This letter was published on May 12, 2021, at NEJM.org.

1. Hawks L, Woolhandler S, McCormick D. COVID-19 in prisons and jails in the United States. JAMA Intern Med 2020;180:1041-2.
2. Snell TL. Capital punishment, 2018 — statistical tables. Washington, DC: Department of Justice, Bureau of Justice Statistics, September 2020 (https://www.bjs.gov/content/pub/pdf/cp18st.pdf).
3. Chin ET, Ryckman T, Prince L, et al. Covid-19 in the California state prison system: an observational study of decarceration, ongoing risks, and risk factors. March 8, 2021 (https://www.medrxiv.org/content/10.1101/2021.03.04.21252942v1). preprint.
4. Gharpure R, Patel A, Link-Gelles R. First-dose COVID-19 vaccination coverage among skilled nursing facility residents and staff. JAMA 2021;325:1670-1.
5. Momplaisir F, Haynes N, Nkwihoreze H, Nelson M, Werner RM, Jemmott J. Understanding drivers of COVID-19 vaccine hesitancy among Blacks. Clin Infect Dis 2021 February 9 (Epub ahead of print).

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PF4 Immunoassays in Vaccine-Induced Thrombotic Thrombocytopenia

TO THE EDITOR: In a recent study, Greinacher et al.[1] reported thrombotic complications, mostly cerebral vein thrombosis, associated with thrombocytopenia in 11 patients after they had been vaccinated with ChAdOx1 nCoV-19 (AstraZeneca). Although none of these patients had received heparin, the authors detected high titers of anti–platelet factor 4 (PF4)–heparin antibodies that strongly activated platelets in vitro without heparin and in the presence of PF4. This syndrome, which resembles autoimmune heparin-induced thrombocytopenia, was called vaccine-induced immune thrombotic thrombocytopenia (VITT), and an algorithm for the management of this syndrome was proposed on the basis of immunoassays detecting anti–PF4–heparin antibodies.

Between March 19 and April 1, 2021, plasma samples from nine patients (median age, 44 years) with suspected VITT after vaccination with ChAdOx1 nCoV-19 were analyzed in our laboratory (Table S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). Cerebral vein thrombosis (in six patients) and splanchnic vein thrombosis (in five patients) were the most common events. All the patients had severe thrombocytopenia (median platelet count nadir, 29,000 per cubic millimeter; range, 9 to 61,000) except for one woman with both cerebral vein thrombosis and splanchnic vein thrombosis. Two rapid immunoassays widely used for the diagnosis of heparin-induced thrombocytopenia (STic Expert HIT and HemosIL AcuStar HIT-IgG) were performed on plasma samples to detect PF4-specific antibodies, and the results were negative in all the patients. Two other rapid tests had been performed in some patients by the referring laboratories and were negative, except in one patient, who had an equivocal result (Fig. 1A).

We also tested all plasma samples with three different PF4-specific enzyme-linked immunosorbent assays and obtained variable results (Fig. 1B). Significant levels of IgG antibodies to PF4 were detected in seven patients only by the assay that used PF4–poly(vinyl sulfonate) (PVS) complex as the antigenic target. In addition, optical density values were variable and lower than those previously reported with a similar test.[2] The diagnosis of VITT was confirmed by PF4–serotonin release assay[3] in all seven patients with IgG antibodies to PF4–PVS (Fig. 1C), whereas a standard serotonin release assay was negative in two patients. Platelet activation was suppressed by IV.3, a monoclonal antibody that binds FcγRIIA receptors, but also by IdeS (IgG-degrading enzyme derived from *Streptococcus pyogenes*) (Fig. 1C), a protease that also inactivates heparin-induced thrombocytopenia IgG antibodies.[4] Intravenous immune globulins may be inappropriate for severe cerebral vein thrombosis with intracranial hypertension. IdeS (imlifidase) may be an effective treatment and needs to be evaluated.

Our results provide further support to show that rapid immunoassays should be avoided in the detection of PF4-specific antibodies in patients with suspected VITT. Therefore, the use of a sensitive, quantitative, immunologic test is strongly recommended, because according to the recently proposed algorithm,[1,5] nonheparin anticoagulants should be preferred when clini-

A

	STic Expert HIT (N=9)	HemosIL AcuStar HIT-IgG (N=9)	ID-PaGIA H/PF4 (N=4)	HemosIL HIT-Ab (N=2)
	no. of patients			
Negative	9	9	3 (◆, ○, ◇)	2 (●, □)
Positive	0	0	1 (✲, but then tested negative)	0

