally proven, thrombosis after vaccination could result from hypercoagulability, which is already present in some vulnerable groups, like young women.[8] Interestingly, laboratory profile of the patients presenting with CVST after immunization with AVBV resembled that of heparin-induced thrombocytopenia (HIT). The most important finding regards the presence of platelet factor 4-specific antibodies, which is a hallmark of HIT.[9,10] A detailed analysis of the laboratory profile of these patients in the already-published studies has recently been discussed.[11] However, a majority of patients suffering from CVST after vaccination did not receive heparin. In addition, mortality rate among these patients was much higher than in normal CVST, which is at the level of 4%.[12] This suggests that such events are not regular thromboses, but rather another kind of thrombotic disorder, perhaps immunothrombosis resulting from an excessive production of neutrophil extracellular traps (NETs) triggered by the vaccine. Immunothrombosis is a pathological process of the release of NETs by activated neutrophils, which is accompanied by activation of the coagulation cascade. NETs are web-like structures composed of DNA and proteins of the nuclear and granular origin. These webs are released by activated neutrophils that mobilize their chromatin, which expands outside these cells and decondensates. Since NETs activate platelets, an exaggerated formation of these extracellular webs can result in local thrombosis. Formation of NETs is an important part of the innate immune system, because they can ensnare and kill microorganisms. On the other hand, formation of NETs has also been found in the settings of many diseases associated with inflammation and thrombosis, such as sepsis, antiphospholipid syndrome, acute respiratory distress syndrome, deep venous thrombosis, rheumatoid arthritis, lupus, psoriasis, and even in some cancer patients. [13,14] NETs are also produced in the settings of HIT,[15] which may explain the laboratory profile of the above-discussed CVST patients. It has already been found that the SARS-CoV-2 spike protein can

evoke release of NETs.[16] A role for the spike protein in the pathogenesis of CVST has already been suggested by some researchers, but it has also been resolved that thrombotic events after vaccinations are not related to the antibodies directed against the spike protein.[7,17] Still, it is known that in the settings of severe COVID-19 the spike proteins are responsible for platelets activation.[18] These findings do not exclude a primarily role for NETs in the pathogenesis of AVBV-associated thrombosis, since the formation of NETs depends on pathological interplay between neutrophils and platelets. Activation of platelets mediated by platelet factor-4 antibodies is probably a part of the entire pathological process.[10,19] However, it seems that an important piece of the puzzle is still missing. Taking into account very high mortality rate in patients presenting with CVST after vaccination with AVBVs, NETs seem to be this missing critical element. Neither standard, nor modified (e.g. with fondaparinux) anticoagulant therapy is ineffective for the management of thrombi containing NETs, which are webs composed mostly of DNA instead of fibrin.[13] Thus, immunothromboses associated with the formation of NETs are associated with a high morbidity and mortality. If the role for NETs were confirmed, then these life-threatening adverse event should be managed differently, perhaps with DNases.[20]

The question why CVST can develop after AVBV and not after mRNA-based products is very intriguing. The gene coding for spike protein is a part of nearly all COVID-19 vaccines, including AVBV and mRNA-based ones. Recently, an interesting and perhaps correct explanation has been proposed.[7] A hypothesis, published as a preprint article, has pointed out that there is a substantial difference between mRNA-based vaccines and AVBVs in terms of how the spike protein is produced by the host cells. In the case of an mRNA-based vaccine, mRNA encoding this protein is transferred in a lipid nanoparticle to the cytosol of muscle cell, where the spike proteins are produced and then transported to the outer membrane of this cell. By contrast, in the case of AVBV, the spike protein gene is transferred to the nucleus, here it is transcribed into mRNA, and is exported to the cytosol. From this stage the subsequent events are the same for both types of vaccines. However, the former step is potentially prone to aberrations. It is possible that in some cases the process of transcription into mRNA that takes place in the nucleus is disrupted and consequently an abnormal shorter spike protein develops. These aberrant spike proteins are soluble and instead of being anchored to the outer membrane of the cell, can migrate to the bloodstream and in rare cases can evoke immunothrombosis.[7] This hypothesis, albeit promising, undoubtedly needs to be validated by other researchers and by differently designed experiments, since there are several aspects of this preprint work that remain to be explained.

Besides, it cannot be ruled out that not only the spike protein is responsible for CVST, but also the adenoviral vector can contribute to these rare thrombotic events. Although adenoviral vectors used in the AVBVs are replication-defective, early events of the vaccine-host interactions can initiate immune processes that in combination with the above-proposed spike protein-mediated immunothrombosis in susceptible individuals can result in these life-threatening albeit extremely rare complications.

It is also intriguing why AVBV-associated thromboses particularly affect cerebral sinuses. An increased rate of thromboembolism after the Vaxzevria vaccine has not been revealed in the Danish survey.[21] Perhaps, this complication in this unique venous territory occurs in patients with anatomical variants of the cerebral veins and sinuses, which evokes venous stasis in the cerebral circulation in the decubitus body position (in the upright body position a substantial part of blood flows out of the cranial cavity through the vertebral venous pathway, thus should not be affected, even is a case of anatomical anomalies).[22,23] An activation of neutrophils in the settings of blood stasis is a well-known phenomenon.[24] However, if such a pathomechanism was actually playing a role, immunothrombotic occlusions could also occur in other veins with stagnant flow, such as varicose veins. But thrombosis of varicosities is not a serious complication. It is associated with a low risk of pulmonary embolism and it is unlikely that all of such varicose vein thromboses were reported. On the contrary, thrombosis of the cerebral veins is a highly symptomatic and life-threatening disease, thus even rare cases were reported. Retrospective analysis of the venograms of CVST patients who developed this complication after AVBV could validate this conjecture regarding anatomical variants being responsible. In the recently published case series describing a group of patient presenting with CVST after vaccination with the Vaxzevria vaccine an asymmetric thrombosis of intracranial sinuses has been reported, which supports the hypothesis of anatomical background of these complications.[10]

There is also a question if thrombotic events after AVBVs could be avoided. Hypothetically, a prophylaxis against potentially fatal CVST with dipyridamole could be a solution to the problem with these vaccines. This pharmaceutical agent is characterized by an agonistic activity on the neutrophil adenosine A2A receptor, which in turn reduces release of NETs.[25] This drug is inexpensive and such a prophylaxis should not significantly increase the costs of vaccination, even in low-income countries. Currently, three clinical trials evaluating efficacy of dipyridamole for the treatment of COVID-19 have been registered (identifiers: NCT04424901; NCT04391179; NCT04410328), since the formation of NETs is a major contributor to COVID-19-associated morbidity and mortality. Results of these studies are expected to be released in a few months. Perhaps, this pharmaceutical agent should also be studied in people vaccinated with AVBV in order to validate its hypothetical protective activity. Such studies on such a use of dipyridamole should also check if this pharmaceutical agent does not affect clinical efficacy of the vaccine since neutrophils may play a role in the entire process of the immunization.

Another aspect associated with CVST after AVBVs regards targeted approach to the use of this class of vaccines in the prophylaxis against COVID-19. Theoretically, with more data on potentially vulnerable groups, such a tailored use of AVBVs would be possible. Perhaps a combination of the vaccine with dipyridamole or another protective pharmaceutical agent would be necessary in the selected groups of people only. Still, as has been already discussed in the first part of this paper, it seems that clinical practice worldwide would be different. Currently people, if they have a choice, prefer to be

vaccinated with mRNA-based vaccines. Consequently, in the countries where logistic issues associated with the transportation of mRNA-based vaccines is not a problem, a majority of future vaccinations will be performed with this class of vaccines. On the other hand, AVBVs will still be needed in the rest of world, however, with the risk that because of a fear of complications in the society (irrespective of the extremely low frequency of such adverse events) a substantial percentage of the population will not be vaccinated at all. Such a phenomenon is already seen in a number of countries. It is not clear if a targeted approach to the prophylaxis, even if scientifically sound, would be beneficial from the perspective of promoting vaccinations against COVID-19. Therefore, definitely more studies addressing the above-presented issues are urgently needed. Particularly, the mechanism responsible for extremely rare yet fatal thrombotic events after vaccination with AVBVs should be elucidated, and possible measures aimed at reducing the frequency of such events should be found.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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