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Original Article

Influenza Vaccine Type-Dependent Antibody Response in Patients with Autoimmune Inflammatory Rheumatic Diseases

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Abstract

Background: The study aimed to explore influenza antibody response in patients with autoimmune inflammatory rheumatoid diseases (AIIRDs) stratified by the different vaccine types applied in Denmark during the 2018–2019 influenza season.

Methods: Included patients were diagnosed with rheumatoid arthritis, psoriatic arthritis, or spondyloarthritis receiving biological disease-modifying antirheumatic drugs (bDMARDs) with or without conventional synthetic disease-modifying antirheumatic drugs. Influenza vaccination status in the 2018–2019 season and vaccine type received were reviewed in the Denmark. Blood samples were drawn ≥ 14 days post vaccination, and antibody titers were determined by the hemagglutinin inhibition (HAI) assay for the serotypes A/Michigan/H1N1, A/Singapore/H3N2, and B/Colorado included in the influenza vaccines in the 2018–2019 season. An overall serotype HAI geometric mean titer (GMT) was calculated from the 3 serotype-specific HAI titers. An overall serotype HAI GMT ≥ 40 was considered protective.

Results: Of the 205 included patients, 105 (51%) had received influenza vaccination. One-quarter of vaccinated patients achieved post-vaccination overall serotype HAI GMT ≥40. For patients vaccinated with Influvac, a significantly higher proportion had HAI titers ≥ 40 for 2 serotypes, namely, A/Michigan/H1N1 and A/Singapore/H3N2, than patients vaccinated with Vaxigrip or VaxigripTetra. The same applied to all serotypes HAI GMT, where significantly more patients who received Influvac achieved postvaccination HAI GMT≥40 versus patients who received Vaxigrip (p=0.02) or VaxigripTetra (p=0.002). The latter outcome was explored in a multivariable logistic regression analysis and remained significant when including the following variables: age, sex, treatment with methotrexate and/or prednisolone, type of influenza vaccine, time interval from vaccination to antibody measurement, and previous vaccination status.

Conclusion: Influenza antibody levels following vaccination with Influvac in bDMARD-treated patients with AIIRDs were superior to Vaxigrip and VaxigripTetra. Treatment with methotrexate (MTX) did not reduce the antibody response.

Keywords: Rheumatic diseases, biological therapies, methotrexate, influenza vaccines

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Introduction

Patients with rheumatoid arthritis (RA) are at higher risk of acquiring influenza and influenza-associated complications compared to age- and gender-matched controls.[1] In accordance with the European Alliance of Associations for Rheumatology recommendations, annual influenza vaccination should be considered for patients on immunosuppressive treatment with RA or other autoinflammatory rheumatic diseases (AIIRDs).[2] In the 2018–2019 influenza season, the Danish Healthcare System provided influenza vaccination free of charge to patients with chronic diseases and immunocompromised status, including patients treated with biological disease-modifying antirheumatic drugs (bDMARDs) and elderly persons ≥ 65 years of age.[3] Despite recommendations regarding influenza vaccination for patients with AIIRDs, uptake has historically been inadequate.[4] One concern has been that patients with AIIRDs would not benefit from influenza vaccination because immunosuppressive treatments might negatively affect the intended protection from infection. In a population, influenza vaccine effectiveness (VE) can be calculated from data on confirmed influenza cases in addition to information about influenza vaccination status, but often antibody response is used as a surrogate marker of VE.[5] Despite treatment with bDMARDs, most studies have found

that patients with AIIRDs have acceptable rates of antibody response, except when treated with rituximab or abatacept.[6-14] Moreover, there is discrepancy regarding the effect of methotrexate (MTX) treatment on influenza vaccination; 1 study has shown a decreased effect of influenza vaccination and simultaneous MTX treatment,[15] whereas other studies have not detected such an adverse outcome.[16,17]

Due to the frequent change of influenza virus surface antigens, the strains included in the seasonal influenza vaccines are evaluated yearly and possibly replaced to comply with the strains in current circulation. Influenza VE wanes if the circulating strains differ from the strains included in the vaccines.[18] In Denmark, the 2018–2019 influenza season was mainly dominated by the influenza A virus, responsible for 99.7% of registered cases, and of these, 60% of cases were of the influenza A subtype H1N1pdm09 and 40% of the subtype H3N2. The prevalence of influenza B was very low during this season (0.3%). Overall influenza activity, measured by the prevalence of influenza symptoms in the general population as well as microbiologically confirmed influenza cases and influenza-related admissions, was at a moderate level compared to earlier seasons. During the 2018–2019 influenza season, vaccination coverage in Denmark among the older population aged ≥ 65 years was 52%, and the overall VE was estimated to be approximately 30%.[19]

The influenza vaccines applied in Denmark during the 2018/2019 season were Influvac, Vaxigrip, and VaxigripTetra,[3] and all 3 vaccine types belong to the group of egg-derived inactivated influenza virus vaccines (IIVs). Vaxigrip and VaxigripTetra are split-virion vaccines, and Influvac is a subunit vaccine. The differences rely on the manufacturing process regarding purification steps and are described elsewhere.[18] There have not been observed major differences in VE or antibody response between split-virion vaccines and subunit vaccines,[20] but vaccine comparison studies are scarce.