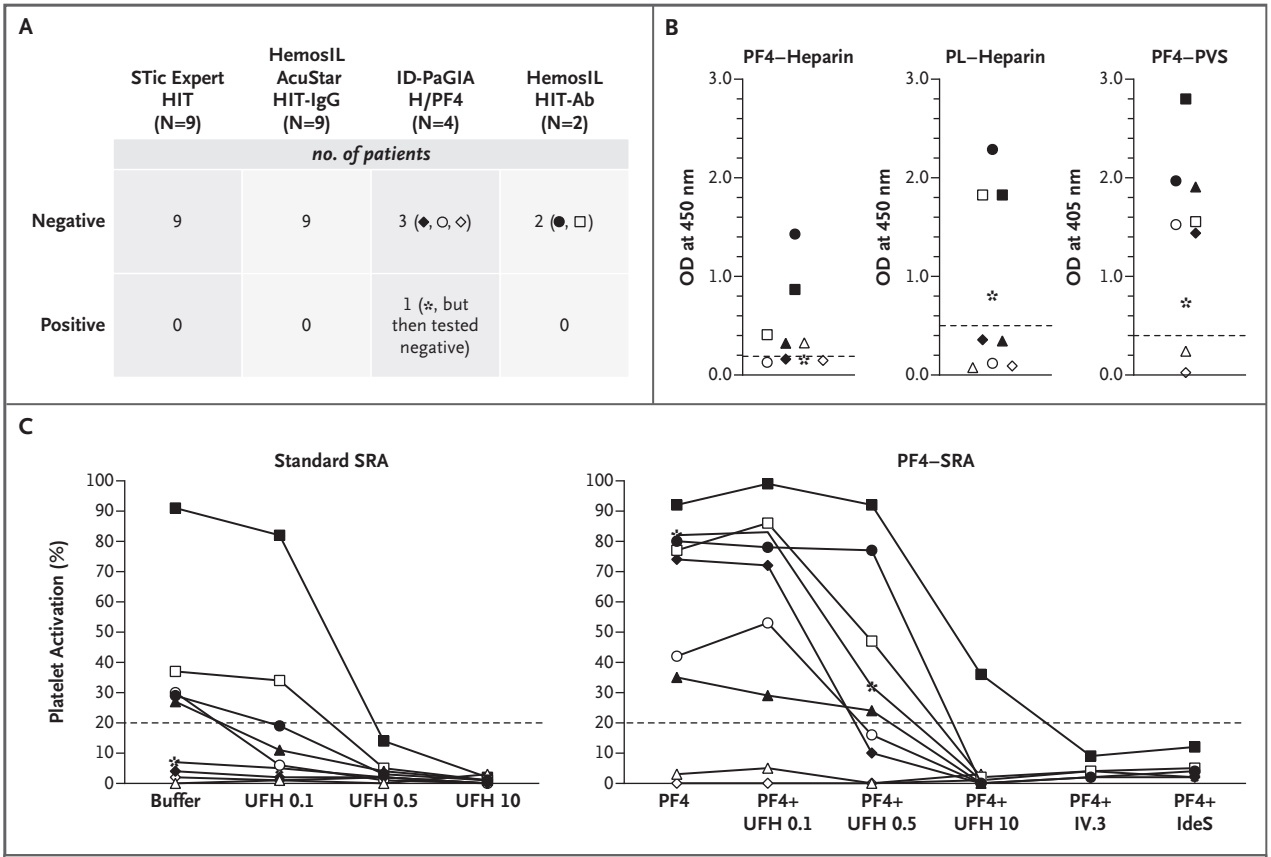

Figure 1. PF4-Specific Immunoassays and SRA in Patients with Suspected VITT.

Panel A shows that rapid immunoassays do not detect antibodies to platelet factor 4 (PF4) associated with vaccine-induced immune thrombotic thrombocytopenia (VITT). All the plasma samples from the patients with findings suggestive of VITT were tested with two rapid immunoassays (STic Expert HIT, Stago, and HemosIL AcuStar HIT-IgG, Werfen), and negative results were obtained in every case. Two other rapid assays had also been performed in some patients (each represented by a specific symbol) in the referring centers (ID-PaGIA H/PF4, DiaMed, and HemosIL HIT-Ab, Werfen), and they also showed negative or doubtful (in patient *, who had an initial positive result but then tested negative) results.

Panel B shows that the sensitivity of enzyme-linked immunosorbent assays (ELISAs) to detect PF4-specific IgG antibodies depends on the antigen target. Levels of PF4-specific IgG antibodies were evaluated in plasma samples from the nine patients with suspected VITT (each represented by a specific symbol) with the use of three different ELISAs with varying antigen targets (PF4–heparin complexes [Asserachrom HPIA, Stago], PF4 released from a platelet lysate [PL] and complexed with heparin [Zymutest HIA IgG, Hyphen], and PF4–poly[vinyl sulfonate] [PVS] complexes [Lifecodes PF4 IgG, Immucor]). OD denotes optical density.

