A large case-control study on vaccination as risk factor of multiple sclerosis

Alexander Hapfelmeier, PhD[1*], Christiane Gasperi, MD[2*], Ewan Donnachie[3], and Bernhard Hemmer, MD[2,4 †]

[1]Institute of Medical Informatics, Statistics and Epidemiology, Technical University of Munich, Ismaninger Str. 22, 81675 Munich, Germany.

[2] Department of Neurology, Klinikum rechts der Isar, Technical University of Munich, Ismaninger Str. 22, 81675 Munich, Germany.

[3] National Association of Statutory Health Insurance Physicians of Bavaria, Elsenheimer Straße 39, 80687 Munich, Germany.

[4] Munich Cluster for Systems Neurology (SyNergy), Germany.

[†] Contact Information:
Bernhard Hemmer, Department of Neurology, Klinikum rechts der Isar, Technical University of Munich, Ismaninger Str. 22, 81675 Munich, Tel. +49-89-4140-4600, Fax. +49-89-4140-7681

Statistical analysis was performed by Alexander Hapfelmeier

Word Count Paper/Abstract: 3033/215

Character Count Title: 78

Number of References/Tables/Figures: 32/3/3

Study Funding: The study was supported by a grant from the German Federal Ministry of Education and Research Competence network Multiple Sclerosis (BMBF, 01GI1601D) and is associated with DIFUTURE (Data Integration for Future Medicine, BMBF 01ZZ1804[A-I]). The authors take on the responsibility for the contents of the present publication. Bernhard Hemmer received funding for the study from the MultipleMS EU consortium and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy within the framework of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy – ID 390857198).

Search Terms: [41] Multiple Sclerosis, [53] Case control studies, [59] Risk factors in epidemiology, [115] Harm/risk (analysis), [132] Autoimmune diseases

[*]These authors contributed equally to the manuscript

Disclosure

Dr. Alexander Hapfelmeier received a speaker honorarium from Biogen for the Biogen Symposium on Statistical Methods in Real World Evidence 2017.

Dr. Christiane Gasperi reports no disclosures.

Ewan Donnachie reports no disclosures.

Dr. Bernhard Hemmer has served on scientific advisory boards for Novartis; he has served as DMSC member for AllergyCare and TG therapeutics; he or his institution have received speaker honoraria from Desitin; holds part of two patents; one for the detection of antibodies against KIR4.1 in a subpopulation of MS patients and one for genetic determinants of neutralizing antibodies to interferon β. All conflicts are not relevant to the topic of the study.

Abstract

Objective: To investigate the hypothesis that vaccination is a risk factor of multiple sclerosis by use of German ambulatory claims data in a case-control study.

Methods: Using the ambulatory claims data of the Bavarian Association of Statutory Health Insurance Physicians (BASHIP) covering the years 2005 to 2017, logistic regression models were used to assess the relation between MS (n=12,262) and vaccinations in the five years before first diagnosis. Subjects newly diagnosed with Crohn's disease (n=19,296), psoriasis (n=112,292) and subjects with no history of these autoimmune diseases (n=79,185) served as controls.

Results: The odds of MS was lower in subjects with a recorded vaccination (OR=0.870, $p<0.001$ vs. subjects without autoimmune disease; OR=0.919, $p<0.001$ vs. subjects with Crohn's disease; OR=0.973, $p=0.177$ vs. subjects with psoriasis). Lower odds were most pronounced for vaccinations against influenza and tick-borne encephalitis. These effects were consistently observed for different time frames, control cohorts and definitions of the MS cohort. Effect sizes increased towards the time of first diagnosis.

Conclusions: Results of the present study do not reveal vaccination to be a risk factor of MS. On the contrary, they consistently suggest that vaccination is associated with a lower likelihood of being diagnosed with MS within the next five years. Whether this is a protective effect needs to be addressed by future studies.

Introduction

In recent years, various environmental risk factors for the development of Multiple Sclerosis (MS) have been suggested some of which could be confirmed in large studies [1, 2]. Vaccination has been discussed as a risk factor for the development of MS and for the occurrence of relapses. Different case reports and smaller studies on the relation to vaccination showed conflicting results, however [3]. The authors of a recent systematic literature review [4] concluded that there was no overall change in the risk of developing MS following most of the investigated vaccinations including hepatitis B, human papilloma virus (HPV), seasonal influenza, measles-mumps-rubella and others. Interestingly, they reported a possible preventive potential of tetanus and diphtheria vaccine, which was however mostly based on studies with few cases and insufficient statistical power.

Vaccinations are the most effective tools to prevent many infectious diseases. Thus it is important to carefully investigate and clarify any risks believed to be associated with vaccination to prevent unwarranted reservations.

The objective of the present case-control study was therefore to investigate the hypothesis that vaccination is a risk factor of multiple sclerosis in a systematic retrospective analysis of ambulatory claims data of 223,035 subjects (12,262 MS patients and 210,773 controls), held by the Bavarian Association of Statutory Health Insurance Physicians (BASHIP).

Methods

Ambulatory claims data held by the *Bavarian Association of Statutory Health Insurance Physicians* (BASHIP) covers all members of the statutory health insurance, that is approximately 85% of the population of Bavaria[5]. It was available for each quarterly billing

period between the years 2005 and 2017. We defined a cohort of new onset MS subjects with at least two secured ICD-10 diagnoses G35 in separate quarterly periods and two control cohorts of subjects diagnosed with other autoimmune diseases, Crohn's disease and psoriasis using the ICD-10 diagnoses K50 and L40 in the same manner, respectively. Inclusion of these control cohorts into the analysis enables the identification of effects that are specific to MS and are not shared by other autoimmune diseases. In order to facilitate the observation of all subjects in the five years prior to diagnosis, we restricted all cohorts to subjects with a first diagnosis in 2010 or later. Subjects without record of these ICD-10 diagnoses were randomly selected without replacement from the BASHIP data and matched to the MS cohort in a 5:1 ratio according to year of birth, gender and district of residence. For the control group, we considered the quarter of "first diagnosis" to be that of their matching partner. All subjects of all cohorts had to be younger than 70 years of age and resident in Bavaria during the entire 5 years period prior to the first diagnosis. Subjects in the MS cohort were further required to have a recorded visit with a neurologist at any time and no ICD-10 diagnosis G04, that is no diagnosis of a clinically isolated syndrome (CIS), before or after MS diagnosis. To enable sensitivity analyses we defined three additional, stricter and therefore more conservative definitions of the MS cohort. These required that there was no diagnosis of optic neuritis (ICD-10 H46), at most one MRI of the head or at most one visit with a neurologist during the investigated 5 years period, respectively.

Reimbursement claims are coded using a five-digit code called the "*Gebührenordnungspositionen*" (GOP). Each recorded GOP is uniquely linked to a subject, a quarterly period and a specific physician consultation. All GOP records coding vaccinations were used to explore the incidence and frequency of vaccinations in the investigated cohorts. As vaccinations are also administered as combination vaccines, we grouped them accordingly into ten sets of vaccinations against: (1) tick-borne encephalitis (TBE) virus, (2) human papilloma virus, (3) pneumococci, (4) imeningococci, (5) influenza virus, (6) hepatitis A, (7)

hepatitis B, (8) mumps, measles, rubella and varicella zoster viruses, (9) clostridium tetani, corynebacterium diphtheriae, poliovirus, bordetella pertussis and haemophilus influenzae type b, and (10) any vaccination. The infrequent combination of haemophilus influenzae type b plus hepatitis B was allocated to each of the sets (7) and (9). The same holds for the rare combination of six vaccines in a single administration which was given by the five vaccines listed in set (9) plus hepatitis B. Combinations of vaccinations against hepatitis A and B were allocated to each of the groups (6) and (7). The listed vaccinations belong to the set of vaccinations recommended by the German Standing Committee on Vaccination (STIKO) and are therefore free of charge for members of the statutory health insurance.

Data Availability Statement

For reasons of data protection, the authors are unable to distribute the underlying data. Interested researchers may contact the corresponding author or the BASHIP to request access.

Standard Protocol Approvals, Registrations, and Patient Consents

In this retrospective case-control study we analyzed anonymous claims data held by the BASHIP. Approval by an ethical standards committee on human experimentation (institutional or regional) for any experiments using human participants was not needed according to the Guidelines and Recommendations for Good Practice of Secondary Data Analysis (GPS)[6]. Approval was obtained from the responsible data protection officer of the BASHIP. Likewise there was no need for written informed consent from participants. Neither photographs, videos, nor other information of recognizable persons is used in this article. Authorization for disclosure was therefore not necessary.

Statistical Analysis

To manage the high computational burden of processing the very large data we used unconditional logistic regression models to assess the association between vaccination and MS by means of odds ratios. Recent findings show that unconditional logistic regression is a proper method to perform for loose-matching data, that is when only a few matching variables are used and matching between cases and controls is therefore not unique [7]. Separate models were built to contrast the MS cohort against each of the control cohorts. The modelled binary outcome was MS (yes/no) and the factorial covariates were vaccination (yes = at least once vs. no = never) and the main effects as well as the interaction effect of gender and age categories (0-20 yrs., 21-30 yrs., 31-40 yrs., …, 61-70 yrs.). The effect measures of the analyses, given by the odds ratios and corresponding confidence intervals, are therefore adjusted for any combination of gender and age categories. The crude numbers of subjects with any vaccination are given along the size of the cohorts.

We repeated each analysis for the three additional definitions of the MS cohort and for different time frames before first diagnosis to be able to explore the robustness of the results and the variation in time. The time frames comprise the whole 5-year period excluding the quarter immediately prior to the first diagnosis (= overall), the quarter before first diagnosis (= -1st qtr.), the quarters 2 to 4 before first diagnosis (= -234th qtr.) and each of the whole years before first diagnosis, starting with the second year and ending with the fifth year (= -2nd yr., -3rd yr., -4th yr. and -5th yr.).

The multiple analyses conducted in this study create a multiple testing problem. This is a rather strict interpretation of the present testing situation which does formally not necessitate a correction for multiple testing because of its exploratory nature. We still addressed this problem by defining a single primary analysis which covers all kinds of vaccinations (any/none). Hypothesis testing was performed on an exploratory two-sided 5% significance

level and corresponding two-sided 95% confidence intervals were computed for this primary analysis. Additional secondary analyses that addressed specific vaccinations were adjusted by Sidak's correction for multiple testing. The correction was applied to p-values as well as to confidence intervals to provide a familywise error control on a 5% significance level. There were nine secondary analyses s in total, resulting in an adjusted significance level of 0.568% and two-sided 99.4% confidence intervals. We did not adjust hypothesis testing for repeated analyses across the investigation of different control groups and time frames. All analyses were performed using R 3.5.0 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

A data query in the BASHIP database identified 15,046 subjects with a first diagnosis of MS, 21,189 subjects with a first diagnosis of Crohn's disease and 133,961 subjects with a first diagnosis of psoriasis in 2010 or later. Another 83,610 controls without any of the three autoimmune diseases were matched to the MS cohort. These numbers decreased to 12,262 in the MS cohort, 19,296 in the Crohn's disease cohort, 112,292 in the psoriasis cohort and 79,185 in the cohort of subjects without these autoimmune diseases after removal of subjects that were older than 70 years at the quarter of first diagnosis and by exclusion of subjects that did not fulfill the definition of an MS patient as outlined in the Methods section. Concerning comorbidities, there were 456 subjects with Crohn's disease and psoriasis, 216 subjects with MS and psoriasis, 48 subjects with Crohn's disease and MS and two subjects with Crohn's disease, psoriasis and MS. These subjects were allocated to each of the respective cohorts and were not treated differently due to the comparatively small sample sizes.

The three additional definitions of MS cohorts which required that there was no diagnosis of optic neuritis (ICD-10 H46), at most one MRI of the head or at most one visit with a

neurologist during the investigated 5-year period lead to sample sizes of 11,675, 11,663 and 9,272, respectively.

Descriptive statistics of the distribution of age at first diagnosis, gender and the number of vaccinations in the five years prior to first diagnosis are presented in Table 1. These statistics are purely illustrative and should not be used to infer on the relation between vaccinations and MS or the other two investigated autoimmune diseases. This research question has been addressed more thoroughly by the computation of age and gender adjusted odds ratios as presented along the following lines.

The ability to accurately define the quarter of first diagnosis for each subject is crucial to this study. It determines the individual 5-year periods that are to be analyzed and that are also required for the inclusion of a subject into the study cohorts. Due to the study design, any preceding diagnoses of the same kind as the respective first diagnosis (i.e. ICD-10 G35, K50 or L40) could be ruled out for a period of at least 5 years for each subject. For 70% of the subjects it was even possible to observe a period of more than 7.5 years before first diagnosis. Further percentiles of the distribution of individual observation periods before first diagnosis are given in Table 1.

We analyzed the occurrence of vaccinations in MS patients and the three control groups during the five years before first diagnosis (Figure 2). The overall effects, which are computed for the 5-year period excluding the quarter before first diagnosis, were OR = 0.870 (95% CI: 0.837 to 0.904), OR = 0.919 (95% CI: 0.876 to 0.963) and OR = 0.973 (95% CI: 0.936 to 1.012) for the control cohorts of subjects without autoimmune disease, of subjects with Crohn's disease and of subjects with psoriasis, respectively. Considering a possible trend in time the Odds Ratios of the respective control cohorts steadily decrease from the -5th year to the -2nd year and the -1st quarter before first diagnosis. Overall the odds of MS was lower in subjects with any vaccination (Figure 2). This result is consistent across all studied time

frames and control cohorts. Statistical significance is almost always reached with only a few exceptions for the Crohn's disease and psoriasis control cohorts. Corresponding vaccination frequencies and cohorts sizes are given in Table 2.

Further sensitivity analyses involving more strict definitions of the MS cohort are provided in Figure 2. Interestingly, these results mirrored those obtained in the primary analysis with decreased odds of MS in subjects with any vaccination and a trend towards stronger effects in periods that are closer to first diagnosis. The strongest effects were observed for the stricter definition of the MS cohort, which requires that subjects had at most one neurological consultation during the investigated 5 years period (Figure 2). In this analysis, significant differences were observed between MS and all control groups even 5 years before first diagnosis.

Next we analyzed the effect of specific vaccinations on the occurrence of MS. Sufficient numbers for meaningful analyses were available for vaccinations against tick-borne encephalitis (TBE) virus, human papilloma virus, pneumococci, meningococci, influenza, hepatitis A and B, meningococci, mumps, measles, rubella and varicella viruses. We found a consistently negative relation between the development of MS and the incidence of vaccinations with a single non-significant exception for the vaccination against human papilloma virus using the Psoriasis control group which shows the inverse effect (Figure 3 and Table 3). Most pronounced were the negative relations for vaccinations against TBE, hepatitis B and influenza viruses while the effect was less pronounced for the other vaccinations. Sensitivity analyses with strict definitions of the MS cohort showed even more pronounced effects especially in the subgroup which requires that subjects had at most one visit with a neurologist during the investigated 5-year period (data not shown).

Discussion

Despite the large number of studies conducted, the role of vaccination as a risk factor for the development of multiple sclerosis is still uncertain. The present study did not reveal vaccination to be a risk factor for MS. It differs from recent research in two major aspects [8-11]. Firstly, it provides substantial evidence from a large population-based sample of 223,035 subjects (12,262 MS patients and 210,773 controls). Secondly, it is focused on a single specific risk factor. It does therefore not share the risk of spurious findings which is present in studies that explore a large set of potential risk factors.

Inconsistent findings have been reported with respect to the association of human papilloma virus, influenza, measles, mumps, typhoid, VZV, rubella and hepatitis B vaccinations with MS risk [12-28]. Meta analyses have lacked sufficient precision to prove or disprove an association of these different vaccinations with MS risk. However, the majority of these studies suggest that vaccinations are not associated with a higher risk of developing MS. For some of the vaccines (e.g. human papilloma virus, tetanus toxoid) some studies even reported a lower likelihood of a subsequent MS diagnosis.

Our findings, derived from a very large number of patients and three matched control cohorts, are in line with previous studies and support the assumption that vaccinations are not associated with a higher likelihood of an MS diagnosis during the following 5 years. Our findings rather suggest a lower likelihood of an MS diagnosis after vaccination. Additionally, the low rate of vaccination during the months before MS diagnosis also argues against a major role of vaccination in the induction of MS relapses. Several reasons may account for the lower rate of vaccinations during the 5 years prior to MS diagnosis.

Previous studies have reported altered behaviour and an increased prevalence of neuropsychiatric symptoms up to five years before MS diagnosis which might be related to an awareness of the subjects of their disease even before the diagnosis is made [11]. For instance, a lower rate of pregnancies has been found in women with MS as compared to controls. A causal relation of lower pregnancy rates with the development of MS seems unlikely. On that note it has been assumed that the onset of disease might have started before the actual first diagnosis and that subjects change their behavior along the course of the disease, even before the diagnosis is made.

Similarly, increased levels of sick leave and disability pension have been reported for MS patients even 15 years before first diagnosis of the disease[29]. In this respect, disease burden may affect a MS patient's lifestyle long before it is diagnosed. Additionally, higher rates of physician services utilization as compared to the general population have been observed at least five years before diagnosis[30].

These findings are of relevance for the interpretation of the results of the present study. Possible explanations of the lower vaccination rates in MS patients up to five years before diagnosis might be that the patients are aware of their disease or are affected by the burden of disease even before the first diagnosis, which may lead to altered behaviour.

Similar results would be expected if the MS group was inaccurately defined and wrongfully included patients who have already been diagnosed with MS at an earlier time point. It could be expected that patients with MS do get vaccinated less often as compared to healthy individuals. However, as the observed effects were even stronger in patients with not more than one neurologist consultation within the five years before diagnosis, we consider it unlikely that our findings are due to the contamination of the MS group with previously diagnosed patients.

Vaccinations might also directly impact the immunopathogenesis of MS. Infections are associated with MS relapses and the prevention of infections might thus decrease relapses and MS risk. In this study, the negative relation of vaccination with the development of MS is most pronounced for influenza. The conclusion that the prevention of influenza infection might decrease the risk of developing MS or relapses in MS patients however cannot be drawn from the present findings. Moreover, vaccination itself might influence the autoimmune response in MS. Stimulation of the immune system with vaccine antigens might have an ameliorating effect on the autoimmune response underlying onset and progression of disease [31, 32].

A limitation of the present study is the subjective definition of the MS cohort, potentially leading to flawed findings. This issue was addressed by multiple strict definitions of the MS cohort, enabling the implementation of sensitivity analyses. Another limitation is given by the data source per se itself. Entry errors and incorrect coding cannot be ruled out, even for databases with high quality standards like the BASHIP database.

In summary, our data show a negative association of vaccinations with MS. This could be seen when considering all reported vaccinations and specifically for influenza, hepatitis B and TBE vaccinations. This data alone does not allow for any conclusion regarding a possible protective effect of vaccinations regarding the development of MS. However, our results do not support the assumption that vaccinations are a risk factor for the development of MS.

Appendix 1

Name	Location	Role	Contribution
Alexander Hapfelmeier, PhD	Technical University of Munich, Germany	Author	Analyzed the data; interpreted the data; drafted and revised the manuscript for intellectual content
Christiane Gasperi, MD	Technical University of Munich, Germany	Author	interpreted the data; drafted and revised the manuscript for intellectual content
Ewan Donnachie	BASHIP, Germany	Author	Major role in the acquisition of data; revised the manuscript for intellectual content
Bernhard Hemmer, MD	Technical University of Munich, Germany	Author	Designed and conceptualized study; interpreted the data; drafted and revised the manuscript for intellectual content

References

1. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. Nature Reviews Neurology 2016;13:25.
2. Marrie RA. Environmental risk factors in multiple sclerosis aetiology. The Lancet Neurology 2004;3:709-718.
3. Kalincik T. Multiple Sclerosis Relapses: Epidemiology, Outcomes and Management. A Systematic Review. Neuroepidemiology 2015;44:199-214.
4. Mailand MT, Frederiksen JL. Vaccines and multiple sclerosis: a systematic review. Journal of Neurology 2017;264:1035-1050.
5. Daltrozzo T, Hapfelmeier A, Donnachie E, Schneider A, Hemmer B. A Systematic Assessment of Prevalence, Incidence and Regional Distribution of Multiple Sclerosis in Bavaria From 2006 to 2015. Frontiers in Neurology 2018;9.
6. Swart E, Gothe H, Geyer S, et al. Gute Praxis Sekundärdatenanalyse (GPS): Leitlinien und Empfehlungen. Gesundheitswesen 2015;77:120-126.
7. Kuo C-L, Duan Y, Grady J. Unconditional or Conditional Logistic Regression Model for Age-Matched Case-Control Data? Frontiers in public health 2018;6:57-57.
8. Disanto G, Zecca C, MacLachlan S, et al. Prodromal symptoms of multiple sclerosis in primary care. Annals of Neurology 2018;83:1162-1173.
9. Högg T, Wijnands JMA, Kingwell E, et al. Mining healthcare data for markers of the multiple sclerosis prodrome. Multiple Sclerosis and Related Disorders 2018;25:232-240.
10. Wijnands JMA, Kingwell E, Zhu F, et al. Health-care use before a first demyelinating event suggestive of a multiple sclerosis prodrome: a matched cohort study. The Lancet Neurology 2017;16:445-451.
11. Wijnands JMA, Zhu F, Kingwell E, et al. Five years before multiple sclerosis onset: Phenotyping the prodrome. Multiple Sclerosis Journal 2018:1352458518783662.
12. Amini Harandi A, Pakdaman H, Sahraian MA, Hosseini SK. Infectious Diseases, Related Vaccinations, and Risk of Multiple Sclerosis Later in Life: A Case-Control Study (P03.242). Neurology 2012;78:P03.242-P203.242.
13. Ascherio A, Zhang SM, Hernán MA, et al. Hepatitis B Vaccination and the Risk of Multiple Sclerosis. New England Journal of Medicine 2001;344:327-332.
14. Baumhackl U, Franta C, Retzl J, Salomonowitz E, Eder G. A controlled trial of tick-borne encephalitis vaccination in patients with multiple sclerosis. Vaccine 2003;21:S56-S61.
15. DeStefano F, Verstraeten T, Jackson LA, et al. Vaccinations and Risk of Central Nervous System Demyelinating Diseases in Adults. Archives of Neurology 2003;60:504-509.
16. Eftekharian MM, Mousavi M, Hormoz MB, Roshanaei G, Mazdeh M. Multiple sclerosis and immunological-related risk factors: Results from a case-control study. Human antibodies 2014;23:31-36.
17. Grimaldi-Bensouda L, Rossignol M, Koné-Paut I, et al. Risk of autoimmune diseases and human papilloma virus (HPV) vaccines: Six years of case-referent surveillance. Journal of Autoimmunity 2017;79:84-90.
18. Hernán MA, Jick SS, Olek MJ, Jick H. Recombinant hepatitis B vaccine and the risk of multiple sclerosis. A prospective study 2004;63:838-842.
19. Kurtzke JF, Hyllested K, Arbuckle JD, et al. Multiple sclerosis in the Faroe Islands. 7. Results of a case control questionnaire with multiple controls. Acta Neurol Scand 1997;96:149-157.
20. Lai YC, Yew YW. Severe Autoimmune Adverse Events Post Herpes Zoster Vaccine: A Case-Control Study of Adverse Events in a National Database. Journal of drugs in dermatology: JDD 2015;14:681-684.

21. Langer-Gould A, Qian L, Tartof SY, et al. Vaccines and the Risk of Multiple Sclerosis and Other Central Nervous System Demyelinating DiseasesVaccines and the Risk of Multiple SclerosisVaccines and the Risk of Multiple Sclerosis. JAMA Neurology 2014;71:1506-1513.
22. Mahmud SM, Bozat-Emre S, Mostaço-Guidolin LC, Marrie RA. Registry Cohort Study to Determine Risk for Multiple Sclerosis after Vaccination for Pandemic Influenza A(H1N1) with Arepanrix, Manitoba, Canada. Emerging infectious diseases 2018;24:1267-1274.
23. McNicholas N, Chataway J. Relapse risk in patients with multiple sclerosis after H1N1 vaccination, with or without seasonal influenza vaccination. Journal of Neurology 2011;258:1545-1547.
24. Miller AE, Morgante LA, Buchwald LY, et al. A multicenter, randomized, double-blind, placebo-controlled trial of influenza immunization in multiple sclerosis. Neurology 1997;48:312-314.
25. Mokhtarian F, Shirazian D, Morgante L, Miller A, Grob D, Lichstein E. Influenza virus vaccination of patients with multiple sclerosis. Multiple Sclerosis Journal 1997;3:243-247.
26. Ramagopalan SV, Valdar W, Dyment DA, et al. Association of Infectious Mononucleosis with Multiple Sclerosis. Neuroepidemiology 2009;32:257-262.
27. Scheller NM, Svanström H, Pasternak B, et al. Quadrivalent HPV Vaccination and Risk of Multiple Sclerosis and Other Demyelinating Diseases of the Central Nervous SystemQuadrivalent HPV Vaccination and Demyelinating DiseasesQuadrivalent HPV Vaccination and Demyelinating Diseases. JAMA 2015;313:54-61.
28. Zorzon M, Zivadinov R, Nasuelli D, et al. Risk factors of multiple sclerosis:a case-control study. Neurological Sciences 2003;24:242-247.
29. Landfeldt E, Castelo-Branco A, Svedbom A, Löfroth E, Kavaliunas A, Hillert J. Sick leave and disability pension before and after diagnosis of multiple sclerosis. Multiple Sclerosis Journal 2016;22:1859-1866.
30. Marrie RA, Yu N, Wei Y, Elliott L, Blanchard J. High rates of physician services utilization at least five years before multiple sclerosis diagnosis. Multiple Sclerosis Journal 2012;19:1113-1119.
31. Ristori G, Buzzi MG, Sabatini U, et al. Use of Bacille Calmette–Guèrin (BCG) in multiple sclerosis. Neurology 1999;53:1588-1588.
32. Ristori G, Romano S, Cannoni S, et al. Effects of Bacille Calmette-Guérin after the first demyelinating event in the CNS. Neurology 2014;82:41-48.

385 **Figure 1** Illustration of studied time frames before first diagnosis.

386

387

388 **Figure 2** Odds Ratio of MS for any vaccination

389

390 Odds Ratios of MS for subjects with at least one recorded vaccination compared to subjects
391 without any recorded vaccination. Results are presented for each of the investigated time
392 frames, control cohorts and the three stricter definitions of the MS cohort. The overall effect,
393 excluding the quarter before first diagnosis, is given in the respective top rows. CI = two-
394 sided 95% confidence intervals.

395

Figure 3 Odds Ratio of MS for specific vaccinations

Odds Ratios of MS for subjects with at least one recorded vaccination compared to subjects without such a vaccination. Results are presented for each of the investigated time frames, control cohorts and for specific vaccinations. The overall effect, excluding the quarter before first diagnosis, is given in the respective top rows. CI = two-sided 99.4% confidence intervals. Wide confidence intervals exceeding the limits 0.5 or 2.0 are clipped to arrows.

404 **Table 1** Descriptive statistics of the cohorts.

Cohort	Size	Observable time* (median yrs., IQR)	Women	Age at first diagnosis (yrs.)	vaccinations* (categories 0, 1-2, >2)
MS	12,262	8.75, 7.00 – 10.50	8,528 (69.5%)	39.3±12.5	6,800, 3,662, 1,800 (55.5%, 29.9%, 14.7%)
Crohn's disease	19,296	8.75, 7.25 – 10.75	10,734 (55.6%)	37.5±15.4	9,814, 5,986, 3,496 (50.9%, 31.0%, 18.1%)
Psoriasis	112,292	8.75, 7.25 – 10.50	58,169 (51.8%)	44.8±15.9	57,644, 31,793, 22,855 (51.3%, 28.3%, 20.4%)
Controls	79,185	9.00, 7.25 – 10.75	54,915 (69.4%)	39.5±12.7	41,062, 25,451, 12,672 (51.9%, 32.1%, 16.0%)

405 * prior to first diagnosis, IQR = interquartile range

406

Table 2 Number of subjects with vaccination and cohort sizes

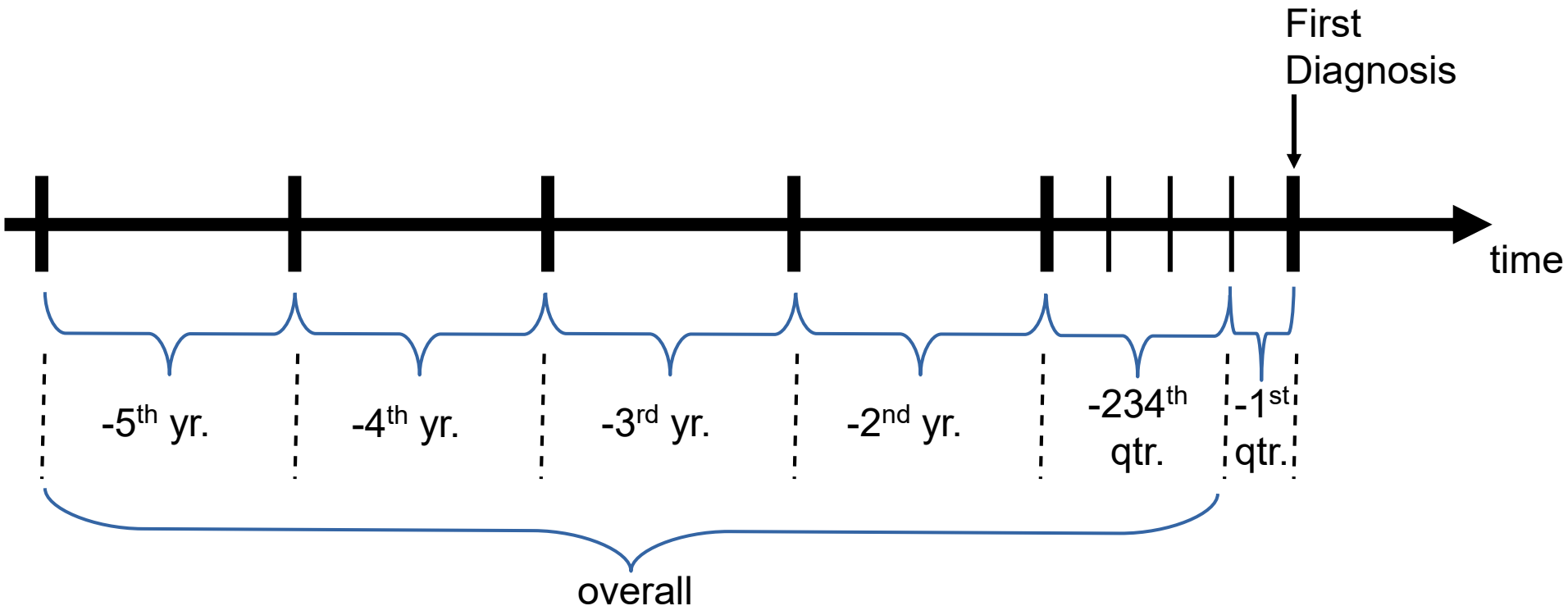
	MS		Control		Chrohn‘s diseases		Psoriasis	
N	12,262		79,185		19,296		112,292	
overall*	5,462	44,5%	38,123	48,1%	9,482	49,1%	54,648	48,7%
-1st qtr.	457	3,7%	3,589	4,5%	893	4,6%	6,382	5,7%
-234th qtr.	1,428	11,6%	9,907	12,5%	2,527	13,1%	16,530	14,7%
-2nd year	1,784	14,5%	12,873	16,3%	3,384	17,5%	21,646	19,3%
-3rd year	1,940	15,8%	13,978	17,7%	3,608	18,7%	22,861	20,4%
-4th year	2,177	17,8%	14,831	18,7%	3,885	20,1%	23,766	21,2%
-5th year	2,185	17,8%	15,343	19,4%	3,974	20,6%	24,245	21,6%

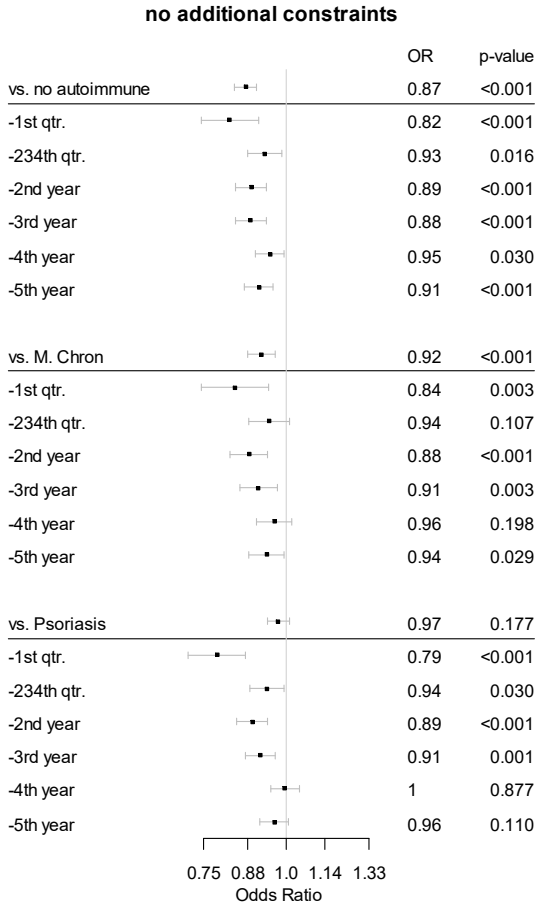
Absolute and relative frequencies of patients with at least one vaccination in the displayed time frames. *overall number of subjects with at least one vaccination in the investigated 5-year period excluding the quarter before first diagnosis.

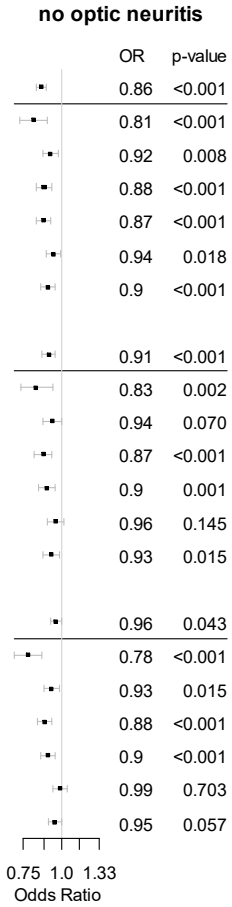
Table 3 Odds Ratio of MS vs. controls by type of vaccination