This study aimed to explore the influenza antibody response between the different vaccine types applied in Denmark during the 2018–2019 influenza season with a focus on AIIRD patients receiving biological disease-modifying antirheumatic drugs (bDMARDs).

Main Points

- One-quarter of influenza-vaccinated patients achieved a postvaccination overall serotype hemagglutination inhibition (HAI) of geometric mean titer (GMT) ≥ 40, which is considered to provide adequate protection from influenza.
- Patients receiving Influvac achieve higher antibody responses compared with patients receiving Vaxigrip or VaxigripTetra.
- Treatment with methotrexate (MTX) seems not to affect antibody formation among autoimmune inflammatory rheumatoid disease patients treated with biological disease-modifying antirheumatic drugs.

Material and Methods

This hospital-based study was conducted in the Department of Rheumatology at the North Denmark Regional Hospital. Adult patients aged ≥ 18 years with RA, psoriatic arthritis (PsA), and spondyloarthritis (SpA) receiving bDMARDs, except rituximab, with or without conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), were included in the study. Patients were registered in the Danish Rheumatology database (DANBIO), where all AIIRD patients are registered systematically.

In conjunction with the launch of the annual influenza vaccination campaign in October 2018, patients were advised to receive the seasonal influenza vaccination. For each patient, vaccination status for the season of 2018–2019 between September 1, 2018, and January 1, 2019, and for the previous two seasons in 2016–2017 and 2017–2018, respectively, was reviewed in the Danish Vaccination Register. Clinical data from the patients were collected from the patient medical files, DANBIO and the Danish Electronic Medicine Module.

Influenza Vaccination and Rheumatic Treatment

In the 2018–2019 influenza season, Influvac and Vaxigrip contained 3 virus antigen components: 2 subtypes of influenza A virus H3N2 and H1N1pdm09 (A/Michigan/45/2015(H1N1) pdm09-like virus (A/Michigan/H1N1), A/Singapore/INFIMH-16-0019/2016(H3N2)-like virus (A/Singapore/H3N2), respectively), and 1 influenza B virus [B/Colorado/06/2017-like virus (B/Colorado)]. Also, the tetravalent VaxigripTetra influenza vaccine used for vaccination of a smaller group of the Danish population during the 2018–2019 influenza season contained the mentioned antigens earlier in addition to the influenza B virus antigen (B/Phuket/3073/2013-like virus).[3]

The rheumatic treatment of the patients was unaffected by the vaccination procedure, and patients receiving MTX had been treated for a stable period of time of several months before and after influenza vaccination.

Blood Sampling and Laboratory Analysis

Postvaccination blood samples of vaccinated patients as well as blood samples from unvaccinated patients were collected between November 1, 2018, and March 1, 2019, for the detection of antibodies against influenza vaccine antigens. Because influenza vaccination was not carried out at the Department of Rheumatology, it was not possible to collect a prevaccination blood sample prior to influenza vaccination. Analysis of prevaccination antibodies was possible from previously drawn blood samples collected between June 1, 2017, and May 31, 2018, as a part of a quality assurance study of pneumococcal antibody response after vaccination.[21]

The antibody analyses were conducted at the National Influenza Center, Statens Serum Institut (SSI), Denmark as prescribed by the World Health Organization in the manual for the laboratory diagnosis of influenza.[22] Antibody titers in serum against the antigens included in the trivalent 2018–2019 seasonal influenza vaccine were determined by the hemagglutination inhibition (HAI) assay ≥ 14 days after vaccination. Each blood sample was analyzed in duplicate, and the mean of the antibody titers against each of the 3 antigens was calculated and registered in whole numbers.

Data Protection and Ethical Approval

According to the Danish Health Care Act the project was classified by the Regional Research Committee as a quality assurance study, which does not require any additional ethical assessment or informed consent according to Danish legislation. The study was conducted in compliance with the General Data Protection Regulation and is therefore part of North Denmark Region's record of processing activities (ID-number 2019-14).

Statistics

For each patient, an overall serotype HAI geometrical mean titer (GMT) was calculated from the 3 serotype-specific HAI titers. An overall serotype of HAI GMT ≥ 40 was considered protective. For group comparisons, the GMT with 95% confidence interval (CI) was calculated for the 3 serotype-specific HAI titers and for the overall serotype of HAI GMT. Continuous data is presented as median + interquartile range (IQR), and categorical data is presented

as frequencies with percentages. The nonparametric Mann–Whitney *U*-test was used to compare continuous variables, χ^2 test for categorical variables, and the Kruskal–Wallis nonparametric test for categorical variables with more than 2 groups. Fisher's exact test was used for categorical variables when expected counts were less than 5. The significance level was 5%. A multivariable logistic regression analysis was applied to the group of vaccinated patients to investigate variables of relevance to achieve an overall serotype HAI GMT ≥ 40. The calculations were carried out using Sas Enterprise Guide 8.1 (Cary, North Carolina, USA).

Results

In total, 248 patients with AIIRDs were eligible for participation in the study, and of those, 120 (48%) participants had been vaccinated against influenza in the 2018–2019 season, as opposed to 128 (52%) participants who had not been vaccinated. However, 26 patients were excluded due to blood collection taking place prior to the prespecified interval of ≥ 14 days after vaccination or due to missing blood samples. Moreover, 17 people who were found nonvaccinated in 2018–2019 were excluded because they had been vaccinated for influenza in the 2016–2017 or the 2017–2018 seasons. Hence, 205 participants were included in the influenza HAI antibody titer comparisons: 105 (51%) vaccinated and 100 (49%) nonvaccinated, respectively. In 3 out of the 105 vaccinated patients, the vaccine type in the 2018–2019 influenza season was unknown. These patients were omitted from comparisons of antibody response where vaccine type was a variable in the analyses.

The group of vaccinated participants (n = 105) had all received their influenza vaccination between October 1, 2018, and November 23, 2018, and their postvaccination blood samples had been collected in a range from 14 to 113 days after the vaccination. Review of the Danish Vaccination Register revealed that among the 105 patients vaccinated in the 2018–2019 influenza season, 87 (73%) had also been vaccinated in both seasons 2016–2017 and 2017–2018.

Table 1 shows the demographic and clinical characteristics of the 2 study subgroups. The median age and disease duration of the subgroup of influenza-vaccinated participants were significantly higher than those of the nonvaccinated subgroup, with figures being 67 years (IQR 58-72) compared to 56 years (IQR 49-64) ($P < .0001$) and 17.4 (IQR 9.8-26.5) versus 13.6 (IQR 8.8-19.9) ($P = .02$), respectively.

Table 1. Patient Demographic and Clinical Characteristics in Accordance with History of Influenza Vaccination in the 2018–2019 Season

	Vaccinated n = 105	Nonvaccinated n = 100	*P*
Median age (IQR), years	67 (58-72)	56 (49-64)	<.001
Male/female, n (%)	35 (33)/70 (67)	50 (50)/50 (50)	.016
Rheumatic disease diagnosis, n (%)			.002
Rheumatoid arthritis	79 (75)	57 (57)	
Psoriasis arthritis	15 (14)	15 (15)	
Spondyloarthritis	11 (11)	28 (28)	
Median disease duration (IQR), years	17.4 (9.8-26.5)	13.6 (8.8-19.9)	.02
Treatment at time of vaccination, n (%)			
Methotrexate	67 (64)	-	
Prednisolone	7 (7)	-	
bDMARDs			
TNF-α inhibitors	87 (83)	-	
Other bDMARDs	14 (13)	-	
No bDMARD treatment	4 (4)	-	
bDMARD treatment duration in years, median (IQR)	9.3 (3.6-12.6)	-	
Days from vaccination to blood sample, median (IQR)	62 (44-68)	-	-

P-values were calculated with χ^2 test for dichotomous variables, the nonparametric Mann–Whitney *U*-test for continuous variables and the Kruskal–Wallis nonparametric test for categorical variables with more than 2 groups.
bDMARDs, biological disease-modifying antirheumatic drugs; IQR, Interquartile range; TNF-α, tumor necrosis factor alpha.

Influenza vaccination uptake was also found to be influenced by gender. Among the 120 female participants, 70 had been vaccinated versus 35 out of 85 male participants ($P = .016$). Regarding AIIRD diagnosis, the influenza vaccination uptake was significantly higher among participants with versus without rheumatoid arthritis (75% vs. 57%, respectively) and lower in participants with versus without spondyloarthritis (11% vs. 28%, respectively)

Table 2. Demographic and Clinical Characteristics Among the Vaccinated Patients in Accordance with Vaccine Type (n = 102) in the 2018–2019 Season

	Influvac (n = 25)	Vaxigrip (n = 54)	VaxigripTetra (n = 23)	*P*
Males, n (%)	6 (24)	21 (39)	6 (26)	
Median age (IQR[1])	68 (60-72)	68 (61-74)	58 (51-68)	.001 (Vaxigrip vs. VaxigripTetra), .01 (Influvac vs. Vaxigripetra)
Treatment at time of vaccination, n (%)				
Methotrexate	18 (72)	35 (65)	13 (57)	
Prednisolone	2 (8)	4 (8)	1 (3)	
Interval (days) between vaccination and postvaccination blood sample, median (IQR)	63 (56-69)	59 (43-65)	62 (49-69)	
Previous vaccination 2016–2017, n (%)	21 (84)	48 (89)	20 (87)	

P-values are calculated with χ^2 test, Fisher's exact test, or nonparametric Mann–Whitney *U*-test. Only $P \leq 0.05$ are reported.
IQR, Interquartile range.

Table 3. GMT of HAI Titers in Influenza Vaccinated (n = 105) Versus Nonvaccinated Patients (n = 100), and in Accordance to Vaccine Type

		Vaccine types, n (%)					
	Vaccinated n = 105	Influvac 25 (24)	Vaxigrip 54 (51)	VaxigripTetra 23 (22)	Unknown 3 (3)	Nonvaccinated n = 100	*P*
Overall HAI serotype GMT, GMT (95% CI)	24.9 (21.7-28.5)	40.2 (30.3-53.3)	21.7 (18.4-25.5)	20.5 (14.8-28.3)	14.6 (3.1-70.0)	11.9 (10.5-13.4)	
Titer ≥ 40, n (%)	26 (25)	12 (48)	11 (20)	3 (13)	0	8 (8)	.0013
Titer < 40, n (%)	79 (75)	13 (52)	43 (80)	20 (87)	3	92 (92)	
Serotype Colorado, HAI GMT (95% CI)	20.5 (17.4-24.2)	25.1 (16.5-38.1)	17.0 (14.2-20.2)	25.6 (16.6-39.3)	12.6 (2.1-75.1)	9.5 (8.4-10.6)	
Titer ≥ 40, n (%)	36 (34)	9 (36)	16 (30)	10 (44)	1	7 (7)	< .001
Titer < 40, n (%)	69 (66)	16 (64)	38 (70)	13 (56)	2	93 (93)	
Serotype Michigan, HAI GMT (95% CI)	18.3 (15.5-21.6)	34.4 (23.5-50.5)	16.2 (13.3-19.8)	12.2 (8.9-16.6)	10.0 (3.1-32.2)	10.9 (9.6-12.5)	
Titer ≥ 40, n (%)	34 (32)	14 (56)	15 (28)	5 (22)	0	11 (11)	< .001
Titer < 40, n (%)	71 (68)	11 (44)	39 (72)	18 (78)	3	89 (89)	
Serotype Singapore, HAI GMT (95% CI)	41.4 (34.7-49.4)	75.7 (55.0-104.3)	37.3 (30.0-46.8)	27.5 (18.3-41.3)	25.2 (2.2-286.9)	16.5 (13.7-19.9)	
Titer ≥ 40, n (%)	73 (70)	23 (92)	36 (67)	12 (52)	2	29 (29)	< .001
Titer < 40, n (%)	32 (30)	2 (8)	18 (33)	11 (48)	1	71 (71)	

P-values between vaccinated and nonvaccinated patients were calculated with the χ^2 test when all counts were ≥ 5 and Fisher's exact test when one or more counts were < 5. Only $P \leq .05$ are shown.
GMT, geometric mean titer; HAI, hemagglutinin inhibition.

(*P* = .002). In the influenza-vaccinated subgroup, 67 patients (64%) received methotrexate, 87 (83%) patients received tumor necrosis factor alpha (TNF-α) inhibitors, and 14 (13%) patients received other bDMARDs. Four patients were temporarily without bDMARD treatment at the time of vaccination because of newly occurred medical conditions.

As presented in Table 2, patients who received VaxigripTetra were significantly younger than patients who received Vaxigrip or Influvac (*P* = .001 and *P* = .01, respectively). No other significant differences were found.

Postvaccination GMT of the overall serotype HAI GMT in the vaccinated group was 24.9 (95% CI 21.7-28.5) compared to 11.9 (95% CI 10.5-13.4) in the nonvaccinated group, corresponding to 25% versus 8% having an overall serotype HAI GMT ≥ 40 (*P* = .0013) (Table 3). In the influenza-vaccinated group, the prevalence of a postvaccination HAI titer ≥ 40 for vaccine antigens B/Colorado, A/Michigan/H1N1, and A/Singapore/H3N2 were 34%, 32%, and 70%, respectively, as opposed to 7% (*P* < .0001), 11% (*P* = .0002) and 29% (*P* < .0001), respectively, in the nonvaccinated group.

In the vaccinated group, 25 patients had received Influvac, 54 (51%) Vaxigrip, and 23 (22%) VaxigripTetra (Table 3). In 3 patients (3%), the vaccine type was not registered. In patients who had received Influvac, 48% achieved a postvaccination overall serotype HAI GMT ≥ 40 versus 20% in the Vaxigrip group (*P* = .01) and 13% in the VaxigripTetra group (*P* = .009) (Figure 1). Higher proportions of the Influvac-vaccinated patients achieved an HAI titer ≥ 40 for the serotypes Michigan (*P* = .02 Influvac versus VaxigripTetra, *P* = .02 Influvac versus Vaxigrip) and Singapore (*P* = .002 Influvac versus VaxigripTetra, *P* = .02 Influvac versus Vaxigrip), but the difference was non-significant for the B/Colorado serotype (Figure 2). As seen in Table 4, 3 patients

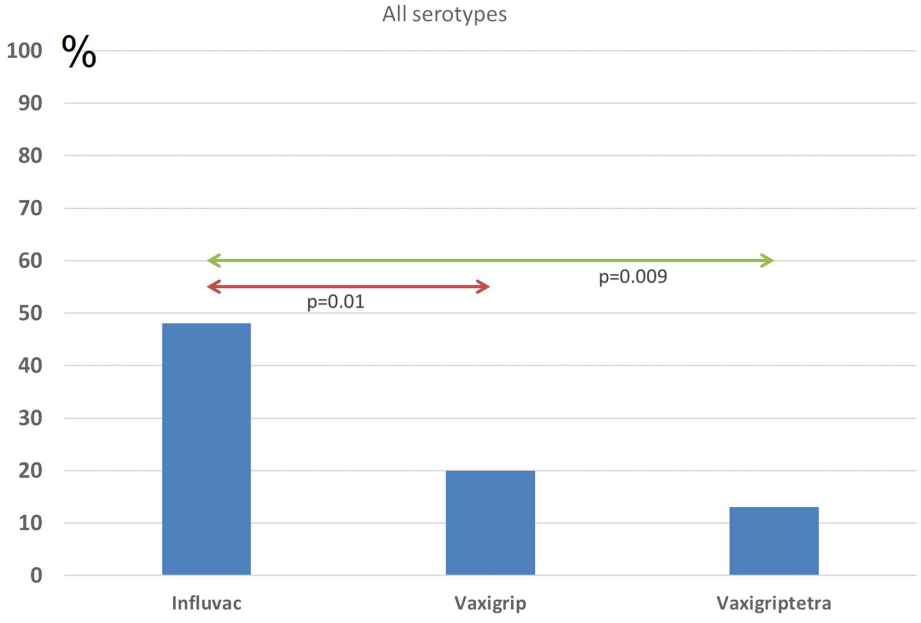

Figure 1. Percentage of patients with a postvaccination all serotypes HAI GMT ≥ 40 stratified by vaccine type received in the 2018–2019 influenza season. Abbreviations: hemagglutinin inhibition assay (HAI), geometric mean titer (GMT). Group comparisons were performed with the χ^2 test. Only $P \leq .05$ are shown.

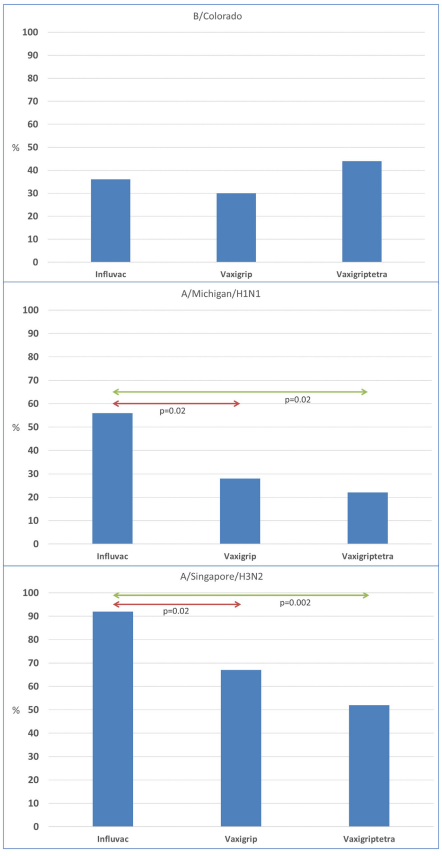

Figure 2. Percentage of patients with a postvaccination HAI titer ≥ 40 for the serotypes A/H1N1/Michigan, A/H3N2/Singapore and B/Colorado and stratified by type of vaccination received in the 2018–2019 influenza season. Group comparisons were performed with the χ^2 test. Only $P \leq .05$ are shown. HAI, hemagglutinin inhibition assay.

only had a prevaccination HAI GMT titer ≥ 40 in the vaccinated group of patients (1 in the Vaxigrip group, 2 in the Influvac group) versus 0 in the group of unvaccinated patients, the difference being nonsignificant. In the VaxigripTetra group, significantly fewer patients had a Singapore HAI titer ≥ 40 compared to the Vaxigrip and Influvac groups ($P = .002$ Vaxigrip versus VaxigripTetra, $P = .003$ Influvac versus VaxigripTetra). For the Colorado serotype, significantly more patients had an HAI titer ≥ 40 when compared to unvaccinated patients ($P = .005$). However, seroconversion rates between pre- and postvaccination were not measured and included in the final analyses due to major and uneven time intervals between pre- and postvaccination antibody measurements.

Multivariable logistic regression analysis was performed by exploring predictors achieving an overall HAI GMT ≥ 40 in influenza-vaccinated patients by using the variables age, sex, treatment with MTX and/or prednisolone, type of influenza vaccine, time interval from vaccination to influenza antibody measurement, previous vaccination status, and bDMARD therapy (Table 5). The probability of achieving an overall serotype HAI GMT ≥ 40 was found to be significantly higher if participants were vaccinated with Influvac compared to vaccination with Vaxigrip or VaxigripTetra.

Discussion

Patients included in this study closely represent patients seen daily in our rheumatology outpatient clinic. The study was defined as a clinical quality assurance study and thus included all patients in normal clinic care without any study-related loss of representativeness. The study findings suggest that patients receiving Influvac achieve higher antibody responses compared with patients receiving Vaxigrip or VaxigripTetra. Moreover, it was also shown that treatment with MTX seems not to affect antibody formation among AIIRD patients treated with bDMARD.

In addition to assessing antibody protection by an absolute cutoff value such as an HAI titer ≥ 40, most studies also determine protection by measuring seroconversion from pre- to post vaccination. In this study, prevaccination antibody measurement was only possible from a blood test taken at least 6 months before influenza vaccination in the 2018–2019 season, whereas measurement of prevaccination antibodies just before vaccination in the 2018–2019 influenza season was not feasible. Therefore, seroconversion measurement was not possible, which is the primary limitation of our study. Although we measured generally low prevaccination overall serotype HAI GMT levels both in the group of vaccinated and unvaccinated patients, we cannot be sure that this would ensue had prevaccination antibody values been measured just before influenza vaccination in the 2018–2019 season. Because of the long and uneven interval between pre- and postvaccination measurements in this study, some patients might have been influenza vaccinated twice (in the 2017–2018 influenza season and in the study season of 2018–2019) in this period of time. However, the proportion of previously vaccinated patients was similar regardless of vaccine type received in the study season, implying that a possible change in antibody titer would have affected each vaccination group equally.

The patients unvaccinated in the 2018–2019 season were also not vaccinated in the preceding season, whereas the majority of the patients vaccinated in the 2018–2019 season were vaccinated in the preceding seasons. Although not statistically significant, this might explain that 3 patients had prevaccination overall serotype HAI GMT ≥ 40 in the vaccinated group of patients versus 0 in the unvaccinated group of patients, suggesting cross-reactivity from previous vaccinations. For unknown reasons, the Singapore serotype had the greatest prevaccination difference in titer, especially between the vaccination groups. Although the Singapore serotype was not included in the influenza vaccines preceding the 2018/2019 season, high prevaccination coverage was seen in the Influvac and Vaxigrip groups as opposed to statistically significant lower values in the VaxigripTetra group. Regarding the unvaccinated group of patients, a rise in antibody titer was seen, especially for the Singapore serotype, between pre- and postvaccination measurements. A potential explanation is that some patients in our study could have been misclassified as unvaccinated in the 2018–2019 season due to failure to register a vaccination in the Danish Vaccination Register.

Postvaccination A/Michigan/H1N1 and A/Singapore/H3N2 antibody levels were lowest and highest, respectively, in both unvaccinated and vaccinated patients. These results are in concordance with a Polish study conducted in an elderly population,[23] but zero percent in this study had been detected with prevaccination protective levels of A/Singapore/H3N2, in contrast to our results of high prevaccination Singapore serotype titers (except for the VaxigripTetra group). This discrepancy might reflect differences in circulating strains between the different countries as well as the combined effects of previous vaccinations.[24] Infection with the influenza virus of any strain is expected to provide a broader and more long-lived antibody response than does vaccination.[5] Influenza infection between our pre- and postvaccination measurements could have affected the antibody measurements in the 3 vaccine groups differently.

The finding in this study that antibody response is superior in an AIIRD population vaccinated with Influvac compared to Vaxigrip and VaxigripTetra, has not previously been reported to our knowledge. We found no previous studies that directly compared Vaxigrip or VaxigripTetra with Influvac in terms of influenza antibody response, or VE. A review from 2018 found no evidence of difference in antibody response or VE between subunit and split-virion vaccines.[25] One comparative study of 6 European influenza vaccines showed both subtle and pronounced

Table 4. Prevaccination GMT of HAI Titers in Influenza Vaccinated (n = 105) Versus Nonvaccinated Patients (n = 100), and in Accordance to Vaccine Type

		Vaccine types, n (%)					
	Vaccinated n = 105	Influvac 25 (24)	Vaxigrip 54 (51)	VaxigripTetra 23 (22)	Unknown 3 (3)	Nonvaccinated n = 100	*P*
Overall HAI serotype GMT, GMT (95% CI)	10.5 (9.4-11.7)	11.8 (9.2-15.0)	10.4 (9.0-12.0)	9.3 (7.5-11.4)	12.7 (3.3-49.0)	6.9 (6.4-7.4)	
Titer ≥ 40, n (%)	3 (3)	2 (8)	1 (2)	0 (0)	0 (0)	0 (0)	
Titer < 40, n (%)	102 (97)	23 (92)	53 (98)	23 (100)	3 (100)	100 (100)	
Serotype Colorado, HAI GMT (95% CI)	9.1 (7.9-10.5)	8.7 (6.7-11.3)	8.3 (6.9-9.9)	11.6 (8.0-16.9)	12.6 (2.1-75.1)	6.2 (5.7-6-8)	
Titer ≥ 40, n (%)	14 (13)	2 (8)	6 (11)	5 (22)	1 (33)	3 (3)	Vaccinated vs. nonvaccinated: .005
Titer < 40, n (%)	91 (87)	23 (92)	48 (89)	18 (78)	2 (67)	97 (97)	
Serotype Michigan, HAI GMT (95% CI)	8.4 (7.4-9.4)	9.7 (7.4-12.7)	8.7 (7.0-10.0)	7.3 (5.7-9.4)	6.3 (3.2-12.4)	6.6 (6.1-7.3)	
Titer ≥ 40, n (%)	11 (10)	3 (12)	6 (11)	2 (9)	0 (0)	4 (4)	
Titer < 40, n (%)	94 (90)	22 (88)	48 (89)	21 (91)	3 (100)	96 (96)	
Serotype Singapore, HAI GMT (95% CI)	15.3 (12.8-18.2)	19.2 (12.5-29.6)	16.3 (12.7-20.8)	9.6 (7.6-12-0)	25.2 (1.7-374.4)	8.0 (7.0-9.1)	Influvac vs. VaxigripTetra: .003. Vaxigrip vs. VaxigripTetra: .002. Vaccinated versus nonvaccinated: .0003
Titer ≥ 40, n (%)	32 (30)	10 (40)	20 (37)	1 (4)	1 (33)	10 (10)	
Titer < 40, n (%)	73 (70)	15 (60)	34 (63)	22 (96)	2 (100)	90 (90)	

P-values were calculated with the χ^2 test when all counts were ≥5 and Fisher's exact test when 1 or more counts were < 5. Only $P \leq .05$ are shown.
GMT, geometric mean titer; HAI, hemagglutinin inhibition.

differences in the active viral components not only between the different vaccines but also between different batches of the same vaccine, which could affect the VE of the different vaccines.[26] Various biases could unknowingly be present in our study, which could have contributed to our finding of a superior antibody response after vaccination with Influvac. Both Vaxigrip and Influvac were in use in the influenza seasons preceding 2018–2019, and previous vaccination with either vaccine could have affected the choice of vaccination during the 2018–2019 influenza season. As we do not have information about which vaccine type previously vaccinated patients received, we cannot investigate whether the immunogenicity of Vaxigrip and VaxigripTetra might be attenuated by repeated vaccination with a different or the same vaccine type in comparison with Influvac. Also, previous influenza vaccination might negatively interfere with antibody response in repeated vaccination,[27,28] and it is not known whether this effect could be more pronounced in some influenza vaccine types compared to others. In our study, the effect of previous vaccination on antibody response was insignificant when included in the multivariable logistic regression. However, only 13 patients were vaccinated in the study season, but not at least once in the 2 preceding seasons. We therefore have limited power to detect a possible difference in the chance of achieving a protective antibody level according to previous vaccination status and if 1 vaccination

Table 5. Exploration of Predictors of Achieving Overall Serotype HAI GMT ≥ 40 in Influenza Vaccinated Patients in the 2018–2019 Influenza Season with Known Vaccine Type (n = 102)

	Crude OR	95% CI	*P*	Adjusted OR	95% CI	*P*
Age (per 1 year increase)	0.99	0.95-1.03	.50	0.97	0.93-1.02	.21
Sex (females versus males)	1.38	0.51-3.72	.53	1.08	0.36-3.3	.88
Vaccination types						
Vaxigrip versus influvac	0.28	0.10-0.77	.01	0.29	0.1-0.87	.03
VaxigripTetra versus influvac	0.16	0.04-0.69	.01	0.13	0.03-0.63	.01
Interval since vaccination (per 1 day increase)	1.00	0.98-1.03	.89	1.00	0.97-1.03	.97
Treatment at the time of vaccination						
Methotrexate (yes versus no)	2.17	0.78-6.04	.14	2.08	0.66-6.60	.21
Prednisolone (yes versus no)	1.18	0.22-6.50	.85	0.92	0.11-7.49	.94
bDMARD treatment (none versus TNF-α inhibitors)	0.74	0.06-8.58	.81	2.18	0.10-48.27	.62
bDMARD treatment (other than TNF-α inhibitors versus TNF-α inhibitors)	2.23	0.46-10.72	.32	0.53	0.10-2.86	.46
Previous vaccination (yes versus no)	0.74	0.21-2.64	.64	1.23	0.28-5.49	0.62

Multivariable logistic regression analysis adjusted for all the variables in the table.
bDMARDs, biological disease-modifying antirheumatic drugs; GMT, geometric mean titer; HAI, hemagglutinin inhibition; OR, odds ratio; TNF-α, tumor necrosis factor alpha.

type is superior in previously vaccination-naive patients.