Panel C shows that a serotonin release assay (SRA) should be performed with the use of PF4 to detect platelet-activating antibodies to VITT. SRA was performed by incubating 75 μl of washed platelets obtained from healthy persons with 20 μl of plasma obtained from each patient, either in the absence (standard SRA) or the presence (PF4–SRA) of 10 μg per milliliter of PF4. All tests were performed with or without unfractionated heparin at 0.1 IU per milliliter (UFH 0.1), 0.5 IU per milliliter (UFH 0.5), and 10 IU per milliliter (UFH 10). Clinically significant and strong platelet activation, with maximum release ranging from 36 to 99%, was measured in seven of the nine patients only when PF4 was present in the reaction mixture. Moreover, platelet activation was not inhibited by therapeutic concentrations of UFH 0.1 or UFH 0.5. In contrast, platelet activation was completely abolished by 10 μg per milliliter of IV.3, a monoclonal antibody specific for FcγRIIA, or 6 U of IdeS, an IgG-degrading enzyme of *Streptococcus pyogenes*, preincubated for 15 minutes at 37°C in the plasma sample of each patient tested (five patients) before SRA.

cally significant levels of anti-PF4 antibodies are detected.

Caroline Vayne, Pharm.D.
Jérôme Rollin, Ph.D.
Yves Gruel, M.D.
Claire Pouplard, Pharm.D.
Tours University Hospital
Tours, France
gruel@med.univ-tours.fr

Hubert Galinat, Pharm.D.
Olivier Huet, M.D.

Brest University Hospital
Brest, France

Vincent Mémier, M.D.
Thomas Geeraerts, M.D.

Toulouse University Hospital
Toulouse, France

Raphael Marlu, M.D.
Gilles Pernod, M.D.

Grenoble Alpes University
Grenoble, France

Guillaume Mourey, M.D.

Etablissement Français du Sang
Besançon, France

Alexandra Fournel, M.D.

Besançon University Hospital
Besançon, France

Charlotte Cordonnier, M.D.

Sophie Susen, M.D.

Lille University Hospital
Lille, France

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1. Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination. N Engl J Med 2021;384:2092-101.
2. Schultz NH, Sørvoll IH, Michelsen AE, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. N Engl J Med 2021;384:2124-30.
3. Vayne C, Guery EA, Kizlik-Masson C, et al. Beneficial effect of exogenous platelet factor 4 for detecting pathogenic heparin-induced thrombocytopenia antibodies. Br J Haematol 2017;179:811-9.
4. Kizlik-Masson C, Deveuve Q, Zhou Y, et al. Cleavage of anti-PF4/heparin IgG by a bacterial protease and potential benefit in heparin-induced thrombocytopenia. Blood 2019;133:2427-35.
5. Oldenburg J, Klamroth R, Langer F, et al. Diagnosis and management of vaccine-related thrombosis following AstraZeneca COVID-19 vaccination: guidance statement from the GTH. Hamostaseologie 2021 April 01 (Epub ahead of print).

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Glycemic Index, Glycemic Load, and Cardiovascular Disease and Mortality

TO THE EDITOR: The Prospective Urban Rural Epidemiology (PURE) study investigators (April 8 issue)[1] report that a diet with a high glycemic index and high glycemic load is associated with an increased risk of cardiovascular disease or death, on the basis of an analysis involving more than 137,000 participants from five continents. Their statistical models were adjusted for age, sex, geographic region, study center, urban or rural location, socioeconomic status, education level, physical activity, smoking status, waist-to-hip ratio, history of diabetes, statin use, use of antihypertensive agents, total daily energy intake, and consumption of whole-grain cereals and dietary fiber. We were surprised that neither a family history of premature coronary artery disease or related death[2] nor ethnic group[3] appears to have been factored into their calculations. We note that the risk ratios for death from cardiovascular disease that were estimated in two other large population-based cohorts — from studies by Levitan et al. (risk ratio, 1.23)[4] and Nagata et al. (risk ratio, 1.22),[5] which adjusted for family history and ethnic group, respectively — are substantially lower than that estimated in the PURE study (risk ratio, 1.32). We hope that the researchers would control rigorously for such strong confounders, which can disproportionately inflate the magnitude of the association being studied.

Melvin K. Leow, M.D., Ph.D.

Lee Kong Chian School of Medicine
Singapore, Singapore
melvin_leow@ntu.edu.sg

Christiani J. Henry, Ph.D.

Singapore Institute of Food and Biotechnology Innovation
Singapore, Singapore

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1. Jenkins DJA, Dehghan M, Mente A, et al. Glycemic index, glycemic load, and cardiovascular disease and mortality. N Engl J Med 2021;384:1312-22.
2. Bachmann JM, Willis BL, Ayers CR, Khera A, Berry JD. Association between family history and coronary heart disease death across long-term follow-up in men: the Cooper Center Longitudinal Study. Circulation 2012;125:3092-8.
3. D'Agostino RB Sr, Grundy S, Sullivan LM, Wilson P. Validation of the Framingham coronary heart disease prediction scores: