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Editorial

Amateurism

Review

Poor evidence of direct anticoagulant (DOAC)

DOAC and increased thrombosis without APS

Candidates of New Products

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Amateurism

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Some readers of this bulletin say that the content is difficult and too specialized, or they are tired of reading articles with too many letters. Since this is a drug bulletin that covers medical and pharmaceutical sciences, it requires readers to have some basic knowledge about these areas. In addition, our articles include unfamiliar statistical terms and numbers, and names of recently developed drugs, which are often long and confusing. That may be one of the reasons why they are discouraged from reading our articles.

The opening message of the first issue of the predecessor of this bulletin, "*Check-up your medicines to save your life* (launched in January, 2001), said "this book explains about diseases and medicines used for them, and whether they are good or bad in plain language." In addition, the editorial of the succeeding bulletin, *Med Check* No. 57 (January, 2015) states that "We would like to make it easy to understand so that lay people and the mass media can also read our bulletin."

We will do our best to describe complicated contents in a plain and easy-to-understand language, keeping our original intention in our mind.

However, the article on vaccine (candidate) in this issue contains full of unfamiliar terms again and again: subunit, spike, lipid nanoparticle, vector, adjuvant, etc. We feel like we can hear our readers screaming “I have a headache”. However, please take enough time and read them carefully. Even if you don’t understand it by reading it once, you would understand the important points if you go back to the articles from time to time and read them again.

The translator (from English to Japanese) of "Representation of the Intellectual" by Palestinian-American literary critic Edward W. Said, said in his postscript that "What kind of person does Said think as a desirable intellectual? The person is not an expert who is bound by one field but an amateur who can freely cross each field." Said does not say that training of professional skills is wasteful. He said "In the current education system, the higher the level of education, the more those who receive education are confined to a narrow area of knowledge." He warns of professional idiots.

We would like to publish easy-to-understand articles in this bulletin, cherishing the spirit of amateurism that "can cross each field freely".

The 2020 annual theme: Criticism on treatment guidelines series (13)

Warfarin is the standard treatment for non-valvular atrial fibrillation

The usefulness of direct anticoagulants (DOACs) has not been proven

Translated and revised from Med Check(in Japanese) Mar 2020 ; 20 (88):30-34

Med Check Editorial Team

Summary

- Until 2010, warfarin, a vitamin K antagonist, was the only oral anticoagulant for the management of non-valvular atrial fibrillation. The dose of warfarin is titrated by the level of the prothrombin time-international normalized ratio (PT-INR). Although PT-INR range of 2.0 to 3.0 is recommended as optimal in general, we recommend the rage of 1.5 to 2.2 in order to minimize the overall risk of stroke (including hemorrhagic and ischemic) and major hemorrhage.
- There are serious limitations in randomized controlled trials (RCTs) of direct oral anticoagulants (DOACs), including dabigatran, apixaban, rivaroxaban, and edoxaban. The U.S. Food and Drug Administration (FDA) identified falsified data especially in the RCTs of apixaban (ARISTOTLE). After excluding the RCTs on apixaban, meta-analysis results of RCTs on DOAC denied many of the initial analysis results which showed superiority of DOAC to warfarin.
- We found 6 studies and 12 cohorts that compared the outcomes including hemorrhagic and ischemic stroke or other major bleeding between DOAC and warfarin with 6 months or more of follow-up period and were not directly funded by pharmaceutical companies. Meta-analysis of these studies showed that DOAC did not significantly reduce the risk of ischemic stroke, systemic thrombosis or overall mortality. The risk of major bleeding and hemorrhagic stroke was significantly reduced, but that of gastrointestinal bleeding was increased. It is highly possible that increased bleeding is related to the recommended PT-INR range of 2.0 to 3.0, which is too high, and more bleeding might have occurred in the warfarin group due to excessive use of warfarin.
- Controlling PT-INR at the range of 1.5 to 2.2 with warfarin is most appropriate for the treatment of non-valvular atrial fibrillation.

Conclusion: There is no evidence that direct oral anticoagulants (DOACs) are superior to warfarin to prevent ischemic stroke in the management of non-valvular atrial fibrillation. It is appropriate to use warfarin to control PT-INR at the range of 1.5 to 2.2.

Keywords:

vitamin K inhibitor, apixaban, dabigatran, rivaroxaban, ischemic stroke, hemorrhagic stroke, gastrointestinal bleeding, total death

Introduction

Prevalence of patients with non-valvular atrial fibrillation increases with age [1,2]. Incidence of stroke and coronary heart disease, cardiovascular mortality and overall mortality increases with each additional risk factor, such as heart failure, hypertension, old age (65 years and older, especially 75 years and older), diabetes, history of stroke and vascular diseases. If non-valvular atrial fibrillation is added, risk of various diseases especially of ischemic stroke increases greatly [1-4].

Warfarin has traditionally been the only oral anticoagulant used for reducing ischemic stroke in patients with atrial fibrillation in Japan. However, since March 2011, direct oral anticoagulants (DOAC) (or non-vitamin K antagonist oral anticoagulants: NOAC) have been marketed one after another in Japan.

We reviewed their efficacy and safety, and found that all DOACs had major flaws in the methods of the randomized controlled trials (RCTs) and that they could not be used safely [5-7]. In addition, we critically reviewed the evidence base of recommending the range of 2.0 to 3.0 as the optimal PT-INR for warfarin use and found that the optimal range of PT-INR should be 1.5 to 2.2 [5,8].

The Japanese Circulation Society stated that "DOAC is preferable to warfarin if there is an equivalent level of indication" [1]. European guidelines have also expressed a preference for NOACs over VKA in stroke prevention for AF patients, especially if newly initiated [9]. DOACs include a direct thrombin inhibitor, dabigatran, and direct factor Xa inhibitors, such as apixaban, rivaroxaban and edoxaban. About five years have passed since we examined these drugs in 2015, so we tried to update the review.

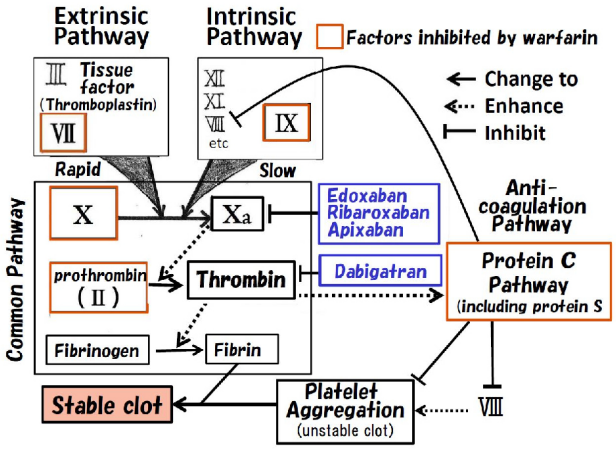
Atrial fibrillation is a risk factor for stroke and ischemic disease

Patients with atrial fibrillation were 2.8 times more likely to have a stroke than those without, but were also 2.3 times more likely to die of cardiovascular disease and 1.4 times more likely to die from all causes [4]. This is because non-valvular atrial fibrillation is the result of ischemia of the myocardium, and hypertension and coronary diseases are also caused by ischemia that damages the endothelium of blood vessels causing inflammation and thrombi. Therefore, it is not surprising that people with atrial fibrillation are more likely to have other heart diseases and higher total mortality.

The fundamental treatment should be stress reduction with adequate sleep

Therefore, reducing tissue ischemia due to sustained excessive stress is very important not only for atrial fibrillation itself but also for the prevention of its complications. For that purpose, it is important to take enough rest with sufficient sleep time without using sleeping pills, and regular break during work or sports and diaphragmatic breathing are also recommended.

Figure 1: Differences in action between warfarin and DOAC



The appropriate PT-INR is 1.5-2.2.

In addition to such non-pharmacological treatment, anticoagulant therapy should be given to prevent ischemic stroke. In order to determine the true optimal range of PT-INR, we searched and found 3 Japanese studies (5 papers) [10-14] and 5 randomized controlled trials (RCTs) [15-19] analyzed in the Cochrane Review [20] and a series of studies by Hylek et al. [21-23]. We examined these in detail to identify the optimal range of PT-INR at which incidence of ischemic stroke + systemic embolic event + major hemorrhage (IS / SEE / MH) was lowest.

From Japanese papers, the optimal range of PT- INR was estimated to be 1.5 to 2.2. In the Cochrane review, the optimal range was stated as 2.0-3.0. However, the target range of PT- INR of the report (BAATAF study) [16] which showed the lowest incidence of IS/SEE/MH was 1.5 to 2.7. In this study PT-INRs of the warfarin group were in the target range 83% of the time. In a retrospective cohort study by Hylek et al., the lowest incidence of stroke (ischemia + bleeding) was between 2.0 and 2.5 [23].

In addition, the weighted average of the annual rate of major bleeding in RCTs in which target PT-INR was mild was 1.1 % per year [16,19]. In particular, in a study [16] in which targeted PT-INR was 1.5 to 2.7 and 83% of the time was within the range, annual rate of major bleeding was the lowest (0.86%). On the other hand, the weighted average of the annual rate of major bleeding in RCTs in which target PT-INRs were in the rage of 2.0 to 3.0 or

higher [15,17,18] and the RE-LY study [24], the annual rate of major bleeding was 3.2%.

This shows that not only in Japan but also in Europe and the United States, where the target PT-INR is in the range of 2.0 to 3.0, there is a great risk of bleeding. In the RCT using the warfarin group with the target PT-INR range of 2.0 to 3.0 as the control group, the risk of major bleeding or cerebral hemorrhage was **about 3 times higher** than that of the warfarin group with the milder target PT-INR range.

Therefore, it is considered that the RCT with 2.0 to 3.0 as the target PT-INR range of warfarin use will definitely give an advantageous result for DOAC. Moreover, since the PROBE method was used in the RE-LY study, the warfarin group was poorly controlled, and the trial could be discontinued should there be any signs of bleeding in the dabigatran group, which is further advantageous for the DOAC group.

Meta-analysis results "DOAC is better" is misleading

The results of a meta-analysis of RCTs on DOAC comparing with warfarin published in 2014 [25] and the results of a systematic review and meta-analysis of Cochrane [26,27] also conclude that DOAC is better than warfarin. These results are also introduced in Japanese review articles [28,29].

There are 2 reports on meta-analysis of observational studies comparing DOAC with warfarin [30,31]. Both focused solely on bleeding, and concluded that among DOACs, apixaban and dabigatran had less risk of bleeding than warfarin, and no difference with rivaroxaban. We will examine whether this information is true or not, because it contradicts with our previous meta-analysis.

There are 3 points to be considered.

(1) Were the studies conducted in a fair manner ?

(2) Were ischemic stroke and total mortality reduced in addition to bleeding?

(3) Target PT-INR range of 2.0 to 3.0 is too high and induces more bleeding.

Results changed by excluding apixaban RCT

The Phase 3 RCT that was the basis for the approval of apixaban (Bristol Myers Squibb and Pfizer) is the ARISTOTLE trial. There is a detailed report [32] which analysed the US FDA's report on the ARISTOTLE trial, including any violations such as data falsification or false information reporting, protocol violations, violation of record collection, adverse event reporting and safety assessments.

From their reanalysis of the 22 meta-analyses, they found that 32 of 99 analyses (32%) yielded results that would change the conclusions of the initial analysis (Table1). Of the 32 affected estimates, 31 (97%) no longer favored apixaban for the prevention of serious medical issues, and 1 (3%) favored the control. [33]

As shown in Table 1, our reanalysis results of the direct Xa factor inhibitor RCTs from the Cochrane's review excluding the ARISTOTLE study show that the assessment of DOAC in ischemic stroke/systemic embolism, cerebral hemorrhage and all strokes (ischemic stroke + hemorrhagic stroke) changed from "significantly effective" to "no significant difference"..

Table 1: Impact of exclusion of ARISTOTLE (from RCTs reviewed by Cochrane)

Diseases/Death	Fixed/ randome	Including ARISTOTLE		Excluding ARISTOTLE		Interpretation
		OR (95%CI)	p value	OR (95%CI)	p value	
Ischemic stroke (IS)	Fixed	1.03 (0.92, 1.14)	0.640	1.07 (0.95, 1.21)	0.301	NS:→NS, slightly increased
	Random	0.94 (0.77, 1.14)	0.498	0.94 (0.73, 1.20)	0.604	NS:→NS no change
IS+ systemic embolism	Fixed	0.87 (0.78, 0.97)	0.011	0.93 (0.84, 1.03)	0.158	favour DOAC → NS
	Random	0.87 (0.78, 0.98)	0.020	0.93 (0.84, 1.03)	0.152	favour DOAC → NS
Hemorrhagic stroke	Fixed	0.50 (0.42-0.59)	<0.0001	0.54 (0.44, 0.65)	<0.0001	favour DOAC→favour DOAC
	Random	0.57 (0.40, 0.82)	0.002	0.69 (0.42, 1.14)	0.1462	favour DOAC → NS
All stroke (Ischemic +hemorrhagic)	Fixed	0.86 (0.73, 0.98)	0.012	0.93 (0.83, 1.03)	0.169	favour DOAC → NS
	Random	0.86 (0.73, 0.98)	0.024	0.93 (0.83, 1.03)	0.162	favour DOAC → NS
Major hemorrhage (mainly gastrointestinal and intracranial hemorrhage)	Fixed	0.78 (0.73, 0.84)	<0.0001	0.82 (0.75, 0.89)	<0.0001	favour DOAC→favour DOAC
	Random	0.88 (0.66, 1.17)	0.373	0.92 (0.64, 1.34)	0.681	NS:→NS, slightly increased
Death from any cause	Fixed	0.89 (0.83, 0.95)	0.0002	0.89 (0.82, 0.96)	0.002	favour DOAC→favour DOAC *a
	Random	0.89 (0.83, 0.94)	0.0002	0.89 (0.82, 0.96)	0.002	

OR: Odds ratio, 95% CI: 95% confidence interval, NS: not significant, DOAC: Direct oral anticoagulant.

*a: In the edoxaban trials, the total mortality was greatly lower than that in the warfarin group, so this may have affected the overall results.

No observational study shows superiority of DOAC

Two meta-analysis reports of observational studies comparing DOAC with warfarin [30,31] focused only on bleeding. Moreover, many of the reports collected and analyzed were those that reported bleeding events after short-term observation and were funded by apixaban manufacturers (Bristol Myers Squibb and Pfizer). Therefore, we extracted 12 cohorts from 6 reports [34-39] and analyzed them after excluding those that reported only bleeding, those that followed-up for less than 6 months, and those that were directly funded by pharmaceutical companies. As a result of comparing the DOAC group and the warfarin group, there was no significant difference in ischemic stroke / systemic embolism, ischemic stroke, and total mortality while the incidence of gastrointestinal hemorrhage was significantly higher in DOAC, and the incidence of hemorrhagic stroke (subarachnoidal hemorrhage and cerebral hemorrhage) was significantly lower in DOAC (**Table 2**).

Especially in the elderly aged 80 years and over (or 85 years and over), DOACs significantly increased gastrointestinal bleeding by 2-fold and total mortality by 24% compared with warfarin group (**Table 3**).

There may be 2 major reasons why hemorrhagic stroke was less common in the DOAC group. One is that gastrointestinal bleeding is generally more frequent than hemorrhagic stroke, so it is possible that gastrointestinal bleeding occurred earlier and hemorrhagic stroke was apparently less while using DOAC.

Secondly, as we already pointed out several times in this paper, hemorrhagic events occur about **3 times more frequently** in the warfarin group if the target PT-INR is set at the range of 2.0 to 3.0 than milder target such as 1.5 to 2.7 as in the BAATAF trial.

Table 2: Meta-analysis results of observational studies.

	Fixed/ randome	Odds ratio (95%confidence interval)	p value	Interoretation
Ischemic stroke (IS)	Fixed	0.99 (0.93, 1.05)	0.656	no difference
	Random	1.00 (0.90, 1.12)	0.995	
IS+ systemic embolism	Fixed	0.93(0.86, 1.003)	0.061	no difference
	Random	0.93 (0.77, 1.11)	0.413	
Major hemorrhage (mainly gastrointestinal and intracranial hemorrhage)	Fixed	0.88 (0.84, 0.92)	<0.0001	significant reducion in DOAC group
	Random	0.83 (0.74, 0.95)	0.005	
Gastrointestinal hemorrhage	Fixed	1.23 (1.13, 1.33)	<0.0001	significant increase in DOAC group
	Random	1.17 (1.02, 1.36)	0.026	
Hemorrhagic stroke	Fixed	0.50 (0.44, 0.56)	<0.0001	significant reduction in DOAC group
	Random	0.51 (0.42, 0.61)	<0.0001	
Death from any cause	Fixed	1.00 (0.96, 1.04)	0.914	no difference
	Random	0.81 (0.65, 1.01)	0.061	

DOAC might have been discontinued at the time when gastrointestinal hemorrhage was noticed. This may be the reason why hemorrhagic stroke was apparently reduced in the DOAC group. Moreover, if warfarin was used with the targeted PT-INR range of 1.5 to 2.2 instead of 2.0 to 3.0, hemorrhagic events might have occurred much less frequently, without increasing ischemic stroke,systemic embolism and deaths from all causes.

Table 3: Meta-analysis results of elderly subgroup (≧ 80 or 85)

	Fixed/ randome	Odds ratio (95%confidence interval)	p value	Interpretation
Ischemic stroke (IS)	Fixed	0.97 (0.86, 1.09)	0.602	no difference
	Random	0.96 (0.72, 1.29)	0.807	
Gastrointestinal hemorrhage	Fixed	1.98 (1.60, 2.45)	<0.0001	significant 2-fold increase in DOAC
	Random	1.91 (1.38, 2.64)	0.0001	
intracranial hemorrhage	Fixed	0.56 (0.42, 0.76)	0.0001	significant reduction in DOAC
	Random	0.58 (0.36, 0.91)	0.018	
Death from any cause	Fixed	1.24 (1.16, 1.32)	<0.0001	No difference by random effect model is due to the results of small size study. Fixed effect is appropriate. * a
	Random	1.15 (0.92, 1.42)	0.215	

Gastrointestinal bleeding is common among people aged 80 to 85 and over, leading to an increase in total mortality. Controlling PR-INR at the range of 1.5 to 2.2 with warfarin treatment may further reduce hemorrhage such as gastrointestinal bleeding. *a: See reference [40]

DOAC is only harmful to antiphospholipid antibody syndrome

As mentioned in another article in this issue **[41]**, DOAC increases thrombosis and even bleeding in antiphospholipid antibody syndrome.

In practice

No randomized controlled trials nor observational studies with no bias or low risk of bias have shown evidence that DOAC prevents ischemic stroke and reduces hemorrhagic events more than warfarin. Because the RCTs based on which apixaban was approved have falsified data, by excluding such trials, evidence for the efficacy and safety are greatly changed. Not only the Japanese but also the European Guidelines which recommend DOAC over warfarin are not reliable. For the prevention of ischemic stroke in patients with non-valvular atrial fibrillation, we strongly recommend treatment with warfarin with target PT-INR range of 1.5 to 2.2 in order to minimize the harm of hemorrhage and prevent ischemic stroke.

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Review

DOAC may induce more thrombosis than warfarin in patients without APS

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Med Check Editorial Team

Summary

- Randomized controlled trials (RCTs) and observational studies have confirmed that direct oral anticoagulants (DOAC) increase thrombosis, especially arterial thrombosis, in patients with antiphospholipid antibody syndrome (APS).
- We examined whether this also applies to the evaluation of DOAC when used for non-valvular atrial fibrillation (NVAF).
- A living body works with a well balanced excitatory and inhibitory systems, and receptors also have subtypes of excitatory and inhibitory systems. A selective agonist or antagonist of excitatory system likely to cause harm by disrupting the balance.
- Blood coagulation function has a coagulation system as excitatory system and an anticoagulation system as inhibitory system. In the early stage of bleeding, positive feedback promotes coagulation, and as coagulation progresses, negative feedback ends coagulation. DOAC is a selective inhibitor of Xa or thrombin, namely the selective inhibitor of the excitatory system, while warfarin inhibits both the excitatory system (Factors II, X, VII and IX in coagulation pathways) and the inhibitory system (protein C pathway).
- Strong platelet aggregation effect of DOAC has been shown in animal experiments. This is suspected to be associated with selective inhibitory action of DOAC.
- Antiphospholipid antibodies promote coagulation. Individuals with any antiphospholipid antibodies are also present at a substantially high rate in the general population, and higher in the elderly or those with heart diseases. Therefore, even if APS is not diagnosed, patients with NVAF, which is common in the elderly, may be prone to have thrombosis like those with APS.

Keywords:

antiphospholipid antibody syndrome (APS), rivaroxaban, warfarin, non-valvular atrial fibrillation, coagulation, anticoagulation, protein C, feedback, platelet aggregation

Introduction

It has been reported that when DOAC is used for antiphospholipid antibody syndrome (APS), the risk of thrombosis is 7.4 times higher than that of warfarin [1] in RCT and 12.1 times higher [2] in observational studies, mainly for arterial thrombosis such as ischemic stroke and myocardial infarction.

Considering the evidence, DOACs are not recommended in patients with antiphospholipid syndrome, particularly high-risk patients (those who test positive for all 3 antiphospholipid tests--lupus anticoagulant, anticardiolipin antibodies, and anti-beta 2 glycoprotein I antibodies) in many countries including UK [3].

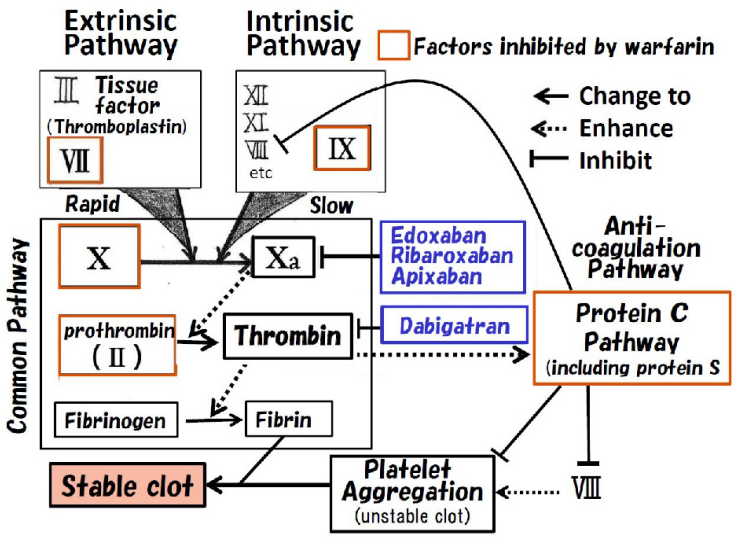
However, none of the guidelines for the treatment of non-valvular atrial fibrillation in many countries

,including in Japan, change the indication of DOAC in prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation with one or more risk factors [4].

Considering that the risk of arterial thrombosis is extremely high (about 10 times higher), the onset of thrombosis in APS is mainly related to pharmacological action rather than pharmacokinetics.

Therefore, we investigated whether DOAC might increase thrombosis compared with warfarin in indications other than APS patients.

Figure 1: Differences in action between warfarin and DOAC



(same as Figure1 in P.55)

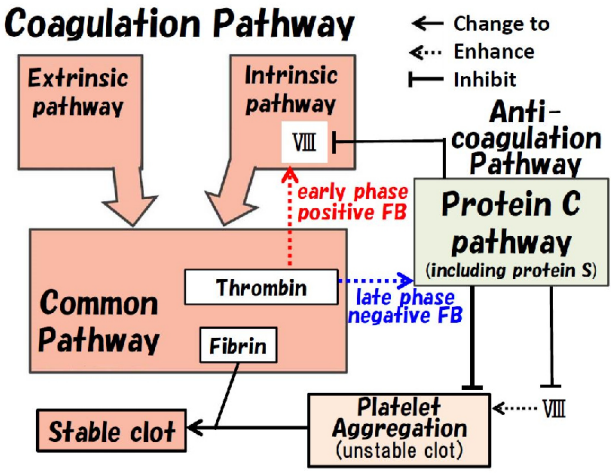
Selectivity of drug action and feedback function

A living body works with a well balanced excitatory system (catecholamine, serotonin, etc., or sympathetic nervous system) and inhibitory system (GABA, etc., or parasympathetic nervous system). For example, catecholamine predominates in emergencies, but GABA is also secreted to reduce overexcitement to prevent excitotoxicity.

Also, when a substance acts on a receptor in vivo, another subtype of receptor usually suppresses the main action so that it does not work too strongly.

A selective agonist or antagonist of excitatory system do not act on the receptors of the inhibitory system, so the balance of action may be lost and the harm may increase. This may be the major reason why angiotensin receptor blockers (ARB) are more harmful than angiotensin converting enzyme inhibitors.

Figure 2: Positive and negative feedback of hemostatic function



FB: feedback, The hemostatic / coagulation function consists of the interaction between the coagulation pathways (left) and the anticoagulation pathway (right). Initially, the resulting thrombin promotes coagulation with positive feedback (omitted in the figure), and as coagulation progresses, thrombin stimulates the protein C pathway (anticoagulation pathway) to suppress VIII, VII, and V, and also suppresses platelet aggregation (negative feedback).

Positive and negative feedback in coagulation and anticoagulation pathway

In the blood coagulation function, a coagulation pathways is the excitatory system. On the other hand, an anticoagulant pathway is the inhibitory system that works to control not to coagulate too much. Hemostasis can be completed normally by both pathways working in a well-balanced manner when necessary (Figure. 1 and Figure. 2).

In the early stages of bleeding, thrombin mainly activates factors VIII and V, and provides positive feedback to promote the intrinsic pathway.

As hemostasis progresses, when thrombin is sufficiently produced, the anticoagulant protein C pathway is activated and the aggregation of factors VIII and V, and platelets is suppressed. This is a function of negative feedback [5,6].

When it is necessary to stop bleeding rapidly, positive feedback promotes hemostasis, and when hemostasis progresses, negative feedback ends coagulation.

Differences in action between DOAC and warfarin

Let's confirm the sites of action of DOAC and warfarin (Figure. 1 and Figure. 2).

Of the DOACs, Xa inhibitor such as rivaroxaban, apixaban and edoxaban inhibits only the factor Xa which produces the final product, thrombin. Dabigatran inhibits only thrombin. Both are selective inhibitors only for the excitatory system that do not inhibit the anticoagulant pathway.

On the other hand, warfarin inhibits some factors of coagulation pathway (excitatory system), such as factors II and X (in common pathway), VII (in extrinsic pathway), and IX (in intrinsic pathway), as well as protein C pathway which plays pivotal role in anticoagulant pathway (inhibitory system). In other words, warfarin is a non-selective inhibitor.

Proposed mechanisms of thrombosis by APS

Antiphospholipid syndrome (APS) is defined by clinical manifestations that include thrombosis and/or fetal loss or pregnancy morbidity in patients with antiphospholipid antibodies (aPL). Antiphospholipid antibodies are directed primarily toward phospholipid binding proteins rather than phospholipid per se, with the most common antigenic target being β2-glycoprotein I (β2GPI). Laboratory diagnosis of aPL depends upon the detection of a lupus anticoagulant (LA), which prolongs phospholipid dependent anticoagulation tests, and/or anticardiolipin and anti-β2-glycoprotein I antibodies [7].

Anti-β2GPI antibodies are central to the pathogenesis of APS, and recognize β2GPI bound to the surface of endothelial cells, monocytes, and immobilized platelets, in some cases leading to cellular activation and expression of procoagulant activity. Other mechanisms by which aPL have been proposed to effect a hypercoagulable state include inhibition of the anticoagulant activity of protein C and S, inhibition of the ability of β2GPI to inhibit von Willebrand factor-dependent platelet aggregation, and in animal models causes tissue factor and complement-mediated neutrophil activation etc [7].

Inhibiting thrombin and factor Xa

DOAC does not suppress extrinsic or intrinsic pathways, but it eventually inhibits thrombin, so coagulation to the common pathway is suppressed. However, when thrombin is suppressed by DOAC, the protein C pathway is not activated indefinitely, so various factors that are normally suppressed (particularly factor VIII) are not suppressed, and platelet aggregation promoted by factor VIII is not suppressed. This is probably the number one reason why DOACs increase thrombosis with or without a thrombus predisposition such as APS or protein C deficiency.

DOAC strongly aggregates platelets

DOAC caused frequent arterial thrombosis especially in APS patients [1]. Experiments have been conducted using normal mice to ligate blood vessels or injure the inner surface of vessels and compare the effects of dabigatran, warfarin and control on on the formation of arterial thrombi, mainly thrombi caused by platelets t [8].

No difference was observed between warfarin the control, but dabigatran enhanced platelet aggregation, arterial thrombus formation and stabilization [8]. The result of the experiment reportedly coincides with the discussion in the previous section that DOAC can enhance platelet aggregation with or without thrombus predisposition.

On the other hand, as mentioned at the beginning, warfarin inhibits the extrinsic, intrinsic, common, and anticoagulant pathways of coagulation in a well-balanced manner, and also suppresses platelet aggregation. Because of this, it can be thought that excessive thrombus formation is avoided with warfarin.

Patients with non-valvular atrial fibrillation also has a risk of thrombosis.

DOAC is likely to cause platelet aggregation with or without predisposition of thrombosis. Moreover, even without positive for all 3 antiphospholipid antibody tests, just positive for only one of them increase a risk of thrombosis [9]. APS is diagnosed if you have some antiphospholipid antibody and have arterial or venous thrombosis, thrombocytopenia, recurrent miscarriage, etc. However if you have antiphospholipid antibody but have not developed thrombosis, you are not diagnosed with APS.

In northern Italy, some antiphospholipid antibody is detected in 15.1% of the general population, and it is more frequent in older people, especially if they have heart disease [10]. Therefore, the percentage of potential

antiphospholipid antibody-positive individuals should be higher in patients with non-valvular atrial fibrillation than in the general population, and they are more likely to develop thrombosis with DOAC.

Conclusions

Even if APS is not diagnosed, many elderly patients with non-valvular atrial fibrilation are presumed to be potentially antiphospholipid antibody-positive, and are prone to form thrombi like patients with APS. Therefore, the use of DOAC is inappropriate in patients with non-valvular atrial fibrilation.

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COVID-19 Vaccine Candidates: Efficacy and Safety

Is frequent neurotoxicity related to new technologies?

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Med Check Editorial Team

Summary

●There are over 200 vaccine candidates against COVID-19 under development, including those produced by new technologies, and several phase III trials are now ongoing.

●In an infection experiment with animals including macaques, infection was not well prevented, while antibody was produced in the blood, largely preventing aggravation. However, long-term efficacy is unknown.

●Only up to phase II trials have been published by Oct. 20, 2020 in which only increase in circulating antibody and adverse events were examined for 2 months. It is totally unknown whether the vaccine candidates actually prevent infection or aggravation, and increased antibody is sustained over a long time.

●In a clinical trial for one vaccine candidate, 2 cases of transverse myelitis or multiple sclerosis were reported. Neurotoxicity is often associated with vaccines, but in this trial, extremely high incidence, 120/100,000 person-years, was observed. It is highly likely that the new technology for vaccine production is closely associated with the neuropathy, and thus causal relation should be greatly suspected.

Conclusion: There is no evidence for preventive effect yet, but high incidence of neuropathy. Stringent monitoring on safety and efficacy is essential.

Keywords:

subunit, inactivation, RNA, DNA, viral vector, vaccine candidate, adjuvant, transverse myelitis, multiple sclerosis

Introduction

Vaccine candidates against COVID-19 are developed at an unprecedented speed. According to the recent information from London School of Hygiene and Tropical Medicine (as of Oct. 12), currently 248 vaccine candidates are under development and 49 clinical trials are ongoing in the world [1].

The biggest characteristic of the vaccine and vaccine candidates under development is that many are produced by new technologies. One of them is to use viral vector against Ebola virus [2,3], which is approved only tentatively for human. The other technologies use RNA or DNA, which is approved for veterinary medicine, but not for preventive vaccines against infection in human.

Technologies for producing antigen for COVID-19 vaccine candidates are roughly classified into 4 traditional and 3 new ways. This article explains about these 7 technologies and status of development, and examine their safety.

Vaccine and vaccine candidates (number of vaccine or candidate as of Dec.17, 2020)

Traditional technologies

① **Using live attenuated virus** (4 candidates)

② **Using inactivated and non-proliferative whole virus particle** (18 candidates, 1 approved)

③ **Using virus-like particle** which is composed of proteins synthesized by gene recombination with no genetic materials (19 candidates)

④ **Using proteins (subunits),** which are believed to play the most crucial role in infection and aggravation. They are produced through genetic engineering and are used as antigens (80 candidates). As SARS-CoV-2 infects human cells by binding its spike to receptor ACE2, a part of the spike or the whole spike is used as antigen.

Note 1: In humans, protein is usually produced based on a blueprint, mRNA, which is made by translating genetic information in DNA. However, as genes in SARS-CoV-2 is RNA, for DNA vaccines, fragments of DNA is produced by reverse translation (reverse transcription) of single-stranded RNA to double-stranded RNA.

- Protein production in humans: DNA → mRNA → protein
- Protein production in SARS-CoV-2 : (m)RNA → protein
- RNA method: mRNA in lipid nanoparticle → protein
- DNA method: mRNA → DNA → mRNA → protein
- Viral vector method: mRNA in virus → protein

New technologies

There are roughly 3 new technologies. In all of them, genetic material (RNA or DNA) is inserted into the human body to synthesize the whole spike protein or a main part of the spike protein. The difference among the three is as follows.

① Using RNA itself

For RNA vaccine candidates (35 candidates, 1 approved), messenger RNA (mRNA) (Note 1), which carries genetic information of the virus, is used. If naked mRNA is injected into the body, it is degraded by ribonuclease (RNase, an enzyme which breaks RNA). In order to prevent it, mRNA is encapsulated in lipid nanoparticle and injected. Lipid nanoparticle and RNA itself act as adjuvants (see Harm of adjuvants).

② DNA vaccine candidates (23 candidates)

Transcription (or one kind of reverse translation) of RNA of viral protein to DNA is performed by reverse transcriptase. Plasmid DNA (circular DNA) encoding the spike gene under a mammalian promoter is injected to the human body. A target protein is synthesized in the human cell when mRNA is translated from the DNA.

③ Viral vector vaccine candidates (non-replicating viral vector: 34 candidate and 1 approved, replicating viral vector: 23 candidates)

Genetic information (RNA) of target protein is inserted to virus with low toxicity (non-replicating or replicating), such as adenovirus. It acts as a vector when it is infected to the body to synthesize target protein in the human cells.

Findings from animal experiments

In animal experiments, vaccine candidates for human are injected to mice or macaque, and increase in IgG level, neutralizing antibody in the blood, is mainly studied. Each animal is infected with SARS-CoV-2 about 4 weeks post inoculation, and viral loads of SARS-CoV-2 in nasal swab and broncho-alveolar lavage (BAL) fluid were measured. Animals were dissected 1 week post infection for pathological examination of the lungs and were compared with the control (saline, vehicle or other vaccine candidates).

Since SARS-CoV-2 enters the body and infects through the nose, IgA antibody (Note 2) at nasal and oral mucosa is supposedly essential for preventing infection. However for most vaccine candidates, only IgG antibody (Note 2) in the blood was measured in animal infection experiments, but not secretory IgA antibody at nasal mucosa.

For example, in a viral vector vaccine candidate developed by the University of Oxford and AstraZeneca [5], almost no difference in viral load in the nose and pharynx was found between the vaccinated and control macaques, while viral load in BAL fluid was reduced, and pneumonia improved significantly at 7 days post exposure. Animals were infected with the virus 4 weeks after vaccination, which is the time when antibody titer reached its peak. No animal study confirmed how long antibody titer is sustained after inoculation.

For a vaccine candidate developed by Can Sino, China [6], an animal experiment was conducted, in which mice and ferrets were infected with the virus after they received the candidate not only by the intramuscular route, but also by the oral and intranasal routes. Intramuscular inoculation suppressed viral replication in the lungs, but not adequately in the nose. Intranasal and oral administration controlled virus almost totally in nose swabs and the lungs especially of ferrets. In this study, symptom scores and pathological changes in the

lungs were not reported, and secretory IgA antibody in the nose was not measured.

The authors discuss that intranasal administration should be considered for human clinical trial. However, in the phase I and phase II trials (Note 3), intramuscular administration was used, implying that there was some kind of inconvenience with intranasal administration.

Moreover, in most animal experiments, animals were infected with the virus at about 4 weeks post inoculation, and none of them has confirmed the long-term effect. From these experiments, it is unknown how long neutralizing antibody persists.

Note 2: Immunity in the body includes innate immunity, cell-mediated immunity, in which lymphocytes (T cells) kill virus-infected cells, and humoral immunity, in which antibodies developed in the mucosa and blood protect against infection. Antibodies are made of protein called immunoglobulin (Ig) and are produced by one of the lymphocytes, B cells. In infectious diseases, immunoglobulin A (IgA), G (IgG) and M (IgM) are mainly produced. IgA is mainly secreted on the surface of the mucosa in the nose, bronchus, mouth and gastrointestine, and prevents viral invasion. IgM increases in the blood at the beginning of infection and decreases when infection is cured. IgG neutralizes virus in the blood. The frequently used term "neutralizing antibody" refers to an antibody that neutralizes (inactivates) virus in the blood, mainly IgG, and does not mean IgA antibody secreted in the mucosa.