In the 2018–2019 influenza season, VaxigripTetra was reserved for special risk groups, which included the severely obese or people with other serious health conditions who had not yet turned 60 years old.[3] This is in contrast to the Vaxigrip and Influvac groups, where the same vaccination indications applied.[3] In our study, patients vaccinated with VaxigripTetra had higher pre- and postvaccination HAI titers for serotype B/Colorado. Other studies have also shown that vaccination with a tetravalent influenza vaccine elicits higher antibody response to the influenza B components of the vaccine compared with trivalent vaccines.[29] It has been shown in other studies, that including a fourth serotype to the vaccine should not affect the antibody response of the remaining 3 serotypes.[30] Only 13% of the VaxigripTetra vaccination group reached overall serotype HAI GMT ≥ 40, although the mean age was younger in comparison with the Vaxigrip and Influvac vaccination groups. The assumed greater comorbidity in this group could be one cause of a generally lower antibody response following influenza vaccination, and when the observed difference in the Singapore prevaccination serotype compared to Influvac and Vaxigrip is added, it means that comparison of this group with the latter 2 should include those reservations. It is a limitation of this study that we do not have information about comorbidities, which could also affect the antibody response.

A protective antibody level is often defined as a cutoff value of serum HAI titer ≥ 40, which, according to previous studies, corresponds to approximately 50% clinical protection from influenza infections.[31] This value was suggested from data originating mainly from young, healthy adults. However, sufficient evidence does not exist to extrapolate this assumption to immunosuppressed patients. Also, it has been argued that cell-mediated response is a better measure of the immune response following influenza vaccination in the elderly.[32] In this study, a postvaccination overall serotype of HAI GMT ≥ 40 was found in one-quarter of influenza-vaccinated patients. Proportions of serotype-specific HAI titers ≥ 40 were higher, suggesting that an overall serotype HAI GMT ≥ 40 is a conservative marker of protection, and some patients below this limit might achieve adequately protective antibody titers to 1 or 2 serotypes. Due to differences in past influenza virus exposure and receipt of different seasonal vaccines, response rates after influenza vaccination might vary and are presumably not directly comparable between studies. Since patients included in this study previously showed a lower degree of pneumococcal antibody protection after pneumococcal vaccination,[33] they could inherently have less likelihood of producing antibodies at sufficient levels.

Unlike our previous studies where pneumococcal antibody response was found to be suppressed by MTX,[21,33] no attenuation of influenza antibody response was seen in rheumatic patients on treatment with bDMARDs in combination with MTX compared to those who were on bDMARD therapy only. In a study by Kapetanovic et al, MTX was not found to have any detrimental effect on the serological response to influenza vaccination in RA patients.[17] Fomin et al also found that various immunosuppressive treatments, including MTX and TNF-α inhibitors, did not decrease the humoral response to the influenza vaccine.[16] Our finding of non-hindrance of influenza vaccination response by MTX treatment in AIIRD patients is consistent with these studies. In a Korean study, temporary discontinuation of MTX for 2 weeks after influenza vaccination significantly improved the immunogenicity of influenza vaccination in patients with RA without a flare in disease activity.[15] The divergent results show that the specific influenza vaccines, although belonging to the same subclass of vaccines and thus seemingly similar in pro-immune modulatory effect, might be differently affected by immunomodulatory drugs such as MTX. Alternatively, inherent immune host factor variations in the populations investigated have an effect on vaccination response.[34]

Because antibody response is only a surrogate marker of VE, it is not possible to draw any firm conclusions from the finding that more patients with AIIRD have a protective antibody level following vaccination with Influvac. However, if the finding is reproduced in future studies, it could have clinical implications for the choice of vaccine in the investigated group of patients. Also, the divergent results regarding the effect of MTX on influenza response in different studies imply that the complexity of immune responses following influenza vaccination might be more pronounced than assumed so far. The coronavirus disease 2019 pandemic and the resulting awareness of vaccinations in general might warrant further investigation into these topics.

Ethics Committee Approval: This study was classified as a quality assurance study by the Regional Research Committee of North Denmark, which do not require any additional ethic assessment or informed consent according to Danish legislation (ID-number 2019-14, Date: 21.01.2019).

Informed Consent: According to the Danish Health Care Act, the project was classified as a quality assurance study, where no additional ethic assessment or informed consent is required.

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