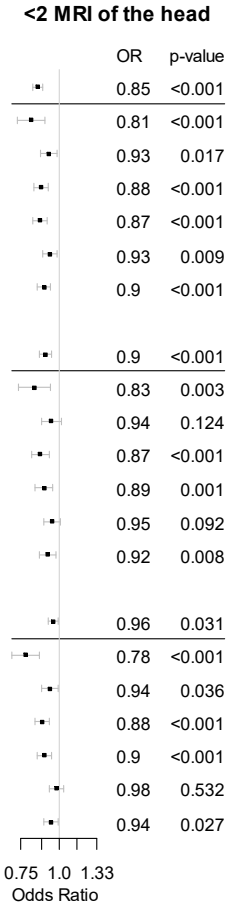
	controls		
vaccination	no autoimmune disease*	Crohn's disease	Psoriasis
tick-borne encephalitis (TBE)	**0.901 [0.844, 0.961]**	**0.899 [0.831, 0.972]**	0.981 [0.919, 1.047]
human papilloma virus	0.852 [0.641, 1.133]	0.824 [0.604, 1.124]	1.284 [0.965, 1.709]
pneumococci	0.900 [0.673, 1.203]	**0.640 [0.465, 0.882]**	**0.638 [0.482, 0.844]**
meningococci	0.977 [0.713, 1.339]	0.751 [0.537, 1.051]	**0.620 [0.456, 0.843]**
influenza	**0.892 [0.834, 0.955]**	**0.905 [0.833, 0.983]**	**0.877 [0.819, 0.938]**
hepatitis A	0.932 [0.726, 1.196]	0.961 [0.709,1.301]	0.952 [0.741, 1.223]
hepatitis B	**0.837 [0.721, 0.972]**	0.849 [0.710, 1.015]	**0.771 [0.664, 0.894]**
mumps, measles, rubella and varicella	0.878 [0.742, 1.039]	0.983 [0.801, 1.206]	0.844 [0.712, 1.000]
tetanus, diphtheria, mumps, poliomyelitis, pertussis, and haemophilus influenzae type b	**0.924 [0.865, 0.987]**	0.977 [0.901, 1.059]	0.999 [0.934, 1.068]

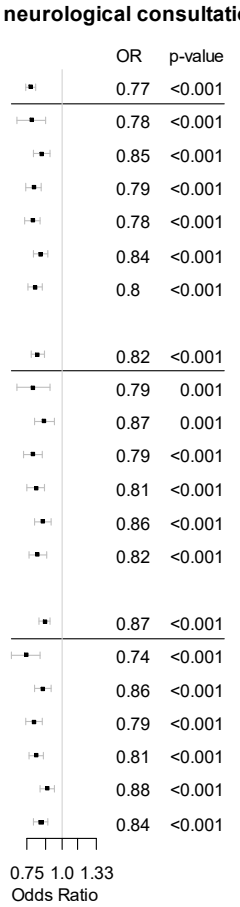
Odds Ratio [two-sided 99.4% confidence interval using Sidak's correction for multiple testing of nine hypotheses] of MS for subjects with at least one recorded vaccination compared to subjects without any recorded vaccination. The overall effect, excluding the quarter before first diagnosis, is presented for each of the predefined subsets of vaccinations and control cohorts. Bold figures indicate statistically significant results. *persons not diagnosed with MS, Crohn's disease or psoriasis.

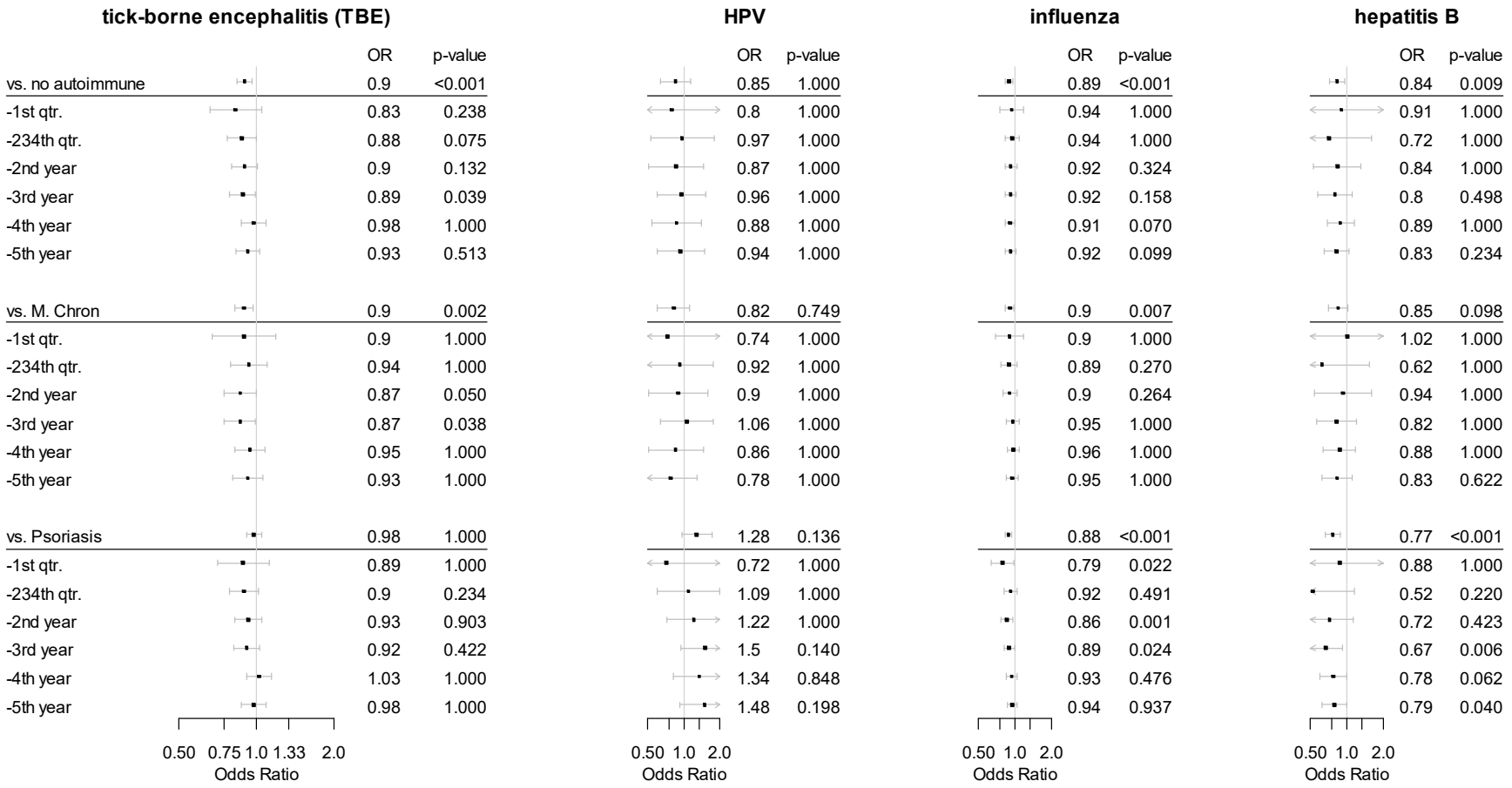

First
Diagnosis
time
-5th yr.
-4th yr.
-3rd yr.
-2nd yr.
-234th
qtr.
-1st
qtr.
overall


no additional constraints
OR
p-value
vs. no autoimmune 0.87 <0.001
-1st qtr. 0.82 <0.001
-234th qtr. 0.93 0.016
-2nd year 0.89 <0.001
-3rd year 0.88 <0.001
-4th year 0.95 0.030
-5th year 0.91 <0.001
vs. M. Chron 0.92 <0.001
-1st qtr. 0.84 0.003
-234th qtr. 0.94 0.107
-2nd year 0.88 <0.001
-3rd year 0.91 0.003
-4th year 0.96 0.198
-5th year 0.94 0.029
vs. Psoriasis 0.97 0.177
-1st qtr. 0.79 <0.001
-234th qtr. 0.94 0.030
-2nd year 0.89 <0.001
-3rd year 0.91 0.001
-4th year 1 0.877
-5th year 0.96 0.110
0.75 0.88 1.0 1.14 1.33
Odds Ratio


no optic neuritis
OR
p-value
0.86 <0.001
0.81 <0.001
0.92 0.008
0.88 <0.001
0.87 <0.001
0.94 0.018
0.9 <0.001
0.91 <0.001
0.83 0.002
0.94 0.070
0.87 <0.001
0.9 0.001
0.96 0.145
0.93 0.015
0.96 0.043
0.78 <0.001
0.93 0.015
0.88 <0.001
0.9 <0.001
0.99 0.703
0.95 0.057
0.75 1.0 1.33
Odds Ratio


<2 MRI of the head
OR
p-value
0.85 <0.001
0.81 <0.001
0.93 0.017
0.88 <0.001
0.87 <0.001
0.93 0.009
0.9 <0.001
0.9 <0.001
0.83 0.003
0.94 0.124
0.87 <0.001
0.89 0.001
0.95 0.092
0.92 0.008
0.96 0.031
0.78 <0.001
0.94 0.036
0.88 <0.001
0.9 <0.001
0.98 0.532
0.94 0.027
0.75 1.0 1.33
Odds Ratio


<2 neurological consultation
OR
p-value
0.77 <0.001
0.78 <0.001
0.85 <0.001
0.79 <0.001
0.78 <0.001
0.84 <0.001
0.8 <0.001
0.82 <0.001
0.79 0.001
0.87 0.001
0.79 <0.001
0.81 <0.001
0.86 <0.001
0.82 <0.001
0.87 <0.001
0.74 <0.001
0.86 <0.001
0.79 <0.001
0.81 <0.001
0.88 <0.001
0.84 <0.001
0.75 1.0 1.33
Odds Ratio


tick-borne encephalitis (TBE)
OR p-value
vs. no autoimmune 0.9 <0.001
-1st qtr. 0.83 0.238
-234th qtr. 0.88 0.075
-2nd year 0.9 0.132
-3rd year 0.89 0.039
-4th year 0.98 1.000
-5th year 0.93 0.513
vs. M. Chron 0.9 0.002
-1st qtr. 0.9 1.000
-234th qtr. 0.94 1.000
-2nd year 0.87 0.050
-3rd year 0.87 0.038
-4th year 0.95 1.000
-5th year 0.93 1.000
vs. Psoriasis 0.98 1.000
-1st qtr. 0.89 1.000
-234th qtr. 0.9 0.234
-2nd year 0.93 0.903
-3rd year 0.92 0.422
-4th year 1.03 1.000
-5th year 0.98 1.000
0.50 0.75 1.0 1.33 2.0
Odds Ratio
HPV
OR p-value
0.85 1.000
0.8 1.000
0.97 1.000
0.87 1.000
0.96 1.000
0.88 1.000
0.94 1.000
0.82 0.749
0.74 1.000
0.92 1.000
0.9 1.000
1.06 1.000
0.86 1.000
0.78 1.000
1.28 0.136
0.72 1.000
1.09 1.000
1.22 1.000
1.5 0.140
1.34 0.848
1.48 0.198
0.50 1.0 2.0
Odds Ratio
influenza
OR p-value
0.89 <0.001
0.94 1.000
0.94 1.000
0.92 0.324
0.92 0.158
0.91 0.070
0.92 0.099
0.9 0.007
0.9 1.000
0.89 0.270
0.9 0.264
0.95 1.000
0.96 1.000
0.95 1.000
0.88 <0.001
0.79 0.022
0.92 0.491
0.86 0.001
0.89 0.024
0.93 0.476
0.94 0.937
0.50 1.0 2.0
Odds Ratio
hepatitis B
OR p-value
0.84 0.009
0.91 1.000
0.72 1.000
0.84 1.000
0.8 0.498
0.89 1.000
0.83 0.234
0.85 0.098
1.02 1.000
0.62 1.000
0.94 1.000
0.82 1.000
0.88 1.000
0.83 0.622
0.77 <0.001
0.88 1.000
0.52 0.220
0.72 0.423
0.67 0.006
0.78 0.062
0.79 0.040
0.50 1.0 2.0
Odds Ratio