Note 3: For treatment clinical trials, phase I trials involve healthy volunteers, while phase II trials enroll a small number (about 10 to 100) of patients, with illness in order to roughly estimate efficacy and safety. Phase III trials include a large number of participants with illness (about 100 to 1,000 patients) to confirm efficacy and safety. On the other hand, for clinical trials of vaccine candidates, from phases I to III, all the participants are healthy. Phase I and II trials of COVID-19 vaccine candidates enroll several dozens and several hundreds of healthy volunteers, respectively. Phase III trials involved thousands to tens of thousands of healthy volunteers to examine whether the substance would actually prevent infection, and a rare but severe adverse reaction would occur.

No evidence for preventive effect in clinical trials

Currently (as of Oct. 12, 2020), 39 phase I and II trials and 10 phase III trials are ongoing. Total 3 large-scale phase III trials are conducted, all of which with viral vectors. According to the COVID-19 vaccine tracker (https://covid19.trackvaccines.org/vaccines/), Phase III clinical trials are ongoing as of Dec. 17, 2020: 2 subunit, 1 VLP, 1 DNA, 2 RNA, 5 viral vector, 4 inactivated.

Of the 3 large-scale phase III trials, the first one is ChAdOx1 nCoV-19 developed by the University of Oxford and AstraZeneca. The trial started on August 17th in the U.S., involving 30,000 participants [9]. The second is Ad26.CoV.S developed by Janssen in the U.S [10]. A phase III trial has been conducted since September 7th in the U.S., Brazil and other Latin American countries, enrolling 60,000 participants. The third is Ad5-nCoV developed by Can Sino in China [11]. The trial has been conducted since September 15th in Pakistan, involving 40,000 participants.

As of October 12th, results from only phase I and II trials have been published, but not yet from phase III trials.

Outcomes reported in phase I and II trials include IgG antibody titer for up to about 2 months, increase in neutralizing antibody and adverse events. No trial has reported whether vaccine candidate protects against infection or mitigates aggravation.

In the protocol of the phase I/II trials by the University of Oxford and AstraZeneca [9], "vaccine efficacy rate" is included as an outcome, but it is not reported in the study. In this study, IgG antibody titer reached peak at day 28 after the first inoculation, and it was lower at day 56. In participants who received the second dose at day 28, slightly increased IgG antibody titer was observed, but it again went down at day 56 (4 weeks after the second inoculation). It is quite doubtful that IgG antibody persists in the blood.

Incidence rates of COVID-19 and changes in antibody titer should be confirmed over a half year to one year without waiting for results of phase III trials. Secretory IgA antibody at the nasal mucosa should be measured as well.

Multiple sclerosis and transverse myelitis

In the phase I/II trials by the University of Oxford and AstraZeneca [12], headache was reported in 40% participants who were pretreated with paracetamol and 60% participants without paracetamol pretreatment in the vaccine candidate group.

It should be noted that transverse myelitis was reported in 2 participants (one each in July and in September). The affected participant who was reported in July was later diagnosed with multiple sclerosis [13]. The New York Times reported AstraZeneca's comment that the volunteer was later determined to have "a previously undiagnosed case of multiple sclerosis, unrelated to the vaccine" [14]. However, this is questionable.

Multiple sclerosis is a chronic inflammatory demyelinating disease (Note 4) of central nervous system (CNS). It is characterized by multiple lesions with evidence for dissemination in time and disemination in space (Note 5). If there is a demyelination in only a single part, it is not diagnosed as multiple sclerosis.

If the participant has had a demyelinating disease of CNS before vaccination and transverse myelitis occurred after vaccination, this fulfills a diagnostic criteria for multiple sclerosis. This contradicts with the fact that the participant was diagnosed with "transverse myelitis" after vaccination, but not with multiple sclerosis. Therefore, it is highly likely that transverse myelitis occurred after vaccination, followed by demyelinating disorders in other parts of CNS, and this lead to the diagnosis as multiple sclerosis. Or even if transverse myelitis was the second demyelinating disorder, the association with the vaccine candidate should be suspected. In either way, objective assessment is not possible because AstraZeneca has not reported any details about this case.

The New York Times also reported "The condition is rare, but serious, and experts said that finding even one case among thousands of trial participants could be a red flag. Multiple confirmed cases, they said, could be enough to halt AstraZeneca's vaccine bid entirely." . They make reasonable points.

According to the New York Times, as of September 11th, 18,000 participants have received this vaccine candidate [14]. However, considering the time of observation, this is still about 1,600 person-years (Note 6). Two persons per 1,600 person-years is equivalent to about 120/100,000 person-years.

The population of people aged 20-29 in Japan is about 12 million, and their mortality rate from COVID-19 is low (only 2 among 42,246 confirmed COVID-19 patients). If all 12 million received this vaccine candidate, 14,000 of them may develop multiple sclerosis and/or transverse myelitis. Considering that 2 out of 42,246 people infected with COVID-19 have died in this age group, it can be said that the harm far outweighs benefits.

Note 4: Nerve consists of nerve cells and nerve fibers (axon) extending from them. Nerve fibers include myelinated nerves wrapped in sheath called myelin sheath (spiral membrane structure) and unmyelinated nerves without sheath. When autoantibody is developed against myelin sheath, it is melted and nerve fibers become exposed. This is called demyelination, which slows or stops nerve impulses.

Note 5: For example, if firstly, inflammation and demyelination of nerves occur in the spinal cord and secondly, in another time, it occurs at other sites of the central nervous system such as the cerebrum, cerebellum or optic nerve in a patient, it is considered that multiple lesions occureed with evidence for dissemination in time and disemination in space.

Note 6: For calculation of incidence rate, the numerator is the number of cases identified during a specified interval, and the denominator is the number of a defined population. Longer the duration of observation is, greater the number of infected people would become. Therefore, the duration of observation should be taken into account. If 100 persons are observed for 2 years, the denominator should be 100 persons × 2 years=200 person-years. Likewise, if 1,000 persons are observed for a short period, such as 0.2 years, it would also be 200 person-years (1,000 persons × 0.2 years=200 person-years). As trials of COVID-19 vaccine candidates have just started, although 18,000 participants have been enrolled, the duration of observation is still shorter than 0.1 year in average, or only 1,600 person-years.

10-60 times higher incidence

Autoimmune diseases often occur after infection, and also after vaccination, which creates a condition similar to infection.

HPV vaccine is an example of a vaccine with high incidence rate for autoimmune disease, and especially high incidence of neuropathy has been an issue. In a clinical trial for one of the HPV vaccines, Gardasil, female participants around 20 years of age were observed for 2 years (about 40,000 person-years). Total morbidity for multiple sclerosis or optic neuritis was 17/100,000 person-years until the 7th month, and 21/100,000 person-years from the 8th month until the end of the

second year [16,17].

This morbidity is 4-20 times higher than that of women in the same age group who spontaneously develop multiple sclerosis (about 1-5/100,000 person-years).

Total incidence of multiple sclerosis in general population, both men and women in all age groups is about 2-12/100,000 person-years [18]. Therefore, the incidence of multiple sclerosis in the clinical trial of AstraZeneca's COVID-19 vaccine candidate is 10-60 times higher than that of spontaneous multiple sclerosis, suggesting that multiple sclerosis observed in the clinical trial was not spontaneous.

If the vaccine candidate was used for 100 million people in the world, 120,000 people would have transverse myelitis or multiple sclerosis. On the other hand, it is unknown how many serious cases of COVID-19 it would prevent. Without waiting for another case of transverse myelitis, it can be assumed that harm outweighs benefits.

Harm of adjuvants

1) Adjuvants essentially cause tissue damage

In order to produce immunity to prevent infection and aggravation by pathogen, "antigen" is not enough, but in addition, adjuvants are often needed to enhance immunity.

In spontaneous infection, bacteria have strong toxicity and enter the human body by damaging the cells. In viral infection, infected cells are destroyed by cytotoxic T cells and leucocytes which treat them are destroyed. Then intranuclear DNA and RNA are released and bind with protein to act as an adjuvant and enhance immunity.

In vaccines, in order for aluminum (Alum) to act as an adjuvant and enhance immunity, aluminum particles must first injure tissues. Then leucocytes are gathered to treat them and breaks, and DNA and RNA are released to bind with protein forming DNA- or RNA-protein complex. This becomes a stable foreign object to be an actual adjuvant [19,20]. In other words, at first, adjuvants must injure tissues.

2) RNA and DNA are strong and direct adjuvants.

Cervarix contains monophosphoryl lipid A (MPL), a derivative of lipid A (major toxic component of lipopolysaccharide) from Salmonella endotoxin and alum. Gardasil contains "AAHS (amorphous aluminum hydroxyphosphate sulphate)" as adjuvant. It also contains DNA and RNA as impurities which are stabilized and protected from endogenous nuclease digestion by binding to the AAHS [20].

For inactivated vaccine candidates and subunit vaccine candidates, traditional adjuvants, such as Alum adjuvants (aluminum hydroxide) and surfactant, are used.

On the other hand, vaccine candidates produced by new technology, such as RNA, DNA or viral vector vaccines, no specific adjuvant is mentioned. This is because RNA or DNA itself, fragments of vector virus RNA/DNA, and lipid nanoparticles that encapsulate them act as adjuvants [21].

Severe disability caused after receiving HPV vaccine was strongly associated with new adjuvants and residual DNA/RNA. Due to the way vaccine candidates for COVID-19 are manufactured, it is highly likely that unavoidable alien substances, such as DNA, RNA, virus, lipid nanoparticles, would exhibit unknown toxicity as adjuvants. In order not to repeat the tragedy of HPV vaccine, careful toxicity study with animals should be conducted and stringent monitoring is essential.

Other harms

1) Antibody-dependent enhancement (ADE)

A typical case of ADE has been reported with Dengue fever. In Dengue fever, when a person is infected with a certain strain of Dengue virus, she/he would develop antibody. However, if she/he is secondly infected with different strain of Dengue virus, she/he might develop severe symptoms of the disease.

When vaccinated persons are infected with Dengue virus, they developed severer symptoms than when the unvaccinated persons are infected with the same disease. This is just like experiencing severe symptoms when one is naturally infected with a strain of virus slightly different from the first infection.

For vaccine candidates against SARS-CoV and MERS-CoV, cases of ADE were reported in animal experiments. Because of that, safe and effective vaccine has not yet been developed.

As of now, ADE has not been reported in animal experiments for COVID-19 vaccine candidates [23]. However, rigorous monitoring is needed in clinical trials and in practice.

2) Fragments of bases might have cytotoxicity

In the process of manufacturing vaccine candidates by the new technologies, bases that do not exist naturally might be produced as RNA, DNA or viral vectors are degraded [21]. Just like an anticancer agent, 5-FU, and antivirals, favipiravir (Avigan) and remdesivir (Veklury), these bases are taken into the human cells when RNA is synthesized and might act as cytotoxic agents [21,24]. They might exert mitochondrial toxicity, causing myopathy, lactic acidosis, pancreatitis, liver disorder, such as fatty liver and neuropathy, which can be fatal [21]. Such toxicity remains unknown with vaccine candidates against COVID-19 as they have not been applied to human use. However, it has been confirmed in animal experiments for other substances [21].

In practice

As of now, harm of vaccine candidates against COVID-19 seems to outweigh benefit. Therefore, they should not be used until appropriate information is published and their safety and efficacy are confirmed. Even if you are recommended to receive the vaccination, you should refuse it.

Postscript 1:

Just before the final proof of this article, the media reported that clinical trials for COVID-19 vaccine and antibody drug candidates were paused [25,26]. Janssen (Johnson & Jonson's subsidiary company) paused a phase III clinical trial of its vaccine candidate developed by viral vector technology on October 13 [10]. Then, Eli Lilly decided to pause a clinical trial of its monoclonal antibody drug candidate on October 14th.

For Janssen's vaccine candidate, the phase III trial had just started on September 7th, and was designed to continue until March, 2023, involving 60,000 participants [10], but it was paused after one month. The manufacturer has not reported what kind of illness had been observed. However, there is no doubt that it was a serious adverse reaction associated with the vaccine candidate. Reuters introduced a comment of one expert "If it was something like prostate cancer, uncontrolled diabetes or a heart attack – they wouldn't stop it for any of those reasons. This is likely to be a neurological event." [25] We agree with his comment.

As Janssen's trial was paused one month after it entered the third phase, based on the designed number of years and participants, it has reached only 110 person-years. If one person experienced a severe adverse reaction, this is equivalent to 900/100,000 persons, a high incidence rate. More information should be published.

Postscript 2:

According to the New York times
https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html?auth=login-email&login=email
6 vaccines are approved for limited use and 2 vaccines are approved for full use as of Dec. 18, 2020.

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